Effects of green tea powder supplementation on egg production and egg quality in laying hens

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Abstract
The aim of this study was to investigate the effects of green tea powder supplementation on egg production and egg quality in chickens. A total of 240 Xianju chickens (all aged 20 weeks) were randomly allotted to five treatments, control group T0, no diet supplements; test groups T1, T2 and T3 were supplemented with 1%, 2% and 3% green tea powder into basal diet, respectively; test group T4, 3% green tea powder and 1.5% peanut oil were added to the diet. After the rearing test, the eggs from each treatment were collected and later analysed for egg quality. Results suggest that 1% green tea powder supplementation had little effect on egg production and feed conversion, but high amounts of green tea powder (≥2%) treatment significantly decreased the egg production performance. Green tea powder treatment had some beneficial effects on egg quality, especially in the albumen height and Haugh unit. Additionally, green tea powder treatment significantly changed the nutritional composition of eggs.

Introduction
With the improvement of experimental research (Zeng et al. 2014) and production performance (Ren et al. 2009) over the past half century, the growth rate and feed conversion rate of animals have been speeded up and made a breakthrough along with the development of nutritional manipulation techniques (Gao et al. 2013) and genetic improvement (He et al. 2012; Zeng et al. 2013). But it also brought many negative impacts, such as reducing poultry meat quality, increasing fat deposits (Chen et al. 2015) and decreasing egg quality (Kojima and Yoshida 2008).

Egg is one kind of animal product mainly consumed, which contains lots of nutrients and is a very good source of protein and energy. But it also contains some risk materials, such as high amounts of cholesterol (200–300 mg/egg) and is bad for old people and hypercholesterolaemia patients (Hisashi et al. 1986), and excessive intake of cholesterol may result in some diseases, such as atherosclerosis and fatty liver (Chung et al. 2012). In order to improve the egg quality and egg nutrition, researchers have focused on improving the egg quality and changing the nutritional composition of eggs via the use of extra nutrient substances (Liu et al. 2017). In recent years, with constantly improve living standards of people, consumption concept updates of people, people focus more on their health and more people like to care healthy and high quality animal products.

China is the home of tea, especially green tea, attributes its long history and culture. Tea contains lots of functional activated compositions, such as tea polyphenol, alkaloid, polysaccharide, etc. Particularly, tea polyphenol plays roles such as anti-oxidizing (Gramza-Michalowska et al. 2016; Rodrigues et al. 2016), anti-bacterial (Nakayama et al. 2012; Kawarai et al. 2016), anti-aging (Li et al. 2016), anti-cancerous (Shih et al. 2016), etc. Current researches suggest that tea treatment could affect the egg quality (Kojima and Yoshida 2008; Wei et al. 2012) and egg production performance (Uuganbayar et al. 2005; Panja 2007), but the results were inconsistent and even very different from each other. In order to figure out the effects of tea on egg production, this study aims at evaluating and studying the effects of diet supplemented with green tea powder on egg production, egg quality and nutritional composition of eggs in chickens; besides, oil was used in this study to evaluate the effects of oil extra supplementation on egg production and egg quality when compared with the same green tea powder supplementation treatment because oil is an energy substance which is good for energy supply and anti-stress from environment and then provided some basal information for future researches on tea resources application in livestock production.

Material and Methods

Test materials and test facilities

Materials
Two hundred and forty healthy Xianju chickens (the average laying rate is 50%) were provided by Xianju Chicken Development Company. Green tea powder was provided by Xianju Green Tea development Company.
by the Institute of Animal Husbandry and Veterinary Science, and Emperor balance (Mettler Toledo pL203-IC) were provided by the Institute of Animal Husbandry and Veterinary Science, Zhejiang Academy of Agricultural Science, Hangzhou 310020.

**Experimental design and feeding management**

**Experimental design**

The 240 Xianju chickens (all aged 20 weeks) were randomly divided into five treatments, with 3 replicates per treatment and 16 chickens in each replicate, and all the animals were fed with the same basal diet, and fed separately: control group T0 received no diet supplements; test groups T1, T2 and T3 were supplemented with 1%, 2% and 3% green tea powder into basal diet, respectively; test group T4 received 3% green tea powder and 1.5% peanut oil added to the basal diet. Studies included 7 d for prefed and 67 d for the fed. Food and water were provided twice a day, at 8:00 and 15:00. The composition and nutrient analysis of the basal diet are shown in Table 1, the basal diet formulation was according to the National Standards of People’s Republic of China (NSPRC 2008a) and nutrients of the basal diet were analysed according to the standard method GB/T 18868–2003 of the National Standards of People’s Republic of China (NSPRC 2002). The composition of the green tea powder is shown in Table 2.

**Sampling and handling**

Ten eggs from each replicate at the end of this study were randomly selected and later analysed for egg quality (eggshell strength, eggshell thickness, egg shape index, albumen height, Haugh unit and relative yolk-to-egg ratio); this was completed within 12 h of sampling. The other three eggs from each replicate were randomly selected and later analysed for the content of cholesterol, crude fat, vitamin E, triglyceride, lecithin and malonaldehyde in eggs.

**Measurement methods**

**Egg production performance**

Daily feed provided, actual feed intake, number of eggs laid and individual egg weight were recorded. Average daily feed intake, average egg weight and feed-to-egg ratio were calculated at the end of the experiment.

**Egg quality**

The egg shape index was measured (in mm) with a caliper and vertical diameter-diameter−1 was calculated to determine the shape index. Eggshell thickness was measured with a digital micrometer (karl deutsch MODEL-1061); measurements at the blunt and sharp ends and the middle of egg were averaged to determine the overall eggshell thickness. Eggshell strength was measured using an eggshell force gauge (MODEL-T1). Albumen height, yolk colour and Haugh units were measured with an automatic egg analyser (Egg Multi Tester, EMT-5200). Egg weight and yolk weight were measured with an electronic balance (Mettler Toledo pL203-IC).

**Statistical analysis**

Data generated in the present study were subjected to statistical analysis using one-way ANOVA of SPSS17.0 in a randomized complete block design. All data were reported as mean ± SEM. When significant differences were identified among treatments, Duncan’s test was used for multiple comparisons. The replicate was used as the experimental unit for the analysis of egg production, egg quality and the cholesterol, vitamin E, crude fat, triglyceride, lecithin and malonaldehyde contents. P-values < .05 were considered significant.

**Results and discussion**

**Egg production performance**

Compared to group T0, the laying rate decreased by 6.51% (P < .05), 14.32% (P < .05) and 16.99% (P < .05) in groups T2, T3 and T4, respectively. The average egg weight increased by 2.07% (P < .05) in group T1, but decreased by 2.86% (P < .05), 3.84% (P < .05) in groups T3 and T4, respectively. The average

| Ingredients          | Content, % | Nutrient Content, g/kg |
|----------------------|------------|------------------------|
| Maize grain          | 54.00      | Metabolizable energy   |
| Rice bran            | 5.00       | 165.0                  |
| Soybean meal         | 26.00      | 7.0                    |
| Fish meal            | 2.00       | 33.5                   |
| Shell powder         | 3.00       | 7.9                    |
| Calcium hydrophosphate | 1.20   | 4.0                    |
| Calcium phosphate    | 6.50       | Ether extract          |
| Salt                 | 0.30       |                        |
| Premix               |            |                        |

Notes: C: Catechin; EC: Epicatechin; EGCG: Epigallocatechin gallate; EGC: Epigallocatechin gallate.

**Nutritional composition of eggs**

Three eggs from each replicate were randomly selected for detection of the nutritional indexes content of eggs. The contents of crude fat, cholesterol, vitamin E and malonaldehyde in eggs were respectively analysed according to the standard method GB/T 4772–2008a, GB/T 5009.128–2003a, GB/T 5009.82–2003b, GB 5009.181–2016a of the National Standards of People’s Republic of China (NSPRC 2003a, 2003b, 2008b, 2016). The contents of lecithin and triglyceride were detected according to the reference of Saunders and Perrin (1960), and Bao and Zhang (2013).

**Table 2. Composition of the green tea powder.**

| Ingredients | Contents, % | Ingredients | Contents, % |
|-------------|-------------|-------------|-------------|
| Tea polyphenol | 16.52 ± 0.21 | Catechin | 2.03 ± 0.03 |
| Caffeine     | 2.59 ± 0.02 | EC          | 0.13 ± 0.03 |
| Crude fibre  | 13.58 ± 0.02 | EGCG      | 4.29 ± 0.01 |
| Total free amino acids | 0.80 ± 0.01 | EGC | 1.31 ± 0.02 |
| Total contents | 8.59 ± 0.05 | EGC: Epigallocatechin gallate |


Table 3. Effects of green tea powder on production performance of Xianju chickens.

| Items                      | T0          | T1          | T2          | T3          | T4          |
|----------------------------|-------------|-------------|-------------|-------------|-------------|
| Laying rate, %             | 69.92 ± 11.26 <sup>c</sup> | 69.23 ± 12.57 <sup>c</sup> | 65.37 ± 13.83 <sup>b</sup> | 59.91 ± 15.99 <sup>a</sup> | 58.04 ± 12.79 |
| Average egg weight, g      | 44.02 ± 1.66 <sup>b</sup> | 44.93 ± 1.74 <sup>a</sup> | 44.24 ± 2.01 <sup>b</sup> | 42.76 ± 2.51 <sup>a</sup> | 42.33 ± 2.49  |
| Average feed intake, g     | 110.92 ± 10.73 <sup>a</sup> | 110.03 ± 8.38 <sup>a</sup> | 110.48 ± 8.05 <sup>a</sup> | 101.30 ± 11.16 <sup>b</sup> | 96.87 ± 8.80  |
| Feed-to-egg ratio, g/g     | 3.73 ± 0.74 <sup>a</sup> | 3.68 ± 0.84 <sup>a</sup> | 4.02 ± 0.99 <sup>b</sup> | 4.23 ± 1.23 <sup>b</sup> | 4.19 ± 1.27  |

Notes: control group T0, no diet supplements; test groups T1, T2 and T3 were supplemented with 1%, 2% and 3% green tea powder into basal diet, respectively; for test group T4, 3% green tea powder and 1.5% peanut oil were added to the diet. Values are expressed as Mean ± SEM. <sup>a</sup> represents significant differences (P < .05) were found between groups T3 and T4.

Table 4. Effects of green tea powder on egg quality of Xianju chickens.

| Items                      | T0          | T1          | T2          | T3          | T4          |
|----------------------------|-------------|-------------|-------------|-------------|-------------|
| Egg shape                  | 1.31 ± 0.04<sup>bc</sup> | 1.27 ± 0.05<sup>a</sup> | 1.29 ± 0.08<sup>ab</sup> | 1.33 ± 0.09<sup>c</sup> | 1.31 ± 0.06  |
| Eggshell thickness, cm     | 0.46 ± 0.04<sup>a</sup> | 0.44 ± 0.04<sup>b</sup> | 0.43 ± 0.05<sup>ab</sup> | 0.41 ± 0.06<sup>c</sup> | 0.41 ± 0.05  |
| Eggshell strength, kg/cm²  | 4.66 ± 0.93<sup>a</sup> | 4.13 ± 0.69<sup>b</sup> | 3.57 ± 0.90<sup>c</sup> | 3.51 ± 0.92<sup>a</sup> | 3.18 ± 0.87  |
| Egg weight, g              | 37.10 ± 3.36<sup>b</sup> | 38.83 ± 2.95<sup>c</sup> | 36.63 ± 3.60<sup>b</sup> | 34.83 ± 2.86<sup>a</sup> | 34.91 ± 2.39  |
| Albumen height, mm         | 4.44 ± 0.77<sup>a</sup> | 5.72 ± 1.65<sup>b</sup> | 4.39 ± 1.80<sup>c</sup> | 5.63 ± 1.55<sup>b</sup> | 5.20 ± 1.08  |
| Yolk colour                | 7.81 ± 1.08<sup>bc</sup> | 8.31 ± 0.64<sup>a</sup> | 7.08 ± 1.19<sup>c</sup> | 8.31 ± 0.82<sup>b</sup> | 7.67 ± 0.88<sup>a</sup> |
| Haugh unit, HU             | 74.82 ± 5.81<sup>a</sup> | 80.50 ± 10.61<sup>b</sup> | 73.02 ± 12.55<sup>c</sup> | 82.07 ± 8.05<sup>a</sup> | 80.70 ± 6.87  |

Notes: control group T0, no diet supplements; test groups T1, T2 and T3 were supplemented with 1%, 2% and 3% green tea powder into basal diet, respectively; for test group T4, 3% green tea powder and 1.5% peanut oil were added to the diet. Values are expressed as Mean ± SEM for 30 eggs. <sup>a</sup>–<sup>c</sup> Values with different lower case letters in the same row differ significantly (P < .05). <sup>a</sup> represents significant differences (P < .05) were found between groups T3 and T4.
weight. According to this phenomenon, the likely explanation is that green tea supplementation changed the balance of feed nutrition, which resulted in insufficient nutrient supply for egg production. Conversely, these results were not consistent with the studies of Panja et al. (2007) and Hisashi et al. (2011). Tea polyphenols is one of the most important biologically active components and the polyhydroxy mixture of phenolic compounds that tea contains. Matsumoto et al. (1998) have found that black tea polyphenols can decrease cholesterol in egg yolk and whole eggs. And this outcome was similar to the studies of Panja (2007) and Hisashi et al. (1986), which also suggested that green tea can significantly decrease the content of cholesterol, and a feasible amount of green tea was 1.0%–2.0%. Moreover, there is evidence that exterior nutrition matter could change the energy metabolism and material metabolism, and then alter the egg composition. Green tea powder can improve the egg quality, particularly by increasing the albumen height and Haugh unit. But this result was not consistent with Panja’s (2007) study, which concluded that Haugh unit, yolk color and albumen height were not significantly different with increasing amounts of tea supplementation.

**Nutritional composition of eggs**

There were no significant differences (P > 0.05) in levels of triglyceride and lecithin of egg yolk among the treatments (Table 5).

Compared to group T0, the vitamin E contents of egg yolk increased by 1.78% (P < 0.05), 3.86% (P < 0.05), 5.34% (P < 0.05) and 2.67% (P < 0.05) in groups T1, T2, T3 and T4, respectively. The cholesterol contents of egg yolk decreased by 5.56% (P < 0.05), 8.33% (P < 0.05), 11.11% (P < 0.05) and 27.8% (P < 0.05) in groups T1, T2, T3 and T4, respectively. The crude fat contents of egg yolk decreased by 0.90% (P < 0.05), 1.69% (P < 0.05), 1.31% (P < 0.05) and 0.49% (P < 0.05) in groups T1, T2, T3 and T4, respectively. The malonaldehyde contents of egg yolk decreased by 1.53% (P < 0.05), 2.28% (P < 0.05), 3.25% (P < 0.05) and 3.00% (P < 0.05) in groups T1, T2, T3 and T4, respectively (Table 5).

Compared to group T0, the vitamin E contents of whole eggs increased by 2.60% (P < 0.05), 5.39% (P < 0.05), 6.99% (P < 0.05) and 5.59% (P < 0.05) in groups T1, T2, T3 and T4, respectively. The cholesterol contents of whole eggs decreased by 6.25% (P < 0.05) and 12.50% (P < 0.05) in groups T2 and T3, respectively. The lecithin contents of whole eggs increased by 0.79% (P < 0.05), 3.18% (P < 0.05), 7.94% (P < 0.05) and 11.91% (P < 0.05) in groups T1, T2, T3 and T4, respectively. The malonaldehyde contents of whole eggs decreased by 2.27% (P < 0.05) in group T3 (Table 5).

There were no significant differences (P > 0.05) in levels of crude fat of whole eggs among the treatments (Table 5). The triglyceride content of whole eggs in group T4 were significantly (P < 0.05) higher than that in group T3 (Table 5).

The result of this study showed that green tea powder supplementation significantly decreased the content of cholesterol, crude fat, triglyceride and malonaldehyde, and increased the contents of vitamin E and lecithin of eggs, and show a dose-response effect. Moreover, extra oil supplementation increased the concentrations of cholesterol, triglyceride and lecithin. This result was consistent with the studies of Panja et al. (2012) study, which suggested that green tea powder could decrease the cholesterol content in egg yolk and whole eggs. And this outcome was similar to the studies of Panja (2007) and Hisashi et al. (1986), which also suggested that green tea can significantly decrease the content of cholesterol, and a feasible amount of green tea was 1.0%–2.0%. Moreover, there is evidence that exterior nutrition matter could change the energy metabolism and material metabolism, and then alter the egg composition. Green tea powder can improve the egg quality, particularly by increasing the albumen height and Haugh unit. But this result was not consistent with Panja’s (2007) study, which concluded that Haugh unit, yolk color and albumen height were not significantly different with increasing amounts of tea supplementation.

**Table 5. Effects of green tea powder on nutritional composition of eggs.**

| Items                     | T0         | T1         | T2         | T3         | T4         |
|---------------------------|------------|------------|------------|------------|------------|
| Nutritional Composition of Egg Yolks |           |            |            |            |            |
| Cholesterol, %            | 0.36 ± 0.02a| 0.34 ± 0.02ab| 0.33 ± 0.02ab| 0.32 ± 0.01a| 0.35 ± 0.01f|
| V₅, mg/100 g             | 3.37 ± 0.09a| 3.43 ± 0.08ab| 3.50 ± 0.08bc| 3.55 ± 0.05c| 3.46 ± 0.19 |
| Crude fat, %              | 26.69 ± 0.37a| 26.45 ± 0.29abc| 26.24 ± 0.24a| 26.34 ± 0.12ab| 26.56 ± 0.08 |
| Triglyceride, %           | 291.13 ± 8.65| 292.39 ± 7.57| 291.29 ± 9.19| 284.54 ± 11.93| 292.11 ± 11.24|
| Lecithin, %               | 4.34 ± 0.05a| 4.40 ± 0.12a| 4.42 ± 0.15a| 4.43 ± 0.07a| 4.40 ± 0.15a|
| Malonaldehyde, mmol/L     | 28.03 ± 0.48e| 27.60 ± 0.27a| 27.39 ± 0.22ab| 27.12 ± 0.17a| 27.19 ± 0.12a|
| Nutritional Composition of Whole Eggs |           |            |            |            |            |
| Cholesterol, %            | 0.16 ± 0.01c| 0.16 ± 0.01c| 0.15 ± 0.01b| 0.14 ± 0.01e| 0.16 ± 0.01f|
| V₅, mg/100 g             | 5.01 ± 0.11a| 5.14 ± 0.10c| 5.28 ± 0.13c| 5.36 ± 0.09f| 5.29 ± 0.18 |
| Crude fat, %              | 11.89 ± 0.32a| 11.67 ± 0.37a| 11.58 ± 0.30a| 11.62 ± 0.36a| 11.74 ± 0.10a|
| Triglyceride, %           | 193.75 ± 3.88ab| 192.05 ± 8.53ab| 189.67 ± 7.45ab| 185.91 ± 8.99e| 196.27 ± 9.35e|
| Lecithin, %               | 1.26 ± 0.054a| 1.27 ± 0.055b| 1.30 ± 0.045b| 1.36 ± 0.044c| 1.41 ± 0.035a|
| Malonaldehyde, mmol/L     | 14.99 ± 0.24ab| 14.96 ± 0.20ab| 14.81 ± 0.28ab| 14.65 ± 0.37ab| 14.73 ± 0.32 |

Notes: control group T0, no diet supplements; test groups T1, T2 and T3 were supplemented with 1%, 2% and 3% green tea powder into basal diet, respectively; for test group T4, 3% green tea powder and 1.5% peanut oil were added to the diet. Values are expressed as Mean ± SEM for 9 eggs.

- Values with different lower case letters in the same row differ significantly (P < 0.05).
- # represents significant differences (P < 0.05) between groups T3 and T4.
treatment significantly decreased the contents of cholesterol, crude fat and malonaldehyde, increased the content of vitamin E of eggs, and showed a dose-response effect.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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