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Effects of Dietary Skullcap (*Scutellaria baicalensis*) Extract on Laying Performance and Lipid Oxidation of Chicken Eggs

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ABSTRACT : This study was conducted to investigate the effect of dietary domestic Skullcap (*Scutellaria baicalensis*) extracts (SCE) on laying performance and egg quality in laying hens. There were no significant differences in feed intake, egg production, or relative liver and spleen weights. The egg weights in groups fed diets containing SCE were significantly increased as compared with the control. The number of cecal total microbes in the group fed a diet containing 0.5% SCE was significantly reduced as compared with other groups. The malondialdehyde contents in stored eggs were significantly lowered by feeding SCE. The Haugh unit in the groups fed diets containing SCE tended to be increased after 2 weeks storage, but not significantly. This result indicated that dietary domestic SCE may delay lipid oxidation in eggs when added to laying hen diets. (**Key Words :** Skullcap Extract, Egg Weight, Malondialdehyde, Haugh Unit, Laying Hens)

INTRODUCTION

The use of antibiotics as feed additive to promote growth performance and prevent intestine from infectious diseases have led to problem of drug residues in animal products and emerge of new antibiotic-resistance bacteria. As a result, the extensive use of antibiotics in animal feed was less common and endeavors are made to develop alternate means for preventing and treating infectious disease in animal industry. Herbs, spices and various plant extracts have received increased attention, in place of antibiotics, as alternative means to stimulate growth and modulate immune responses (Wenk, 2000). In addition, natural antioxidants such as rosemary, saffron and green tea extracts have been shown to improve the oxidative stability of poultry products (Botsoglou et al., 2005; Florou-Paneri et al., 2006; Rababah et al., 2006). Skullcap (*Scutellaria baicalensis*) is powerful medical herb of member of a mint family from rich woods and moist soils and it is used in alternative medicine as an anti-inflammatory and anti-cancer (Burnett et al., 2007). It has recently been known that the polyhydroxyflavonoids of Skullcap exhibited potent antioxidative and free-radical scavenging properties (Huang et al., 2006). The objective of the present study was to evaluate the effects of dietary domestic Skullcap extracts (SCE) on laying performance, egg quality, cecal microflora profile and oxidative stability of stored egg yolk.

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MATERIALS AND METHODS

A total of four-hundred 42-wk-old Hy-Line Brown layers were divided into four groups and fed one of the four diets containing 0 (as control), 0.1, 0.3 or 0.5% SCE for 6 weeks. The layers were randomly placed in five replicates with 20 birds each per group in wire cage. The powdered *S. baicalensis* (1-mm sieve, sample 5 kg) was extracted overnight with 200 L 75% ethanol using a large scale extractor at 60°C. The extract was filtered 3 times and the filtrate was condensed by vacuum rotary evaporator. Baicalin, baicalein wogonin and oroxylin A are the main active components in *S. baicalensis* (Huang et al., 2006). The experimental diets were formulated to meet or exceed the nutrient requirements of NRC as shown in Table 1 (NRC, 1994). SCE was added at the expense of control diet at 0.1, 0.3 or 0.5% levels on a weight basis. The experimental diets and water were provided for *ad libitum* intake. A room temperature of 22±3°C and a photoperiod of

Table 1. Formula and chemical compositions of the experimental diet

Items (%)	Control
Yellow corn	60.27
Soybean meal	16.18
Hulled lupine	3.00
Corn gluten meal	2.00
Wheat bran	2.50
Canola meal	3.20
Tallow	1.60
Molasses	0.50
Limestone, coarse	9.40
Dicalcium phosphate	0.64
Salt	0.30
DL-Methionine, 98%	0.03
Choline-chloride, 50%	0.05
Mineral mix[1]	0.15
Vitamin mix[2]	0.13
Natuphos	0.03
Total	100
Calculated values	
Dry matter (%)	89.12
Crude protein (%)	16.50
Ether extract (%)	4.45
Crude fiber (%)	3.69
Ash (%)	12.77
Available P (%)	0.21
Ca (%)	3.86
TMEn (kcal/kg)	2,780

[1] Mineral mixture provided following nutrients per kg of diet: Fe, 70 mg; Zn, 60 mg; Mn, 8 mg; Cu, 7.5 mg; I, 1 mg; Se, 0.2 mg; Co, 0.13 mg.

[2] Vitamin mixture provided following nutrients per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,300 IU; vitamin E, 20 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 3.5 mg; vitamin B_{12}, 0.02 mg; biotin, 0.12 mg; niacin, 30 mg; pantothenic acid, 10 mg; folic acid, 0.7 mg.

17/7 h light/dark cycle were maintained throughout the experimental period. All animal care procedures were approved by Institutional Animal Care and Use Committee in Konkuk University.

Egg production was recorded daily and experimental diets were freshly added. The internal egg and eggshell quality were measured biweekly. The eggs were weighed individually and then were exposed to a breaking force by using an eggshell strength tester (FHK, Fugihira, Ltd. Japan). On breaking, the egg contents were poured into a glass plate to measure the albumen height. Haugh units were calculated using the Haugh unit formula. The yolk color was determined with a Roche yolk color fan. The eggs laid for the last three days of experiment were used for analysis of egg qualities and malondialdehyde content. The collected eggs were kept in storage temperature of 18°C during 7 or 14 days to observe the change of Haugh unit. At the end of experimental period, nine birds from each group were randomly selected weighed individually. The blood was drawn from wing vein using sterilized syringes for determination of the various blood profiles. At necropsy, the liver and spleen were immediately removed and weighed. The concentration of total cholesterol and high density lipoprotein-cholesterol (HDL-C), the activity of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were estimated according to the colorimetric method using cholesterol diagnostic kit (Cholesterol E kit, HDL-cholesterol kit, Youngdong Medical Corperation) and GOT-GPT assay kit (BCS GOT-GPT assay kit, Bio Clinical System Corporation), following the manufacturer's direction.

The contents of each lipid fraction in the liver were separated by thin layer chromatography (TLC) on silica gel chromarods (Chromarod-S III, Mitsubishi Kagaku Iatron, Inc., Japan), an exclusive media in the same manner of normal phase TLC, using hexane : diethylether : formic acid (85:15:0.15, *vol/vol*) as developing solvents, and quantified by IATRO SCAN (TH-10 TLC/FID analyzer, Iatron, Ltd., Japan) as previously described (An et al., 1997). The cecal digesta homogenates in PBS were serially diluted from 10^{-1} to 10^{-7}. Dilutions were subsequently plated on duplicate selective agar media for enumeration of target bacterial strains. Total microbes, *coliforms* and *Lactobacillus spp.* were enumerated using nutrient agar, MacConkey agar, and MRS agar, respectively, using the traditional method (Tuohy et al., 2002). Each plate was incubated at 37°C, for 24 to 72 h anaerobically or aerobically, and colonies were then counted. Results obtained were presented as base-10 logarithm colony-forming units per gram of cecal digesta.

Some modified method was used to determine tiobarbituric acid reactive substances (TBARs) values in egg yolk to evaluate lipid oxidation as described by Botsoglou et al. (1994). To study on the oxidative stability of egg yolk, 8 eggs from each group was placed in a high temperature cabinet over 3 days of storage at 35°C. A 1.5 g egg yolk sample was homogenized with 5% aqueous trichloroacetic acid solution containing 0.8% butylated hydroxytoluene and then centrifuged for 3 min at 3,000 g. Following reaction with tiobarbituric acid reagent, malondialdehyde was directly quantified by third-derivative spectrophotometry against blank reaction mixture (Beckman DU-650, Beckman Counter, Inc.).

The main effects between treated groups were subjected to ANOVA using the general linear models procedure of SAS (SAS Institute, 2002), and significant differences were

Table 2. Effects of dietary domestic Skullcap extracts on laying performance and relative organ weight in laying hens[1,2]

	Control	SCE 0.1%	SCE 0.3%	SCE 0.5%
Laying performance				
Feed intake (g/d/bird)	123.08±2.59	126.26±2.38	128.30±7.42	122.32±1.39
Egg production (%)	85.00±1.56	85.65±1.18	85.92±0.88	85.00±1.27
Egg weight (g/egg)	65.61±0.18[c]	66.98±0.18[a]	66.16±0.20[b]	67.07±0.16[a]
Egg mass (g/d/bird)	55.64±1.07	57.03±0.87	55.99±0.69	56.37±0.78
Relative organ weight				
Liver (g/100 g BW)	1.35±0.06	1.33±0.05	1.35±0.04	1.29±0.07
Spleen (g/100 g BW)	0.10±0.01	0.08±0.01	0.10±0.01	0.09±0.01

[1] SCE = Skullcap extracts.
[2] Values are presented Mean±SE (n = 4 for laying performance and n = 8 for relative organ weight, each group).
[a-c] Values with different superscript were significantly differ ($p<0.05$).

determined using Duncan's multiple range test at the level of $p<0.05$ (Duncan, 1955). Percentage data were transformed to arc sine percentages before square root percentages ANOVA was performed.

RESULTS AND DISCUSSION

There were no significant differences in feed intake, egg production, relative liver and spleen weights (g/100 g BW). The egg weights in groups fed diets containing SCE were significantly increased as compared with that of control ($p<0.05$), although there were no significant differences in egg production and daily egg mass (Table 2). Eggshell strength, thickness and yolk color were not also influenced by the dietary treatments (Table 3). There was no significant difference in Haugh unit of fresh eggs, ranging from 91.0 (SCE 0.1% group) - 89.5 (Control). Only limited information is available on the dietary effects of plant extracts on egg production traits. Feeding rosemary powder or marigold extract did not significantly affect egg production and egg weight (Florou-Paneri et al., 2006; Sirri et al., 2007). Further studies using a larger number of layers are suggested in order to clarify effects on egg production traits including the changes of egg weight.

There were no significant differences in the activities of serum GOT and GPT, and the contents of hepatic cholesterol, triacylglycerol and phospholipids (Table 4). Measurement of serum GOT and GPT activities indicative of liver damage in bird is a valuable tool to determine a safe inclusion rate for non-conventional feedstuff or feed additives (Diaz et al., 2003). SCE appeared safe at an inclusion rate of 0.5% and thus will be recommended at this point without having adversary effect on physiological status.

The number of cecal total microbes in group fed diet containing 0.5% SCE was significantly reduced as compared with those of other groups ($p<0.05$), although there were no differences in *coliforms* or lactic acid bacteria (Table 4). Cross et al. (2007) reported that the profile of gut microflora was not affected by feeding herbs or their associated essential oils. On the other hand, Jamroz et al. (2005) observed a decrease in *E. coli* and an increase in *Lactobacillus spp.* of chicks fed plant extracts. In present study, SCE added to the diets modulated the profiles of cecal microflora, reflecting a potential to alter gut micro ecology in laying hens. The reason that the number of cecal *coliforms* was high as compared with those of total microbes except the group fed diet containing 0.3% SCE in this study is still unknown so the exact reason requires further study.

The malondialdehyde contents in stored eggs were significantly lowered by feeding SCE ($p<0.05$) (Table 5). These values in groups fed diets containing SCE ranged from 0.025-0.029 μg/g yolk, suggesting SCE was effective

Table 3. Effects of dietary domestic Skullcap extracts on egg and eggshell qualities in laying hens[1,2]

	Control	SCE 0.1%	SCE 0.3%	SCE 0.5%
Eggshell strength (kg/cm^2)	3.05±0.10	2.91±0.07	2.87±0.09	2.84±0.09
Eggshell thickness (mm/100)	33.98±0.41	33.67±0.37	33.23±0.42	33.37±0.41
Egg shell color	28.47±0.46	27.13±0.57	27.98±0.53	27.88±0.51
Egg yolk color	6.83±0.05	6.82±0.05	6.81±0.05	6.89±0.05
Haugh unit	89.48±0.57	90.08±0.54	90.97±0.64	90.90±0.78

[1] SCE = Skullcap extracts. [2] Values are presented Mean±SE (n = 90, each group).

Table 4. Effects of dietary domestic Skullcap extracts on enzyme activities of serum, the concentration of hepatic lipid fractions and cecal microbial population in laying hens[1,2]

	Control	SCE 0.1%	SCE 0.3%	SCE 0.5%
GOT, U/L	100.26±4.33	101.60±3.24	100.71±6.47	104.23±2.74
GPT, U/L	5.09±0.30	5.06±0.16	5.15±0.26	5.30±0.54
Hepatic lipid fractions	---------- mg/g ----------			
Cholesterol	3.57±0.13	3.26±0.12	3.41±0.20	3.48±0.09
Triacylglycerol	13.75±0.18	12.91±0.17	13.51±0.18	13.56±0.16
Phospholipid	33.68±1.22	34.07±1.05	34.20±1.69	34.18±1.69
Cecal microbial population	---------- log cfu/g ----------			
Total microbes	5.31±0.11[a]	5.34±0.14[a]	5.66±0.11[a]	4.83±0.10[b]
Coliform bacteria	5.70±0.12	5.38±0.13	5.36±0.23	5.84±0.16
Lactic acid bacteria	4.32±0.66	4.31±0.29	5.69±0.13	4.24±0.33

[1] SCE = Skullcap extracts; GOT = Glutamic-oxaloacetic transaminase; GPT = Glutamic-pyruvic transaminase.
[2] Values are presented Mean±SE (n = 8, each group).
[a,b] Values with different superscript were significantly differ ($p<0.05$).

Table 5. Effects of dietary domestic Skullcap extracts on the change of Haugh unit and yolk lipid peroxidation during storage in laying hens[1,2]

	Control	SCE 0.1%	SCE 0.3%	SCE 0.5%
Haugh unit (7d)	71.63±2.81	74.67±2.73	75.55±2.16	76.12±1.80
Haugh unit (15d)	62.33±2.64	64.28±1.79	65.60±2.32	67.50±1.86
MDA (μg/g yolk)	0.052±0.01[a]	0.025±0.00[b]	0.028±0.00[b]	0.029±0.01[b]

[1] SCE = Skullcap extracts; MDA = Malondialdehyde. [2] Values are presented Mean±SE (n = 10, each group).
[a,b] values with different superscript were significantly differ ($p<0.05$).

in minimizing lipid oxidation at relative low levels. Florou-Paneri et al. (2006) also observed that dietary use of ground rosemary has been demonstrated to reduce lipid oxidation of egg yolks. The observed antioxidant effect of dietary SCE agree with other previous reports with eggs and chicken meats (Botsoglou et al., 2005; Rababah et al., 2006). There are demands for using natural products that can prevent lipid oxidation in lipid enriched animal foods, due to consumer preferences for natural feed stuff and toxicological concerns of synthetic antioxidants. The present study demonstrated that SCE could be used as a valuable natural source for reducing lipid oxidation developed during storage. The Haugh units in the groups fed diets containing SCE were tended to be increased after 2 weeks of storage, but not significantly (Table 5). In this study, the Haugh unit of fresh eggs was not influenced by feeding of SCE. The Haugh unit, an indicator of the most widely accepted measure of internal egg quality, tended to be decreased according to the elapsed time of storage as found (Williams, 1992). General nutrients in layer feed did not appear to have any beneficial effect on Haugh unit (Naber, 1979), but it suggested that certain natural antioxidants such as vitamin C, vitamin E and selenium being beneficial to albumen quality by it's antioxidant property (Keshavarz, 1996; Sahin et al., 2003). It may be assumed that at least part of the ingested antioxidant SCE compounds were retained in egg yolk precursor and that they were still active in egg yolk. Because available knowledge on correlation between the change of cecal microflora population and delaying lipid oxidation of stored egg yolk is very few, the interpretation of the present results has remained difficult. Studies are needed to describe the effects of secondary antioxidant SCE compounds in digestive tracks and the extent to which they might be incorporated in egg yolks. In conclusion, SCE could have beneficial effects in reducing lipid oxidation of egg yolks when added in laying hen diets.

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