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Effect of Feeding Diets Containing Green Tea By-Products on Laying Performance and Egg Quality in Hens

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녹차 가공 부산물을 첨가한 산란계 사료의 급여가 난 생산성과 난 성분에 미치는 영향

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ABSTRACT : This study was carried out to evaluate effect of dietary green tea by-products (GTB) on laying performance and quality of eggs in hens. A total of 96 laying hens (22-week-old) "Tetran Brown" were allotted to four dietary treatments, which containing 0, 2, 4 and 6% green tea-by products. The results showed that the egg production increased ($P<0.05$) in layers fed diets containing 4% or 6% green tea-by products compared to the layers fed control no GTB. Egg weight and egg mass in layers fed diets containing 2% green tea by-products significantly decreased compared to those in the other groups ($P<0.05$). The feed intake was lower in layers fed diets containing 2% and 6% green tea by-products. The feed conversion ratio was lower ($P<0.05$) in layers fed diets containing 4% or 6% green tea by-product supplementation respectively. The egg shell thickness was no significantly different between layers fed the control and green tea by-product diets ($P>0.05$). The egg yolk cholesterol tended to decrease when layers fed diets containing green tea by-products, although the differences were not significant ($P>0.05$). The linolenic and docosahexaenoic (DHA) acid contents in egg yolk significantly increased in layers fed diets containing different levels green tea by-products compared to those fed control diet ($P<0.05$). These results suggest that inclusion of green tea by-products in layer diets is suitable for hens without negative effects on egg composition

(Key words: green tea by-product, feed conversion ratio, egg yolk cholesterol and fatty acid)

INTRODUCTION

There is an increasing focus on the health potential of foods, particularly an interest in foods that go beyond supplying adequate nutrients, to influencing systemic physiological functions and processes. Functional foods are those that, by virtue of the presence of physiological active components, are purported to be efficacious in the prevention and /or treatment of disease and the promotion of optimal health (Guenter and Sim, 1998).

The egg , meat of chicken and their products are in low price, easily utilized and accordingly are universally accepted products by people. However, people who are suffering from cardiovascular diseases are afraid of egg and meat consumption because of high content of cholesterol and fat. Scientists already recommended several feed ingredients such alfalfa meal containing saponin and garlic for poultry diet to decrease cholesterol contents in their products (Kendler, 1987).

Green tea by virtue of its scientifically validated healthful effects has potential utility in the management of a variety of disorders by depression of cholesterol absorption from the cut and prevents the oxidation of fat and fat products. Many studies have reinforced that green tea ingestion lowers cancer incidence (Mukhtar and Ahmad, 1999) and inducing lowers blood cholesterol (Muramatsu et al. 1986) and lowers blood pressure (Ikeda et al., 1992). Naber (1976) and Isigaki et al. (1991)

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reported green tea had antitumor and antidiabetic effects. Matussuzaki et al. (1985) and Ryuhei (1990) cited the anti-oxidant effects of green tea.

As we know green tea has a specific chemical and biological property, we expected that properties and inclusion of green tea by-products in layer diets may reduce cholesterol content of egg yolk, it could be reduce rancidity of egg yolks. Also it is one attempt to get "biologically clean" natural feed for laying hens on the way to produce functional eggs.

The objectives of this study were to find the optimum level inclusion of green tea by-products in layer diets and to determinate the potential values of the green tea by-products as a feed ingredient for poultry feed.

MATERIALS AND METHODS

1. Animals and Design

Total of 96 Tetran Brown (22 weeks of age) laying hens was used in this experiment. The layers were divided to 4 treatments and 4 replicates with 6 layers per replicate based on egg production. The layers were kept to battery cages and the cage size was 24 × 36 × 40 cm for 2 layers. Experimental diets and drinking water were provided *ad libitum*. The lighting program and rearing method followed by general instructions for rearing commercial laying hens. The egg collection was done once a day at 4 pm. The layers were given a one week of adjustment period and the trial period was 8 weeks. The egg production was recorded every day and egg weight, egg mass, egg shell thickness and feed intake were determined on a weekly basis.

2. Diets

The green tea by-products used in this experiment were provided from the Green Tea Experimental Station (Bo-sung, Korea). The experimental diets were control (basal diet without green tea by-product), 2% green tea by-product (basal diet +2% GTB), 4% green tea by-product (basal diet + 4% GTB) and 6% green tea by-products (basal + 6% GTB). The basal and experimental diets were formulated to meet or exceed the nutritional requirements for laying hens suggested by the NRC (1994). Formula and chemical composition of experimental diets are given in Table 1. Chemical compositions of green tea-by products were determined and the results are shown in Table 2.

Table 1. Formula and chemical composition of basal diet

Ingredient	%
Corn grain	66.00
Soybean meal-45	11.06
Corn gluten meal-60	5.00
Wheat bran	8.50
Lysine	0.14
Methionine	0.10
Limestone	8.00
Salt	0.25
Vit-min.mix[1]	0.50
Tricalcium phosphate	0.45
Chemical composition[2]	
ME (kcal/kg)	2700,00
Crude protein	15.00
Calcium	3.25
Phosphorous	0.25

[1] Contained per kg diet: 9,000,000 IU; Vitamin D_3, 2,100,000 IU; Vitamin E, 15,000 IU; Vitamin K, 2,000mg; Vitamin B_1, 1,500mg; Vitamin B_2, 4000mg; Vitamin B_6, 3,000mg; Vitamin B_{12}, 15 mg; Pan- Acid-Ca, 8,500mg; Niacin, 20,000mg; Biotin, 110mg; Folic-Acid, 600mg; Fe, 40,000mg; Co, 300mg; Cu, 3,500mg; Mn 55,000mg; Zn, 40,000mg; 1,600mg; Se, 130mg.

[2] Calculated value.

Table 2. Chemical composition of green tea by-product

Content	Green tea by-product
Tannin (%)	5.95
Caffeine (%)	1.29
Chlorophyll (mg/g)	115.00
Moisture (%)	9.05
Crude ash (%)	4.77
Crude fiber (%)	62.03
Crude protein (%)	21.16
Ether extract (%)	2.99

3. Egg Yolk Cholesterol

The egg yolk cholesterol content was determined at the 4th and 8th week of the experiment. Five eggs from each treatment were selected for analysis a total of 20 eggs. To determination of cholesterol content, 0.5 g of egg yolk from each sample was transferred to a test tube and 100 μg of 5α-cholestane was added. The resulting slurry was homogenized with 0.5N KOH solution and saponified for 30 minutes at 55℃. The cholesterol content was extracted through hexane before injecting for gas chromatography (HP5890 series II). The column used with CG was a HP-1 (cross-linked methyl silicone, 25 m×0.32 mm×0.17 μm) capillary column and the temperature of the column was maintained at 290℃ (Brunnekreeft, 1983).

4. Composition of Fatty Acid in Egg Yolk

The fatty acids composition of the egg yolk was determined at 4th and 8th week of the experiment. For fatty acids composition analysis of the egg 5 g of egg yolk were collected. Each egg yolk sample was dissolved to 100ml of Folch solution (Chloroform: methanol 2:1 v/v) and blended for 30 minutes. The samples were flushed with nitrogen gas for 30 minutes in an evaporator then filtrated through a Buchner funnel. The filtrate was transferred into 70 ml of distilled water, blended gently and then kept at 5℃ in a refrigerator until it separated into 2 layers. After phase separation, the bottom layer was evaporated at 35℃ with nitrogen gas. Then the concentrate was dissolved in 3 ml of 5% sulfuric acid methanol. The tubes were heated in a water bath at 95℃ for 45 minutes and cooled. When cooled, fatty acid methyl ester was extracted three times with 3 ml of petroleum ether, dried with nitrogen gas, dissolved again in 100ml of petroleum ether and then injected into gas chromatography.

5. Statistical Analysis

The data from this study were analyzed by SAS Package Program (1989) to estimate variance components. Duncans multiple comparison tests (1955) were used to compare the significant differences between treatment means. Differences were statistically assessed with the significance set at 5%.

RESULTS AND DISCUSSION

1. Productive Performance

The effects of the green tea by-products on egg production, egg weight, egg mass, feed intake, feed conversion ratio and egg shell thickness are shown in Table 3. The laying hens that consumed diets containing 4% and 6% green tea by-products supplementation showed significantly higher egg production rates compared to these fed the control and 2% green tea by-product supplemented diet ($P<0.05$) but there were no significant differences in egg production of layers fed 4% and 6% green tea-by product diet ($P>0.05$). There were observed very significant differences in egg production rates between layers fed different levels of green tea by-product supplemented diet, it could be caused by high temperature condition faced during the experimental period and the low number of laying hens used in the experiment. The egg weight and egg mass were reduced significantly for layers that consumed a diet

Table 3. Effect of dietary green tea by-product on egg laying performance of the hens

Items	Treatments			
	T0[1]	T1[2]	T2[3]	T3[4]
Egg production rate (%)	79.62[b]	75.00[b]	90.37[a]	86.87[a]
Egg weight (g)	61.81[a]	59.68[b]	62.70[a]	61.81[a]
Egg mass (g)	49.51[bc]	45.37[c]	56.63[a]	53.59[b]
Feed intake (g/bird/d)	138.13[a]	117.13[b]	138.13[a]	118.13[b]
Feed conversion (g/g)	3.03[a]	2.69[ab]	2.49[b]	2.30[b]
Egg shell thickness(μm)	397.50	385.00	387.50	385.00

[ab] Means with different superscripts in the same row are significantly different ($P<0.05$).

[1] Control, [2]GTB addition by 2.0%, [3]GTB addition by 4.0%, [4]GTB addition by 6.0%.

containing 2% green tea-by product supplementation compared to layers fed 4% and 6% green tea by-product supplemented diet ($P<0.05$). The reduction of egg mass for the 2% green tea by-product group was due to low egg production and low egg weight obtained from GTB 2% dietary group. The feed intake was increased significantly for layers fed the control and 4% green tea by-product supplemented diet compared to those groups fed 6% and 2% green tea by-product supplementation ($P<0.05$). There was no significant difference in feed intake of the layers fed 2% and 6% green tea by-product supplemented diet ($P>0.05$). Also, high environmental temperatures during the experimental period have affected on feed intake of the layers. Bottjed and Harrison (1985) reported that feed intake of cockerels was decreased at high temperatures in summer. The feed conversion ratio was improved significantly for the green tea by-product groups compared to the control ($P<0.05$) and the highest feed conversion ratio was shown in the 6% green tea by-product supplemented diet. Egg shell thickness slightly decreased when increasing green tea by-product inclusion levels, but there were no significant difference observed among treatments. The egg shell thickness from layers fed diets containing green tea by-products inclusion was much thicker than those fed diets containing hot pepper supplementation (Choi et al., 1988).

2. Egg Yolk Cholesterol

Table 4 shows the effect of green tea by-products on egg yolk cholesterol at the 4th and 8th week of experiment. In the initial period, the cholesterol concentration of egg yolks from layers fed diets containing green tea by-product supplementation tended to decrease when increasing the level of green tea by-products, but it was not significantly different among treatment groups ($P>0.05$). At the 8th week of the experiment the egg yolk cholesterol content varied between green tea by-product and control groups and the lowest egg yolk cholesterol was obtained from the treatment 4% green tea by-product supplemented diet. There was no significant difference in egg yolk cholesterol contents between the 4th and 8th week of the experiment ($P>0.05$) Bair and Marion (1977) reported that egg yolk cholesterol contents tended to decrease at increasing age of laying hens. Balmer and Zilversmit (1974) reported that roughage plants had positive effects on cholesterol absorption, cholesterol turnover excretion in rats. Park and Song (1997) said that the inclusion of Korean medical herbs in broiler diets reduce the cholesterol content of chicken meat. An et al. (1992) reported that alfalfa meal in layer diets decreased egg yolk cholesterol content.

Table 4. Effect of dietary green tea by- product on egg yolk cholesterol

Treatment	4 week	8 week
	(mg/g)	(mg/g)
T0[1]	15.09±1.22[ns]	14.61±0.36[ns]
T1[2]	14.32±0.47	14.48±0.35
T2[3]	14.23±0.62	13.64±0.24
T3[4]	13.85±0.82	14.02±1.13

[ns] Not significant.

[5] Standard error of the mean.

[1] control, [2]GTB addition by 2%, [3]GTB addition by 4%, [4] GTB addition by 6 %.

3. Fatty Acid Composition

The fatty acid composition of egg yolks at the 4th week of the experiment is presented in Table 5. The content of oleic and palmitic acids were highest among all fatty acid groups. The inclusion of green tea by-products in layer diets reduced significantly the palmitoleic acid content of egg yolks. The oleic and linolenic acids were increased significantly in layers fed a diet containing 6% green tea by-product supplementation ($P<0.05$). The docosahexaenoic acid (DHA), the unsaturated fatty acid, was increased significantly in layers fed a diet containing 4% green tea by-products ($P<0.05$).

Table 6 shows the fatty acid composition of egg yolks at the 8th week of the experiment. Most of fatty acid composition of the egg yolk was similar at the 4th week of the experiment for all groups except the oleic acid that was decreased in eggs from layers fed diets containing green tea by-product supplementation. At the 8the week, the palmitoleic acid content was decreased compared to the 4th week of the experiment, but there were no significant differences among green tea by-product treatment groups ($P>0.05$). The oleic acid was decreased significantly in layers fed a diet containing 4% green tea by-product supplementation ($P<0.05$). The linolenic acid was

Table 5. Effect of green tea by-product on fatty acid composition of egg yolk at 4th week

Fatty acid (%)	T0[1]	T1[2]	T2[3]	T3[4]
Myristic (14: 0)	0.44±0.04[a]	0.38±0.05[bc]	0.40±0.06[ab]	0.34±0.03[c]
Palmitic (16:0)	27.92±1.275	26.42±1.24	26.57±1.04	25.75±0.94
Palmitoleic (16:1)	5.66±1.78[a]	4.36±0.91[b]	4.21±0.43[b]	4.03±0.86[b]
Stearic (18:0)	8.80±0.80	9.48±0.65	9.31±0.57	8.88±0.38
Oleic acid (18:1)	42.54±1.64[c]	45.54±1.22[ab]	44.19±3.07[bc]	46.32±1.28[a]
Vaccenic acid (18: 1)	1.06±1.15[a]	0.34±0.96[ab]	0.32±0.90	nd
Linoleic acid (18:2)	10.46±1.29	10.35±1.32	11.56±1.63	11.39±1.38
Linolenic acid (18:3)	0.23±0.02[c]	0.25±0.04[c]	0.33±0.06[b]	0.43±0.05[a]
Eicosamonoenoic (20:1)	0.39±0.08	0.34±0.03	0.38±0.10	0.35±0.09
Eicosadienoic (20:2)	0.22±0.16	0.22±0.22	0.21±0.20	0.13±0.01
Eeicosatrienoic (20:3)	0.22±0.08	0.22±0.18	0.16±0.07	0.14±0.02
Arachidonic acid (20:4)	1.53±0.16	1.66±0.19	1.72±0.75	1.64±0.34
Eicosapentaenoic (20:5)	0.14±0.31	0.02±0.03	0.04±0.04	0.08±0.16
Docosahexaenoic (22:6)	0.34±0.05[d]	0.44±0.06[c]	0.62±0.11[a]	0.55±0.08[b]

[1] Control, [2]GTB addition by 2.0%, [3]GTB addition by 4.0%, [4]GTB addition by 6.0%.

[abcd] Mean with different superscripts in the same row are significantly different (P<0.05).

[nd] Not detected.

[5] Standard error of the mean.

Table 6. Effect of green tea by-product on fatty acid composition of egg yolk at 8th week

Fatty acid (%)	T0[1]	T1[2]	T2[3]	T3[4]
Myristic (14: 0)	0.39±0.06	0.37±0.05	0.37±0.02	0.36±0.03
Palmitic (16:0)	26.03±1.54[5]	26.70±1.06	25.95±0.88	25.79±0.70
Palmitoleic (16:1)	2.67±1.74	3.54±0.63	2.74±0.85	3.13±0.33
Stearic (18:0)	8.62±1.05	8.22±0.78	8.40±0.65	8.42±0.41
Oleic acid (18:1)	41.31±1.33[a]	41.18±2.28[a]	39.16±1.68[b]	40.17±1.20[ab]
Vaccenic (18: 1)	6.94±1.16	2.08±0.25	1.82±0.08	1.89±0.13
Linoleic acid (18:2)	16.35±1.94[b]	14.63±1.43[c]	18.16±1.12[a]	16.77±1.26[ab]
Linolenic acid (18:3)	0.25±1.94[b]	0.28±1.94[b]	0.38±1.94[a]	0.38±1.94[a]
Eicosamonoenoic (20:1)	0.31±0.03	0.35±0.09	0.29±0.09	0.32±0.15
Eicosadienoic (20:2)	0.18±0.03	0.16±0.02	0.27±0.20	0.18±0.03
Eeicosatrienoic (20:3)	0.14±0.01	0.14±0.01	0.15±0.04	0.15±0.02
Arachidonic (20:4)	2.07±0.25	1.98±0.31	1.84±0.18	1.98±0.20
Eicosapentaenoic (20:5)	nd	-	0.07±0.20	-
Docosahexaenoic (22:6)	0.32±0.05[c]	0.40±0.03[b]	0.41±0.06[b]	0.47±0.03[a]

[ab] Means with different superscripts in the same row are significantly different (P<0.05).

[nd] Not detected.

[5] Standard error of the mean.

[1] Control, [2]GTB addition by 2.0%, [3]GTB addition by 4.0%, [4]GTB addition by 6.0%.

increased significantly in layers fed diets containing 4% and 6% green tea-by products compared those fed control and 2% green tea by-product supplemented diet ($P<0.05$). Han et al. (1999) reported that enrichment of ω-3 fatty acids in layer diets increased ω-3 fatty acid contents in the egg yolk. Docosahexaenoic acid (DHA), an unsaturated fatty acid, increases significantly when layers consumed a diet containing 6% green tea green tea by-product supplementation compared those fed the control, 2% and 4% green tea by-product supplemented diet ($P<0.05$). Kim et al. (1999) reported that the inclusion of safflower oils in layer diets reduced significantly docosahexanoic acid. Dyeberg et al. (1975) reported an optimum level of docosahexaenoic acid belonging to the ω-3 group played an important role in control of blood pressure and blood cholesterol content. Our results indicated that 4% and 6% green tea by-products in layer diet had positive effects on egg production rate, egg weight and feed intake of the layer The supplementation with green tea-by products in layer diet improved the feed conversion ratio of laying hens from 22 to 30 weeks of age. Inclusion of green tea by-products in layer diet tended to reduce egg yolk cholesterol. The linoleinic and docosahexaenoic acid contents were increased in layers fed diets containing 4% and 6% green tea by-product supplementation.

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적 요

본 연구는 산란계에서 사료내 녹차 가공부산물을 첨가하여 난 생산성과 난 성분에 미치는 영향과 녹차 부산물의 최적 첨가수준을 알아보고자 연구하였다. 공시동물은 갈색 테트란 산란계 22주령 총 96수를 이용하였다. 녹차 부산물을 첨가를 각각 0%, 2%, 4% 및 6% 수준으로 4처리 4반복 반복당 6수로 완전임의 배치하였으며, 사료급여와 물은 자유 채식토록 하였고 나머지는 일반 관행에 따랐다. 실험 결과로 산란율은 4% 첨가구와 6% 첨가구가 각각 90.37%와 86.87%로 대조구 79.62%보다 높게 나타났으며, 통계적 유의차를 보였다($P<0.05$). 난중, 산란량 및 사료섭취량은 2% 첨가구가 59.68, 45.37 및 117.13 g으로 대조구와 다른 처리구보다 낮은 경향을 보였다 ($P<0.05$). 난각의 두께는 대조구가 397.50 ㎛로 가장 두꺼웠지만 처리구간 통계적 유의차를 보이지 않았다 ($P>0.05$). 4주와 8주때 측정한 난황 콜레스테롤 함량은 녹차 가공부산물의 첨가수준이 증가할수록 낮아지는 경향을 보였으나 처리간 통계적 유의성은 없었다 ($P>0.05$). 계란의 난황내 지방산 함량은 linolenic acid와 docosahexaenoic acid (DHA)가 대조구보다 녹차 가공 부산물을 첨가한 처리구에서 증가하는 경향을 보였다.

(색인 : 녹차 가공 부산물 (GTB), 사료요구율, 난황내 콜레스테롤, 지방산, 산란계)

REFERENCES

AOAC 1990 Official Methods of Analysis, 15th ed. Association official Analytical Chemists. Washington DC.

An BK, Chung TY, Kim CM, Lee SJ, Kim SS, Chung SB 1992 Effect of dietary alfalfa meal on egg. Yolk Cholesterol Content and Productivity in Laying Hens. Kor J Poult Sci 19: 125-136.

Bair CW, Marion WW 1978 Yolk cholesterol in eggs from various avian species. Poultry Sci 57: 1260-1265.

Balmer J, Zilversmit DB 1974 Effects of dietary roughage on cholesterol absorption, cholesterol turnover and steroid excretion in the rat. J Nutr 104: 1319-1328.

Bottjed WG, Harrison PC 1985 The effect of tap water, carbonated water, sodium bicarbonate, and calcium chloride on blood acid-base balance in cockerels subjected to heat stress. Poult Sci 64: 107-113.

Brunnekreeft JWG, Boerma JM, Leijnse B 1983 Direct determination of total cholesterol by column gas chromatographic analysis without previous derivatisation compared with WHO-CDC reference method. Ann Clin Biochem 20: 360-363.

Choi BR, Kim YI, Oh SJ 1988 Effect of hot pepper on egg shell component and egg yolk color in broiler parent stock.

Kor J Poult Sci 15: 281-286.

Duncan DB 1955 Multiple range and multiple F tests. Biometrics. 11 :1.

Dyeberg JH, Bang O, Hjome N 1975 Fatty acid composition of the plasma lipids in greenland eskimos. Am J Clin Nutr 28: 958.

Fuenter W, JS Sim 1998. Production of Special and Modulated Eggs. Proceeding of the 8th World Conference on Animal Production. pp 361 June 28-July 4 Seoul Korea.

Han CK, Lee BH, Lee NH 1999. Analysis of biofunctional components in brand eggs. Kor J Anim Sci 41: 343-354.

Ikeda I, Imasato Y, Sasaki E 1992. Tea catechins decrease micellear solubility and intestinal absorption of cholesterol in rats. Biochem Biophys Acta 1127: 141.

Isigaki K, Takakuwa T, Takeo T 1991 Anti-diabetes mellitus effect of watersoluble tea polysaccharide. Proceeding of the International Symposium on Tea Science. Japan pp 240-242.

Kendler BS 1987 Garlic (*Allium sativum*) and onion (*Allium cepa*): A revies of their relationship to cardiovascular disease. Prev Med 16: 670-685.

Kim EM, Choi JH, Chee KM 1997 Effects of dietary safflower and perilla oils on fatty acid composition in egg yolk. Kor J Anim Sci 39: 135-144.

Kohne HJ, Jones JE 1975 Changes in plasma electrolytes, acid-base balance and other physiological parameters of adult female turkeys under conditions of acute hyperthermia. Poult Sci 54:2034-2038.

Matsuzaki T, Hara Y 1985 Antioxidative activity of tea leaf catechins. Nipon Kaishi Nogeikagaku 59: 129.

Mukhatar H, Ahmad N 1999 Mechanism of cancer chemopreventive activity of green tea. Proc Soc Exp Biol Med. 220: 234-238.

Muramatsu K, Fukuyo M, Hara Y 1986 Effect of green tea catechins on plasma cholesterol level in cholesterol fed rats. J Nutr Sci Vitaminol 32: 613-622.

Naber EC 1976 The cholesterol problem, the egg and lipid metabolism in the laying hen. Poult Sci 55: 14-30.

National Research Council 1994 Nutrient requirements of poultry 9th ed National Academy Press, Washington DC USA.

Park JH, Song YH 1997 Nutritive value of Korean medical herb residue as dietary supplements for broiler chicks. Kor J Anim Nutr Feed. 21: 59-64.

Ryuhei F 1990 A tentative approach to dietary control of again process-antioxidative activity of tea leaf catechins *in vivo* Fragrance Jour 11: 20-23.

SAS/STAT 1990 SAS user guide. Statistics Version 6. Fourth Edition SAS Institute Inc Cary NC.