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Full Length Article

Effects of Dietary Tea Polyphenols on Epigallocatechin Gallate, Catechin, Egg Quality and Production of *Gallus domestiaus*

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Abstract

Effect of dietary tea polyphenol (TP) on the content of epigallocatechin gallate (EGCG), catechin (C) in egg yolk, and impact on egg quality and production was investigated. For this purpose, 315 healthy *Gallus domestiaus* aged 42 weeks were selected and randomly divided into 7 groups. The experimental group added 0.02, 0.05, 0.09, 0.14, 0.19 and 0.24% TP to the basic diet. The control group (CK) was fed basal diet. After 10, 20 and 30 days, 15 eggs were randomly selected from each group for egg quality testing, and 20 eggs were selected for EGCG and C content test in the yolk. The results showed that in the 0.24% TP group, the content of EGCG in egg yolk was significantly higher than that of CK group and the 0.02, 0.05, 0.09 and 0.14% TP groups ($P < 0.05$), and reached the highest on the 30th day (19.171μg/g). The content of C in egg yolk was significantly higher than that in CK group and the 0.02, 0.05, 0.09 and 0.14% TP groups ($P < 0.05$); and reached the highest on 30th day (7.071 μg/g). The egg weight of the 0.14% TP group on the 10th day and of the 0.19% TP group on the 20th day were significantly lower than that of the CK group ($P < 0.05$); the egg yolk weight of 0.14% TP on the 10th and 20th days were significantly lower than that of the CK group ($P < 0.05$). On the 30th day, compared with the control group, the egg weight of the 0.14% TP group was significantly reduced ($P < 0.05$), the egg yolk color of 0.05 and 0.14% TP groups was significantly increased ($P < 0.05$). During the whole experiment, the egg production of 0.24% TP group was significantly higher than that of control group ($P < 0.05$). In conclusion, adding high concentrations of TP can increase the content of EGCG and C in the yolk; however, it has a certain impact on egg weight, yolk color and eggshell thickness. Adding high concentrations of TP may increase egg production. © 2021 Friends Science Publishers

Keywords: Catechin; Epigallocatechin gallate; Egg yolk; Egg quality; *Gallus domestiaus*; Tea polyphenols

Introduction

Tea polyphenol (TP) is the general term for a class of polyhydroxy phenolic compounds contained in tea, accounting for about 30% of the dry weight of tea (Wang 1981), including catechins, flavonoids, anthocyanins and phenolic acids (Wan 2003). Catechins are an important substance in TP, and accounts for about 70% of the total TP. There are 8 monomers of catechin compounds, including epigallocatechin gallate (EGCG), epigallocatechin (EGC), catechin (C), epicatechin (EC), catechin gallate (CG), gallocatechin (GC), gallocatechin gallate (GCG) and epicatechin gallate (ECG) (Lu *et al*. 2018). Catechin compounds have anti-microbial, anti-oxidant, anti-mutation, anti-cancer, anti-diabetic and anti-viral effects (Dorota *et al*. 2004; Khan and Mukhtar 2007; Sharangi 2009). EGCG is the polyphenol with the largest proportion of catechins that has the strongest activity. Studies have shown that the antioxidant activity of EGCG is 20 times that of vitamin E and 6 times that of superoxide dismutase (Huang *et al*. 2010).

The strong antioxidant activity of EGCG is due to the trihydroxy group (on the B ring C3, C4 and C5) and the esterified gallate (on the C ring C3) (Ahmad *et al*. 2014). In vitro studies have found that C can scavenge free radicals, remove cytotoxic hemoglobin, and show obvious antioxidant activity (Lu *et al*. 2011). In addition, C inhibits tumor-specific angiogenesis by regulating the production of pro-inflammatory cytokines, nitric oxide, vascular endothelial growth factor, interleukin-2, etc. (Guruvayoorappan and Kuttan 2008).

At present, many studies focus on the improvement of livestock and poultry production performance, immune function, antioxidant function, regulation of glucose and lipid metabolism, antibacterial by adding TP to the diet (Wang 2017). Adding 300 mg/kg of tea polyphenols to broiler diets can increase the activity of T-AOC in the blood of broilers

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and improve the antioxidant properties of meat quality (Li *et al*. 2012). A proper concentration dietary addition of tea polyphenols can improve the production performance and carcass quality of Partridge shank Chickens, and improve the blood biochemical indicators related to fat metabolism (Xu *et al*. 2011). Other studies have shown that adding green tea powder to the diet can effectively promote the development of immune organs of white feather broilers, enhance the activity of antioxidant enzymes and increase the expression of immunoglobulin (Yin 2017).

However, up to now, there are few reports on the in-depth study of the effect of adding TP to the diet on the enrichment of TP active components in egg yolk. Therefore, this experiment took the local breed of *Gallus domestiaus* laying hens, and added different levels of TP to the diet to explore its effect on the catechin compound EGCG content and C content in egg yolk, as well as egg quality indicators and egg production. This article aims to provide a basis for the production of TP-rich functional eggs and the optimization of dietary additives for animal husbandry.

Materials and Methods

Animals and experimental design

This study was carried out at the chicken breeding base of Tianjin Jinwa Agricultural Technology Development Co., Ltd. from October to November in 2019. 315 healthy *Gallus domestiaus* aged 42 weeks were randomly selected and randomly divided into 7 groups (45 chickens in each group), raised indoors on flat ground (with an activity area of 50 m^2 for each group), with free eating and drinking. The control group was fed with the basal diet, the rest 6 groups were the experimental groups, with 0.02, 0.05, 0.09, 0.14, 0.19 and 0.24% TP added to the basal diet, respectively. The pre-test period of all groups was 3 days, and the formal test period was 30 days. The composition of the basic diet is shown in Table 1.

Materials, regents and equipment

Main materials, regents and equipment are listed in Table 2.

Sample collection

Around the 10th, 20th and 30th days, 35 eggs were randomly collected from each group, 15 eggs of which were used for the determination of egg quality, and the remaining 20 eggs were used for the detection of EGCG content and C content in the eggs. Before testing, the eggs are stored at 4°C for later use.

Egg quality index determination

Eggshell color were measured using an eggshell color tester of the sharp end, blunt end and middle of the eggshell, and take the average value. Use an electronic balance to measure the egg weight, yolk weight, egg white weight and eggshell weight of the eggs. Egg shape index was measured using an egg shape index tester to measure the vertical and horizontal diameters of eggs, egg shape index = vertical diameter / horizontal diameter. The relative density of eggs is measured with sodium chloride aqueous solutions of different specific gravity. The higher the relative density, the fresher the eggs. Use an eggshell thickness tester to measure the thickness of the three parts of the eggshell at the sharp end, the blunt end and the middle, and take the average value. Egg yolk color was measured by the yolk colorimeter. Use the 15 egg yellow grades of the Roche yolk color fan for comparison; higher the grade, the darker the egg yolk. Haugh unit: break the egg and pour it on a glass plate, use a protein height measuring instrument, avoiding the lace to measure the middle of the thick protein layer around the yolk, and take the average of three equidistant points to be the protein height, according to the formula calculate the corresponding Haugh unit.

$$\text{Haugh unit} = 100 \log (H - 1.7W^{0.37} + 7.57)$$

Note: H is the height of concentrated protein (mm), and W is egg weight (g).

Egg production

The egg production of each group was recorded every day, and the egg production of each group on the 10th, 20th and 30th day was counted and the data was analyzed.

Determination of EGCG and C content in egg yolk

High performance liquid chromatography (HPLC) was used to determine the content of EGCG and C in egg yolk. The sample preparation was carried out according to SN/T3848-2014 (SN/T 3848-2014) with slight modifications. Weigh 2g egg yolk sample into a 10 mL graduated centrifuge tube, add methanol to the volume to 10 mL, mix well, vortex for 2 min, sonicate for 20 min, freeze in the refrigerator at -20°C for 10 min, centrifuge at 5000 r/min for 8 min, filter the supernatant with 0.45 μm organic microporous membrane, and the filtrate is used for detection by high performance liquid chromatography. The liquid phase conditions during the determination are: Chromatographic column: C18 (4.6 mm × 250 mm, 5 μm); Mobile phase: 0.1% phosphoric acid aqueous solution: Methanol = 68:32 (V:V); Flow rate 0.9 mL/min; Column temperature: 30°C; Detection wavelength: 279 nm; Injection volume: 15 μL.

Data analysis

The test data are all expressed as "mean ± standard error". Using SPSS 22.0 software, one-way ANOVE analysis (Duncan'D) was used to perform multiple comparisons between means, and a significance test was performed. $P < 0.05$ was considered significant.

Table 1: Basic diet composition

Component	Content (%)	Component	Content (%)
Corn	60	Stone powder	4
Soybean meal	20	Fish meal	2.5
Sorghum	5	Bone meal	2.5
Bran	4	Premix	2

Table 2: Main materials, regents and equipment

Name	Producer
Tea polyphenols	Wuxi Century Biological Engineering Co., Ltd. (Batch No.: FP190702)
Epigallocatechin Gallate (EGCG)	Tianjin Xiensi Biochemical Technology Co., Ltd.(CAS No.989-5-15)
Catechin (C)	China Institute for Food and Drug Control (CAS No.7295-85-4)
Methanol (chromatographically pure)	Merck Inc., Germany
Phosphoric acid (analytically pure)	Tianjin Fengchuan Chemical Reagent Technology Co., Ltd.
Protein height meter (NFN382)	FHK Inc., Japan
Analytical Balances (FB223)	Shanghai Shunning Hengping Scientific Instrument Co., Ltd.
Multi purpose vortex mixer (VORTEX-6)	Haimen qilinbeier Instrument Manufacturing Co., Ltd.
Egg shell reflectivity (TSS-QCR)	Beijing Tianxiang Feiyu Technology Co., Ltd.
Ultrasonic cleaning machine (SB-100D)	Ningbo Xinzhi Biological Technology Co., Ltd.
High performance liquid chromatograph (Agilent-1260)	Agilent Technologies Co., Ltd.
Column: C18 (250 mm × 4.6 mm, 5 μm)	Shimadzu-GL Sciences (Shanghai) Laboratory Supplies Co., Ltd.

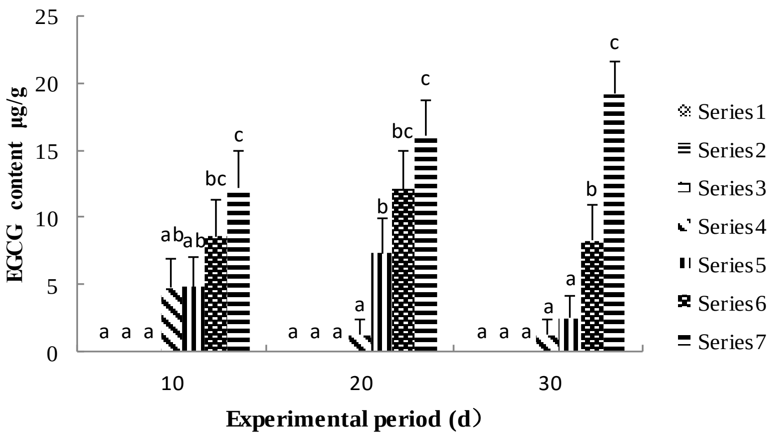

Fig. 1: Effect of adding different content of TP in diet on EGCG content in egg yolk.
Note: The lowercase letters represent the significant differences among different groups ($P < 0.05$). The same applies below

Results

The effect of adding TP in diet on the content of EGCG in egg yolk

It can be seen from Fig. 1 that the EGCG content in the egg yolk of the 0.24% TP group was significantly higher than that of the control group and the 0.02, 0.05, 0.09 and 0.14% TP groups ($P < 0.05$). And reached the highest on the 30th day (19.171 μg/g), which was significantly higher than the 0.19% TP group ($P < 0.05$).

The effect of adding TP in diet on C content in egg yolk

It can be seen from Fig. 2 that the C content in the egg yolk of the 0.24% TP group was significantly higher than that of the CK group and the 0.02, 0.05, 0.09 and 0.14% TP groups ($P < 0.05$); on the 10th day, the 0.19% TP group was significantly higher than that in the 0.02% TP group ($P < 0.05$); on the 20th day, the 0.19%TP group and 0.24%TP group was significantly higher than the other groups ($P < 0.05$). On the 30th day, the 0.19% TP group was significantly higher than that of the CK group and the 0.02, 0.05 and 0.14% TP groups. In addition, on the 30th day, the 0.24% TP group reached the highest, which was 7.071 μg/g.

The effect of adding TP in diet on egg quality

It can be seen from Table 3 that on the 10th day, the egg weight, yolk weight and eggshell weight of the 0.14% TP group were significantly lower than that of the control group ($P < 0.05$); the egg shape index of 0.05, 0.09, 0.14 and 0.24% TP groups were significantly lower than those of control group and 0.02% TP group ($P < 0.05$); the eggshell thickness of all test groups was significantly lower than the

Table 3: Effects of different concentrations of TP on egg quality on the 10th day

Egg quality Index	CK	0.02% TP	0.05% TP	0.09% TP	0.14% TP	0.19% TP	0.24% TP
Egg weight (g)	$54.134^{a} \pm 1.512$	$51.012^{ab} \pm 0.956$	$51.553^{ab} \pm 1.080$	$52.126^{ab} \pm 1.007$	$50.201^{b} \pm 1.030$	$51.133^{ab} \pm 1.184$	$52.066^{ab} \pm 0.959$
Egg yolk weight (g)	$16.467^{a} \pm 0.318$	$16.113^{ab} \pm 0.241$	$15.842^{ab} \pm 0.501$	$15.854^{ab} \pm 0.294$	$15.073^{b} \pm 0.310$	$15.673^{ab} \pm 0.427$	$15.849^{ab} \pm 0.242$
Egg white weight (g)	29.442 ± 1.230	27.470 ± 0.654	28.346 ± 0.703	29.376 ± 0.716	27.539 ± 0.826	28.239 ± 1.208	28.738 ± 0.925
Eggshell weight (g)	$7.052^{a} \pm 0.211$	$6.476^{ab} \pm 0.178$	$6.622^{ab} \pm 0.181$	$6.444^{ab} \pm 0.207$	$6.101^{b} \pm 0.180$	$6.680^{ab} \pm 0.238$	$6.495^{ab} \pm 0.202$
Eggshell color	48.500 ± 1.885	53.371 ± 1.396	48.679 ± 1.492	50.273 ± 1.074	52.740 ± 1.226	49.814 ± 1.802	51.536 ± 1.662
Egg Shape Index	$1.419^{a} \pm 0.019$	$1.413^{a} \pm 0.015$	$1.372^{b} \pm 0.012$	$1.343^{b} \pm 0.012$	$1.345^{b} \pm 0.012$	$1.383^{ab} \pm 0.013$	$1.353^{b} \pm 0.014$
Relative density (g/mL)	1.086 ± 0.001	1.085 ± 0.002	1.086 ± 0.002	1.138 ± 0.054	1.080 ± 0.002	1.084 ± 0.002	1.084 ± 0.001
Yolk color	6.800 ± 0.442	7.267 ± 0.248	7.600 ± 0.235	7.333 ± 0.270	7.333 ± 0.252	7.333 ± 0.211	7.333 ± 0.232
Haugh unit	85.565 ± 4.526	85.066 ± 1.646	85.028 ± 1.890	89.741 ± 1.579	87.606 ± 2.586	87.748 ± 1.742	84.613 ± 1.965
Eggshell thickness (mm)	$0.375^{a} \pm 0.005$	$0.349^{b} \pm 0.006$	$0.349^{b} \pm 0.008$	$0.349^{b} \pm 0.006$	$0.326^{c} \pm 0.005$	$0.349^{b} \pm 0.009$	$0.353^{b} \pm 0.005$

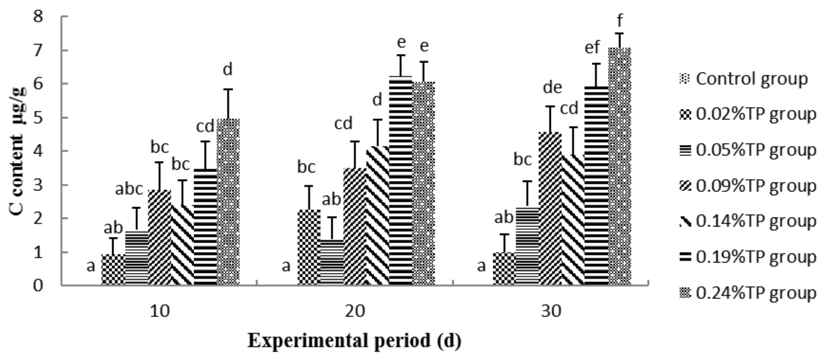

Fig. 2: Effect of adding different content of TP in diet on C content in egg yolk

control group (P < 0.05). There were no significant differences in egg white weight, relative density, Haugh unit, egg shell color, egg yolk color and other egg quality indexes of each group of eggs (P > 0.05).

It can be seen from Table 4 that on the 20th day, the egg weight of the 0.19% TP group was significantly lower than the control group (P < 0.05); the egg yolk weight of the 0.14%TP group was significantly lower than the control group (P < 0.05); except for the 0.02% TP group, the eggshell color of other test groups was significantly lower than that of the control group (P < 0.05); the relative density of the 0.24% TP group was significantly lower than that of the 0.02 and 0.05% TP groups (P < 0.05); in the 0.14 and 0.24% TP groups, the eggshell thickness was significantly lower than that of the CK group (P < 0.05). There were no significant differences in other egg quality indicators among the groups (P > 0.05).

It can be seen from Table 5 that on the 30th day, the egg weight in the 0.14% TP group was significantly lower than that in the control group, and the egg yolk color in the 0.05 and 0.14% TP groups increased significantly (P < 0.05); the relative density of the eggs in the 0.14% TP group was significantly lower than 0.05% TP group (P < 0.05); the Haugh unit of 0.24% TP group was significantly lower than that of 0.14% TP group (P < 0.05); 0.09, 0.14, 0.19 and 0.24% TP groups eggshell thickness is significantly lower than 0.05% TP group (P < 0.05); other egg quality indicators were not significantly different among the groups (P > 0.05).

The effect of adding TP to the diet on egg production

It can be seen from Fig. 3 that on the 10th day, the egg production of the 0.05 and 0.24% TP groups was significantly higher than that of the control group, 0.14% TP, and 0.19% TP groups (P < 0.05); on the 20th day, the egg production of the 0.24% TP group Significantly higher than the control group, 0.09% TP, 0.14% TP groups; on the 30th day, the egg production of 0.02, 0.05, 0.19, 0.24 TP group was significantly higher than that of the control group, 0.09, 0.14% TP groups (P < 0.05).

Discussion

Related studies have shown that dietary special substances may have effect on the physiology of laying hens (Celebi 2019; Demir and Kaya 2020), and thus also may effect the quality and the nutritional components of eggs (Wahab *et al.* 2019; Aydin and Bolukbasi 2020; Liu *et al.* 2020; Mahfuz *et al.* 2020). TP can improve health of the body, so it is widely used in livestock and poultry feed. Studies have shown that EGCG can inhibit the body's oxidative stress and inflammation, lower blood lipids and blood sugar, and regulate gene expression and related signal pathways (Masuda *et al*. 2001; Khan *et al*. 2006; Wolfram *et al*. 2006; Bose *et al*. 2008; Tipoe *et al.* 2010). In addition, EGCG stimulates the production of tumor necrosis factor (TNF-α) and interferon (IFN-γ) by changing the immune response of macrophages, thereby increasing the immune activity of

Table 4: Effects of different concentrations of TP on egg quality on the 20th day

Egg quality index	CK	0.02% TP	0.05% TP	0.09% TP	0.14% TP	0.19% TP	0.24% TP
Egg weight (g)	54.295^{a} ± 0.769	50.764^{ab} ± 1.067	52.174^{ab} ± 0.993	51.462^{ab} ± 1.028	51.559^{ab} ± 1.389	50.165^{b} ± 1.080	52.397^{ab} ± 1.136
Egg yolk weight (g)	16.950^{a} ± 0.304	16.125^{ab} ± 0.365	15.900^{ab} ± 0.224	15.990^{ab} ± 0.240	15.671^{b} ± 0.412	16.195^{ab} ± 0.532	16.337^{ab} ± 0.391
Egg white weight (g)	29.931 ± 0.841	27.289 ± 0.795	28.705 ± 0.712	28.257 ± 0.798	29.853 ± 1.232	27.481 ± 0.824	28.800 ± 1.137
Eggshell weight (g)	6.674 ± 0.233	6.600 ± 0.176	6.859 ± 0.154	6.599 ± 0.149	6.720 ± 0.225	6.414 ± 0.137	6.686 ± 0.277
Eggshell color	55.260^{a} ± 1.689	51.340^{ab} ± 1.336	46.907^{b} ± 1.748	49.973^{b} ± 1.196	49.893^{b} ± 0.989	50.087^{b} ± 1.676	49.807^{b} ± 2.181
Egg Shape Index	1.374 ± 0.015	1.395 ± 0.016	1.391 ± 0.016	1.365 ± 0.016	1.382 ± 0.014	1.381 ± 0.012	1.376 ± 0.010
Relative density (g/mL)	1.084^{ab} ± 0.003	1.090^{a} ± 0.002	1.090^{a} ± 0.002	1.089^{ab} ± 0.002	1.085^{ab} ± 0.002	1.087^{ab} ± 0.002	1.082^{b} ± 0.003
Yolk color	7.300 ± 0.213	7.333 ± 0.159	7.143 ± 0.143	7.400 ± 0.254	7.267 ± 0.228	7.267 ± 0.206	7.000 ± 0.138
Haugh unit	82.640 ± 3.410	83.572 ± 2.988	81.247 ± 1.994	82.374 ± 3.446	85.288 ± 1.402	83.431 ± 1.960	83.646 ± 2.362
Eggshell thickness (mm)	0.371^{a} ± 0.007	0.364^{ab} ± 0.007	0.362^{ab} ± 0.006	0.365^{ab} ± 0.007	0.341^{bc} ± 0.006	0.356^{ab} ± 0.007	0.333^{c} ± 0.011

Table 5: Effects of different concentrations of TP on egg quality on the 30th day

Egg quality index	CK	0.02%TP	0.05%TP	0.09%TP	0.14%TP	0.19%TP	0.24%TP
Egg weight (g)	54.058^{a} ± 0.882	51.419^{ab} ± 1.272	52.170^{ab} ± 0.821	50.788^{ab} ± 1.174	49.913^{b} ± 1.008	51.717^{ab} ± 1.217	51.608^{ab} ± 0.608
Egg yolk weight (g)	17.141 ± 0.219	16.332 ± 0.359	16.304 ± 0.401	16.163 ± 0.341	16.026 ± 0.319	16.267 ± 0.395	16.131 ± 0.361
Egg white weight (g)	30.598 ± 0.872	27.786 ± 0.916	28.245 ± 0.384	27.901 ± 0.998	27.828 ± 1.003	27.981 ± 0.967	28.552 ± 1.110
Eggshell weight (g)	30.598 ± 0.872	27.786 ± 0.916	28.245 ± 0.384	27.901 ± 0.998	27.828 ± 1.003	27.981 ± 0.967	28.552 ± 1.110
Eggshell color	51.260 ± 1.721	49.467 ± 1.811	48.547 ± 1.865	51.247 ± 1.295	50.680 ± 1.799	50.073 ± 1.678	48.414 ± 1.565
Egg Shape Index	1.381 ± 0.020	1.399 ± 0.011	1.387 ± 0.013	1.376 ± 0.016	1.373 ± 0.013	1.383 ± 0.013	1.367 ± 0.013
Relative density (g/mL)	1.081^{ab} ± 0.001	1.085^{ab} ± 0.002	1.086^{a} ± 0.002	1.082^{ab} ± 0.001	1.080^{b} ± 0.002	1.081^{ab} ± 0.002	1.081^{ab} ± 0.002
Yolk color	6.933^{c} ± 0.153	7.286^{abc} ± 0.163	7.867^{a} ± 0.192	7.333^{abc} ± 0.187	7.600^{ab} ± 0.254	7.067^{bc} ± 0.228	7.091^{bc} ± 0.211
Haugh unit	70.898^{ab} ± 2.460	69.974^{ab} ± 2.180	73.249^{ab} ± 2.108	73.062^{ab} ± 2.199	74.646^{a} ± 1.865	69.715^{ab} ± 3.229	66.692^{b} ± 2.678
Eggshell thickness (mm)	0.349^{abc} ± 0.006	0.363^{ab} ± 0.008	0.370^{a} ± 0.007	0.335^{c} ± 0.005	0.329^{c} ± 0.007	0.343^{bc} ± 0.009	0.345^{bc} ± 0.010

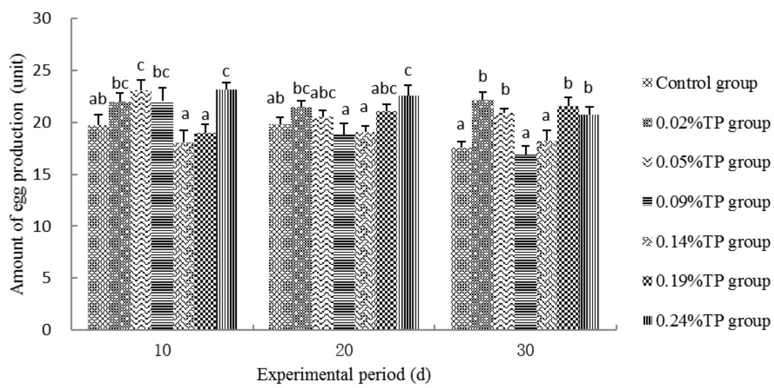

Fig. 3: Effect of adding different content of TP in diet on egg production

macrophages and achieving the purpose of suppressing tumors (Matsunaga *et al.* 2001).

Compared with EGCG, C occupies a smaller proportion in TP, and its antioxidant and anti-inflammatory activities have been confirmed in some studies (Moraes*et al.* 2014). However, there is no report on the analysis of TP composition in eggs by adding TP to the diet. In this experiment, *Gallus domestiaus* was used as the experimental animal to study the effect of dietary supplementation of TP on the content of EGCG and C in eggs. The results showed that the content of EGCG in egg yolk increased with the increase of TP.

On the 30th day, the content of EGCG in egg yolk of 0.24% TP group was significantly higher than that of control group and other experimental groups. On the 30th day, the content of EGCG in egg yolk of 0.24% TP group was the highest, reaching 19.171 μg/g. On the 10th, 20th and 30th day, the content of C in yolk of 0.24% TP group was significantly higher than that of control group and 0.02, 0.05, 0.09 and 0.14% TP groups. On the 30th day, the C content in yolk of 0.24% TP group was the highest, reaching 7.071 μg/g. Comprehensive analysis suggests that the content of EGCG and C in egg yolk increases with the increase of dietary TP. Adding a higher concentration of TP can significantly increase the enrichment of EGCG and C in egg yolk. The results of this study provide a theoretical and practical basis for the development of TP rich functional eggs.

Egg weight is an important indicator for evaluating egg grade. Egg weight is affected by the type of layer, the composition of the diet, the age of the layer, the breeding environment and other conditions. Wang xiaohong and other studies have shown that dietary supplementation of 400 mg/kg TP can significantly reduce the average egg weight in the first 4 weeks of the test period (Wang *et al*. 2017). Other studies have shown that dietary supplementation of catechins can increase the fertilization rate and hatchability of quail and prolong the shelf life of eggs, but it reduces egg weight and egg shell quality (Kara *et al*. 2016). The results of this test show that during the entire test period, compared with the control group, the egg weight of each test group has a tendency to decrease, which is basically consistent with the above research results. The analysis may be due to the anti-nutrients factors such as phenolic acid and caffeine contained in TP that can destroy or hinder the digestion and absorption of certain nutrients in laying hens; or catechins inhibit the intestinal absorption of fat and the activity of fat synthase, affect the formation of egg yolk lipids, thereby reducing egg weight (Yamane *et al*. 1999; Kojlma and Yoshida 2008).

The color of egg yolk is also an important sensory index for evaluating egg quality, it mainly depends on the type and quantity of carotenoids obtained by the laying hens from the diet. In this experiment, on the 10th day, the egg yolk color of each test group was higher than that of the control group. On the 30th day, compared with the control group, the egg yolk color of the 0.05 and 0.14% TP groups were significantly increased by 13.47 and 9.62%, respectively. The results indicate that adding TP to the diet of laying hens can improve the color of egg yolk, and the specific mechanism remains to be further studied.

The thickness of the eggshell is generally 0.3~0.4 mm. If the eggshell is too thin, it will affect the storage, transportation and sales of the egg; if the eggshell is too thick, the hatching rate of the laying hen will be reduced (Zhao *et al*. 2017). During the experiment, the eggshell thickness of each experimental group was 0.3~0.4 mm. On the 10th day, the eggshell thickness of each test group was significantly lower than that of the control group. On the 20th day, the eggshell thickness of the 0.14% TP group and 0.24% TP group was significantly lower than that of the control group. The analysis may be due to the caffeine contained in TP. Studies have shown that the intake of caffeine in the body will lead to the decrease of calcium absorption and loss of calcium in the digestive tract, and reduce bone mineral density (Tsuang *et al*. 2006), which will reduce the thickness of the eggshell and affect the quality of the eggshell.

Egg production is one of the important economic traits of laying hens. Studies have shown that when the Roman layer is 30 weeks old, adding 6000 mg/kg of green tea powder and 1000 mg/kg of TP to the diet can significantly increase the egg production rate of the layer (Xiao 2010). Because TP has anti-oxidant and antibacterial properties, scavenge free radicals, and improve the intestinal microbial environment. It can improve absorption and utilization of nutrients in the diet, thereby increasing feed conversion rate and egg production. The results of this test showed that during the entire test period, the egg production of the 0.24% TP group was significantly higher than that of the control group, and the egg production of other test groups also tended to be higher than that of the control group. The experimental results are similar to the above result, which is, adding higher concentration of TP can improve the egg production of laying hens.

Conclusion

Adding a certain amount of TP to the diet can significantly increase the content of EGCG and C in the yolk, which has a certain impact on the egg weight, yolk color, and eggshell thickness. Adding high concentrations of TP can increase egg production.

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Author Contributions

LA Li designed this study; ZZ Fan, MR Qin, ZM Zhang, KY Zhang and Q Wang conducted the experiments; ZZ Fan, CN Wu, YQ Zhang and SQ Mao analyzed the main data; ZZ Fan and LA Li wrote the manuscript.

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