Effect of dietary supplementation of gallic acid and linoleic acid mixture or their synthetic salt on egg quality

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ABSTRACT

The effect of a dietary supplementation of gallic acid and linoleic acid mixture (MGL) and their synthetic salt, sodium 2,3-dihydroxy-5-[(9E,12E)-octadeca-9,12-diendyloxy]carbonyl]phenolate (NGL), on egg quality was investigated. A total of 120 laying hens were allotted into five groups over 4 weeks of the experimental period. Birds were fed the following diets: (1) control [commercial diet (CD)], (2) 0.05% MGL (w/w, GA:LA = 1:1, equal molar ratio), (3) 0.1% MGL, (4) 0.05% NGL, (5) 0.1% NGL. The performance of the hen, the anti-oxidative potential of egg albumen and yolk, and the fatty acid composition and cholesterol content of egg yolk were measured. The TBARS value of egg yolk from hens fed 0.1% MGL and 0.05% NGL was lower than that fed control diet after storage for 14 days. The ABTS’ reducing activity of egg albumen was significantly improved by MGL and NGL, but only NGL had an effect on yolk (p < 0.05). The dietary supplementation of 0.05% or 0.1% MGL, and 0.05% NGL raised the PUFAs composition in egg yolk. The cholesterol content of egg yolk from hens fed control diet was higher than those fed 0.1% MGL, 0.05% or 0.1% NGL (p < 0.05). In conclusion, a diet consisting of MGL and NGL can improve the antioxidative potential of egg and the fatty acid quality of egg yolk while lowering the cholesterol level.

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1. Introduction

Although eggs are an important source of nutrients due to their high quality protein and variety of vitamins and minerals, it is often recommended that people restrict their consumption since egg yolk contains nearly 30% lipids and high cholesterol content (Milinsk et al., 2003; Weggemans, Zock, & Katan, 2001). Egg consumption is also believed to raise the risk of cardiovascular disease by increasing blood cholesterol levels (Weggemans et al., 2001). However, previous studies have found that dietary cholesterol does not contribute to high blood cholesterol, and there has been little evidence that egg ingestion causes increases in serum lipid or total cholesterol levels (McNamara, 2002).

On the other hand, polyunsaturated fatty acids (PUFAs) contained in the lipids of egg yolk are regarded as a substantial nutrient as they prevent coronary heart disease and other chronic diseases (Russo, 2009). A high ratio of unsaturated fatty acids to saturated fatty acids could diminish the negative effects of high cholesterol intake (Milinsk et al., 2003). For this reason, several studies had been conducted to raise PUFA content in eggs by using dietary fat sources, such as natural and fish oil containing PUFAs (Ronzoni-Esquerra & Leeson, 2000; Lawlor, Gaudette, Dickson, & House, 2010). However, increased content of PUFAs in egg may result in oxidation since they are the primary targets of free radicals prior to initiation of peroxidation (Scisloewski, Bauchart, Gruffat, Laplaud, & Durand, 2005). The oxidation products of PUFAs lead to deterioration of food quality, such as flavour, colour, texture, and nutritional value (Mielnik, Olsen, Vogt, Adeline, & Skrede, 2006). While lipid peroxidation of fresh shell egg is not readily generated, it is facilitated when eggs are preserved and cooked. Therefore, antioxidants of a kind of those natural polyphenols and tocopherols have been used as dietary supplementation (Gladinea, Morandb, Rockb, Baucharta, & Duranda, 2007; Meluzzi, Sirri, Manfreda, Tallarico, & Franchini, 2000).

Octadeca-9,12-diend-3,4,5-hydroxybenzoate (GA–LA) is synthesized from gallic acid (GA) and linoleic acid (LA; C18:2n-6) (Jo, Jeong, Lee, Kim, & Byun, 2006). GA is a representative natural polyno phenol found in wine, grapes, and tea (Hogan, Zhang, Li, Zoeklein, & Zhou, 2009; Kim et al., 2006). As a metabolite of propyl gallate, GA is known to possess several pharmacological and biological activities, including anti-oxidative, anti-carcinogenic, anti-mutagenic, anti-allergic, and anti-inflammatory activities (Jo et al., 2006). LA is an essential fatty acid as well as the primary precursor of all n-6 PUFAs (Russo, 2009). It is
converted to arachidonic acid (AA; C_{20:4}n-6) in animal tissues and has been shown to have anti-inflammatory effects by decreasing the secretion of interleukin (IL)-6 and -1β as well as that of tumor necrosis factor α (Zha et al., 2005). Jo et al. (2006) reported that synthesized GA–LA possesses greater biological function than GA, including antioxidative activity by free radical scavenging ability, skin whitening by tyrosinase inhibition which is more powerful than ascorbic acid, and anti-inflammatory effects by cyclooxygenase 1 and 2 inhibition. Jang et al. (2008) also reported that GA–LA showed a hypolipidemic activity in mice induced by high-fat diet. Jang et al. (2009) indicated that GA–LA had a strong and synergistic inhibitory effect on cancer cell proliferation (in vitro), and had higher enzyme inhibition ability, such as tyrosinase, hyaluronidase, and xanthine oxidase when compared with those of tocopherol. Furthermore, synthesized GA–LA as well as a mixture of GA and LA (MGL) decreases serum triglycerides as well as the total serum cholesterol levels (in vivo) (Jang et al., 2008, 2009). Further, a previous study showed that dietary supplementation with MGL improved the antioxidative potential as well as the ratio of unsaturated fatty acids to saturated fatty acids in broiler meat (Jung et al., 2010).

To be used more conveniently as a functional material, high solubility and bioavailability are key properties in pharmaceutical compounds (Serajuddin, 2007). Therefore, in the present study, the salt form of GA–LA was developed (NGL), and the effect of dietary supplementation with NGL or MGL was investigated on hen performance and egg quality attributes.

2. Materials and methods

2.1. Chemicals

Linoleic acid ethyl ester, gallic acid, diisobutylaluminum hydride (DIBAL-H), tetrahydrofuran (THF), anisole, 2-naphthalene sulfonic acid, sodium methoxide, 2-thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Synthesis

The synthesis was performed by the modified method of Jo et al. (2006), as described by the following steps (Fig. 1). From octadeca-9,12-dienyl-3,4,5-hydroxybenzoate (GA–LA, compound 4), the sodium 2,3-dihydroxy-5-((9E,12E)-octadeca-9,12-dienyl-oxo)carbonyl)phenolate (NGL) was synthesized. A solution of sodium methoxide (1.3 mM) and compound 4 (3 mM) was mixed in with methanol (10 ml) at room temperature. After stirring the solution at room temperature for 4 h, the solvent was removed under reduced pressure. The crude compound was purified by column chromatography (SiO₂, elution with hexane). The final product was obtained as dark green powder with 97% yield; 1H NMR (400 MHz, CDCl₃) δ 0.9 (t, J = 6.9 Hz, 3H, H₁₈), 1.29 (m, 16H, H₃–7, H₁₅–1₇), 1.73 (m, 2H, H₂), 2.05 (m, 4H, H₈, H₁₄), 2.78 (dd, J = 6.1, 6.1 Hz 2H, H₁₁), 4.26 (t, J = 6.3 Hz 2H, –OCH₂), 5.35 (m, 4H), 5.8 (s, 2H, –OH), 7.26 (s, 1H, aromatic); IR (CHCl₃) 3570, 3357, 3013, 2918, 2865, 1691, 1515, 1465, 1390, 1242, 1198 cm⁻¹.

The estimated water solubilities of NGL were 82.0% and 62.4% at 1000 and 10,000 ppm, respectively.

2.3. Animals and experimental design

A total of 120 laying hens (18 wk old, Led Lohman) were obtained from a commercial flock. Laying hens of similar body weight (1.7 ± 0.1 kg) were randomly distributed into five groups (12 hens/treatment, 2 hens/cage). They were reared in accordance with university guidelines for animal experimentation and were maintained under standard conditions of temperature, humidity, and ventilation with 16 h of fluorescent lighting for the entire experimental period. Birds were fed a commercial diet until 90% egg production was obtained. Each treatment consisted of six replicates containing four birds each. During the 4 week experimental period, birds were fed the following diets: (1) control [commercial diet containing four birds each. During the 4 week experimental period, birds were fed the following diets: (1) control [commercial diet (CD)], (2) 0.05% MGL (w/w, GA:LA = 1:1, equal molar ratio), (3) 0.1% MGL, (4) 0.05% NGL, (5) 0.1% NGL. The control diet was a typical commercial diet consisting of approximately 15.2% crude protein, 4.5% crude fat, 3.1% crude fibre, and 2700 ME kcal/kg (Chunhajeil Feed Co., Daejeon, Korea). The treatment diets were based on the commercial diet supplemented with 0.05% MGL, 0.1% MGL, 0.05% GA–LA, or 0.1% GA–LA. Clean drinking water was supplied ad libitum. The experimental diets were mixed every 3 days, at which point the feed intake was calculated. The eggs were gathered daily and weighed individually.

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Fig. 1. Scheme for sodium 2,3-dihydroxy-5-((9E,12E)-octadeca-9,12-dienyl-oxo)carbonyl)phenolate. 1. linoleic acid ethyl ester; 2. octadeca-9,12-diene-ol; 3. gallic acid; 4. octadeca-9,12-dienyl-3,4,5-trimethoxybenzoate; 5. sodium 2,3-dihydroxy-5-((9E,12E)-octadeca-9,12-dienyl-oxo)carbonyl)phenolate. Reagents and reaction conditions: (i) diisobutylaluminum hydride (2–3 M eq. of compound 1) CHCl₃ at 78 °C; (ii) 2-naphthalene sulfonic acid (0.05 M eq.), anisole at 150 °C for 17 h; (iii) sodium methoxide (0.43 M eq.), methanol at room temperature for 4 h.
2.4. Laying hens performance and egg quality

Daily feed intake was recorded, and the feed conversion ratio (FCR, feed intake:mass production) was calculated during the 4 week feeding periods. Daily egg production and individual egg weights were recorded, and the egg mass production (g/d/hen) was also determined. Whole eggs from each group of laying hens were collected at the 27 and 28 day feeding periods in order to measure egg quality, such as yolk colour and Haugh units (HU), during storage for 14 days at room temperature. The yolk colour and HU were determined using the GCM+ System (Technical Services and Supplies, York, England).

2.5. Blood collection and analysis

At the end of the experiment, a hen from each cage was randomly selected. Blood was collected via wing vein puncture using a syringe (Greenject-5, Doowon MediTec Co., Co., Gimje, Korea), placed into vacuum tubes containing ethylenediaminetetraacetic acid, and stored at −4 °C. Samples were centrifuged (Union 32R, Hanil Co., Ltd., Inchun, Korea) at 1500g for 15 min after which the serum was separated. The separated serum was used to measure the contents of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total cholesterol (T. chol), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).

2.6. 2-Thiobarbituric acid-reactive substances (TBARS) value

After measurement of the egg quality, the egg yolks were separated and pooled for analysis of TBARS. 2.2-azinobis-(3 ethylbenzothiazoline-6-sulfphonic acid) (ABTS)’ reducing activity, fatty acid composition, and cholesterol content.

The egg yolk sample (5 g) in 15 ml of distilled water was homogenised (T25b, Ika Works (Asia), Sdn, Bhd, Malaysia) at 1130g for 1 min. The sample homogenate (1 ml) was transferred to a test tube, and the lipid oxidation was determined as the TBARS value by following the method described by Liu et al. (2009a). Briefly, 50 μl of butylated hydroxyanisol (7.2%) and 2 ml of TBA-TCA solution (20 mM TBA in 15% TCA) were added to the test tube. Tubes were then heated (90 °C) in a boiling water bath for 30 min, cooled, and then centrifuged at 2090g for 15 min. Absorbance of the supernatant was measured at 532 nm with a spectrophotometer (Beckman) at 734 nm, with ethanol as a blank. The percentage inhibition was calculated by the following equation:

\[
\text{ABTS reducing activity (\%)} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control} \times 100}
\]

2.8. Fatty acid composition

The total lipid of the samples was extracted using chloroform-methanol (2:1, v/v) according to the procedure of Folch, Lees, and Sloane Stanley (1957). Fatty acid methyl esters were prepared from the extracted lipids with BF3-methanol (Sigma–Aldrich), followed by separation on a gas chromatograph (HP-6890N, Palo Alto, CA, USA). A split inlet (split ratio, 50:1) was used to inject the samples into a capillary column (Omegawax 320, Supelco, Bellefonte, PA, USA; 30 m × 0.25 mm × 0.25 μm), and ramped oven temperature was used (150 °C for 3 min, increased to 180 °C at 2.5 °C/min and maintained for 5 min, then increased to 220 °C at 2.5 °C/min and maintained for 25 min). The inlet temperature was 210 °C. Air served as the carrier gas at a constant flow rate of 0.7 ml/min.

2.9. Cholesterol content

Egg yolks (1 g) were saponified with 20 ml of 33% ethanolic KOH in tightly-capped tubes placed in a 60 °C water bath for 1 h. The mixture was then cooled in ice water, and 5 ml of distilled water was added. Cholesterol in unsaponifiable fractions was extracted twice with 5 ml of hexane. The resulting aliquot of hexane containing cholesterol was dried under nitrogen, redissolved in 5 ml of hexane, and injected into a gas chromatograph (HP-6890 N, Palo Alto, CA, USA). 5α-cholestane (Sigma–Aldrich) was used as an internal standard. A split inlet (split ratio, 100:1) was used to inject samples into a capillary column (HP-5, Agilent, Steven, CA, USA; 30 m × 0.53 mm × 0.5 μm), and the ramped oven temperature was 270 °C isothermal, detector temperature was 300 °C, and inlet temperature was 210 °C. N2 served as the carrier gas at a constant flow rate of 1.0 ml/min.

2.10. Statistical analysis

The experiment was conducted under the completely randomised design. Analysis of variance was performed using the raw data, and the mean values and standard errors of the means (SEM) were calculated by the Statistical Analysis System (SAS, 2000). Differences amongst the means were determined by the Duncan’s multiple range test with a significance defined at \( p < 0.05 \).

3. Results

3.1. Blood profile of laying hen

The levels of serum GOT (also known as aspartate aminotransferase, AST) and GPT (known as alanine aminotransferase, ALT) in the hens fed MGL or NGL were not significantly different from that of the hens fed control diet. Concentrations of T. col, TG, HDL-C, and LDL-C in serum were not affected by the diet supplemented with MGL or NGL (Table 1).

3.2. Hen performance and egg quality

The treatments did not influence egg production, egg weight, egg mass, or feed intake of hens, whereas FCR was significantly lower \( (p < 0.05) \) in the hens fed a diet supplemented with 0.1% MGL compared to that of the other treatments during the 28 day feeding periods (Table 2).
The general results of egg quality are shown in Table 3. The results show significant differences \((p < 0.05)\) in the Haugh unit. Specifically, the Haugh units of the eggs from the hens treated with 0.1\% MGL, 0.05 or 0.1\% NGL were higher than that from the hens fed a control diet after storage for 7 days, and that from the hens fed 0.05\% and 0.1\% NGL were higher compared to the control diet after storage for 14 days. Although the changes in yolk colour after treatment showed no regular trend, the yolk colour values of the eggs were significantly reduced \((p < 0.05)\) in the eggs from hens fed 0.1\% MGL and 0.1\% NGL (8.83 and 8.58 after storage for 0 day), 0.1\% NGL (7.33 after storage for 7 days), and 0.05\% NGL (7.25 after storage for 14 days) compared to that of the control (9.25, 7.75, and 8.00, respectively).

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GOT (U/l)</th>
<th>GPT (U/l)</th>
<th>T. chol (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>191 x,y</td>
<td>2.3 x,y</td>
<td>48</td>
<td>310</td>
<td>31</td>
<td>16</td>
</tr>
<tr>
<td>MGL 0.05%</td>
<td>179 y</td>
<td>2.0 y</td>
<td>64</td>
<td>400</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>MGL 0.1%</td>
<td>206 x,y</td>
<td>3.3 x</td>
<td>64</td>
<td>368</td>
<td>33</td>
<td>15</td>
</tr>
<tr>
<td>NGL 0.05%</td>
<td>187 y</td>
<td>3.3 x</td>
<td>46</td>
<td>234</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>NGL 0.1%</td>
<td>211 x</td>
<td>2.6 x,y</td>
<td>51</td>
<td>228</td>
<td>33</td>
<td>11</td>
</tr>
<tr>
<td>SEM(\a)</td>
<td>6.8</td>
<td>0.30</td>
<td>6.2</td>
<td>62.0</td>
<td>2.8</td>
<td>2.7</td>
</tr>
</tbody>
</table>

\(\a\) Sodium 2,3-dihydroxy-5-\(((9E,12E)-octadeca-9,12-dienyloxy)carbonyl)phenolate.

The general results of egg quality are shown in Table 3. The results show significant differences \((p < 0.05)\) in the Haugh unit. Specifically, the Haugh units of the eggs from the hens treated with 0.1\% MGL, 0.05 or 0.1\% NGL were higher than that from the hens fed a control diet after storage for 7 days, and that from the hens fed 0.05\% and 0.1\% NGL were higher compared to the control diet after storage for 14 days. Although the changes in yolk colour after treatment showed no regular trend, the yolk colour values of the eggs were significantly reduced \((p < 0.05)\) in the eggs from hens fed 0.1\% MGL and 0.1\% NGL (8.83 and 8.58 after storage for 0 day), 0.1\% NGL (7.33 after storage for 7 days), and 0.05\% NGL (7.25 after storage for 14 days) compared to that of the control (9.25, 7.75, and 8.00, respectively).

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Egg production (%)</th>
<th>Egg weight (g)</th>
<th>Egg mass (g/d/hen)</th>
<th>Feed intake (g/d/hen)</th>
<th>Feed conversion efficiency^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>97.64</td>
<td>57.63</td>
<td>56.29</td>
<td>123.11</td>
<td>2.19(\a)</td>
</tr>
<tr>
<td>MGL 0.05%</td>
<td>97.82</td>
<td>57.11</td>
<td>55.86</td>
<td>118.96</td>
<td>2.13(\a)</td>
</tr>
<tr>
<td>MGL 0.1%</td>
<td>97.97</td>
<td>58.54</td>
<td>57.35</td>
<td>117.00</td>
<td>2.04(\a)</td>
</tr>
<tr>
<td>NGL 0.05%</td>
<td>98.59</td>
<td>58.10</td>
<td>57.28</td>
<td>120.15</td>
<td>2.09(\a)</td>
</tr>
<tr>
<td>NGL 0.1%</td>
<td>97.97</td>
<td>57.86</td>
<td>57.48</td>
<td>121.46</td>
<td>2.14(\a)</td>
</tr>
<tr>
<td>SEM(\a)</td>
<td>0.668</td>
<td>0.551</td>
<td>0.691</td>
<td>2.164</td>
<td>0.692</td>
</tr>
</tbody>
</table>

\(\a\) Sodium 2,3-dihydroxy-5-\(((9E,12E)-octadeca-9,12-dienyloxy)carbonyl)phenolate.

The general results of egg quality are shown in Table 3. The results show significant differences \((p < 0.05)\) in the Haugh unit. Specifically, the Haugh units of the eggs from the hens treated with 0.1\% MGL, 0.05 or 0.1\% NGL were higher than that from the hens fed a control diet after storage for 7 days, and that from the hens fed 0.05\% and 0.1\% NGL were higher compared to the control diet after storage for 14 days. Although the changes in yolk colour after treatment showed no regular trend, the yolk colour values of the eggs were significantly reduced \((p < 0.05)\) in the eggs from hens fed 0.1\% MGL and 0.1\% NGL (8.83 and 8.58 after storage for 0 day), 0.1\% NGL (7.33 after storage for 7 days), and 0.05\% NGL (7.25 after storage for 14 days) compared to that of the control (9.25, 7.75, and 8.00, respectively).

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage (day)</th>
<th>SEM(\a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Haugh unit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>99.3 x,y</td>
<td>65.7 x,y</td>
</tr>
<tr>
<td>MGL 0.05%</td>
<td>100.7 x</td>
<td>66.7 x</td>
</tr>
<tr>
<td>MGL 0.1%</td>
<td>101.6 x,y</td>
<td>75.1 x,y</td>
</tr>
<tr>
<td>NGL 0.05%</td>
<td>103.8 x</td>
<td>81.3 x</td>
</tr>
<tr>
<td>NGL 0.1%</td>
<td>103.1 x</td>
<td>80.2 x</td>
</tr>
<tr>
<td>SEM(\a)</td>
<td>1.64</td>
<td>2.37</td>
</tr>
<tr>
<td>Yolk colour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.25 x,y</td>
<td>7.75 x,y</td>
</tr>
<tr>
<td>MGL 0.05%</td>
<td>9.06 x,y</td>
<td>8.08 x</td>
</tr>
<tr>
<td>MGL 0.1%</td>
<td>8.83 x,y</td>
<td>7.58 x,y</td>
</tr>
<tr>
<td>NGL 0.05%</td>
<td>8.92 x,y</td>
<td>7.67 x,y</td>
</tr>
<tr>
<td>NGL 0.1%</td>
<td>8.56 x,y</td>
<td>7.33 x,y</td>
</tr>
<tr>
<td>SEM(\a)</td>
<td>0.115</td>
<td>0.163</td>
</tr>
</tbody>
</table>

\(\a\) Sodium 2,3-dihydroxy-5-\(((9E,12E)-octadeca-9,12-dienyloxy)carbonyl)phenolate.

3.3. ABTS\(^{+}\) reducing activity

The ABTS\(^{+}\) reducing activity of egg albumen and yolk are shown in Fig. 2. The albumen of eggs from hens fed 0.1\% MGL (42.4%), and 0.05 (49.9%) or 0.1\% NGL (48.0%) had significantly higher ABTS\(^{+}\) reducing activity \((p < 0.05)\) compared to the control (38.6%) after 28 days of feeding. Further, the ABTS\(^{+}\) reducing activity of egg yolk from the hens fed 0.05\% (20.8%) and 0.1\% NGL (19.2%) was significantly higher than that of the control (8.1%).
3.4. 2-Thiobarbituric acid-reactive substances (TBARS) of egg yolk

Dietary supplementation with both MGL and NGL inhibited the development of lipid oxidation in egg yolk (Table 4). The TBARS values of egg yolk from hens fed 0.05% and 0.1% MGL as well as 0.05% and 0.1% NGL showed significantly lower values (p < 0.05) than those of the control after storage for 7 days at room temperature. However, only the egg yolk from the hens fed 0.1% MGL and 0.05% NGL differed from that of control after 14 days of storage.

3.5. Fatty acid composition and cholesterol content of egg yolk

There were significant differences in the fatty acid composition amongst the treatments (Table 5, p < 0.05). Dietary supplementation of MGL and NGL decreased the contents of palmitic and palmitoleic acid composition of egg yolk (p < 0.05). The content of stearic acid in the egg yolk from the hens fed 0.1% MGL (10.58%), and 0.05% or 0.1% NGL (10.62% and 11.17%, respectively) were higher than that fed the control diet (10.21%) (p < 0.05). The dietary supplementation with 0.1% MGL and 0.1% NGL increased the content of oleic acid in egg yolk compared to the control diet (p < 0.05). The linoleic acid content of egg yolk was increased by 0.05% MGL (15.93%) and 0.05% NGL (16.02), but 0.1% MGL (14.97%) and 0.1% NGL (14.96%) decreased the linoleic acid content of egg yolk when compared to the control diet (15.24%) (p < 0.05). The dietary supplementation with 0.1% MGL decreased the linolenic acid content of egg yolk, however, 0.05% NGL increased the linolenic acid content of egg yolk compared to other treatments (p < 0.05). Arachidonic and docosahexaenoic acids were not influenced by the dietary treatments. Regarding changes in the fatty acid composition, the ratio of unsaturated:saturated fatty acid of egg yolk was higher in the samples fed 0.05% or 0.1% MGL (1.68% and 1.67, respectively) and 0.05% NGL (1.69) than the control (1.62) (p < 0.05). The cholesterol contents of egg yolk were affected by dietary supplementation with MGL or NGL (Fig. 3). Specifically, cholesterol content of egg yolk from hens fed control diet was 17.69 mg/g, whereas that of egg yolk from hens fed 0.1% MGL, and 0.05% or 0.1% NGL were 15.4, 14.6, and 15.5 mg/g of egg yolk, respectively, which were significantly lower than that of the control diet (p < 0.05).

4. Discussion

The water solubility of a functional material is vital for its wider application. Sodium 2,3-dihydroxy-5-(((9E,12E)-octadeca-9,12-dienyloxy)carbonyl)phenolate (NGL), which is the salt form of octadeca-9,12-dienyl-3,4,5-hydroxybenzoate (GA–LA), is water soluble even though GA–LA is an insoluble material. It is important to determine the effects of feed additives on the biochemistry of the animals when new materials are added to feedstuffs. Serum GOT and GPT activities can be used as biochemical indicators for liver function; an increase in these enzyme activities indicates hepatocellular damage (Han, Huang, Li, Jiang, & Xu, 2008). In this work, serum GOT and GPT activities were not affected by dietary supplementation with either MGL or NGL. This result implies that dietary MGL and NGL may not have a negative effect on liver function.

Table 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage (day)</th>
<th>SEM²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.23³</td>
<td>0.010</td>
</tr>
<tr>
<td>MGL 0.05%</td>
<td>0.23³</td>
<td>0.021</td>
</tr>
<tr>
<td>MGL 0.1%</td>
<td>0.26³</td>
<td>0.018</td>
</tr>
<tr>
<td>NGL 0.05%</td>
<td>0.23³</td>
<td>0.021</td>
</tr>
<tr>
<td>NGL 0.1%</td>
<td>0.21³</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Table 5

<table>
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<th>Fatty acid</th>
<th>Treatment</th>
<th>SEM⁶</th>
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<tbody>
<tr>
<td>C16:0</td>
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<td>26.89⁴</td>
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<tr>
<td>C16:1</td>
<td>3.52⁴</td>
<td>2.94⁴</td>
</tr>
<tr>
<td>C18:0</td>
<td>10.21⁴</td>
<td>10.58⁴</td>
</tr>
<tr>
<td>C18:1</td>
<td>37.98⁴</td>
<td>39.61⁴</td>
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<tr>
<td>C18:2</td>
<td>15.24⁴</td>
<td>14.97⁴</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.25⁴</td>
<td>0.19⁴</td>
</tr>
<tr>
<td>C20:4</td>
<td>3.56⁴</td>
<td>3.48⁴</td>
</tr>
<tr>
<td>C22:6</td>
<td>1.34⁴</td>
<td>1.32⁴</td>
</tr>
</tbody>
</table>

Fig. 3. Cholesterol content of egg yolk from laying hens fed a dietary gallic acid and linoleic acid mixture (MGL), and sodium 2,3-dihydroxy-5-(((9E,12E)-octadeca-9,12-dienyloxy)carbonyl)phenolate (NGL). a,bDifferent letters within albumen differ significantly (p < 0.05).

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effect on the biochemical function of laying hens. Dietary MGL and NGL did not significantly impact the concentrations of serum T. chol, TG, HDL-C, or LDL-C in the hens. These results are in disagreement with a previous study that showed that dietary GA–LA and the mixed form of GA and LA (MGL) reduced the levels of serum TG (22% and 26%) and LDL-C (17% and 26%) in mice fed a high-fat diet supplemented with 1.0% GA–LA and 1.0% MGL (Jang et al., 2008). Additionally, the serum TG level decreased by 56% when dietary 1.0% MGL was fed to the broilers (Jung et al., 2010).

The FCR of hens was improved by dietary supplementation with 0.1% MGL compared to that of control, whereas egg production, egg weight, egg mass, and feed intake were not influenced by either MGL or NGL diets. FCR is generally related to the energy concentration of the diet. In addition, the amount of antioxidants in the diet also affects the FCR by preventing the decrease in utilisation of nutrients caused by environmental stress (Frikha, Safaa, Serrano, Arbe, & Mateos, 2009; Sahin et al., 2006). A diet containing MGL has linoleic acid, which may raise the energy content. However, Safaa et al. (2008) reported that increasing the linoleic acid concentration of a diet from 1.12% to 1.60% had no effect on the FCR. However, the level of dietary linoleic acid in the present study was lower than that of the previous study. Therefore, this improved FCR was not due to the addition of linoleic acid but instead from the antioxidative activity of gallic acid in the MGL diet.

The best index of internal egg quality is the Haugh unit (HU), which is calculated by measuring the height of the inner thick albumen and the egg weight. A reduced HU indicates deterioration of egg freshness. In the present study, the experimental diets did not affect the HU of eggs on the day of lay, but differences in HU were observed in the eggs from hens fed 0.1% MGL or both 0.05 and 0.1% NGL compared to the eggs from hens fed control diet. This result is consistent with previous studies. The HU on the day of lay was not affected by diet supplemented with tallow, sunflower oil, or flaxseed oil as the fatty acid source, or ‘lycopene or vitamin E as the antioxidant’ (Celebi & Macit, 2008; Sahin et al., 2006). Hayat, Cherian, Pasha, Khattak, and Jabbar (2009) reported that the albumen height and HU of the egg were affected by a diet supplemented with flax and antioxidants. However, a reduction in HU in egg during storage was delayed by feeding with organic selenium as an antioxidant (Pappas, Acamovic, Sparks, Surai, & McDevitt, 2005). A reduction in HU in egg caused by storage has already been described by many researchers (Rovinson & Monsey, 1972; Silversides & Scott, 2001). The increased pH of albumen due to the loss of carbon dioxide during storage (ovomucin-lysozyme complex) to break (Silversides & Scott, 2001). Further, Liu et al. (2009b) informed that oxidation of proteins in egg upon irradiation stimulates the denaturation and scission of egg white proteins, resulting in a severe decrease in HU. In this work, the albumen of the eggs from hens fed 0.1% MGL or both 0.05% and 0.1% NGL possessed higher antioxidative activity (ABTS* reducing activity) than that of eggs from hens fed control diet. Gallic acid contained in the MGL and NGL diets is a natural polyphenol (Kim et al., 2006), which have been shown to bind free radicals that play a key role in oxidation and leads to their inactivation (Hogan et al., 2009). A previous study reported that dietary phenolic compounds, such as oregano, rosemary, and grape pomace could improve the antioxidative activities of lamb and broiler meat (Gofii et al., 2007; Simitzis et al., 2008). The results of the present study show that the delayed reduction of HU during storage may hinder protein oxidation by improving the antioxidative activity of egg albumen from hens fed MGL or NGL diets.

The ABTS* reducing activity of egg yolk was different amongst the treatments, and it reflected the level of lipid oxidation of egg yolk in this study. Lipid oxidation of egg yolk was increased by dietary PUFA sources and was inhibited around 55% based on the TBARS value by dietary supplementation of 0.08% vitamin E as an antioxidant (Qi & Sim, 1998). Shahyrr, Salamatidoust, Chekani-Azar, Ahadi, and Vahdatpoor (2010) reported that the TBARS values of stored shell eggs with enriched PUFAAs were alleviated around 24% and 33% after refrigerated storage for 30 and 60 days, respectively, by addition of 0.12% vitamin E in the diet. These results are similar to the present study, which found that dietary supplementation of 0.1% MGL and 0.1% NGL inhibited the increase of the TBARS value (approximately 22% and 33%, respectively) in egg yolk after storage for 7 days, and that the increase of the TBARS values at 14 days was suppressed by the dietary supplementations of 0.1% MGL (12%) and 0.05% NGL (10%). Galobart, Barroeta, Baucells, and Guardiola (2001) found that although the TBARS values were not significantly different, the amount of lipid hydroperoxide (primary oxidation products) in fresh eggs increased upon treatment with sunflower oil compared to linseed oil since the level of natural antioxidants in dietary oil was higher than in linseed oil.

The modification of the fatty acid composition of egg yolk has been attempted ever since PUFAAs have been known as a beneficial substance for human health. Celebi and Macit (2008) reported that the content of n-3 and n-6 PUFAAs in egg yolk increased by dietary fat sources of n-3 and n-6 PUFAAs, respectively. The conjugated linoleic acid in egg yolk is increased by a dietary source of conjugated linoleic acid (Raes et al., 2002). In addition, Gǔçlü, Uyanık, and Işcan (2008) showed that the diet including 4% of n-6 sources, such as sunflower oil, maize oil, sesame oil, and cottonseed oil, and 4% of n-3 source, such as fish oil, raised the contents of linoleic and arachidonic acid and docosahexaenoic acid in egg yolk, respectively. These studies agree that the fatty acid composition of egg yolk is reflected in the fatty acid composition of the diet. However, the results of the present study on the fatty acid composition of egg yolk did not agree with previous studies. Although the linoleic acid concentrations were higher in egg yolk from hens fed 0.05% MGL and 0.05% NGL, those of egg yolk from hens fed 0.1% MGL and NGL were lower compared to the control (p < 0.05). This may be due to the effect of gallic acid in the MGL and NGL diets. Lipids from egg yolk are derived and synthesized in the hen liver through the Δ 9- and Δ 6-desaturase pathway, and the activity of Δ 9- and Δ 6-desaturase is affected by dietary antioxidants (Ozkán, Yilmaz, Ozturt, & Ersan, 2005). Hayat et al. (2009) reported that dietary antioxidants, such as vitamin E and butylated hydroxyl toluene alter the contents of saturated, monounsaturated, and n-6 and n-3 polyunsaturated fatty acids in egg.

The dietary supplementation of 0.1% MGL and NGL increased the stearic acid content of egg yolk. This result concurred with Celebi and Macit (2008) and Jiang, Ahn, and Sim (1991), who confirmed that high levels of linoleic acid in sunflower increased the stearic acid composition of egg yolk. Nonetheless, the effect of dietary MGL and NGL on the fatty acid composition of egg yolk remains unclear due to the observation that dietary 0.1% MGL and 0.1% NGL increased the oleic acid content of egg yolk when compared with that of the control.

Although cholesterol is essential for the development of the embryo, many researchers have tried to decrease the amount of cholesterol in egg yolk due to its risk for cardiovascular disease in humans (Viveros, Centeno, Arjia, & Brenes, 2007). Naber and Biggert (1989) reported the cholesterol-lowering effect of PUFAAs, which affect the activities of enzymes related to lipogenesis, whereas González-Muñoz et al. (2009) observed that the cholesterol levels of egg were higher from the hens fed saturated fat sources than from the hens fed unsaturated fat sources. The cholesterol levels of egg yolk were decreased by dietary 0.1% MGL as well as 0.05% and 0.1% NGL compared to that of control, which is in agreement with previous results. Kang, Kim, Park, and Jang (2006) reported that 5% corn oil, composed of high linoleic acid (57%), decreased the cholesterol content of egg yolk. However,
the dietary supplementation of sunflower oil with high linoleic acid (58%) did not influence the cholesterol content of egg yolk. Güçlü, K., Uyanik, F., & Hayat, Z. (2009). Effect of dietary lycopene and vitamin E on egg production, antioxidant status and Cabernet Franc (Vitis vinifera) grapes. LWT-Food Science and Technology, 42, 1269–1274.


