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Original Article

The Effect of Supplementing Tea Polyphenols on Yolk Cholesterol and Production Performance of Laying Hens During the Egg-laying Period

■Author(s)

Lin PW[I,II*] https://orcid.org/0000-0003-2594-2822
Li XY[I,II*] https://orcid.org/0000-0003-0793-1206
Ma RY[I,II] https://orcid.org/0000-0002-1140-4946
Daijun S[I,II] https://orcid.org/0000-0002-9890-7983

[I] College of Animal Science and Technology, Southwest University, Beibei 400715, China;
[II] Laboratory for Bio-feed and Molecular Nutrition, College of Animal Science and Technology, Southwest University, Chongqing 400715, China.

*These authors contributed equally to this work.

■Mail Address

Corresponding author e-mail address
Daijun Song
24 Buildings in Huangshu Village, Tiansheng Road, Beibei District, Chongqing, 400715, China.
Phone: +23 13983209390
Email: djsong@swu.edu.cn

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Cholesterol content, Laying hens, Production performance, Tea polyphenols.

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ABSTRACT

This study aimed to investigate the effect of supplementing 300 mg/kg tea polyphenols (TP) on yolk cholesterol content and production performance of laying hens during the egg-laying period. A total of 600 Roman laying hens aged 24 weeks were randomly divided into two dietary treatment groups. The feeding experiment lasted for 48 weeks. Layers fed basal diet supplemented with 0 (control group) and 300mg/kg TP (TP group) diet, respectively. The yolk cholesterol content, laying performance, and egg quality were determined at 28, 38, 48, 58, and 68 weeks of age. The yolk cholesterol content in the TP group was significantly decreased at 28-68 weeks of age ($p<0.01$), compared to the control group. There was a significant increase in laying rate in the TP group at 38 weeks of age ($p<0.05$), compared to the control group, while no significant differences during the other laying periods were obtained ($p>0.05$). The FCR significantly decreased in the TP group at 38 weeks of age whereas AEW significantly increased in the TP group at 58 weeks of age ($p<0.05$). Similarly, the eggshell thickness and eggshell strength in the TP group significantly increased ($p<0.05$), compared with the control group at 38 weeks of age. The albumen height and Haugh unit significantly increased at 28 weeks of age ($p<0.05$). In conclusion, the results showed that the diet supplemented with 300 mg/kg TP had positive effects on production performance of layers during the egg-laying period, and could lessen yolk cholesterol content significantly at 28-68 weeks of age.

INTRODUCTION

Eggs are a good source of protein and energy for humans, but the yolk is high in cholesterol and can negatively affect the health of the elderly and people suffering from heart disease. However, it has been reported that eating low-cholesterol eggs can reduce people's cholesterol intake, which is beneficial to both healthy individuals and those suffering from cardiovascular disease (Zhong *et al*., 2019; Xia *et al*., 2020). In recent years, much attention has been focused on plant extracts, which could reduce the yolk cholesterol content by regulating the cholesterol metabolism of laying hens. Bioflavonoids are widely distributed secondary metabolites of plants, among which tea polyphenols (TP) have been investigated more (Arcone *et al*., 2016; Zhang *et al*., 2019; Mine *et al*., 2020). The TP is a catechin-based polyhydroxy compound contained in tea. It has the functions of scavenging reactive oxygen species produced by various systems, inhibiting the proliferation of cancer cells, lowering blood lipids, and improving immunity (Khan & Mukhtar, 2008; Wang *et al*., 2019). A previous study has shown that polyphenols is effective in improving the production performance of poultry and the quality of poultry products, replacing antioxidants, and enhancing the immunity of poultry (Abdel-

Moneim *et al*., 2020). The main application of TP in laying hens is to improve the laying performance and reduce the yolk cholesterol content. Many studies have shown that the diet addition of TP could reduce the yolk cholesterol content, enhance antioxidant enzyme activity (Gramza-Michałowska *et al*., 2016; Rodrigues *et al*., 2016), and improve egg quality and laying performance of laying hens (Luo *et al*., 2018; Wang *et al*., 2018; Zhu *et al*., 2020). However, in the mentioned reports, the effect of TP on lowering yolk cholesterol content was significantly different, with a decrease of 11-42%. Azeke & Ekpo (2008) reported that the yolk cholesterol content reduced by 29% when 1% black tea powder was added to the diet of laying hens, and it decreased by 66% when 2% was added. Bing *et al*. (2018) demonstrated that the addition of 3% of green tea powder to the diet of laying hens could reduce the yolk cholesterol content by 11.1%. Biswas *et al*. (2000) suggested that the addition of added green tea powder to the diet of laying hens could significantly decrease the yolk cholesterol content during the egg-laying period, but the variation of yolk cholesterol content with age was not obvious. These studies indicated that the influence of TP on decreasing yolk cholesterol content was related to the types of tea products, concentration, and duration of TP addition. A feasible amount of TP for the diet of laying hens was 200-400 mg/kg (Azeke & Ekpo, 2008; Bing *et al*., 2018; Wang *et al*., 2018), and the TP was only added to the diets of laying hens for a short period (about 4 weeks). Hence, more experimental studies of long-term feeding of TP are required to provide experimental pieces of evidence for developing TP as feed additives for laying hens.

There is limited information on the long-term addition of TP to the diet of laying hens. Therefore, the purpose of this study was to investigate the stability of TP in lowering yolk cholesterol levels and affecting the production performance of laying hens during the egg-laying period.

MATERIALS AND METHODS

Experimental Materials

The TP was purchased from Chengdu Huagao biological products limited company, which with 20% active ingredients. TP is added at the time of feed preparations, and the test feed is prepared once a week. Sanitary index: plumbum ≤ 2 mg/kg, Hexachlorocyclohexane (HCH) ≤ 0.05 mg/kg, Dichlorodiphenyltrichloroethane (DDT) ≤ 0.02 mg/kg.

Experimental Animals and Design

A total of 600 Roman hens aged 24 weeks were randomly divided into 2 dietary treatment groups, with 6 replicates per group and 50 hens per replicate. The feeding experiment lasted for 48 weeks. All laying hens were from the same hatching batch with similar body weight. All experimental procedures have been approved by the Animal Health and Use Committee of Southwestern University, China. Chickens in the control group were fed with the basal diet (NRC, 1994), and the treatment group (TP group) was fed with the basal diet supplemented with 300 mg/kg TP. Laying hens in two groups were fed experimental diets from 24 to 72 weeks of age. The basal diet composition and nutritional level are shown in Table 1.

Table 1 – Ingredient and nutrient content of the basal experimental diet (%).

Ingredients	Content (%)	Nutrients levels	Content
Corn	63	ME (MJ/kg)	11.676
Wheat bran	4.7	Crude protein (%)	16.0
Soybean	21.24	Calcium (%)	3.23
limestone	7.75	Total phosphorus (%)	0.62
Bicalcium phosphate	0.4	Available phosphorus (%)	0.32
Soybean oil	1.53	digestible DL-Methionine (%)	0.340.38
Sodium chloride	0.195	digestible Lysine (%)	0.850.74
NaHCO3	0.09	digestible Methionine+cystine (%)	0.720.62
Choline chloride	0.09	digestible Isoleucine (%)	0.520.45
Methionine	0.045	digestible Threonine (%)	0.540.47
Vitamin premix[1]	0.03	digestible Tryptophan (%)	0.170.15
Phytase	0.03	digestible Valine (%)	0.680.59
Mineral premix[1]	0.9	digestible Arginine (%)	0.891.00
Total	100		

Legend: Premix per kg compound feed: VA, 12,000 IU; VD3, 1,500 IU; VE, 25 IU; VK3, 1.0 mg; VB1, 1.6 mg; VB2,5.0mg; VB5,20mg; VB6,6.0mg; VB12,0.01mg; biotin,0.2mg; Pantothenicacid,15mg; choline, 500 mg; folic acid, 0.5 mg; Fe, 90 mg; Cu, 20 mg; I, 0.45 mg; Mn, 80 mg; Zn, 80 mg; Se, 0.2 mg.

The metabolic energy was calculated values, and other data were determined values.

Metabolic energy (ME).

Feeding and Management

The experiment was conducted at Luzhou Siwei Poultry Limited Company, Luzhou, China. All laying hens were housed in 3-tiered cages (47 × 37 × 38 cm) with 5 hens per cage. The chicken farm adopted the feeding mode of all in and all out, automatic temperature control equipment, automatic egg collecting device, and automatic feeding system. Fodder and water were provided *ad libitum*. Layers were fed at 9:00 a.m. and 4:00 p.m. during the experiment. The lighting program was 16 hours light/8 hours dark per day, light intensity was 20Lx. Laying hens received the standard hatchery vaccinations against Newcastle disease and infectious bronchitis (ND-VII+ma5, purchased from Qingdao Yibang Bioengineering Company Limited, Qingdao, China) at the hatchery, and no concomitant drug therapy was used during the study.

The Sample Collection

Twelve eggs per replicate were randomly collected at the end of weeks 28, 38, 48, 58, and 68. Six eggs were analyzed for the quality (eggshell strength, eggshell thickness, egg weight, yolk color, Haugh unit, albumen height, and yolk specific gravity), and six eggs were evaluated for yolk cholesterol content, which was completed within 12 hours of sampling.

Determination of Indexes and Methods

The yolk cholesterol content was assayed by the method of O-phthalaldehyde (Bai *et al*., 2010). Average daily feed intake, feed conversion ratio, average egg weight, laying rate, and yolk weight were calculated during the experimental period. The eggshell thickness, eggshell strength, albumen height, and yolk color were measured by eggshell thickness meter (Shenyang Fushiping Industry Limited Company, Shenyang, China), eggshell strength meter (Shenyang Fushiping Industry Limited, Shenyang, China), eggshell height meter (Shenyang Fushiping Industry Company Limited Company, Shenyang, China), and yolk colorimetric fan (F. Hoffmann-La Roche, Limited, United States), respectively. The yolk specific gravity and Haugh unit were calculated by the following formula:

Yolk specific gravity = yolk weight/egg weight

Haugh unit = 100 × log (Albumen height - 1.7 × egg weight0.37 + 7.57)

Statistical Analysis

The Differences among weeks of age experimental results were analyzed by SPSS (version 22) using a one-way analysis of variance followed by Duncan analysis for multiple comparisons. An independent sample T-test was used to evaluate the differences between the control group and the TP group. The results were expressed as mean ± standard error. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Yolk cholesterol

As shown in Figure 1, there were no significant differences in yolk cholesterol content, compared with the control group when the TP was added for two weeks (26 weeks of age, $p>0.05$). The yolk cholesterol content in the TP group significantly decreased at 28-68 weeks of age, compared with the control group ($p<0.01$). At 48, 58, and 68 weeks of age, the yolk cholesterol content reduced by 18.10%, 20.04%, and 18.36%, respectively ($p<0.01$).

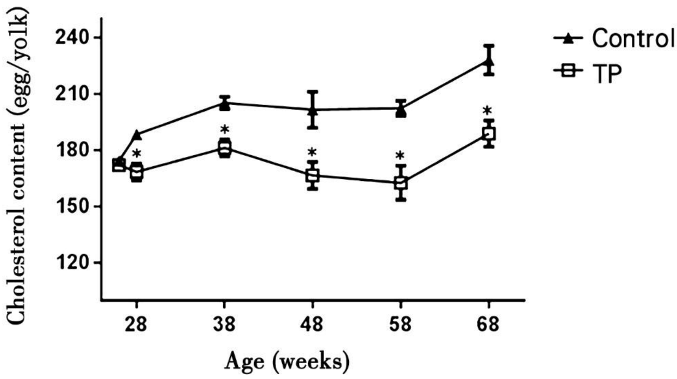

Figure 1 – Effects of dietary supplementation of 300 mg/kg tea polyphenols on yolk cholesterol content of Roman laying hens during the egg-lying period.

Legend: *Indicate a significant difference between the tea polyphenols (TP) group and the control at the same age ($p<0.01$).

Egg is one of the main kinds of animal product consumed with high cholesterol content consumed in human diets (Geiker *et al*., 2018). However, there is a controversy on the increased risk of cardiovascular disease by egg overconsumption since individuals indicate different levels of sensitivity to cholesterol-induced cardiovascular disease (Clayton *et al*., 2017). Moreover, the dietary guidelines for Americans (from 2015 to 2020) suggested that the intake of cholesterol should be as little as possible (USDA\DHHS, 2016). In order to alleviate the progression of cardiovascular disease, the American Heart Association pointed out that the daily intake of cholesterol content should be less than 200 mg, while the American College of Cardiology believed that it should be less than 150 mg (Lichtenstein *et al*., 2002). Therefore, it is beneficial for consumers to reduce the yolk cholesterol content. Studies have shown that the intake of TP is negatively correlated with the occurrence and development of hyperlipidemia (Davies *et al*., 2003). Loest *et al*. (2002) also found that the TP has a positive effect on cholesterol metabolism. It was demonstrated that TP could significantly increase the secretion of cholesterol in bile, reduce the production of very-low-density lipoprotein (VLDL) in the liver, and promote the excretion of cholesterol (Hirsova, *et al*., 2012). The TP also could promote the breakdown of fat in fat cells (Kim *et al*., 2017). Some previous studies confirmed that TP could reduce the plasma cholesterol of obese mice with a high rate of fat in the diet and also the blood cholesterol of people with high blood lipid, however, it could not significantly reduce the serum cholesterol content of laying hens (Azeke & Ekpo, 2008; Bose *et al*., 2008; Frank *et al*., 2009). It is suggested that the TP may reduce the yolk cholesterol content by reducing the amount of VLDL entering oocytes rather than simply promote the decomposition or excretion of cholesterol in the blood of laying hens. Russo *et al*. (2002) reported that it was possible to reduce yolk cholesterol content only by inhibiting the activity of oocyte receptors in laying hens. Wang *et al*. (2021) found that the addition of 200, 250, 300, and 350 mg/kg TP to the diet of laying hens could significantly reduce the yolk cholesterol content by 10.79%, 12.73%, 18.06%, and 21.61%, respectively. Biswas *et al*. (2000) added green tea powder to the diet of laying hens, which could reduce the yolk cholesterol content during the egg-laying period, the maximum reduction was 24%. In this study, 300 mg/kg TP was added to the diet of laying hens during the egg-laying period (from 24 to 72 weeks of age), which could steadily reduce the yolk cholesterol content. This result was similar to that of Bing *et al*. (2018), who suggested that the yolk cholesterol content reduced by 11.1% with 3% green tea powder supplementation, and showed a dose-response effect. From above, this study suggests that TP had good cholesterol-lowering effects as a feed additive for laying hens.

Laying performance

Effects of TP on ADFI, laying rate, AEW, and FCR are presented in Table 2. There were no significant differences in ADFI between the TP and the control groups at 28-68 weeks of age ($p>0.05$). The ADFI in the control group at 48-58 weeks of age and in the TP group at 58-68 weeks of age was significantly lower than that at 28-38 weeks of age ($p<0.05$). The laying rate in the TP group significantly increased at 38 weeks of age, compared with the control group ($p<0.05$), while it had no significant effect on the other laying period ($p>0.05$). The laying rate in both the TP group and the control group at 58-68 weeks of age was significantly lower than that at 28-48 weeks of age ($p<0.05$). The AEW in the TP group significantly increased at 58 weeks of age, compared to the control group ($p<0.05$), while no significant differences during the other laying period were observed ($p>0.05$). Moreover, the FCR in the TP group was significantly decreased at 38 weeks of age compared with the control group ($p<0.05$), and there were no significant differences in FCR among other laying periods.

Generally, commercial laying hens start laying at 20 weeks of age, and the laying period can continue to about 80 weeks of age. Tea polyphenols and their metabolites in the intestinal tract could promote beneficial bacteria and inhibit harmful bacteria to ensure gastrointestinal microflora health, facilitate the absorption and utilization of nutrients, and improve feed efficiency (Zhao & Zhang, 2020). Biswas *et al*. (2000) indicated that long-term feeding of green tea powder to the diet of laying hens had no side effects on laying performance. Similar results were obtained

Table 2 – Effects of dietary supplementation of 300 mg/kg tea polyphenols on laying performance of Roman laying hens during the egg-laying period.

Item	group	Age (weeks)				
		28	38	48	58	68
ADFI (g/d)	Control	110.16 ± 1.41B	112.76 ± 0.35C	106.05 ± 0.53A	105.44 ± 1.00A	108.94 ± 0.65B
	TP	112.22 ± 0.82BC	113.28 ± 0.04BC	109.07 ± 1.03AB	107.22 ± 1.55A	108.72 ± 1.42A
Laying rate (%)	Control	93.53 ± 0.97C	89.03 ± 1.36aB	91.83 ± 0.48C	86.87 ± 1.03A	82.20 ± 1.07A
	TP	94.30 ± 0.75C	93.93 ± 0.62bBC	91.83 ± 1.28B	88.46 ± 0.89A	83.30 ± 1.20A
AEW (g)	Control	57.43 ± 0.17A	60.71 ± 0.16B	59.98 ± 0.08B	62.41 ± 0.34aC	66.38 ± 0.70D
	TP	57.89 ± 0.29A	61.10 ± 0.39B	60.26 ± 0.29B	63.88 ± 0.37bC	67.18 ± 0.92D
FCR (kg/kg)	Control	2.05 ± 0.03B	2.08 ± 0.03aB	1.92 ± 0.01A	1.95 ± 0.04A	2.00 ± 0.03AB
	TP	2.06 ± 0.02C	1.97 ± 0.02bB	1.97 ± 0.04B	1.90 ± 0.03A	1.94 ± 0.02AB

Legend: a, b Means with a column with different superscripts significantly differ (between the control group and tea polyphenols (TP) group, $p<0.05$).

A, B, C, D Means with a row with different superscripts significantly differ ($p<0.05$).

Average daily feed intake (ADFI), Average egg weight (AEW), Feed conversion ratio (FCR).

by Wang *et al*. (2018), which reported that adding 200 mg/kg TP to the diet of laying hens lead to no significant difference in the ADFI and AEW of the treatment group, but the laying rate significantly increased. In the current study, there was no significant difference in the ADFI of the TP group, which was consistent with the results reported above. The laying rate of the TP group significantly increased and the FCR of the TP group significantly decreased at 38 weeks of age, compared with the control group. This result was also consistent with the study conducted by Kaya *et al*. (2014) that added 2% and 4% black tea to the diet of Roman laying hens (24 weeks of age) could increase the laying rate and reduce FCR. It was found that the laying rate was related to the age of laying hens and the age of 33-41 weeks was the golden period for laying hens (Jian *et al*., 2021). Therefore, increasing the laying rate at this time could significantly improve the economic benefits. Besides, in the present study, the AEW in the TP group significantly increased at 58 weeks of age, compared to the control group. However, the findings of Uuganbayar *et al*. (2005) demonstrated that the addition of 1-2% green tea to the diet of laying hens (40 weeks of age) could decrease the AEW. The reason for the decrease of AEW might be the presence of anti-nutrient factors in TP, such as phenolic acid and theophylline, which hindered the digestion and absorption of nutrients by laying hens. In addition, catechin could inhibit the intestinal absorption of fat and the activity of fatty acid synthase, thus impede the formation of lipids in the yolk (Qiu *et al*., 2018). Therefore, high doses of TP could decrease the AEW. Besides, it could be concluded that the appropriate concentration of TP addition could improve the laying performance of laying hens, and long-term addition of TP had a positive effect on production. Hence, there is a huge potential value to develop TP as a feed additive of laying hens to improve the laying performance.

Egg quality

Effects of TP on egg quality are presented in Table 3. There were no significant differences in yolk weight, yolk specific gravity, and yolk color of laying hens between the TP group and the control group. The eggshell strength in the TP group decreased at 28, 48,58, and 68 weeks of age, compared to the control group, but the differences were not significant (p>0.05). The eggshell strength in the TP group significantly increased at 38 weeks of age, compared with the control group (p<0.05). There was no significant difference in eggshell thickness between the TP and control groups at 28, 48, 58, and 68 weeks of age (p>0.05), but the eggshell thickness in the TP group significantly increased at 38 weeks of age (p<0.05). The albumen height and Haugh unit in the TP group significantly increased at 28 weeks of age, compared with the control group (p<0.05).

Egg quality is closely related to the nutritional composition and edible value of eggs and has a positive correlation with the breakage rate, preservation time, and hatchability of eggs. The egg albumen formed in the oviduct magnum, which has a protective effect on egg yolk. Haugh unit is an important index to evaluate the quality of protein and freshness of egg products,

Table 3 – Effects of dietary supplementation of 300 mg/kg tea polyphenols on egg quality of Roman laying hens during the egg-laying period.

Item	group	Age (weeks)				
		28	38	48	58	68
Eggshell strength(kgf)	Control	5.35 ± 0.19B	4.58 ± 0.14Aa	4.73 ± 0.43A	4.62 ± 0.41A	4.73 ± 0.38A
	TP	5.24 ± 0.13B	4.98 ± 0.10Bb	4.35 ± 0.24A	4.18 ± 0.45A	4.45 ± 0.20AB
Eggshell thickness (mm)	Control	0.404 ± 0.008B	0.385 ± 0.007Aa	0.395 ± 0.009AB	0.38 ± 0.010A	0.41 ± 0.007B
	TP	0.395 ± 0.006B	0.409 ± 0.006Bb	0.395 ± 0.008B	0.37 ± 0.014A	0.40 ± 0.011B
Egg yolk weight (g)	Control	15.36 ± 0.18A	16.99 ± 0.31B	17.22 ± 0.50B	17.03 ± 0.57B	18.68 ± 0.56C
	TP	15.20 ± 0.18A	17.26 ± 0.24B	17.37 ± 0.48B	17.12 ± 0.59B	18.95 ± 0.69C
Yolk specific gravity (%)	Control	26.58 ± 0.30A	28.01 ± 0.47B	28.50 ± 0.75B	28.03 ± 0.69B	28.22 ± 0.56B
	TP	26.62 ± 0.38A	27.69 ± 0.24AB	29.27 ± 0.78C	28.12 ± 0.87BC	28.33 ± 0.88BC
Yolk color	Control	9.6 ± 0.22AB	10.07 ± 0.04B	9.50 ± 0.21AB	9.16 ± 0.37A	9.44 ± 0.29A
	TP	9.6 ± 0.04A	9.73 ± 0.48B	9.58 ± 0.21A	9.60 ± 0.44A	9.39 ± 0.20A
Albumen height (mm)	Control	8.78 ± 0.13aB	8.41 ± 0.20AB	7.97 ± 0.25A	8.79 ± 0.21B	8.31 ± 0.20AB
	TP	9.30 ± 0.10bB	8.47 ± 0.19A	8.28 ± 0.29A	8.63 ± 0.14A	8.36 ± 0.12A
HaughtHarding unit	Control	94.05 ± 0.62aB	91.31 ± 1.03AB	89.19 ± 1.32A	93.31 ± 0.96AB	89.34 ± 0.93A
	TP	96.73 ± 0.45bB	91.34 ± 0.99A	90.43 ± 1.67A	92.33 ± 0.72A	89.54 ± 0.78A

Legend: a, b Means with a column with different superscripts significantly differ (between the control group and tea polyphenols (TP) group, p<0.05).

A, B, C Means with a row with different superscripts significantly differ (p<0.05).

it is positive correlation with the content of thick albumen and the freshness of egg products. Haugh unit and albumen height were affected by many factors, including storage time of eggs, temperature, age of laying hens, nutrition, and medicines (Roberts, 2004; Marques *et al*., 2011). The nutrients (lysine, methionine, and feed enzymes) and supplements (vitamin E, ascorbic acid) were the main factors affecting Haugh unit and albumen height under the same environmental conditions (Marques *et al*., 2011). The reason for the decrease of Haugh unit and albumen height during egg storage appears to be changes occurring in ovomucin, particularly the thick albumen (Roberts, 2004). Biswas *et al*. (2000) found that dietary supplementation with green tea powder significantly elevated the albumen height and Haugh unit of hens at 26 weeks of age. Similarly, dietary supplementation with 300 mg/kg TP significantly elevated the albumen height and Haugh unit of laying hens at 28 weeks of age. Therefore, it could be concluded that the storage time of eggs could be prolonged by adding TP to the diet of laying hens. In the current study, the eggshell thickness and eggshell strength in the TP group significantly increased at 38 weeks of age, compared to the control group. This result was consistent with the study conducted by Yuan *et al*. (2016) that dietary supplementation of 600 and 1000 mg/kg TP could increase eggshell strength and eggshell thickness. However, Uuganbayar *et al*. (2005) reported that the addition of Japanese green tea to the diet of laying hens significantly decreased the eggshell thickness, but there were no significant differences in eggshell thickness of laying hens in the group supplied with the same dose of Korean green tea and Chinese green tea. Kaya *et al*. (2014) found that the eggshell strength and eggshell thickness decreased with the increase in the addition of black tea. It could be concluded that the effect of TP on egg quality was related to the tea source and concentration of TP addition. In the present study, adding 300 mg/kg TP to the diet of laying hens played an active role in improving egg quality during the egg-laying period.

In conclusion, using 300 mg/kg long-term supplementation of 300 mg/kg TP had positive effects on the laying performance and egg quality of laying hens, and has a significant effect on lowering yolk cholesterol content. In addition, the TP is a natural plant extract, which is safer than other cholesterol-lowering drugs. This provides a new idea for solving the problem of high cholesterol content in egg yolk and producing low cholesterol eggs. However, the cholesterol-lowering mechanism of TP in laying hens is unclear, the effects of TP on the digestive tract morphology, intestinal microflora, and cholesterol metabolism of laying hens still need further study.

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