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


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. Phytoergic 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, hypocholesterolemic and anti-inflammatory effects (Cowan, 1999; Craig, 1999; Faleiro et al., 2003). Recoilillay (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...
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-bergapten, β-bisabolene, linalool (8%), linalyl acetate (28%), nerol, neryl acetate, geraniol, geraniol acetate and α-terpineol (Cum et al., 1991). Kırbaşlar et al. (2001) indicated that the chemical composition of the bergamot oil was strongly influenced by harvesting time. It was declared 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: (Folch et al., 1957) yolk samples were methylated for gas chromatographic analysis (GC- Agilent 6890 Mass, a fused silica capillary column, and film thickness of 0.25 μm). Oven temperature was from 165°C to 200°C at 5°C/min. Detector temperature was 200°C; 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 ml/kg bergamot oil had induced decreases in feed intake by 13.5%. There were no effects of the ex-
The addition of bergamot oil to the basal diet had no impact on albumen percentage of egg, 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 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.

The results showed that the addition of bergamot oil to the basal diet had no impact on albumen percentage of egg, eggshell thickness and Haugh unit in this study. The yolk had induced decreases in Ca concentration by 1.06±0.07 mg/dL. The diet with 0.50 ml/kg bergamot oil showed the highest yolk percentage of egg. Shell thickness and breaking strength in this study were shown in Table 2. The addition of 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.
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 (Langout, 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 affected 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; Aydin et al., 2006; Çabuk et al., 2006; Böyükbaş et al., 2007, 2008). In accordance with the present findings, Böyükbaş 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 Böyükbaş et al. (2008) reported that addition of essential oils(thyme, sage, rosemary) to diet significantly reduced proportion of yolk compared with the control. However, Böyükbaş 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, Böyükbaş 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 thymociene 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 Böyükbaş 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

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

<table>
<thead>
<tr>
<th>Fatty acids (%)</th>
<th>Control</th>
<th>Bergamot oil 0.25 ml/kg</th>
<th>Bergamot oil 0.50 ml/kg</th>
<th>Bergamot oil 0.75 ml/kg</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic</td>
<td>22.77 ± 0.29</td>
<td>21.79 ± 0.60</td>
<td>25.09 ± 0.46</td>
<td>22.21 ± 0.58</td>
<td>NS</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>3.00 ± 0.09</td>
<td>2.60 ± 0.21</td>
<td>2.75 ± 0.01</td>
<td>2.48 ± 0.15</td>
<td>NS</td>
</tr>
<tr>
<td>Stearic</td>
<td>9.25 ± 0.19</td>
<td>10.08 ± 0.21</td>
<td>13.58 ± 0.25</td>
<td>10.33 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Oleic</td>
<td>39.67 ± 0.9</td>
<td>39.60 ± 1.4</td>
<td>41.07 ± 0.9</td>
<td>37.15 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Linoleic</td>
<td>18.49 ± 1.3</td>
<td>18.07 ± 0.6</td>
<td>15.63 ± 1.3</td>
<td>17.64 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Linolenic</td>
<td>0.15 ± 0.03</td>
<td>0.13 ± 0.02</td>
<td>0.26 ± 0.03</td>
<td>0.22 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>0.35 ± 0.07</td>
<td>0.20 ± 0.06</td>
<td>0.29 ± 0.02</td>
<td>0.31 ± 0.07</td>
<td>NS</td>
</tr>
<tr>
<td>EPA</td>
<td>2.20 ± 0.09</td>
<td>2.44 ± 0.11</td>
<td>2.86 ± 0.12</td>
<td>2.82 ± 0.08</td>
<td>*</td>
</tr>
<tr>
<td>DHA</td>
<td>0.80 ± 0.02</td>
<td>1.32 ± 0.06</td>
<td>1.38 ± 0.13</td>
<td>1.16 ± 0.14</td>
<td>**</td>
</tr>
<tr>
<td>SFA</td>
<td>32.03 ± 0.87</td>
<td>31.87 ± 0.98</td>
<td>30.60 ± 0.73</td>
<td>32.54 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>MUFA</td>
<td>42.67 ± 0.87</td>
<td>42.20 ± 1.58</td>
<td>43.34 ± 0.55</td>
<td>39.64 ± 1.10</td>
<td>NS</td>
</tr>
<tr>
<td>PUFA</td>
<td>22.00 ± 1.07</td>
<td>22.17 ± 1.03</td>
<td>20.44 ± 0.68</td>
<td>22.16 ± 0.35</td>
<td>NS</td>
</tr>
<tr>
<td>n-3</td>
<td>3.15 ± 0.14</td>
<td>3.89 ± 0.15</td>
<td>4.51 ± 0.27</td>
<td>4.20 ± 0.25</td>
<td>**</td>
</tr>
<tr>
<td>n-6</td>
<td>18.84 ± 1.66</td>
<td>18.27 ± 1.35</td>
<td>15.78 ± 0.48</td>
<td>17.95 ± 1.54</td>
<td>NS</td>
</tr>
<tr>
<td>n-6/n-3</td>
<td>5.97 ± 0.49</td>
<td>4.69 ± 0.62</td>
<td>3.52 ± 0.16</td>
<td>4.26 ± 0.28</td>
<td>*</td>
</tr>
</tbody>
</table>

\*: \( P<0.05 \), **: \( P<0.01 \), NS: not significant, \( a,b,c \): Column means with no common superscript differ significantly.
hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity which is a key regulatory enzyme in cholesterol synthesis by essential oils (Crewell, 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 ml/kg bergamot oil in basal diet did not affect eggshell strength. Occhiuto and Circosta (1996, 1997) explained that bergamottin, another important component of the non-volatile fraction of bergamot essential oil, might be endowed with Ca 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 ml/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. Moreover, 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 (Bölükbaşi 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 ml/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 ml/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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