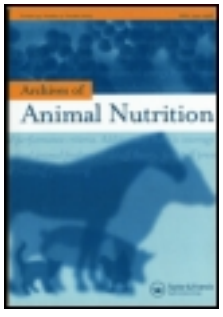

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Effect of adding extracted hesperetin, naringenin and pectin on egg cholesterol, serum traits and antioxidant activity in laying hens

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Effect of adding extracted hesperetin, naringenin and pectin on egg cholesterol, serum traits and antioxidant activity in laying hens

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In this study three feed additives (hesperetin, naringenin and pectin) for laying hens were investigated on their influence on the egg yolk cholesterol, serum traits and antioxidant activities in hens. Additives were extracted from citrus and grapefruit peels and contained 31.5% crude hesperetin, 39% crude naringenin and 60% galacturonic acid (pectin). Eighty 30-week-old Leghorn laying hens were randomly assigned to four groups and received, for two months, a control diet or diets with 0.05% hesperetin, 0.05% naringenin or 0.5% pectin. All additives reduced the egg yolk cholesterol level significantly. Feeding diets with added flavonoids (hesperetin and naringenin) increased the yolk weight and the ratio of yolk weight/egg weight and the blood serum superoxide dismutase (SOD) activity was elevated. Total antioxidation capacity, the level of thiobarbituric acid-reactive substances and superoxide scavenging capacity in the naringenin group were greater than in the control group. Supplemented flavonoids reduced the serum cholesterol level significantly, while serum triglyceride concentration in the naringenin and pectin groups was reduced. Addition of flavonoids resulted in an enhanced cholesterol level in excreta. The results of this study indicated that intake of hesperetin, naringenin and pectin extracted from citrus and grapefruit peel in laying hens diet, may exhibit positive effects.

Keywords: hesperetin; naringenin; pectin; egg; antioxidation; hens

1. Introduction

Large quantities of citrus and grapefruits are produced in Taiwan during summer. These fruits are often used for juice extraction creating problems in disposing the peels. Citrus peels contain abundant flavonoids called ''hesperetin'' while grapefruit peels contain ''naringenin''. Larrauri et al. (1996) reported that citrus peels contain high levels of hesperetin (904 μg/g), which plays a role in reducing cholesterol, based on the structure of flavonoids (Figure 1). It contains numerous OH groups, which can supply H atoms to quench free radicals, making it a strong antioxidant (Deng et al. 1997; Jeon et al. 2001). Many diseases are triggered by free radicals that damage cells. Flavonoids are known for health-promoting effects in humans and animals. In this study, we investigated hesperetin and naringenin as potential antioxidants.

Peels of citrus and grapefruit also contain large amounts of pectin, which can reduce cholesterol in rats (Sudheesh and Vijayalakshmi 1999). Pectin (partially methoxylated polygalacturonic acid) is a kind of carbohydrate gel, a component of the plant cell wall.

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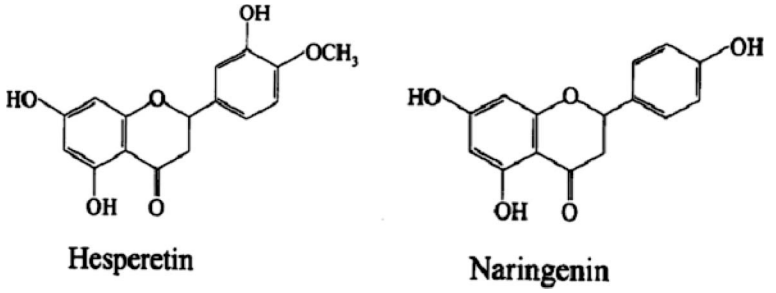

Figure 1. Chemical structure of hesperetin and naringenin.

Because pectin has a high ability to absorb water, its viscosity has significant health benefits and it can be used for treating diarrhea. In the intestine, pectin can also absorb lipids, cholesterol and bile acids, and thus it interferes with the absorption of those substances and gut-liver recycling, and consequently, this binding capacity increases lipid and cholesterol excretion (Sudheesh and Vijayalakshmi 1999; Roy et al. 2002). Therefore, a reducing effect on yolk cholesterol level can be expected.

In this study, the dietary effects of hesperetin and naringenin on egg quality, serum traits and the antioxidant ability of Leghorn laying hens were investigated.

2. Materials and methods

2.1. Hesperetin and naringenin extraction

This study followed the extraction procedure described by El-Nawawi (1995). Citrus or grapefruit peels (2 kg) were cut into small pieces and 3 l of water was added. The mixture was boiled and stirred for 90 min. Then lime was added to pH 11, and then the mixture was allowed to stand at room temperature for 30 min. It was then filtered and HCl was added to pH 4.7, the mixture was heated to 50°C and stirred for 90 min. Thereafter, the mixture was chilled overnight and the precipitate was separated and dried. The end product was the crude hesperetin and naringenin, respectively.

2.2. Pectin extraction

According to the extraction method by Kar and Arslan (1999), 1 kg of citrus or grapefruit peels were added to 2.5 l of 0.03 N HCl with pH 2.5, heated to 90°C for 90 min, filtered and allowed to cool to 37°C. Then the mixture was supplemented with 0.1 M Na_3PO_4 (pH 7.5) and 5 mg protease, stored overnight at room temperature before being filtered after which 95% ethanol was added. The floating pectin was picked out, dried and ground.

2.3. Determination of hesperetin, naringenin and pectin concentration

Hesperetin and naringenin were measured following the method described by Bronner and Beecher (1995) and Kanaze et al. (2004) using HPLC. Briefly, standards for narigenin and hesperetin (Sigma, USA) were diluted to various concentrations with methanol and water (1:1). Samples and standards were added to 100 µl 1 M sodium acetate buffer (pH 5) and

40 µl β-glucuronidase for 18 h at 37°C for degradation. Subsequently 2 ml ethanol was added, and the samples were filtered (0.45 µM membrane), dried at 45°C, and dissolved with mobile phase solvent.

HPLC analysis was performed using a C18 cartridge column, preconditioned with 6 ml methanol and 6 ml 0.01 M HCl. The mobile phase was methanol/water/acetic acid (40:58:2) (flow rate 1 ml/min, UV detector set at 280 nm, and sample injected volume of 20 µl).

Pectin was analysed by examining the galacturonic acid content in accordance with the procedure of Cho et al. (2003). Samples and standard (0–100 µl galacturonic acid) were mixed with 200 µl H_2O, 3 ml concentrated H_2SO_4, 100 µl, carbazole (0.1%), then incubated at 60°C for 1 h, after which galacturonic acid content was determined at 530 nm using a spectrophotometer (Hitachi, U 2000, Tokyo, Japan).

2.4. Animals and treatments

Eighty 30-week-old white Leghorn laying hens were selected and randomly divided into four groups, including a control group (basal diet) (Table 1). The diets of the other three groups were supplemented with 0.05% hesperetin, 0.05% naringenin or 0.5% pectin. Layers were caged individually, with free access to water and received equal amounts of feed (per hen about 110 g/d). The average room temperature was 29.6°C in the daytime. Light was controlled by a timer and provided 15 h light daily. The study was conducted for two months and egg production and egg weights were recorded. At the final stage of the experiment blood was sampled via the wing vein (fasting overnight before blood sampling), and serum was prepared. Additionally, excreta and egg samples were taken from each hen at the end of the experiment. Animals used in this experiment were reared following the guidelines in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies [FASS] 1999).

Table 1. Composition of basal diets.

Ingredients	Contents [%]	Nutrients	Contents
Yellow corn meal	60.75	*Calculated*	
Soybean meal (44% CP)	24.00	Crude protein [%]	17.80
Fish meal (65% CP)	3.00	ME [MJ kg^{-1}]	11.82
Soybean oil	2.00	Calcium [%]	3.40
Dicalcium phosphate	1.30	Available phosphorous [%]	0.47
Limestone, pulverised	4.25	Lysine [%]	0.98
Salt	0.30	Methionine + cystine [%]	0.62
Vitamin premix*	0.05		
Mineral premix[#]	0.20	*Analysed*	
Oyster shells	4.00	Crude protein [%]	17.52
DL-methionine	0.05	GE [MJ kg^{-1}]	15.06
Choline chloride (50% purity)	0.10	Calcium [%]	4.1
		Total phosphorous [%]	0.62

*Supplied per kg diet: vitamin A, 12,500 IU; vitamin D_3, 3125 IU; vitamin E, 37.5 mg; vitamin K_3, 6.25 mg; vitamin B_1, 3.75 mg; vitamin B_2, 12.5 mg; vitamin B_6, 10.0 mg; pantothenate, 18.8 mg; niacin, 50 mg; biotin, 0.06 mg; folic acid, 1.25 mg; vitamin B_{12}, 0.05 mg; [#]Supplied per kg of diet: Cu ($CuSO_4 \cdot 5H_2O$, 25.45% Cu) 6 mg; Fe ($FeSO_4 \cdot 7H_2O$, 20.29% Fe) 50 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn) 40 mg; Zn (ZnO, 80.35% Zn) 60 mg; Se ($NaSeO_3$, 45.56% Se) 0.075 mg.

2.5. Determination of traits and methods

Egg quality parameters involved egg shell thickness, egg shell strength, yolk weight and yolk cholesterol content. Egg shell strength was evaluated using a press meter (FHK, Japan) to measure the pressure each egg could sustain before shell breakage. Egg shell thickness was examined at both the side and middle of a small piece of eggshell using a micrometer. The extraction of egg yolk cholesterol followed the method of Beyer and Jensen (1989) by homogenising an entire yolk, and then 0.5 ml of homogenated yolk was mixed with 5 ml of 2% (wt/vol) KOH-alcohol. This mixture was incubated at 60°C in a shaking water bath (100 strokes per min) for 1 h. After cooling to room temperature, 10 ml of petroleum ether was added, and the supernatant was taken for cholesterol measurement by a commercial kit.

Cholesterol content of excreta was determined by extraction of steroids with petroleum ether, and saponification with KOH-alcohol, and then the cholesterol was measured by a commercial kit (Dongowski and Lorenz 2004). Serum cholesterol was determined with an enzyme kit (Roche Co. No. 07-36635, Switzerland) using an automatic serum biological analyser (Roche, Co. Switzerland).

Serum lipoproteins including HDL (high density lipoprotein), LDL (low density lipoprotein) and VLDL (very low density lipoprotein) were determined by electrophoresis. Each band percentage was then measured by a densitometer (Helena Co. 8JF00105, USA).

Serum total globulin and γ-globulin were measured by an electrophoresis method and then scanned by a densitometer (Helena Co. 8JF00105, USA). Serum total protein was determined with a commercial kit (Roche Co., Switzerland).

2.6. Antioxidant activity traits examination

Serum superoxide dismutase (SOD) activity was determined according to Ellerby and Bredesen (2000): 15 μl of a stock solution of 6-hydroxydopamine were added to 1 ml of 0.05 M sodium phosphate, 0.01 mM diethylenetriaminepentaacetic acid (pH 7.4) and 100 μl serum. The autooxidation of 6-hydroxydopamine was recorded at 490 nm in triplicate. One unit of enzyme activity is presented as mg of protein resulting in 50% inhibition of 6-hydroxydopamine autooxidation per min. A purified SOD (Sigma Co, USA) used as standard.

Catalase activity was measured according to the method described by Ellerby and Bredesen (2000). Briefly, in a quartz cuvette containing 100 μl 1 M Tris and 5 mM EDTA buffer (pH 8.0), 100 μl saturated thymol, 100 μl aminoantipyrine, 100 μl peroxidase (1 U/ml), 50 μl saturated thymol H_2O_2 and 540 μl H_2O, mix with 10 μl serum sample, then measured at 505 nm for 5 min at 25°C by a spectrophotometer. One unit of catalase decomposes 1.0 mM of H_2O_2 per min. Purified catalase was used as standard. The enzyme activity is expressed as unit/mg protein.

Glutathione peroxidase (GSH-Px) activity examination was conducted following the method reported by Bhat et al. (1992): 0.8 ml substrate contain 1 mM EDTA, 1 mM $NaHCO_3$, 0.2 mM NADPH, 1 U/ml glutathione reductase, 1 mM glutathione and 100 mM KH_2PO_4 (pH 7.0) are mixed with 25 μl serum sample and kept at room temperature for 5 min. The reaction is started by addition of 0.1 ml 2.5 mM H_2O_2 and then measured using a spectrophotometer (Hitachi U-2000, Tokyo, Japan) at 340 nm for 3 min at 25°C. One unit is defined as 1 nM NADPH oxidised per mg protein and min.

Trolox (a derivate of water-soluble vitamin E) equivalent (some papers also term it as total antioxidant ability) was examined using the method described by Erel (2004).

The measurement was performed using 200 µl reagent 1 (0.4 M acetate buffer, pH 5.8), containing 0.4 M CH_3COONa and 0.4 M acetic acid) mixed with 20 µl reagent 2 (30 mM acetate buffer, pH 3.6, containing 30 mM CH_3COONa and 30 mM acetic acid), then 2.86 M H_2O_2 and 5 µl sample were added. After incubation at 25°C for 5 min, absorbance was determined at 660 nm with a spectrophotometer. Authentic trolox (Sigma Chem. Co.) was used as standard.

Thiobarbituric acid-reactive substance (TBARS) was determined according to the procedure reported by Fraga et al. (1988). The peroxidative damage of serum lipids results in the production of malondialdehyde (MDA), which reacts with thiobarbituric acid under conditions of high temperaturen (80°C, 90 min) and acidity (trichloroacetic acid) resulting in a chromogen that can be measured spectrophotometrically at 535 nm. The unit is expressed as nM MDA/ml. Scavenging superoxide activity was assessed following the method described by Shi and Dalal (1991): A cuvette containing 0.95 ml of reaction solution (50 mM potassium phosphate, pH 7.8; 1 mM EDTA; 100 µM nitroblue tetrazolium (NBT); 0.025% triton x-100; 100 µM hypoxanthine and 50 µl serum sample); the reaction was started by addition of 0.025 U/ml xanthine oxidase, and the end product was measured using a spectrophotometer at 560 nm for 5 min under 25°C. One unit was defined as the amount that was required to inhibit the reduction rate of NBT by 50%. The enzyme specific activity was expressed in unit per mg protein per min. Serum total protein concentration was examined by Lowry method (Lowry et al. 1951).

2.7. Statistical analysis

The experimental data were subjected to statistical analysis by GLM using the SAS (statistical analysis system) software for variance analysis while the significant differences among groups were determined using Duncan's New Multiple Range test (SAS 1998) according to the model $Y = \mu + T_i + e_{ij}$. Where Y denotes the dependent variable, µ represents the mean, T is the treatment effect and e denotes the random residual error term. All values were presented as means and SEM, the level of significantly different was set at $p < 0.05$.

3. Results

The hesperetin and naringenin contents in the dried extracts were 31.5% and 39%, respectively. The extracted pectin contained 60% galacturonic acid.

Table 2 shows the effects of hesperetin, naringenin and pectin supplementation on egg quality. The egg yolk cholesterol was significantly reduced in all treatment groups. The egg yolk weight and yolk/egg ratio in the hesperetin and naringenin supplemented groups were higher than in the control group ($p < 0.05$). Egg production, egg shell strength and thickness and egg weight did not differ significantly among groups.

Table 3 shows the effects of hesperetin, naringenin and pectin supplementation on serum traits. Serum cholesterol level was reduced by hesperetin and naringenin supplementation ($p < 0.05$). Concentration of serum triglycerides (TG) was lower in the naringenin and pectin groups than the control group ($p < 0.05$). The serum γ-globulin and total globulin percentages were markedly greater in both the hesperetin and pectin groups than in the control group ($p < 0.05$). Cholesterol level in excreta was markedly higher in the hesperetin and the naringenin groups than in the control group ($p < 0.05$). The percentage of serum lipoproteins did not differ significantly among groups.

Table 4 shows the effects of hesperetin, naringenin and pectin supplementation on antioxidation activity. Serum SOD activity in both the hesperetin and the naringenin groups was markedly increased compared to the control group ($p < 0.05$). Furthermore,

Table 2. Effect of supplemented hesperitin, naringenin and pectin on the egg traits of laying hens ($n = 20$).

	Experimental groups				
	Control	Hesperitin	Naringenin	Pectin	SEM
Egg production [%]	92.1	87.4	93.0	88.0	3.09
Eggshell strength [kg/cm^2]	3.19	3.56	3.57	3.34	0.13
Eggshell thickness [mm]	4.54	4.64	4.65	4.71	0.06
Egg weight [g]	61.9	60.2	61.1	61.3	0.89
Yolk weight [g]	17.95^b	19.1^a	19.2^a	18.1^b	0.39
Yolk weight/egg weight	0.29^b	0.32^a	0.31^a	0.29^b	0.01
Yolk cholesterol [mg/g]	12.31^a	10.22^b	9.80^b	9.55^b	0.71
Yolk total cholesterol [mg]	221.0^a	195.1^b	187.7^b	172.9^b	14.02

Means in the same row with different superscripts differ significantly ($p < 0.05$).

Table 3. Effect of supplemented hesperitin, naringenin and pectin on serum traits of laying hens ($n = 20$).

	Experimental groups				
	Control	Hesperitin	Naringenin	Pectin	SEM
Cholesterol [mg/dl]	142^a	116^b	112^b	124^{ab}	5.95
Triglyceride [mg/dl]	1746^a	1475^{ab}	1064^c	1219^{bc}	112
γ-globulin [g/dl]	0.53^c	0.87^b	0.65^{bc}	1.10^a	0.09
Total-globulin [g/dl]	3.08^c	3.29^a	3.12^{bc}	3.25^{ab}	0.06
Excreta cholesterol [mg/100 g DM]	7.43^b	14.18^a	16.73^a	11.66^{ab}	1.64
HDL* [%]	16.6	15.3	16.3	19.3	1.66
LDL§ + VLDL$^{\#}$ [%]	83.4	84.7	83.7	80.7	1.66

*HDL, high-density lipoprotein; §LDL, low-density lipoprotein; $^{\#}$VLDL, very low-density lipoprotein; Means in the same row not sharing the same superscript differ significantly ($p < 0.05$).

Table 4. Effect of supplemented hesperitin, naringenin and pectin on the antioxidant activity of laying hens ($n = 20$).

	Experimental groups				
	Control	Hesperitin	Naringenin	Pectin	SEM
SOD* [unit]‡	24.0^b	47.4^a	42.37^a	34.9^{ab}	4.64
Catalase [unit]	95.2	84.5	76.8	94.2	8.68
GSH Px$^{\#}$ [unit]	18.7	27.6	35.1	28.7	6.21
Trolox equivalent [mM/l]	30.2^b	33.9^b	56.0^a	34.31^b	5.10
TBARS§ [nM MDA†/ml]	11.95^a	7.69^{ab}	5.89^b	12.99^a	1.94
Scavenging O_2^- [unit]	84.4^b	91.8^{ab}	95.2^a	92.0^{ab}	2.82

*SOD, superoxide dismutase; ‡Unit, 1 n mole substrate consumed per mg protein and min; $^{\#}$GSH Px, glutathione peroxidase; §TBARS, thiobarbituric acid-reactive substance; †MDA, malondialdehyde; Means in the same row not sharing the same superscript differ significantly ($p < 0.05$).

both the trolox equivalent and the scavenging superoxide ability were significantly increased while the TBARS level was decreased in the naringenin supplemented group compared to the control group ($p < 0.05$). There were no differences in the catalase and the GSH Px activities among groups ($p > 0.05$).

4. Discussion

4.1. Effects on serum traits

The experimental results indicated that serum cholesterol was reduced by either hesperetin or naringenin supplementation. Other reports also demonstrated that Sprague-Dawley rats' diets supplemented with hesperetin or naringenin had decreased serum cholesterol level (Lee et al. 2003; Seo et al. 2003; Kim et al. 2004). This phenomenon occurs because liver cholesterol synthesis rate limiting enzyme-HMG-COA reductase, and acyl-CoA:cholesterol acyltransferase (ACAT) activities were reduced by hesperetin and naringenin supplementation (Lee et al. 1999; Bok et al. 2000; Kim et al. 2003; Lee et al. 2003; Seo et al. 2003; Kim et al. 2004), thus liver cholesterol synthesis was decreased. Consequently, serum and egg yolk cholesterol may also be reduced. As for pectin, some investigators also indicated that rat diets supplemented with 5–15% pectin reduced serum cholesterol (Suprijana et al. 1997; Vergara-Jimenez et al. 1999; Roy et al. 2002). Pectin is a soluble fibre and the monogastric animal's intestine cannot digest it by its own digestive enzymes, or absorb it. Pectin has a viscosity property and is a carrier of negative electrons (OH groups) that can absorb bile acids and bind with bile Na^+ salt, thus interfering in bile acid re-absorption by the gut (Sudheesh and Vijayalakshmi 1999). Therefore, excretion of bile acid, lipid and cholesterol was increased. Furthermore, in order to have sufficient bile acid, more cholesterol is necessary for bile acid synthesis that will reduce serum cholesterol level (Sudheesh and Vijayalakshmi 1999; Roy et al. 2002).

After supplementation with hesperitin or naringenin the serum cholesterol concentration was significant decreased, but pectin did not show the same effect. This may be due to the relative low supplementation level of pectin. Lee et al. (1999), Bok et al. (2000) and Lee et al. (2003) indicated that supplementation with hesperetin or naringenin could reduce serum TG level. However, in the present experiment, only the naringenin group exhibited this effect. On the other hand, the hesperetin group did not differ significantly from the control group. The pectin group also showed significantly reduced serum TG in laying hens, consistent with studies conducted on rats (Sudheesh and Vijayalakshmi 1999; Vergara-Jimenez et al. 1999; Roy et al. 2002; Seo et al. 2003).

Such results may be due: (1) To the viscosity property of pectin (negative charge), which enables it to absorb intestinal bile salt (positive charge), interferes in bile acid re-absorption of lipids (Sudheesh and Vijayalakshmi 1999); (2) Pectin can also decrease the activities of liver lipid synthesis enzymes such as glucose-6-phosphate dehydrogenase and malate dehydrogenase, and thus lipid synthesis can also be reduced (Sudheesh and Vijayalakshmi 1999); (3) The lipoprotein lipase (LPL) activity in the heart and the adipose tissue also increased. The function of LPL is to degrade the blood VLDL-TG, thus resulting in reducing serum TG (Sudheesh and Vijayalakshmi 1999); and (4) Grizard et al. (2001) additionally noted that pectin supplementation could reduce serum insulin level while increasing glucagon, which would depress lipid conversion from glucose. This may explain why pectin supplementation could reduce serum TG level.

This experiment showed that neither hesperetin nor naringenin nor pectin supplementation had an influence on the lipoprotein profile. The published literature on this

topic is scarce. Kurowska et al. (2000) reported that rabbits fed citrus and grapefruit juice showed reduced serum LDL levels.

This study found that supplementation with hesperetin or naringenin increased excreta cholesterol level, which is consistent with the report of Kim et al. (2004), which indicated that both hesperetin and naringenin supplementation increased neutral, acidic and total sterol excretion in rat faeces. In contrast, Lee et al. (2003) reported that naringenin supplementation in the diets of Sprague-Dawley rats resulted in less faeces sterol compared to the control group.

Both hesperetin and pectin supplementation increased serum γ-globulin and total globulin (including α, β and γ-globulin). Because γ-globulin contains a majority of IgG, this may indicate that hesperetin and pectin supplementation could promote antibody production and enhance immune capacity.

4.2. Effect on egg quality

Supplementation of hesperetin, naringenin and pectin in the layers' diet did not influence egg production, egg shell strength, shell thickness and egg weight. Orban et al. (1993) reported that egg weight enhanced up to 5% and egg specific gravity was increased in hens fed ascorbic acid (antioxidant) at 2–3 g/kg feed for 4 weeks. Zapata and Gernat (1995) observed similar results. After feeding hens with ascorbic acid at 250 mg/kg for 12 weeks, Keshavarz (1996) also observed increased egg weight, but no beneficial effects on shell quality. However, Kassim and Norziha (1995) indicated that a supplementation of 400–600 mg ascorbic acid per kg layer feed significantly reduced egg weight and had no effects on egg production. Thus, the supplementation of antioxidant to layers' diets does not give consistent results on egg production and egg quality.

This work showed that both hesperetin and naringenin supplementation could increase egg yolk weight and yolk/egg ratio, but the reasons remain unclear. Egg yolk cholesterol content can be reduced by hesperetin, naringenin and pectin supplementation. Egg quality is enhanced with reducing egg cholesterol content. This may increase consumer demand and thus the market price. The use of hesperetin and naringenin to produce low cholesterol eggs may be more acceptable to consumers because they are natural organic materials. Egg cholesterol is synthesised in the liver, and then combined with TG, phosphate and apolipoprotein to synthesise VLDL, and transferred to oocyte for yolk formation. Hesperetin and naringenin inhibit enzymes for cholesterol synthesis such as HMG-CoA reductase and ACAT activities (Lee et al. 1999; Bok et al. 2000; Kim et al. 2003, 2004; Lee et al. 2003), thus depressing cholesterol synthesis. Consequently, the transfer of cholesterol to oocytes is also reduced, and therefore also yolk cholesterol.

4.3. Effect on antioxidant activity

This investigation demonstrated that serum SOD level was increased by hesperetin and naringenin supplementation. Additionally, total antioxidant activity (trolox equivalent) and scavenging superoxide ability were enhanced, and TBARS level was decreased by naringenin supplementation. This indicated that these additives have an antioxidant activity, which was in accordance with the results of Jeon et al. (2001), Seo et al. (2003) and Kim et al. (2004). They also indicated that diets for rats or mice supplemented with naringenin caused an enhanced liver and serum SOD, catalase and glutathione peroxidase activities and reduced liver TBARS level. Deng et al. (1997) also indicated that hesperetin

and naringenin supplementation depressed superoxide and fat oxidation induced by Fe/H_2O_2. However, this study found no influence on catalase and GSH Px activities.

Both hesperetin and naringenin are flavonoids and have structures that are rich in hydroxyl (OH) groups capable of supplying hydrogen atoms for free radicals to block the oxidation chain reaction, and this moiety is comparable to vitamin E (α-tocopherol) (Van Acker et al. 2000; Miyake et al. 2003). Another antioxidant mechanism of flavonoids may result from the ability to produce chelates with metal ions such as iron and copper. Redox reactions are observed through the change of the oxidation state of the metal, jointly with the oxidation of the flavonoid by loss of hydrogen, rendering them inactive to participate in free radical generating through the Fenton reaction (Fernandez et al. 2002). Thus, hesperetin and naringenin are very good antioxidants. Erkoc and Erkoc (2002) compared the antioxidant property of naringenin and genistein and indicated that genistein may interact with its environment, especially in polar conditions. On the other hand, naringenin is more apolar, leading to its possibly more accelerated interaction with lipids. Miranda et al. (2000) have shown experimentally that naringenin exerts pro-oxidant effects on LDL oxidation. Oxidation of LDL is thought to play a central role in atherosclerosis. Regarding pectin, this work showed that it has no antioxidant activity. Related reports were also not found.

5. Conclusions

Supplementation of hesperetin, naringenin and pectin to the diet of laying hens reduced egg yolk cholesterol. Hesperetin and naringenin also decreased serum cholesterol and increased excreta cholesterol. Notably, naringenin and pectin supplementation reduce serum TG, while hesperetin and pectin could enhance serum total and γ-globulin. Additionally, hesperetin and naringenin supplementation increased serum SOD activity and TBARS level was reduced. Naringenin can also promote total antioxidant activity and scavenging superoxide ability. Therefore, from this study it is concluded that hesperetin, naringenin and pectin supplementation has a positive effect on lipid-related traits and antioxidant activity, and has a potential to produce low cholesterol eggs. However, dose responses still need further investigation.

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