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Effect of green tea and tea catechins on the lipid metabolism of caged laying hens

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ABSTRACT

The present study was conducted to evaluate the effects of supplementing different levels of green tea or tea catechins on total cholesterol (TC), total triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels in the plasma of laying hens, as well as the TC, TG and total fatty acid (TFA) levels in breast and thigh meat and egg yolk. Laying hens (1620), 16-week-old, were randomly assigned to 9 dietary groups. Each treatment had 180 birds with 3 replicates. The basal diet was respectively supplemented with green tea powder (0, 2, 4, 6, 8 g/kg) and tea catechins (0.5, 1, 1.5, 2 g/kg). Hens were then reared in battery cages for 60 days. Supplementation with 6 g/kg green tea and 1 g/kg tea catechins at day 56 lowered TC, TG and LDL levels in both plasma and meat, increased plasma HDL levels and decreased yolk TG levels, where statistical significance was considered quadratically. TC levels in yolk were not affected. The addition of green tea and tea catechins had no effect on the levels of myristic acid (C14: 0), stearic acid (C18: 0), oleic acid (C18: 1), linoleic acid (C18: 2n-6), arachidonic acid (C20: 4n-6) and TFA content in yolk, but increased the unsaturated fatty acid content. Dietary green tea and tea catechins increased the excretion of TFA in faeces compared to the control. These results suggest that the addition of green tea and/or tea catechins to the diet of laying hens can improve lipid metabolism and the fatty acid composition of egg yolk.

Key words: Green tea, HDL, Laying hens, Low-density lipoprotein,Tea catechins, Triglycerides

The cholesterol and triglyceride present in chicken meat and eggs may constitute a health hazard, if consumed regularly over long periods of time (Bragagnolo and Rodrigues-Ameya 2003) as high cholesterol is a major risk factor for coronary heart disease, heart attack, and stroke (Angelin *et al.* 2010). High triglyceride levels were found associated with an increased risk of ischemic stroke (Shahar *et al.* 2003, Psaty *et al.* 2004, Freiberg *et al.* 2008), coronary health problems (Sarwar *et al.* 2007) and symptoms of depression (Herva *et al.* 2006, McCaffery *et al.* 2003). Reducing the cholesterol and triglyceride levels in processed products is not a cost-effective process and may also reduce product quality (Hand *et al.* 1987, Hensley *et al.* 1995). Studies were carried out to reduce the cholesterol and fat contents in eggs (Elkin *et al.* 1999, Raes *et al.* 2002) or in broiler meat (Ahn *et al.* 1995).

Green tea has received considerable attention over claims that it has specific health benefits and antioxidant properties (Graham 1992). Studies revealed that ECG extracted from green tea lowers cholesterol and triglyceride concentrations in plasma (Lee *et al.* 2008). Tang *et al.* (2001) showed that adding 200–300 mg/kg tea catechins to chicken feed delayed lipid oxidation in chicken breast and thigh meat. However, little information is available about the effect of green tea and tea catechins on cholesterol and triglycerides levels. The objectives of our research were: (i) to evaluate the effect of high doses of dietary green tea and tea catechins on cholesterol and triglycerides levels in chicken meat and eggs, and (ii) to investigate the possibility of using green tea or tea catechins as an additive in chicken feed in the future.

MATERIALS AND METHODS

Dietary compounds: Tea catechins were purchased commercially and according to the company's certificate the composition was as follows: 415 g/kg EGCG; 113 g/kg EGC; 94 g/kg ECG; 54 g/kg EC; 70 g/kg gallocatechin gallate (GCG) and 3 g/kg caffeine. The composition of green tea, also obtained from the same source, was determined (Wu *et al.* 2004). As a percentage of wet weight, the composition was 45.8 g/kg moisture; 20.8 g/kg ECG; 17.6 g/kg EC; 58.3

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g/kg EGC; 200.1 g/kg EGCG; 10.3 g/kg GCG and 105.1 g/kg caffeine. Commercial test kits were used for cholesterol, triglycerides. Standard samples were used at concentrations of 0.0125, 0.025, 0.05, and 0.1 mg/ml to construct standard curves to assess levels of cholesterol and triglyceride.

Animal management: Lohmann (LSL) laying hens (1,620), whose beaks had been trimmed at day-old age and who had received relevant vaccinations were used. At 16 weeks of age, hens of similar body weight were randomly divided into 9 groups with 180 hens in each. The hens were housed in battery cages (60 cm × 60 cm × 44 cm) with five hens per cage, and the experiment began at 18 weeks of age. A control group (C) was fed a basal diet. The other eight groups were fed basal diets supplemented with either 2, 4, 6 or 8 g green tea powder/kg feed, or 0.5, 1, 1.5 or 2 g tea catechins/kg feed. Feeds (Table 1) were prepared weekly and the daily intake was 110–115 g per hen. The birds had *ad lib.* access to water.

Table 1. Composition and nutrients of diets fed to laying hens[A]

Ingredients	Compositions g/kg	Nutrition	Contents
Corn	625	ME (Kcal/kg)	10.83
Soybean meal	250	Calcium (g/kg)	32
Wheat bran	26.5	Total phosphorous (g/kg)	6
Limestone	60	Crude protein (g/kg)	161.2
(Phosphorous) P/ (Calcium) Ca	1.5	Methionine (g/kg)	15.8
Salt	3.5	Cystine (g/kg)	28.9
Additives	20	Lysine (g/kg)	90.5
		Threonine (g/kg)	65.2
		Tryptophan (g/kg)	17.0

[A] Ingredient and nutrient composition reported on an as-fed basis.

Additives supplied (per kg of diet): vitamin B_{12}, 16g; menadione, 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; folic acid, 1.62 mg, Vitamin A 15 000 IU; vitamin D3 800 IU; Vitamin E 30 mg; Mn, 80 mg; Fe, 60 mg; Zn, 90 mg; Cu, 12 mg; Se, 0.147 mg.

Sampling procedure: The hens and eggs were weighed every 2 weeks during the experimental period. Nine laying hens in each group were slaughtered every 2 weeks during the rearing period, and their blood was collected into tubes containing EDTA (final concentration, 1 g/l). Plasma was isolated by centrifuging at 1500 *g* for 10 min at 4°C, and 1-ml aliquots were frozen at -20°C for later analysis (Lee *et al.* 2003). After feathers were removed, breast and thigh meat were immediately cut from each carcass, vacuum packaged and stored at -20°C until analysis (Tang *et al.* 2001). Every 2 weeks, 50 eggs were collected from each group and maintained at refrigerator temperature for analysis. Before analysis, samples were heated at 105°C for 2 h in an oven to calculate cholesterol and triglyceride levels on a dry weight basis. Feces that fell onto a smooth plastic plate placed under the cages were collected prior to slaughter and cooled. Fecal samples from three cages in each group were lyophilized and then vacuum packaged for analysis.

Determination of total cholesterol and total triglycerides in plasma samples: The concentrations of TC, TG, HDL and LDL were measured enzymatically using an auto-analyzer. Very low-density and low-density lipoproteins were precipitated from plasma with phosphotungstic acid/$MgCl_2$ according to Lee *et al.* (2003), and the supernatant (HDL) was assayed for cholesterol.

Determination of total cholesterol and total triglycerides in chicken breast and thigh: Lipids were extracted from thigh and breast muscle according to Folch *et al.* (1956). The cholesterol and triglyceride content were determined using kits according to the manufacturer's instructions.

Determination of total cholesterol, total triglycerides and fatty acid composition in egg yolk: Eggs (5 large, 5 medium and 5 small eggs) were obtained for each group. Eggs were boiled for 10 min before the yolk was removed. All yolks were then lyophilized and ground into powder, and yolks from the same group were mixed and vacuum packaged for analysis. The constituents of the yolk samples (5±0.01 g) were extracted with chloroform-methanol (2: 1, v/v) according to the methods of Folch *et al.* (1956) and Bragagnolo *et al.* (2003) with some modifications. A 3 ml aliquot of the lipid extract was dried under nitrogen and saponified with 4 ml of absolute ethanol and 10 ml 50% KOH (w/w) for 90 min at 65°C. The sample was then cooled under running water, followed by the addition of 5 mL of distilled water. The unsaponifiable sample was extracted twice with 10 mL of hexane, from which 3 ml was dried under nitrogen. Cholesterol and triglyceride content was determined commercial kits according to the manufacturer's instructions. Fatty acids were extracted from the yolk according to AOCS (1990). The dry samples were re-dissolved in hexane for gas chromatographic (GC) analysis. The fatty acid methyl esters obtained were analyzed with a GC equipped with a flame ionization detector (FID) and fitted with a fused capillary column. The apparatus was programmed with an initial temperature of 150°C for 4 min, with incremental increases of 1.3°C/min, until a final temperature of 210°C was reached. Injector and detector temperatures were 220°C and 240°C, respectively. The carrier gas was hydrogen (1.5 ml/min) and the make-up gas was nitrogen (25 ml/min). C19: 0 was used as an internal standard for fatty acid quantification.

Determination of total fatty acids in feces: Concentrations of neutral and acidic sterols, the only TFAs present in the feces, were determined according to Chan *et al.* (1999). Stigmasterol and hyodeoxycholic acid (0.5 mg each) were used as internal standards for quantification of fecal neutral and acidic sterols, respectively. Both neutral and acidic sterol trimethylsilyl derivatives were subjected to GC analysis.

Statistical analysis: In each group, 3 replicates were performed for all measurements. Results are expressed as the mean±SEM. A statistical package SPSS12.0 (SPSS Inc., Chicago, IL, USA) was used for all analysis. One-way analysis of variance (ANOVA) was used to determine the significance of differences among the groups. A value of P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Blood: No significance differences were observed among the groups at day 0. Plasma TC, TG, HDL, and LDL concentrations gradually increased from 0 to 42 days (Table 2). Plasma TC and TG levels were lower in groups that were administered green tea and tea catechins, and the best results were quadratically obtained with 6 g/kg green tea at 42 d (P = 0.04), 56 d (P = 0.03), and with 1 g/kg tea catechins at 42 d (P = 0.04), and 56 d (P = 0.04). Plasma TC and TG levels of the group given 6 g/kg green tea were 0.94 mmol/l and 3.13 mmol/l lower (P<0.05), respectively, compared to the control group at day 42. For the group given 1 g/kg tea catechins, TC and TG levels were 0.85 mmol/l and 2.26 mmol/l lower (P<0.05), respectively. Plasma HDL levels were quadratically higher in the groups given green tea (P = 0.03) and tea catechins (P=0.04) at 56 d. LDL levels were 0.22 mmol/l lower (P = 0.03) in the group given 6 g/kg green tea and 0.18 mmol/l lower (P = 0.04) in the group given 1 g/kg tea catechins at 56 d. These groups showed a quadratic effect. The decrease in plasma TG levels was greater than the decrease observed in plasma LDL levels. The effect of green tea on TC, TG, HDL, and LDL levels was similar to that of tea catechins, but the changing trend did not show a linear relationship among the supplemented levels during breeding periods.

Chicken breast and thigh: The levels of TG and TC in breast and thigh gradually increased from 0 to 42 d (Table 3). In both thigh and breast meat at 42 and 56 d, TG levels and TC levels were significantly less than control values (P<0.05), but TC levels were only slightly reduced. The decrease in TG levels was significantly lower than that of TC. Our results indicated that 6 g/kg green tea had a greater effect than 2 or 8 g/kg, and that 1g/kg tea catechins had a greater effect than 0.5 or 2 g/kg. However, these treatment groups did not display a quadratic dose response during breeding period.

Egg yolk: There was no significant difference among the groups for yolk TC levels (P>0.05) (Table 3), but TG levels decreased by 2.42 mg/g at day 42 and 2.48 mg/g at day 56 for 4 g/kg green tea (P<0.05), and by 2.69 mg/g at day 42 and 56 for 6 g/kg green tea (P<0.05). Tea catechins @ 1 g/kg were the most effective in reducing TG levels, since they led to a decrease of 2.74 mg/g and 2.76 mg/g at days 42 and 56, respectively. The effect of green tea was similar to that of dietary tea catechins, but these groups did not show a quadratic dose response during breeding period.

The fatty acid content of yolks (Table 4) was determined at day 56. Consumption of dietary supplements had no effect on myristic acid (C14: 0), stearic acid (C18: 0), oleic acid (C18: 1), linoleic acid (C18: 2n-6) or arachidonic acid (C20: 4n-6) levels (P>0.05), but led to a decrease (P<0.05) in the level of palmitic acid (C16: 0) and increased (P<0.05) levels of palmitoleic acid (C16: 1), alfa-linolenic acid (C18: 3n-3) and n-6, n-3 when compared with the control. No significant differences were found amongst treatment groups in terms of saturated fatty acid (SAFA) or total fatty acids (TFA) except in the group that received 1 g/kg tea catechins, where levels were slightly lower. C16: 0 levels in yolk were lower than the control group by 83 mg/kg yolk in the 6 g/kg green tea group (P<0.05) and by 85 mg/kg yolk in the 1 g/kg tea catechins group (P<0.05). Yolk levels of C16: 1, C18: 3n-3, adrenic acid (C22: 4n-6), n-6 and n-3 polyunsaturated fatty acids (PUFA) were greater than the control group in both the green tea and tea catechins groups.

Total fatty acids in fecal excretion: The levels of TFA excreted in the feces averaged across all groups at day 56. Compared with the controls, the excretion of total fat in feces was greatest in the groups that received supplements, with the exception of 2 g/kg green tea. The fat in feces excreted increased by 1% for 6 g/kg green tea and by 0.8% for 1 g/kg tea catechins when compared with the control.

Although there have been numerous studies on the effects of green tea and tea catechins on lipid metabolism in rats (Lee *et al.* 2008, Kuo *et al.* 2005, Muramatsu *et al.* 1986), there have been relatively few studies into their effect in chickens. Furthermore, most of the published reports have focused on the antioxidative effect of these supplements on lipid oxidation in chicken meat systems. Tang *et al.* (2001) reported that a tea catechin supplement at levels of 50 to 300 mg/kg feed could reduce lipid oxidation in chicken breast and thigh meat during frozen storage for 9 months, and that the most effective level was 200 mg/kg. The addition of 200 or 400 mg/kg tea catechins to chicken feed was found to inhibit lipid oxidation in chicken meat and improve its surface color (Mitsumoto *et al.* 2005). However, direct evidence for the regulatory action of green tea and tea catechins on lipid metabolism in chickens has not previously been reported.

The addition of 2–8 g/kg green tea and 0.5 to 2 g/kg tea catechins partially inhibited the increase in plasma levels of TC, TG and LDL, meat and yolk levels of TC and TG, and also partially enhanced plasma HDL levels. Bursill *et al.* (2006) reported that administration of 0.5 to 2% (w/w) crude catechin extract from green tea significantly lowered plasma cholesterol and enhanced plasma HDL in rats. Based on this experiment, our results indicated that the effects of dietary green tea and tea catechins on TC, TG, HDL, and LDL levels were not proportional to levels of supplement given, suggesting that there may be an optimum concentration of supplement.

Compared with the control group, there was no significant

Table 2. Plasma TC, TG, HDL, and LDL content of laying hens[A]

Indices		Green tea groups (g/kg)[C]				SEM	P value		Tea catechins groups (g/kg)[D]				SEM	P value	
	0[B]	2	4	6	8		Linear	Quadratic	0.5	1	1.5	2		Linear	Quadratic
TC (mmol/L)															
0 d	3.30	3.22	3.27	3.35	3.27	0.25	0.12	0.14	3.16	3.33	3.22	3.24	0.13	0.14	0.19
14 d	3.49	3.52	3.42	3.49	3.80	0.10	0.13	0.10	3.41	3.28	3.39	3.56	0.11	0.10	0.21
28 d	4.50	4.04	3.66	3.56	4.14	0.06	0.11	0.09	3.68	3.50	3.29	3.93	0.12	0.11	0.10
42 d	4.63[c]	4.36[c]	3.78[b]	3.69[b]	4.21[c]	0.09	0.10	0.04	4.15[c]	3.78[b]	3.91[b]	4.16[c]	0.08	0.12	0.04
56 d	4.65[c]	4.22[c]	3.98[b]	3.84[b]	4.21[c]	0.07	0.15	0.03	4.37[c]	4.09[c]	4.18[c]	4.27[c]	0.10	0.11	0.04
TG (mmol/L)															
0 d	10.58	10.22	10.66	10.24	10.35	2.76	0.57	0.29	10.96	10.0 5	10.22	10.89	2.15	0.46	0.27
14 d	12.87	12.40	11.28	11.56	12.25	0.34	0.19	0.15	12.14	12.77	11.55	11.94	0.31	0.21	0.17
28 d	14.68	14.08	11.73	11.87	14.07	0.57	0.21	0.13	14.05	13.51	13.21	13.87	0.52	0.19	0.14
42 d	16.39[d]	16.27[d]	14.13[b]	13.26[a]	14.29[b]	0.09	0.10	0.02	15.52[c]	14.13[b]	14.69[b]	14.69[b]	0.10	0.11	0.05
56 d	16.72[d]	16.62[d]	14.64[b]	14.23[b]	14.63[b]	0.21	0.13	0.03	15.96[c]	14.29[b]	14.72[b]	14.81[b]	0.19	0.14	0.04
HDL (mmol/L)															
0 d	2.58	2.45	2.61	2.46	2.50	0.37	0.22	0.26	2.73	2.39	2.45	2.70	0.36	0.22	0.29
14 d	3.02	3.07	3.21	3.18	3.12	0.32	0.17	0.13	3.08	3.26	3.38	3.37	0.34	0.26	0.18
28 d	3.15	3.23	3.54	3.79	3.28	0.48	0.27	0.16	3.17	3.70	3.64	3.54	0.42	0.19	0.15
42 d	3.46	3.53	3.81	4.01	3.45	0.23	0.13	0.05	3.63	3.94	3.85	3.69[a]	0.20	0.21	0.05
56 d	3.42[a]	3.69[a]	4.02[b]	4.14[b]	3.43[a]	0.38	0.12	0.03	3.64[a]	4.17[b]	4.05[b]	3.90[a]	0.37	0.17	0.04
LDL (mmol/L)															
0 d	0.81	0.82	0.82	0.83	0.84	0.35	0.28	0.27	0.84	0.88	0.87	0.86	0.36	0.29	0.23
14 d	0.83	0.80	0.79	0.81	0.81	0.24	0.17	0.19	0.87	0.82	0.86	0.89	0.27	0.26	0.21
28 d	0.84	0.84	0.75	0.73	0.86	0.15	0.12	0.09	0.84	0.76	0.80	0.87	0.18	0.19	0.10
42 d	0.91[d]	0.81[c]	0.73[b]	0.71[b]	0.82	0.19	0.13	0.05	0.81[c]	0.73[b]	0.76[b]	0.83[c]	0.15	0.14	0.06
56 d	0.91[d]	0.82[c]	0.74[b]	0.69[a]	0.83	0.23	0.10	0.03	0.82[c]	0.73[b]	0.77[b]	0.85[c]	0.21	0.11	0.04

[A]Values are the mean±SEM. Each value is an average value from three replicate groups, and each replicate sample is determined in triplicate. TC: total cholesterol; TG: total triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

[a-d]The values in the same row with different letters are significantly different ($P < 0.05$). [B]indicates control laying hen fed with a basal diet; [C]indicates a basal diet supplemented with 2, 4, 6, 8 g green tea powder/kg feed, respectively;

[D]indicates a basal diet supplemented with 0.5, 1, 1.5, 2 g tea catechins/kg feed, respectively.

Table 3. Change in TC and TG levels of chicken breast, thigh (g/kg meat), and egg yolk (mg g^{-1} yolk)[A]

Samples	Indices		Green tea groups (g/kg)[C]				SEM	P value		Tea catechins groups (g/kg)[D]				SEM	P value	
		0[B]	2	4	6	8		Linear	Quadratic	0.5	1	1.5	2		Linear	Quadratic
Breast	TC															
	0 d	3.56	3.53	3.58	3.54	3.49	0.02	0.19	0.21	3.57	3.51	3.56	3.53	0.02	0.23	0.18
	42 d	4.18^{c}	3.87^{b}	3.73^{b}	3.69^{b}	3.94^{b}	0.03	0.13	0.06	3.81^{b}	3.76^{b}	3.83^{b}	3.87^{b}	0.03	0.16	0.08
	56 d	4.22^{c}	4.08^{c}	3.91^{b}	3.78^{b}	4.12^{c}	0.04	0.11	0.05	3.84^{b}	3.79^{b}	3.91^{b}	3.94^{b}	0.04	0.11	0.06
	TG0 d	17.78	17.82	17.80	17.79	17.81	0.02	0.20	0.11	17.78	17.81	17.76	17.79	0.01	0.23	0.15
	42 d	21.48^{c}	19.39^{b}	18.32^{a}	18.11^{a}	19.29^{b}	0.06	0.12	0.01	18.67^{a}	18.71^{a}	18.78^{a}	19.16^{b}	0.07	0.12	0.02
	56 d	21.65^{c}	19.75^{b}	18.43^{a}	18.25^{a}	19.31^{b}	0.10	0.10	<0.01	18.76^{a}	18.72^{a}	18.93^{a}	19.28^{b}	0.11	0.13	0.01
Thigh	TC															
	0 d	2.34	2.38	2.37	2.32	2.35	0.02	0.22	0.14	2.36	2.37	2.34	2.36	0.03	0.21	0.19
	42 d	3.18^{b}	3.05^{b}	2.84^{a}	2.78^{a}	2.89^{a}	0.03	0.13	0.10	2.80^{a}	2.77^{a}	2.87^{a}	2.96^{a}	0.04	0.19	0.12
	56 d	3.22^{b}	3.12^{b}	2.96^{a}	2.83^{a}	3.08^{b}	0.05	0.11	0.09	2.98^{a}	2.85^{a}	3.01^{b}	3.02^{b}	0.06	0.12	0.10
	TG0 d	12.84	12.81	12.79	12.85	12.83	0.05	0.24	0.16	12.81	12.85	12.82	12.83	0.03	0.25	0.18
	42 d	17.39^{d}	16.20^{c}	14.63^{a}	14.27^{a}	14.72^{a}	0.12	0.11	0.02	14.68^{a}	14.65^{a}	14.83^{a}	14.81^{a}	0.11	0.10	0.03
	56 d	17.85^{d}	16.34^{c}	14.76^{a}	14.34^{a}	14.84^{a}	0.13	0.10	<0.01	14.74^{a}	14.63^{a}	15.04^{b}	15.12^{b}	0.12	0.11	0.01
Egg yolk	TC															
	0 d	3.69	3.65	3.69	3.63	3.61	0.01	0.09	0.16	3.63	3.62	3.65	3.62	0.01	0.17	0.22
	42 d	3.78^{b}	3.69^{b}	3.66^{b}	3.68^{b}	3.64^{b}	0.02	0.16	0.10	3.70^{b}	3.65^{b}	3.60^{b}	3.62^{b}	0.02	0.14	0.13
	56 d	3.74^{b}	3.72^{b}	3.61^{b}	3.66^{b}	3.69^{b}	0.01	0.12	0.08	3.64^{b}	3.64^{b}	3.66^{b}	3.68^{b}	0.01	0.14	0.12
	TG0 d	19.35	19.56	19.34	19.46	19.16	0.03	0.23	0.12	19.26	19.15	19.15	19.21	0.16	0.14	0.19
	42 d	22.62^{d}	21.48^{c}	20.22^{b}	20.04^{b}	21.18^{c}	0.12	0.13	0.03	20.16^{b}	19.68^{a}	20.63^{b}	21.31^{c}	0.11	0.10	0.04
	56 d	22.67^{d}	21.52^{c}	20.18^{b}	20.09^{b}	21.24^{c}	0.10	0.10	0.01	20.72^{b}	19.81^{a}	21.07^{b}	21.83^{c}	0.11	0.10	0.02

[A]Values are the mean±SEM. Each value is an average value from three replicate groups, and each replicate sample is determined in triplicate. TC: total cholesterol; TG: total triglycerides.

[a-d]The values in the same row with different letters are significantly different ($P < 0.05$).

[B]indicates control laying hen fed with a basal diet;

[C]indicates a basal diet supplemented with 2, 4, 6, 8 g green tea powder/kg feed, respectively;

[D]indicates a basal diet supplemented with 0.5, 1, 1.5, 2 g tea catechins/kg feed, respectively.

Table 4. Fatty acid content (mg kg^{-1} yolk) of eggs laid at 26 weeks[A]

Fatty acids		Green tea groups (g/kg)[C]				SEM	P value		Tea catechins groups (g/kg)[D]				SEM	P value	
	0[B]	2	4	6	8		Linear	Quadratic	0.5	1	1.5	2		Linear	Quadratic
C14: 0	16.9	17.1	16.2	16.1	16.7	4.2	0.12	0.16	16.6	16.2	16.7	16.8	4.3	0.11	0.21
C 16: 0	1104[d]	1095[c]	1084[c]	1021[b]	1087[c]	63.2	0.16	0.04	1065[c]	1019[c]	1071[c]	1086[c]	62.5	0.18	0.04
C 16: 1	182[a]	180[a]	191[b]	201[c]	187[a]	31.5	0.24	0.03	190[b]	195[b]	186[a]	186[a]	30.2	0.21	0.03
C 18: 0	356	354	357	351	357	41.3	0.31	0.16	356	352	351	349	42.5	0.27	0.19
C 18: 1	1684	1679	1685	1687	1682	78.1	0.46	0.05	1679	1678	1683	1683	67.9	0.37	0.05
C 18: 2n-6	497	501	506	517	513	20.5	0.27	0.06	501	510	510	513	16.9	0.26	0.07
C 18: 3n-3	1.7[a]	2.1[b]	2.6[b]	4.3[d]	2.7[b]	0.1	0.11	0.04	3.8[c]	4.1[d]	3.7[c]	3.5[c]	0.2	0.12	0.03
C 20: 4n-6	96.0	94.3	96.5	92.8	94.6	2.7	0.13	0.29	95.0	94.7	94.0	94.5	3.4	0.15	0.21
C 20: 5n-3	nd	nd	nd	nd	nd				nd	nd	nd	nd			
C 22: 4n-6	16.5[b]	15.3[a]	18[d]	18.5[d]	17.2[c]	1.4	0.11	0.04	16[b]	18.2[d]	17.4[c]	16.8[b]	1.6	0.13	0.04
C 22: 6n-3	9.5	9	10	11.6	10	0.2	0.12	0.05	11	12.5	10.6	10.3	0.5	0.12	0.05
“SAFA	1478	1467	1458	1390	1462	69.5	0.48	0.07	1436	1387	1439	1452	71.3	0.30	0.08
“MUFA	1868	1859	1876	1885	1869	78.5	0.61	0.05	1869	1873	1870	1870	66.7	0.47	0.07
“n – 6[A]	610[a]	611[a]	622[b]	630[c]	635[c]	40.6	0.21	0.03	623[b]	620[b]	622[b]	625[b]	34.1	0.26	0.04
“n – 3[A]	12[ab]	11[a]	13[b]	16[c]	13[b]	0.1	0.14	0.04	15[bc]	17[d]	15[bc]	14[c]	0.1	0.14	0.02
TFA	3965	3950	3964	3920	3978	70.3	0.48	0.24	3935	3897	3945	3960	70.6	0.57	0.23

[A]Values are the means of 15 eggs, each replicate with 5 eggs.

[a-d]Values with different letters within the same rows are significantly different ($P < 0.05$).

nd, not detectable.

C14: 0, myristic acid; C16: 0, palmitic acid; C16: 1, palmitoleic acid; C18: 0, stearic acid; C18: 1, oleic acid; C18: 2n - 6, linoleic acid; C18: 3n-3, alfa-linolenic acid; C20: 4n - 6, arachidonic acid; C20: 5n - 3, eicosapentanoic acid; C22: 4n - 6, adrenic acid; C22: 6n -3, docosahexaenoic acid. SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; TFA, total fatty acids.

[B]indicates control laying hen fed with a basal diet;

[C]indicates a basal diet supplemented with 2, 4, 6, 8 g green tea powder/kg feed, respectively;

[D]indicates a basal diet supplemented with 0.5, 1, 1.5, 2 g tea catechins/kg feed, respectively.

difference in yolk TC or TFA levels, although there was a small effect on yolk unsaturated fatty acid content. The results of fecal analysis indicated that the excretion of TFA in hens fed the dietary supplements was higher than in the control group, with the greatest effect from 6 g/kg green tea and 1 g/kg tea catechins. However, we still do not have a clear understanding of the metabolic pathway underlying the observed effects of these dietary supplements, nor do we have a physiological theory to explain them. Therefore, further studies are needed to elucidate how dietary green tea and tea catechins influence the lipid metabolism of laying hens.

It is concluded that dietary green tea and tea catechins can affect the lipids of laying hens, which significantly reduce the TG contents in plasma, breast and thigh meat and egg yolk, and increase the excretion of TFA in feces. In addition, it is interesting to note that the addition of green tea and tea catechins can slightly enhance the levels of unsaturated fatty acids in egg yolk. However, the effect is not proportional to the concentrations of the added green tea and tea catechins.

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