Changes in egg production, egg quality and egg nutritional values of laying hens in response to dietary supplementation with green tea water extracts

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**ABSTRACT**

Our previous studies demonstrated that low-grade tea had some beneficial effects on egg production performance and egg quality. Extracts derived from low-grade tea had high levels of active ingredients. Thus, this study investigated the effects of green tea water extracts (GTWE) supplementation on egg production, egg quality and egg nutritional value of laying hens. A total of 192 laying hens were randomly allotted to four groups T0, T1, T2 and T3, which fed with basal diet supplemented with 0%, 0.3%, 0.5% and 1.0% GTWE, respectively. Results showed that 0.3% GTWE supplementation increased egg production and improved feed conversion ratio significantly, while 0.5% and 1.0% GTWE supplementation resulted in no significant effects. Furthermore, 1.0% GTWE had adverse effects on feed intake. GTWE treatment improved egg quality, as shown by the increased albumen height and Haugh unit. At the same time, GTWE treatment improved the egg nutritional value, as presented by the increased content of vitamin E and lecithin. On the other hand, GTWE treatment decreased cholesterol, triglyceride, and malonaldehyde levels. However, the strength and thickness of eggshell were negatively affected. The comprehensive analysis suggested low dose of GTWE could be used to developing a desired functional feed supplement.

**Introduction**

Egg is one of the most popular animal products in our daily life, and is a good source of protein and energy. Several researches focus on the nutritional improvement of eggs. Previous studies reported that addition of exogenous nutrients, such as \textit{B. subtilis} (Liu et al. 2017), green tea (Yuan et al. 2016; Xia et al. 2018), saccharicterpenin (Liu et al. 2016) improved the egg quality and nutritional value. Thus, the dietary supplementation may serve as a pathway to improve the quality and nutritional value of eggs.

It is widely known that tea is a healthy beverage and has many health benefits. With the development of the tea industry, plenty of tea resource waste are generated in commercial tea garden. It contains high concentration of nutrients that can be used in feeding animals and especially poultry. It is important to take full advantage of tea by-products. In other words, it is urgent to regularly apply the utilization of tea waste leaves. How to convert tea by-products into feed additive has been a focus in today’s research field. In our early experimentation, we found that low dose of green tea powder (1.0%) significantly improved quality and nutritional value of eggs. At the same time, the composition of eggs was improved. However, high doses of green tea powder decreased the production performance, which suggesting that low doses of green tea could be more appropriately applied as an ideal feed additive. Besides, tea extracts are derived from tea leaves, which contains higher levels of active ingredients than tea leaves of the same weight. Particularly, tea polyphenols (one common active ingredients in tea) have received widely concern for their antioxidant (Rodrigues et al. 2016), antibacterial (Kawarai et al. 2016), anticancer (Shih et al. 2016) properties. Up to now, although many studies investigated the effects of tea on egg production performance, the results were inconsistent and controversial (Uuganbayar et al. 2005; Panja 2007). In addition, few researchers dealt with the effects of tea by-products on quality and nutritional value of eggs. Thus, green tea water extracts (GTWE) were selected as the examined feed additive. This study investigated the effects of different doses of GTWE supplementation on production, quality and nutritional value of eggs.

**Material and methods**

**Materials**

One hundred and ninety-two healthy Xianju chickens (the average laying rate was 50%) were provided by Xianju Chicken Development Company (Huzhou, China).
GTWE was extracted from Chinese low-grade green tea by our lab. High temperature extraction method is used to extract water-soluble substance (Zhang et al. 2018). It contains 22.7% total tea polyphenols, 8.4% epigallocatechin gallate, 2.3% Epicatechin gallate and 4.8% caffeine.

Experimental design and feeding management

Experimental design

The 192 Xianju chickens (20-week aged) were randomly allotted to four treatments, with three replicates per treatment and sixteen chickens in each replicate. Control group T0 fed with basal diet, test groups T1, T2 and T3 fed with basal diet supplemented with 0.3%, 0.5% and 1.0% GTWE, respectively.

Studies included 7 day as a pre-experimental period that served as acclimation period and 67 day for the main experimental period. All the chickens had free access to water and feed. The hen house provided 16 h of light per day, with natural light during the day and artificial light from 1700 to 2400 HRS. The composition and main characteristics of the basal diet are shown in Table 1. The basal diet formulation was in accordance with 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 18868-2002 of the National Standards of People’s Republic of China (NSPRC 2002). The animal care and use protocol were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University and performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (China, 1988) and the Standards for the administration of experimental practices (Jiangsu, China, 2008).

Sampling and handling

Ten eggs from each replicate at the end of this study were randomly selected and later used for egg quality analysis. The analyses were completed within 12 h. Three eggs from each replicate were randomly selected and later used for the egg nutrition analysis.

Measurement methods

Egg production performance

Egg production including number and weight, feed intake and mortality were recorded daily. Average daily feed intake, average egg weight and feed-to-egg ratio were calculated at the end of the trial.

Egg quality

The egg shape index was measured (in mm) with a calliper, and vertical diameter-diameter-1 was calculated to determine the shape index. Haugh units, albumen height, and yolk color were measured with an automatic egg analyser (Egg Multi Tester, EMT-5200). Eggshell thickness was measured with a digital micrometre (karl deutsch MODEL-1061). Eggshell strength was measured with the eggshell force gauge (MODEL-T1). Egg and yolk weight were measured with the electronic balance (Mettler Toledo pL203-IC).

Egg nutritional value

Three eggs from each replicate were randomly collected for the estimation of the nutritional value of eggs. The content of crude fat, cholesterol, vitamin E and malonaldehyde were detected according to the reference of GB/T 4772-2008 (NSPRC 2008b), GB/T 5009.128-2003 (NSPRC 2003a), GB/T 5009.82-2003 (NSPRC 2003b), GB 5009.181-2016 (NSPRC 2016). The content of lecithin and triglyceride were detected according to the reference of Bao and Zhang (2013).

Statistical analysis

Data were reported as mean ± SEM, and all the data were analysed using the one-way ANOVA of SPSS17.0 in a randomized complete block design. When significant differences were found, Duncan’s test was used for multiple comparisons. P-values <0.05 were considered significant.

Results and discussion

Analysis of egg production performance changes

No mortality was observed during the experiment. The results of different levels of GTWE supplementation on egg production performance are shown in Table 2. Compared with group T0, the laying rate increased by 8.41% (P < 0.05), and the feed-to-egg ratio decreased by 7.51% (P < 0.05) in group T1. The average egg weight decreased by 0.93% (P < 0.05), 0.64% (P > 0.05), 0.89% (P < 0.05) in groups T1, T2 and T3, respectively. The average daily feed intake decreased by 2.88% (P < 0.05) in group T3.

The results revealed that 0.3% GTWE treatment significantly improved egg production and feed conversion, while 0.5% GTWE had slight effects. Furthermore, 1.0% GTWE had
adverse effects on the feed intake. In the case of the increased laying rate, but had no significant changes in feed intake, it was clear that 0.3% GTWE supplementation improved feed conversion ratio, and this phenomenon may be related to the action of tea (Yuan et al. 2016; Xia et al. 2018). Previous studies found that tea’s functional ingredients had a lot of health benefits, such as anti-oxidation (Rodrigues et al. 2016), antibacteria (Kawarai et al. 2016), and contribute to the improvement of body healthy status (Lin and Lin 2010; Periandavan et al. 2017). But on the other hand, high doses of GTWE supplementation had some bad effects on feed intake. Possibly because GTWE supplementation influenced and altered the palatability of the feed. The GTWE used in this study contains 22.7% total tea polyphenols, 8.4% epigallocatechin gallate, 2.3% epicatechin gallate, and 4.8% caffeine. Tea polyphenols and caffeine are the two bitter factors, and it reduced the palatability and as a result of reduced feed intake (Table 2). Energy and protein supply are vitally important for the farmed animal industries, especially in laying hens breeding industry. It is important that chronic insufficient nutrition and energy absorption will result in the decreased production performance in animal production (Trindade Neto et al. 2011; Pérezbonilla et al. 2012).

### Analysis of changes in egg quality

The effects of different doses of GTWE supplementation on egg quality are shown in Table 3. There were no significant differences (P > 0.05) in egg yolk color among the treatments. Compared with group T0, the egg shape index decreased by 3.05% (P < 0.05) in group T1. The eggshell thickness decreased by 6.52% (P < 0.05), 6.52% (P < 0.05) and 6.52% (P < 0.05) in groups T1, T2 and T3, respectively. The strength of eggshell decreased by 13.09% (P < 0.05), 16.31% (P < 0.05) and 19.31% (P < 0.05) in groups T1, T2 and T3, respectively. The albumen height increased by 21.40% (P < 0.05), 28.83% (P < 0.05) and 10.14% (P > 0.05) in groups T1, T2 and T3, respectively. The Haugh unit increased by 7.97% (P < 0.05), 11.29% (P < 0.05) and 3.64% (P < 0.05) in groups T1, T2 and T3, respectively.

### Analysis of changes in egg nutritional value

The effect of different doses of GTWE supplementation on egg nutritional value changes is shown in Table 4. There was no significant difference (P > 0.05) between crude fat in egg yolk and whole egg among the treatments. Compared with group T0, the contents of cholesterol in egg yolk were decreased by 8.33% (P < 0.05), 8.33% (P < 0.05) and 11.11% (P < 0.05) in group T1, T2 and T3, respectively. The triglyceride decreased by 6.21% (P < 0.05) in group T3. The vitamin E increased by 1.19% (P > 0.05), 3.86% (P < 0.05) and 5.64% (P < 0.05) in group T1, T2 and T3, respectively. The lecithin increased by 3.69% (P > 0.05) in group T3. The malonaldehyde decreased

### Table 2. Effects of GTWE on egg production performance of laying hens.

| Items                        | T0     | T1     | T2     | T3     | p-value |
|------------------------------|--------|--------|--------|--------|---------|
| Laying rate (%)              | (69.42 ± 11.26) | (75.26 ± 11.61) | (69.45 ± 11.70) | (69.87 ± 12.39) | 0.000  |
| Average egg weight (g)       | (44.02 ± 1.66)  | (43.61 ± 1.86)  | (43.74 ± 1.67)  | (43.63 ± 1.48)  | 0.024  |
| Average feed intake (g)      | (110.92 ± 10.73) | (110.37 ± 12.21) | (110.70 ± 11.67) | (107.73 ± 8.07) | 0.091  |
| Feed to egg ratio (g·egg⁻¹)  | (3.73 ± 0.74)   | (3.45 ± 0.66)   | (3.76 ± 0.78)   | (3.68 ± 0.94)   | 0.001  |

Notes: T0, T1, T2, T3 represent the test groups supplemented with 0, 0.3%, 0.5%, and 1.0% green tea water extracts (GTWE) into basal diet, respectively. Values are expressed as Mean ± SEM. bValues with different lowercased letters in the same row differ significantly (P < 0.05).

### Table 3. Effects of GTWE on egg quality of laying hens.

| Items                        | T0     | T1     | T2     | T3     | p-value |
|------------------------------|--------|--------|--------|--------|---------|
| Egg shape index              | 1.31 ± 0.04   | 1.27 ± 0.05   | 1.30 ± 0.05   | 1.29 ± 0.08   | 0.545   |
| Eggshell thickness (cm)      | 0.46 ± 0.04   | 0.43 ± 0.04   | 0.43 ± 0.05   | 0.43 ± 0.06   | 0.003   |
| Eggshell strength (kg·cm⁻³)  | 4.66 ± 0.93   | 4.05 ± 0.64   | 3.90 ± 0.84   | 3.76 ± 0.90   | 0.041   |
| Albumen height (mm)          | 4.44 ± 0.77   | 5.39 ± 1.45   | 5.72 ± 1.45   | 4.89 ± 1.05   | 0.626   |
| Yolk colour                  | 7.81 ± 1.08   | 7.85 ± 0.97   | 7.54 ± 1.14   | 7.38 ± 1.02   | 0.042   |
| Haugh unit (HU)              | 74.82 ± 3.81  | 80.78 ± 3.93  | 83.27 ± 7.54  | 77.54 ± 6.71  | 0.804   |

Notes: T0, T1, T2, T3 represent the test groups supplemented with 0, 0.3%, 0.5%, and 1.0% GTWE into basal diet, respectively. Values are expressed as Mean ± SEM. bValues with different lowercased letters in the same row differ significantly (P < 0.05).
Table 4. Effects of GTWE on nutritional values of eggs.

| Items                        | T0          | T1          | T2          | T3          | p-value |
|------------------------------|-------------|-------------|-------------|-------------|---------|
| Nutritional Composition of Egg Yolks |             |             |             |             |         |
| Cholesterol (%)              | 0.36 ± 0.02b| 0.33 ± 0.02a| 0.33 ± 0.03a| 0.32 ± 0.03a| 0.003   |
| Vitamin E (mg 100g⁻¹)        | 3.37 ± 0.09a| 3.41 ± 0.10ab| 3.50 ± 0.09b| 3.56 ± 0.14c| 0.002   |
| Crude fat (%)                | 26.69 ± 0.37| 26.88 ± 0.45| 26.56 ± 0.44| 26.54 ± 0.27| 0.196   |
| Triglyceride (%)             | 291.13 ± 8.65ab| 293.33 ± 6.91b| 291.71 ± 9.15ab| 283.80 ± 11.39a| 0.111   |
| Lecithin (%)                 | 4.34 ± 0.05a| 4.45 ± 0.14ab| 4.42 ± 0.15ab| 4.50 ± 0.12b| 0.048   |
| Malonaldehyde (mmol L⁻¹)     | 28.03 ± 0.48c| 27.41 ± 0.34b| 27.30 ± 0.28b| 26.89 ± 0.28a| 0.000   |
| Nutritional Composition of Whole Eggs |             |             |             |             |         |
| Cholesterol (%)              | 0.16 ± 0.01c| 0.15 ± 0.01b| 0.15 ± 0.01b| 0.14 ± 0.01a| 0.000   |
| Vitamin E (mg 100g⁻¹)        | 5.01 ± 0.11a| 5.14 ± 0.12b| 5.27 ± 0.11c| 5.40 ± 0.09d| 0.000   |
| Crude fat (%)                | 11.89 ± 0.32| 11.72 ± 0.31| 11.66 ± 0.32| 11.66 ± 0.28| 0.272   |
| Triglyceride (%)             | 193.75 ± 3.88b| 191.33 ± 9.40b| 188.58 ± 10.24ab| 181.72 ± 11.31a| 0.035   |
| Lecithin (%)                 | 1.26 ± 0.05a| 1.29 ± 0.04ab| 1.32 ± 0.03b| 1.33 ± 0.06b| 0.003   |
| Malonaldehyde (mmol L⁻¹)     | 14.99 ± 0.24b| 14.95 ± 0.11b| 14.77 ± 0.27ab| 14.63 ± 0.35a| 0.013   |

Notes: T0, T1, T2, T3 represent the test groups supplemented with 0, 0.3%, 0.5%, and 1.0% GTWE into basal diet, respectively. Values are expressed as Mean ± SEM. a–cValues with different lowercased letters in the same row differ significantly (P < 0.05).

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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by 2.21% (P < 0.05), 2.60% (P < 0.05), 4.07% (P < 0.05) in group T1, T2 and T3, respectively.

In whole eggs, compared with group T0, the contents of cholesterol decreased by 6.25% (P < 0.05), 6.25% (P < 0.05) and 12.50% (P < 0.05) in group T1, T2 and T3, respectively. The triglyceride decreased by 6.21% (P < 0.05) in group T3. The lecithin increased by 2.38% (P < 0.05), 4.76% (P < 0.05), 5.56% (P < 0.05) in group T1, T2 and T3, respectively. The vitamin E increased by 2.60% (P < 0.05), 5.19% (P < 0.05) and 7.78% (P < 0.05) in groups T1, T2 and T3, respectively. The malonaldehyde decreased by 2.40% (P < 0.05) in group T3.

The results showed that GTWE treatment decreased the content of cholesterol, triglyceride and malonaldehyde. At the same time, it increased the vitamin E and lecithin of egg yolk and whole eggs which showed a dose-dependent potency. These findings were consistent with the studies of Panja (2007), Wei et al. (2012) and Xia et al. (2018). Current studies have shown that intake some exterior nutrients such as substances with physiological activity could affect and alter body metabolism, including energy metabolism and material metabolism (Oliveira et al. 2011). Tea polyphenols is one of the major categories of biological active compounds found in tea or tea extracts. They could reduce the lipid levels and increase the faecal excretion of total lipids and cholesterol depending on the dose (Matsumoto et al. 1998). Eventually, changes in body metabolism finally altered the egg composition and egg nutritional values (Du et al. 2016; Liu et al. 2017; Xia et al. 2018).

Conclusion

Low dose of GTWE (0.3%) treatment increased the egg production, decreased feed to egg ratio, but intermediate dose of GTWE (0.5%) had minor effects. More importantly, 1.0% GTWE had adverse effects on feed intake. GTWE treatment increased the quality and nutritional value of eggs, as shown by the increased values of albumen height and Haugh unit. In addition, the contents of vitamin E and lecithin increased, while the cholesterol, triglyceride and malonaldehyde decreased which was dose-dependent effect. However, it had adverse influence on strength and thickness of eggshell. By combing the changes of production performance, quality and nutritional value of eggs, low dose of GTWE had a great potential use for developing new kinds of feed additives.
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