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Full Length Research Paper

Influence of *Epimedium koreanum* on the performance of laying hens, egg quality, and fat soluble vitamin and cholesterol contents in the yolk

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This study was conducted to evaluate the effects of feeding *Epimedium koreanum* to laying hens on performance, as well as to determine its effects on interior and exterior egg quality along with fat soluble vitamin and cholesterol contents in the egg yolk. The experimental diets contained 0.0, 1.0, 5.0, or 10.0 g/kg *E. koreanum*. No significant changes were observed in the rate of egg production, with all treatments exceeding 95%. Linear increases in egg weight and mass ($p < 0.01$) were found with dietary *E. koreanum* supplementation. A dramatic increase ($p = 0.05$) was found in the number of extra large (60 to 70 g) size eggs with a concomitant reduction ($p = 0.06$) in the number of small (50 to 55 g) size eggs. Feed consumption and feed conversion were cubically ($p < 0.01$) affected by *E. koreanum* supplementation. Albumen weight and yolk color linearly increased ($p < 0.01$). The percentage of albumen linearly increased ($p < 0.01$) whereas yolk percentage decreased ($p < 0.01$). Vitamin E content in the egg yolk was quadratically ($p = 0.02$) affected by the treatments. In addition, cholesterol content of the egg yolks was quadratically ($p < 0.01$) increased. In conclusion, dietary inclusion of *E. koreanum* can have beneficial effects on the performance of laying hens in terms of improving egg weight, albumin weight, yolk color, and vitamin E content. However, these advantages may be more than offset by a significant increase in the cholesterol content of the egg yolk.

Key words: *Epimedium koreanum*, layer, egg quality, egg production, vitamin, cholesterol.

INTRODUCTION

Epimedium is a genus of about sixty or more species of herbaceous flowering plants in the family Berberidaceae, and commonly known as Barrenwort, Bishop's Hat, Fairy Wings, Horny Goat Weed, Rowdy Lamb Herb, or Ying Yang Huo. It is widely used as a traditional medicinal herb in China, Korea, and Japan (Yang, 1985). The major pharmacologically active component of this herb is icariin (Liu and Xu, 1984) This compound can improve the condition of reproductive organs (Liu et al., 2005; Zhao et al., 2006) and has antioxidant effects (Xie, 2008), potential activity against osteoporosis (Huang, et al., 2007;Zhang et al., 2008), and enhances immune function (Luo et al., 2009; Zhang et al., 2009). It also raises ovarian and uterine weight and increases days of oestrus (Ye and Lou, 2005) and weight gain in mice (Zhao et al., 2006). Such studies showed that *E. koreanum* could be considered as an alternative natural growth promoter for poultry.

Although *E. koreanum* is a potent performance modifier, its role in laying hens has not been studied. Energy metabolism and hormonal conditions strictly control egg production in hens. Differences in laying performance have been related to differences in plasma levels of reproductive hormones such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and progesterone (Wilson,

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Table 1. Ingredients and analyzed chemical composition of the formulated basal diet (g/kg as-fed basis).

Yellow corn, USA	**504.5**	**Moisture**	**122.2**
Wheat, EU	80.0	Ether extract	39.7
Lupin kernel	20.0	Crude protein	170.5
Soybean meal	184.0	Crude fiber	27.2
Maize gluten meal	29.0	Ash	132.8
DDGS, USA	40.0	Ca	40.8
Meat and bone meal	25.0	P	4.4
Animal fat	8.0	MEn, MJ/kg (calculated)	11.62
Sodium bicarbonate	0.6	Lysine	8.5
L-Lysine HCl	0.5	Methionine	4.3
Methionine	1.7	Methionine + Cystine	7.2
Limestone	85.6		
Oyster shell	15.0		
Phytase, Optiphos-1000®	0.50		
Carbohydrase, Endopower®	1.00		
Premix[a]	2.36		
Choline	0.24		
Salt	2.0		

[a]Provided the following nutrients per kg of diet: vitamin A, 12,000 IU; vitamin D 3,3,500 IU; vitamin E, 30 IU; vitamin K 3, 3.0 mg; vitamin B1, 3.0 mg; vitamin B2, 7.0 mg; vitamin B6, 5.0 mg; vitamin B12, 0.025 mg; niacin, 40.0 mg; patothenic acid, 10 mg; folic acid, 1.0 mg; biotin, 0.15 mg; Fe, 75.0 mg; Zn, 97.5 mg; Mn, 97.5 mg; Cu, 7.5 mg; I, 1.5 mg; Se, 0.2 mg.

1978; Wang and Johnson, 1993; Vanmontfort et al., 1995; Onagbesan et al., 2006). The aim of the present study was to evaluate whether the supplementation of a diet with *E. koreanum* may assist in laying hen performance and to determine its effects on interior and exterior egg quality as well as fat soluble vitamin and cholesterol contents in the egg yolk.

MATERIALS AND METHODS

Preparation of *E. koreanum*

The *E. koreanum* used in the present experiment was obtained from Yaksan Farmer's Cooperation (Wando, Korea) and is marketed under the name Ying Yang Huo. All parts of the *E. koreanum* were air dried and then ground to a fine powder using an electric blender. The average icariin content of the *E. korenum* was 7.47 mg/g.

Animals and housing

One-hundred and twenty-eight, 18 week-old Lohmann Brown-Lite layers were obtained from a commercial source (Join farm, Choongju, Korea). After 6 weeks of adaptation, 64 hens with an average laying rate of 96.4 ± 6.95% (mean ± S.D.) were selected and allocated randomly into 4 treatments, with 8 replicates per treatment and 2 hens per replicate. A basal diet (Table 1) was fed in mash form and all nutrient levels met or exceeded the nutrient requirements suggested in the Lohmann Brown Management Guide (Lohmann Tierzucht GmbH, 2008). The four experimental groups received an identical basic mixture supplemented with different concentrations of *E. koreanum* (0%, 0.1, 0.5 and 1.0%). All hens were housed in windowless and environmentally controlled rooms and offered feed and water *ad libitum*. The cages were galvanized metal wire (approximately 25 x 35 x 50 cm) in double-decker rows providing 430 cm^2 /hen. For this experiment, one upper deck of cages and one lower deck of cages were used. Each cage had a nipple waterer. A continuous plastic feed trough was divided by replicate to insure that the hens were not able to consume feed assigned to the adjoining replicate. A wire egg collector was installed in front of the cage to prevent eggs from separate replicates from being mixed. The room temperature was kept at 21-23° and the light cycle consisted of 16 h of light (incandescent lighting, 10 lx) and 8 h of dark. All procedures used in this experiment were approved by the Animal Ethics Committee of Sungkyunkwan University and by the Guidelines for the Care and Use of Animals in Research (Korean Ministry for Food, Agriculture, Forestry and Fisheries, 2008).

Sampling and analyses

The eggs were collected and weighed every day and feed consumption was measured on a weekly basis. Egg production was calculated on a replicate basis. Egg weight (g of egg/hen per day) and feed conversion (g of feed/g of egg) were calculated from egg production, egg weight, and feed consumption. Egg components were measured using two eggs of average weight in a week, from each cage on the last 2 days of each week. Yolk color and Haugh units were measured using an egg multi tester EMT-5200 (Robotmation Co. Ltd., Tokyo, Japan). Haugh units were calculated from the records for egg weight and albumen height using the formula: $HU = 100 \log_{10} (H - 1.7 W^{0.37} + 7.56)$, where HU = Haugh unit, H = height of the albumen (mm), and W = egg weight (g). The yolks were separated from the tester tray (yolk, albumen, and tray) using a Teflon spoon. Before yolk weight was determined, the chalaza was removed by a spatula. The shells were weighed without drying. Albumen weight was calculated by subtracting the weight of the tester tray. Daily egg content was calculated (% egg production x (daily yolk weight + daily albumen weight) from egg production, yolk weight, and albumen weight.

Total cholesterol was determined without saponification according to Zhang et al. (1999).

Table 2. Performance of laying hens fed experimental basal diets supplemented with *E. koreanum*.

	Level of *E. koreanum*				SEM	P-value		
	0 g/kg	1.0 g/kg	5.0 g/kg	10.0 g/kg		L	Q	C
Egg production (%)	97.5	96.5	95.4	98.5	1.12	0.68	0.07	0.40
Egg weight (g/hen/d)	54.7	54.9	55.0	58.4	0.81	<0.01	0.05	0.26
Egg content (g/hen/d)	47.0	47.7	47.7	50.7	0.72	<0.01	0.10	0.27
Feed consumption (g/hen/d)	106.5	107.1	103.5	106.8	0.82	0.23	0.10	<0.01
Feed conversion (g feed/g egg)	1.96	1.97	1.89	1.86	0.025	0.08	0.48	<0.01

The extraction and HPLC analysis of fat soluble vitamins in the egg yolk were performed using very similar methods of Gong and Ho (1997) used for analysis in milk.

The feed was analyzed in triplicate according to AOAC methods (1995). Analyses were conducted for moisture (method 930.15), ether extract (method 920.39), crude protein (method 984.13), crude fiber (method 978.10), and ash (method 942.05). Calcium was determined by a Shimazu AA625 Atomic Absorption Spectrophotometer (Shimazu, Kyoto, Japan), and phosphorus was analyzed using a UV-vis. Spectrophotometer (Hitachi, Tokyo, Japan). An amino acid analysis of the feed was performed using a L8500-Hitachi Amino Acid Analyzer (Hitachi, Tokyo, Japan) after hydrolysis for 24 h in 6 N HCl. Performic acid (85%) hydrolysis was conducted to analyze sulfur-containing amino acids. Eggs from each cage were sorted by six size groups (jumbo, extra large, large, medium, small, and miniature).

Statistical analysis

The data were analyzed as a randomized block design (Snedecor and Cochran, 1989), using the appropriate General Analysis of Variance procedures of STATISTIX (1996). The hens were blocked on the basis of hen-day egg production during the adaptation period and the cage was considered the experimental unit for all analyses. The model included the effects of replication (that is, block), treatment, and replication x treatment (error). Moreover, we constructed polynomial contrasts to determine the nature of response variables to increasing levels of supplemental *E. koreanum*.

RESULTS AND DISCUSSION

The effects of different levels of dietary *E. koreanum* on performance are presented in Table 2. No significant changes were observed for rate of egg production with increasing dietary *E. koreanum*. However, a quadratic trend (p = 0.07) existed for egg production. Since the rate of egg production exceeded 95% for all treatments, there was a very small window of opportunity for *E. koreanum* to increase the rate of production. In commercial farms, peak production usually occurs when hens reach 24 to 26 weeks of age, and production steadily declines until the flock is taken out of production at approximately 76 weeks of age (Bell, 2002). The present study was conducted close to the period of peak production, and it would be interesting to repeat the study to determine whether or not *E. koreanum* has any impact on the productivity of laying hens during later stages of the production cycle.

Linear increases in egg weight and egg content (p < 0.01) were found with increasing dietary *E. Koreanum*. The general increases in egg weight and content were associated with a dramatic increase (P = 0.05) in the number of extra large (60 - 70 g) size eggs with a concomitant reduction (p = 0.06) in the number of small (50 - 55 g) size eggs (Table 5). Since many countries pay a premium for larger sized eggs (Food and Agriculture Organization of the United Nations, 2003), there may be an economic incentive for producers to utilize *E. Koreanum* as a means of increasing the percentage of large size eggs produced. Feed consumption and feed conversion were cubically (p < 0.01) affected by *E. koreanum* supplementation. As dietary *E. koreanum* increased, feed conversion improved because egg weight increased significantly (p < 0.01) with increased dietary levels of E. koreanum. In contrast to our result, Botsoglou et al. (2005) found that the supplementation of rosemary, oregano and saffron to a layer diet had no significant effect on egg production, feed intake and feed conversion ratio. The determined egg components and quality for laying hens fed the experimental basal diet supplemented with *E. koreanum* are shown in Table 3. Albumen weight and yolk color linearly increased (p < 0.01) as dietary *E. koreanum* increased. This is consistent with data from Ghasemi et al. (2010) who reported that yolk color responded linearly with increasing levels of medicinal herbs. Poltowicz and Wezyk (2001) had also showed that the herbs used significantly increased yolk color intensity in the experimental groups. The percentage of albumen linearly and cubically increased (p < 0.01), whereas yolk percentage linearly and cubically decreased (p < 0.01) as dietary *E. koreanum* level increased. Yolk weight was cubically affected (p < 0.01) by increases of dietary *E. koreanum*. A quadratic increase in egg shell weight (p < 0.01) was found with increasing dietary *E. koreanum*, whereas egg shell percentage was not affected by the dietary

Table 3. Egg components and egg quality for laying hens fed experimental basal diets supplemented with *E. koreanum*.

	Level of *E. koreanum*				SEM	P-value		
	0 g/kg	1.0 g/kg	5.0 g/kg	10.0 g/kg		L	Q	C
Albumin weight (g)	33.7	34.0	34.8	36.8	0.58	<0.01	0.14	0.76
Albumin (%)	61.4	60.8	62.8	62.0	0.30	<0.01	0.68	<0.01
Yolk weight (g)	13.4	13.7	12.9	13.9	0.24	0.38	0.16	<0.01
Yolk (%)	25.8	25.1	24.5	25.0	0.28	<0.01	0.72	<0.01
Egg shell weight (g)	7.6	7.3	7.2	7.7	0.16	0.93	0.01	0.45
Egg shell (%)	12.8	13.1	12.7	13.0	0.14	0.74	0.87	0.05
Yolk colour	6.9	6.9	7.3	7.3	0.10	<0.01	0.82	0.06
Haugh units	88.7	87.7	88.8	88.0	0.73	0.73	0.88	0.23

Table 4. Fat soluble vitamin and cholesterol content in egg yolk for laying hens fed experimental basal diets supplemented with *E. koreanum*.

	Level of *E. koreanum*				SEM	P-value		
	0 g/kg	1.0 g/kg	5.0 g/kg	10.0 g/kg		L	Q	C
Vitamin A (mg/100 g)	0.93	0.94	0.95	0.94	0.005	0.13	0.46	0.37
Vitamin D (µg/100 g)	43.9	43.9	44.1	43.9	0.24	0.85	0.76	0.59
Vitamin E (mg/100 g)	1.95	1.98	2.02	1.98	0.015	0.30	0.02	0.14
Cholesterol (mg/100 g)	1106	1323	1346	1247	51.6	0.06	<0.01	0.75

treatments. The Haugh unit was also not affected by the addition of *E. koreanum*.

The effects of *E. koreanum* supplementation on the fat-soluble vitamin content of the egg yolk are presented in Table 4. Vitamin E (p = 0.02) content was quadratically affected by the dietary treatments. Eggs are considered a good source of Vitamin E in the human diet, as two large eggs can contribute 6% of the recommended daily allowance of this nutrient (Applegate, 2000).

Increases as a result of *E. koreanum* supplementation can enhance the reputation of eggs as a good source of vitamin E. There was a quadratic ($p < 0.01$) increase in the cholesterol content of the egg yolks with increasing levels of *E. koreanum* supplementation (Table 5). This finding has broad implications for the potential adoption of *E. koreanum* as a feed additive for use in the layer industry, as consumers are very concerned about the cholesterol content of foods (Applegate, 2000). This concern is based on more than 40 years of speculation regarding the association between the consumption of high cholesterol diets, blood cholesterol, and coronary heart disease (Masironi, 1970; Dawber et al., 1982; Paik and Blair, 1996; Steinberg, 2004). Current human dietary guidelines recommended that in order to reduce the risk of cardiovascular disease, consumers should limit their intake of dietary cholesterol to less than 300 mg per day (American Heart Association Nutrition Committee, 2006). For most people trying to lowerblood cholesterol, however, limiting saturated fat is more effective than limiting cholesterol intake. Most foods that are high in cholesterol are also high in saturated fat, but eggs are an exception. An egg contains only one gram of saturated fat. Eggs are a valuable part of the diet because they are a source of high quality protein and other nutrients.

Conclusion

The effects of dietary *E. koreanum* supplementation on performance and egg quality parameters of laying hens have not been previously reported. The current results indicate that dietary inclusion of *E. koreanum* can have beneficial effects on laying hen performance in terms of

Table 5. Exterior egg quality for laying hens fed experimental basal diets supplemented with *E. koreanum*.

	Level of *E. koreanum*				SEM	P-value		
	0 g/kg	1.0 g/kg	5.0 g/kg	10.0 g/kg		L	Q	C
Defected eggs (%)	0.96	1.00	2.50	1.21	0.826	0.55	0.43	0.26
Dirty eggs (%)	4.75	3.62	3.00	2.53	1.371	0.24	0.81	0.95
Egg grade (%)								
Jumbo (70 g and over)	1.13	0.98	1.12	0.64	0.503	0.55	0.75	0.68
Extra large (65 - 70 g)	1.32	3.59	6.74	8.47	2.629	0.05	0.92	0.85
Large (60-65 g)	14.73	19.08	23.81	29.07	5.984	0.09	0.94	0.99
Medium (55-60 g)	42.11	37.08	33.98	45.42	5.059	0.76	0.11	0.58
Small (50-55 g)	30.58	35.35	23.77	14.94	6.687	0.06	0.32	0.53
Miniature (50 g and under)	10.13	3.93	10.58	1.46	3.982	0.29	0.72	0.12

improving egg weight, albumin weight, yolk color, and vitamin E content. However, these advantages may be more than offset by a significant increase in the cholesterol content of the egg yolk. However, further research needs to be conducted in future with emphasizing utilization of *E. koreanum* and its appropriate concentration.

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