Introduction

Gamma fatty acids are newly emerging lipid-derived biomaterials that can help to improve lipid metabolism and prevent metabolic disease and inflammation. Atopic skin disease, which is prevalent in children and is believed to result from a combination of genetic and environmental factors, is recognized as a serious illness. Therefore, functional foods, which help to prevent this condition, are required to be developed. Gamma fatty acids refer to gamma linolenic acid (GLA, 18:3n-6) and dihomo-gamma linolenic acid (DGLA, 20:3n-6) which are the intermediary metabolites of linoleic acid (18:2n-6), the parent fatty acid of omega-6 (Kim et al., 2012; Tso et al., 2012). It is known that human blood contains 5~6 g of lipid per liter, including 1,500 mg L⁻¹ of linolenic acid and its metabolites, 25 mg L⁻¹ of GLA, 100 mg L⁻¹ of DGLA and 400 mg L⁻¹ of arachidonic acid, and human breast milk is known to be high in GLA (Miles et al., 2004). It has been reported that GLA content of human breast milk after a week of lactation is about 0.35~1.0% per liter of breast milk lipid, and colostrum, the breast milk produced by the mammary glands of mammals in late pregnancy, also contains similar level of GLA (Demmelmair et al., 2001). Since human breast milk contains about 33g of lipid per liter, a 5kg breastfed infant, who consumes about 800ml of breast milk per day, takes in about 25~65 mg of GLA per kg (Park, 2008). Human breast milk is also high in DGLA, occupying 0.3~0.4% of total lipid. Therefore, a breastfed baby takes in about 20~26 mg of
It can be thought that atopic dermatitis is more prevalent in formula-fed infants because the incidence of atopic dermatitis increases in people who have lack of GLA (Andreasen et al., 1997; Yasumoto et al., 1997; Thijs et al., 2000). It has been newly demonstrated that GLA supply is effective in drastic reduction of dysmenorrhea, prevention of skin disease such as eczema, anti-aging protection of skin in women using cosmetics, prevention of cardiovascular disease, cerebrovascular disease and hypertension by improving blood lipid metabolism, hypertension, obesity, rheumatoid arthritis, atopic dermatitis, enhancement of immunity, prevention of obesity and prevention of osteoporosis in menopause women (Barre, 2001; Demmelmaier et al., 2001; Park, 2002; Park and Zammitt, 2003; Menendez et al., 2005; Kim et al., 2012). The maximum daily dose of gamma fatty acids, considering the safety of gamma fatty acids, is reported to be 300–360 mg, or 6–7 mg kg<sup>-1</sup> in an adult woman who is 50 kg in weight (Leicer et al., 2000). Gamma fatty acids were first found in breast milk, they have been also found in hemp seed oil (1-6%), evening primrose oil (7–10%), black currant oil (15–20%) and borage oil (18–25%) in a form of GLA (Fan and Chapkin, 1998; Miles et al., 2004; Foster et al., 2010; Nykforuk et al., 2012). There are some reports about the effect of GLA on egg, specially on the quality. For instance it has been suggested that GLA is rapidly metabolized to arachidonic acid in the body and incorporated into the yolk (Furuse et al., 1992).

It was reported that gamma fatty acids of chickens could be reinforced with hemp seed oil and evening primrose oil (Park, 2007). Gamma fatty acids are hardly found in animal food such as eggs, and it was also difficult to find results of such study on gamma fatty acids as conducted with animal food. If GLA is insufficient in a human body due to a lack of Δ<sub>6</sub>-desaturase activity and various factors suppressing Δ<sub>6</sub>-desaturase activity even though GLA is normally produced, he will be susceptible to a variety of diseases (Duche and Rkste, 2001; Kim et al., 2012). To increase GLA intake through everyday food, GLA of eggs can be reinforced. This study was conducted to investigate the feeding effects of dietary GLA on haying performance and egg quality and to contribute the production of highly functional eggs with reinforced GLA.

Materials and Methods

Experimental design: One hundred twenty Hyline brown hens which were 16 weeks old were purchased from Eunkwan farm (Gyunggido Ahnsung, Republic of Korea). Hens were completely randomized into four treatment groups with three replicates of 10 animals per group in three-layer wire cages. These groups were: Control group (beef tallow as saturated fatty acids), CO (corn oil as n-6 fatty acids), HSO (hemp seed oil as low GLA 1.68%) and EPO (evening primrose oil as high GLA 8.03%). When they were 30 week old reaching 98% of egg production, laying performance and egg quality were evaluated for 5 weeks. Lighting during the laying period was adjusted to 17 hr, and the experimental diet was provided ad libitum and they had free access to water.

Preparation of diet: Experimental diet was prepared according to NRC feeding standard (1994), and composed of corn grain 51.30%, soybean meal 11.70%, wheat bran 18.00%, corn gluten meal 5.20%, fat 5.00%, limestone 7.30%, tricalcium phosphate 0.80%, salt 0.30%, DL-methionine 0.10% and growth promoters 0.30%. Beef tallow and CO were obtained from conventional markets, and HSO and EPO were purchased from a Chinese company (Rolf M. Wunder & Co.) via Sinwoo Trading Co. Ltd. (Seoul Republic of Korea). To prevent oxidation of unsaturated fatty acids in vegetable oils, vitamin E (α-tocopheryl acetate) was used as a natural antioxidant. 50 mg of vitamin E was added to 1 kg of oil. Each content of crude protein (16.25%) and metabolizable energy (2,700 kcal kg<sup>-1</sup>) was adjusted to same level.

Laying performance and egg quality: Feed intake was assessed on weekly basis to calculate the average daily feed intake per hen, and daily egg production was checked at 15:00 hour every day. The eggs produced daily were weighed together each day. Three eggs, whose weight were close to the average weight, were selected from each replicate pen of each treatment group to evaluate the egg quality. The automatic egg quality tester (Roboshan Egg Multi Tester-EMT-5200, Japan) was used to evaluate egg shell breaking strength, egg shell thickness, Haugh unit and York color.

Determination of blood lipid profile: Blood samples were collected from wing veins of laying hens (5 hens per pen in each treatment group) using heparinized syringes. The blood samples were centrifuged for 15 min at 3,000 rpm to separate plasma. Triglyceride and total cholesterol content in the plasma were determined by commercial enzyme kits (Asan pharmaceutical, Seoul, Republic of Korea) (Park, 2011).

Determination of fatty acid composition: 10 eggs per replicate pen in each treatment group were boiled, and egg yolks were separated to analyze fatty acids. Fatty acid composition was determined by commercial enzyme kits (Asan pharmaceutical, Seoul, Republic of Korea) (Park, 2011).
After cooling the mixture to room temperature, 1 ml of heptane and 2 ml of NaCl saturated solution were added to it, which was then mixed for 1 min. This mixture was left for 30 min at room temperature. Supernanant (1~2 μl) was taken, and it was injected to GLC (ACEM 6000 model, Young In Scientific Co., Ltd., Korea) for analysis of fatty acids. PUFA No. 2, animal source (Supelco, USA) as a standard solution and FFAP capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) as a column were used for fatty acid analysis. Nitrogen (1 ml min⁻¹) was used as a carrier gas. Injection port temperature, detector temperature and oven temperature were set up at 240 °C, 250 °C and 160 °C, respectively. The split ratio was 10:1.

**Statistical analysis**: ANOVA was performed by means of the statistical software SAS using the GLM procedure (SAS, 2000). Statistical significance between the average values of the treatment groups was tested at 95% using Duncan's multiple range test (p<0.05).

**Results and Discussion**

Feed intake and changes in egg production during the study are shown in Table 1. There was no statistical significance in feed intake between the treatment groups. On the other hand, the egg production of the control group was significantly lower than that of other groups (p<0.05). Haugh unit, eggshell thickness, eggshell breaking strength and yolk color are shown in Table 2. Haugh unit was significantly higher in CO, HSO and EPO groups than control group (p<0.05). There were no statistically significant differences noted in Haugh unit among CO, HSO and EPO groups, and also control, CO and HSO groups. There were no statistically significant differences in eggshell thickness and eggshell breaking strength among the treatment groups. Yolk colors were classified into 14 grades according to its light and shade. As compared to control group, egg yolk color was significantly higher in CO, HSO and EPO groups (p<0.05), while there were no statistically significant differences observed among CO, HSO and EPO groups.

The treatment group fed with diet containing HSO showed least changes in plasma total cholesterol (Fig. 1) and triglyceride (Fig. 2). There was a significant difference found in total cholesterol and triglyceride between control and other treatment groups (p<0.05). It was estimated that this resulted due to synergistic effect between n-3 fatty acid and GLA in HSO, which would have consequently reduced the cholesterol level of the eggs (not analyzed) (Leicer et al., 2000; Özpinar et al., 2003).

Changes in the blood fatty acid composition of laying hens are shown in Table 3. GLA was not detected in the blood samples of control group and treatment group fed with the diet containing CO. In contrast, a significantly higher level of GLA was found in the treatment group fed with the diet containing HSO and the treatment group fed with the diet containing EPO, compared with the control group (p<0.05). This shows that GLA, which was taken through the experimental diet, was absorbed into the blood.

**Table 1**: Feed intake and egg production for laying hens fed with experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>CO</th>
<th>HSO (1.68% GLA)</th>
<th>EPO (8.03% GLA)</th>
<th>SEM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (g hen⁻¹)</td>
<td>121.59</td>
<td>121.87</td>
<td>121.23</td>
<td>121.60</td>
<td>2.8867</td>
</tr>
<tr>
<td>Egg production (%)</td>
<td>96.07*</td>
<td>97.54</td>
<td>97.86*</td>
<td>97.75*</td>
<td>0.2501</td>
</tr>
</tbody>
</table>

*CO: com oil, HSO: hemp seed oil, EPO: evening primrose oil; GLA: gamma-linolenic acid; SEM: standard error of means; *Values with different superscripts within a row differ significantly (p<0.05)
Changes in the fatty acid compositions of eggs produced by the laying hens are shown in Table 4. GLA in the eggs had a cumulative effect as observed in the blood samples of the treatment groups fed with the diet containing HSO and the diet containing EPO. GLA retention significantly increased the eggs produced by laying hens having ingested lipids containing GLA (p<0.05). This means that the ingested GLA was absorbed into the blood and transmigrated to the eggs and accumulated in them. Although it is widely known that GLA is not accumulated in animals, fatty acid compositions of chicken eggs can be easily
Effect of GLA on laying performance and egg quality

changed by supplying the feed containing a certain amount of specific unsaturated fatty acids because monogastric animals such as chickens don’t have any microorganisms in their stomach (Fruse et al., 1992; Özpinar et al., 2003). The most significant finding of this study was that the GLA content of egg yolk could be increased by adding HSO or EPO as a GLA source to the feed. This result is supported by Fruse et al. (1992) who reported that fatty acid compositions can be changed if the lipid feed source for monogastric animals is changed. Gamma fatty acids are synthesized from linoleic acid; linoleic acid is first converted into GLA and then DGLA by Δ-6 desaturase, one of the rate-limiting enzymes, and is also converted into arachidonic acid by Δ5-desaturase at a speedy rate; therefore, accumulation of GLA in human or animal bodies cannot be obtained naturally (Park, 1997; Duché and Rkste, 2001; Kim et al., 2012) but can be obtained by GLA supply. GLA is insufficient in animals and humans due to lack of Δ6-desaturase activity and various factors suppressing Δ6-desaturase activity although GLA is normally produced, and gamma fatty acids are rapidly converted into arachidonic acid in human bodies (Park, 2008; Johnson et al., 2013; Sato et al., 2013). For this reason GLA was not accumulated in the eggs produced by the laying hens fed with diet containing CO, even though CO contained linolenic acid.

In conclusion, researchers could identify the new fact that supplementing the layer diets with hemp seed oil or evening primrose oil resulted in egg production enriched with gamma-linolenic acids.

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References


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