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Original Article

The Effect of Supplementing Tea Polyphenols in Diet of Laying Hens on Yolk Cholesterol Content and Production Performance

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■Keywords

Antioxidant, laying hens, production performance, tea polyphenols, yolk cholesterol content.

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ABSTRACT

This study was conducted to evaluate the influence of supplementing tea polyphenols (TP) in diet of laying hens on yolk cholesterol content and production performance. A total of 1800 Lohmann laying hens aged 48 weeks were randomly allocated to 6 groups. Each group consisted of 6 replicates with 50 layers. The feeding experiment was 4 weeks including one-week acclimatization. Layers fed basal diet supplemented with 0, 150, 200, 250, 300 and 350 mg TP/kg diet, respectively. The results showed that average daily feed intake (ADFI), feed conversion ratio (FCR), average egg weight (AEW), laying rate and the indicators of egg quality were not significantly affected by the diet supplemented with 300 mg/kg TP ($p>0.05$). However, yolk cholesterol content decreased by increasing TP concentration ($p<0.01$), with 18.06% reduction in layers fed diet supplemented with 300 mg TP/kg. Also, the diet supplemented with 300 mg/kg TP significantly decreased plasma triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) level ($p<0.05$). The activity of serum glutathione peroxidase (GSH-Px) was enhanced by increasing TP concentration, while the content of serum methane dicarboxylic aldehyde (MDA) was decreased by increasing TP concentration. The highest activity of GSH-Px and the lowest serum MDA content were both determined in 300 mg/kg TP group ($p<0.01$). In conclusion, this study suggests that the addition of 300 mg TP/kg basal diet had no negative effect on the production performance laying hens, yet decreased the egg yolk cholesterol content and enhanced the antioxidant capacity of laying hens at the same time.

INTRODUCTION

Eggs are one of the most nutritious food for humans, which can provide the recommended daily requirement of nutrients for humans of all ages. However, nearly 30% lipids composition and high cholesterol content of egg yolk of chicken eggs is a primary health concern for consumers (Jung e*t al.*, 2011). Excessive intake of cholesterol may result in diseases such as atherosclerosis and fatty liver (Chung e*t al.*, 2012). What's more, dietary cholesterol intake with pancreatic cancer (Chen *et al.,* 2015), II diabetes (Tajima *et al.,* 2014) are also related. Therefore, it is necessary to control and limit cholesterol intake. Thus, many studies have been made to decrease the cholesterol content in egg-yolk such as adding garlic (Kaya & Macit, 2012), ginger (Wen *et al.,* 2019), and grape seeds (Sun *et al.*, 2018), etc.

There is a growing interest in the role of green tea in health and disease (Li e*t al.*, 2020). Tea contains many natural compounds, particularly polyphenols, which have anti-oxidative (Gramza-Micha Owska e*t al.*, 2016; Rodrigues e*t al.*, 2016), anti-bacterial (Nakayama e*t al.*, 2012; Kawarai e*t al.*, 2016), anti-aging (Li *et al.*, 2016), anti-cancerous (Shih

et *al.*, 2016) and other biological activities. Inclusion of TP in the diet often results in higher animal production performance due to its antioxidant activity in animal body (Luo et *al.*, 2017; Sahin et *al.*, 2010). The positive influence of TP on lipid and cholesterol are getting more and more well-known. In both animal experiments and human experiments, TP significantly lowered blood lipid level and fat deposition in liver and peripheral tissues (Ashida et *al.*, 2008; Basu et *al.*, 2010; Axling et *al.*, 2012; Bogdanski et *al.*, 2012; Chung et *al.*, 2012). Studies indicated that TP can reduce yolk cholesterol content, and the effective addition amount of tea polyphenols is mostly in the range of 200-400mg/kg, which is quite different. We assume that within this range, with the increase of the concentration of tea polyphenols, the content of egg yolk cholesterol will decrease. But, when the added amount of tea polyphenols reaches a certain level, its cholesterol-lowering effect will also decrease. Therefore, the aim of this study was to evaluate the cholesterol-lowering effects of TP and identify the most effective dose, which provided new ideas for solving the problem of high cholesterol content of yolk and producing low cholesterol eggs.

MATERIALS AND METHODS

Experimental Materials

Tea polyphenol was purchased from Chengdu Huagao biological products limited company, which with more than 20% active ingredients. Sanitary index: plumbum ≤ 2 mg/kg, Hexachlorocyclohexane (HCH) ≤ 0.05 mg/kg, Dichlorodiphenyltrichloroethane (DDT) ≤ 0.02 mg/kg.

Experimental Animals and Design

In total, 1800 48-week-old Lohmann laying hens were obtained from zhengda company and randomly divided into 6 groups, with 6 repetitions in each group and 50 layers in each repetition. The hens for each group were obtained from the same hatchery which were in similar body weight. The animal care and use protocol (SYXK (CQ) 2014-0002) was approved by the Animal Care and Use Committee of Southwest University. Six groups were supplemented with 0, 150, 200, 250, 300 and 350 mg TP /kg basal diet (NRC, 1994), respectively, as shown in Table 1.

Table 1 – Design of experiment.

Group	1	2	3	4	5	6
Treament	0	150 mg/kg	200 mg/kg	250 mg/kg	300 mg/kg	350 mg/kg

The basal diet composition and nutrient levels are shown in Table 2.

Table 2 – Ingredient and nutrient content of the basal experimental diet (%).

Ingredients	Content (%)	Nutrients levels	Content
Corn	63	(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	Methionine(%)	0.38
Sodium chloride	0.195	Lysine(%)	0.85
$NaHCO_3$	0.09	Methionine+cystine(%)	0.72
Choline chloride	0.09	Isoleucine(%)	0.52
Methionine	0.045	Threonine(%)	0.54
Vitamin premix[1]	0.03	Tryptophan(%)	0.17
Phytase	0.03	Valine(%)	0.68
Mineral premix[1]	0.9	Arginine(%)	1.00
Total	100		

Legend: Premix per kg compound feed: VA, 12,000 IU; VD_3, 1,500 IU; VE, 25 IU; VK_3, 1.0 mg; VB_1, 1.6 mg; VB_2,5.0mg;VB_5,20mg;VB_6,6.0mg;VB_{12},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

All laying hens were kept in 3-tiered cages (47×37×38cm) with 5 hens per cage and under the same management, hygienic, and environmental conditions. The chicken farm adopted the feeding mode of all in and all out, automatic temperature control equipment, automatic egg collector and automatic feeding system. We fed the hens at 9:00 a.m. and 4:00 p.m. during the test, feed and water were provided *ad libitum* during the entire experimental period. The light program was constant and consisted of 16 hours of light per day. The feeding experiment was 4 weeks, which included one-week acclimatization and feeding trial.

The Sample Collection

At the end of the experiment, 12 eggs were randomly selected from each replicate group, 6 eggs were determined for egg quality and 6 eggs were determined for egg yolk cholesterol content. One hen in good condition was randomly selected from each replicate for blood collection. We collected 5 ml blood by the blood collection needle and put it into a heparin sodium anticoagulation tube, gently inverted it twice, then immediately centrifuged at 3000 r/min for 15 min to separate the serum. Three tubes were placed in a 1.5 ml centrifuge tube in a foam box with an ice pack and brought back to the laboratory to quantify serum biochemical markers.

Determination of Indexes and Methods

Production Performance. During the experiment, we fed the hens at 9:00 a.m. and 4:00 p.m. every day, and the weight of feed was determined by observing that there was almost no feed left in the feed trough in the next day. ADFI was determined by the total of feeding weight in the morning and afternoon. Eggs were collected at 11:00 a.m. every day, the number of eggs and total egg weight were recorded. Taking repetition as the unit and the formal period as the test days, ADFI, AEW, FCR and laying rate were calculated.

ADFI = total daily intake / (test days x total birds)

Laying rate = total number of eggs / (test days x total birds)

AEW = total egg weight / total eggs

FCR = feed intake / (egg weight x laying rate)

Egg Quality. Three eggs were selected from each replicate group to weigh the egg weight, eggshell weight and yolk weight. The eggshell thickness, eggshell strength, albumen height and yolk color were measured with eggshell thickness gauge, eggshell strength meter and albumen height micrometer (Shenyang Fujipin Industrial Co., Ltd.) and Roche egg yolk color fan (Roche, USA). The shell specific gravity, the yolk specific gravity and the Haugh unit were calculated by the following formula:

Shell specific gravity = shell weight / egg weight

Yolk specific gravity = yolk weight / egg weight

Haugh unit = 100 x log (albumen height - 1.7 x egg weight $^{0.37}$+7.57)

Yolk Cholesterol Content. Six eggs were selected from each replicate group to determine the egg yolk cholesterol content. The yolks were separated from the albumen and 1g samples of yolk were weighed after the eggs boiled. The egg yolk was dissolved with 10ml methanol-chloroform solution (methanol: chloroform = 2:1), stirred evenly and then filtered. The cholesterol content of 0.1ml filtrate (10mg/mL) was determined by spectrophotometer through the method which was consistent with the previous published article (Sun e*t al.*, 2018).

Yolk Cholesterol content= Cholesterol (mg/g yolk) x the egg yolk weight

Serum Biochemistry. One serum was taken from each repeat group to determine TC, TG, LDL-C, high density lipoprotein cholesterol (HDL-C), GSH-Px, total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and MDA. The above indicators were measured by commercial assay kits, which were purchased from Nanjing Jiancheng biology Co., Ltd.

Statistical Analysis

The experimental results were analyzed by SPSS 22.0 that used one-way analysis of variance and Duncan analysis was used for multiple comparisons. The results were expressed as "mean ± standard error".

RESULTS AND DISCUSSION

Production Performance and Egg Quality

Table 3 showed that there was no significant effect on the feed intake and laying rate among groups which were supplied with 150-350 mg/kg TP (p>0.05). Compared with the control group and 300 mg/kg TP group, there was a significant decrease in egg weight in 250 mg/kg and 350 mg/kg TP groups (p<0.05), and there was no difference among other groups. Compared with the control group, there was no difference in feed-egg ratio in each treatment, but in the 300 mg/kg TP group there was a significant decrease compared with the 200 mg/kg, 250 mg/kg and 350 mg/kg TP groups (p<0.05). According to the above indicators, the diet supplemented with 300 mg/kg TP had the best production performance in this study.

Table 3 – Effect of TP addition on performance of laying hens.

Items	TP supplemented level (mg/kg)						
	0	150	200	250	300	350	*p*
Feed intake (g/d)	106.05±0.53	105.31±0.81	106.37±0.70	105.13±0.74	106.79±0.85	105.72±0.71	0.486
Laying rate (%)	92.33±0.68	91.54±0.66	91.76±0.49	91.15±0.44	92.47±1.06	91.83±0.39	0.733
Egg weight (g)	59.98±0.08^{bc}	59.25±0.44^{ab}	58.78±0.37^{ab}	58.64±0.45^{a}	60.26±0.29^{bc}	58.57±0.65^{a}	0.005
FCR (Kg/Kg)	1.92±0.01^{ab}	1.94±0.02^{ab}	1.97±0.02^{b}	1.96±0.02^{b}	1.90±0.02^{a}	1.96±0.03^{b}	0.077

Legend: Different shoulder marks (a, b) in the same row of data indicates significant differences (p<0.05).

Tea polyphenols (TP), feed conversion ratio (FCR).

As shown in Table 4, 150-350 mg/kg TP had no significant effect on eggshell strength, yolk weight, yolk specific gravity, yolk color, albumen height and Haugh unit (p>0.05). Compared with the control group, the eggshell specific gravity significantly decreased in group supplied with 200 mg/kg TP (p<0.05); the

Table 4 – Effect of TP addition on egg quality of laying hens.

Items	TP supplemented level (mg/kg)						
	0	150	200	250	300	350	*p*
Eggshell strength (kgf)	4.73±0.43	4.58±0.42	4.16±0.40	3.97±0.40	4.35±0.24	4.42±0.38	0.429
Eggshell thickness (mm)	0.397±0.009^{b}	0.390±0.011^{ab}	0.373±0.018^{ab}	0.367±0.011^{a}	0.395±0.008^{b}	0.385±0.006^{ab}	0.046
Eggshell specific gravity (%)	12.78±0.29^{b}	12.84±0.41^{b}	11.97±0.40^{a}	12.15±0.31^{ab}	12.57±0.27^{ab}	12.86±0.26^{b}	0.036
Yolk weight (g)	17.46±0.40	18.00±0.65	17.37±0.48	17.37±0.42	17.40±0.49	16.93±0.31	0.494
Yolk specific gravity (%)	28.91±0.67	29.81±0.99	29.00±0.41	28.18±0.80	29.20±0.78	28.43±0.65	0.361
Yolk color	9.50±0.21	9.50±0.21	9.33±0.27	9.33±0.20	9.58±0.21	9.63±0.27	0.717
Albumen height (mm)	7.84±0.39	8.43±0.33	8.17±0.39	8.38±0.34	8.25±0.37	8.15±0.39	0.802
Haugh unit	88.45±2.09	91.31±1.59	91.01±2.33	90.77±1.76	90.43±1.87	90.14±1.97	0.9

Legend: Different shoulder marks (a, b) in the same row of data indicates significant differences (p<0.05).

Tea polyphenols (TP).

eggshell thickness significantly decreased in group supplied with 250 mg/kg TP (p<0.05); and in the 300 mg/kg TP group was closest to the control group.

The production performance indicators of laying hens include ADFI, AEW, FCR, laying rate, etc. Wang *et al.* (2018) reported that adding 200 mg/kg TP to the diet of laying hens could improve the production performance of laying hens. This study found that egg weight in the 250 mg/kg and 350 mg/kg TP groups was decreased, the FCR in the 350 mg/kg TP group was higher than that in the 300 mg/kg TP group. Because tea has a certain bitter taste, it will reduce the chicken's feed intake to a certain extent. In addition, catechins inhibit the intestinal absorption of fat and lipase activity, which hinders the formation of egg yolk lipids and also affects egg weight (Sadao & Yuko, 2008). In terms of production performance, the group supplied with 300 mg/kg TP was better than other groups. These results indicated that high concentration of TP might not be conducive to the production performance of laying hens. The addition of 300 mg/kg of TP can keep a good production performance level of laying hens.

Investigators obtained similar results by adding green tea powder to the diet of layers (Uuganbayar e*t al.*, 2005), when the addition amount was greater than 1.5%, the eggshell thickness was significantly decreased. Xia *et al.* (2018) added 1%-3% green tea powder to the diet of twenty-week-old Xianju chicken, and found that the thickness and strength of the eggshell significantly decreased by increasing the green tea powder concentration. However, contrary results have also been reported by Yuan *et al.* (2016) that investigators added 600 mg/kg and 1000 mg/kg TP to Lohmann laying hens' diet for 5 weeks, and the eggshell thickness, eggshell strength, albumen height and Haugh unit significantly increased. Bing *et al.* (2018) also found that the albumen height and the Haugh unit increased. These differences might be caused by the different nutrition levels and basic production performance of laying hens. In summary, this study suggests that adding 300 mg/kg TP to the diet of Lohmann laying hens could maintain the quality of eggs.

The Content of Yolk Cholesterol

As shown in Table 5, compared with the control group, increasing supplement of TP concentration lowered the cholesterol content of egg yolk by 1.87% (p>0.05), 10.79% (p<0.05), 12.73% (p<0.05), 18.06% (p<0.05) and 21.61% (p<0.05), respectively. The cholesterol content of egg yolk was decreased by increasing additive amount of TP. And the cholesterol content of egg yolk was mostly decreased in group supplied with 300 mg/kg, 350 mg/kg TP.

Eggs are the primary source of food cholesterol. Early researches have shown that increased food cholesterol

Table 5 – Effect of TP addition on cholesterol content in egg yolk, serum cholesterol and triglycerides of laying hens.

Items	TP supplemented level (mg/kg)						
	0	150	200	250	300	350	*p*
Serum TG (mmol/L)	22.77±0.65^{b}	18.92±1.96^{ab}	19.03±0.91^{ab}	16.87±1.67^{a}	17.27±1.05^{a}	23.84±1.04^{b}	0.033
TC (mmol/L)	3.74±0.27^{b}	3.54±0.18^{ab}	3.18±0.23^{ab}	3.21±0.28^{ab}	2.94±0.19^{a}	3.24±0.18^{ab}	0.225
HDL-C (mmol/L)	0.712±0.050	0.711±0.086	0.539±0.048	0.619±0.093	0.578±0.062	0.533±0.038	0.279
LDL-C (mmol/L)	2.27±0.12^{d}	2.22±0.10^{d}	1.86±0.10^{c}	1.56±0.0065^{ab}	1.44±0.045^{a}	1.78±0.061^{bc}	<0.001
LDL-C/HDL-C	3.09±0.20	3.86±0.36	3.42±0.28	3.27±0.37	3.01±0.36	3.53±0.12	0.356
Yolk TG (mg/egg)	201.72±3.90^{a}	205.50±4.64^{a}	179.96±4.85^{b}	176.03±2.40^{b}	165.28±1.75^{c}	158.13±2.70^{c}	<0.001

Legend: Different shoulder marks (a, b) in the same row of data indicates significant differences (p<0.05).

Tea polyphenol (TP), total plasma triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C).

intake would increase plasma total cholesterol and LDL-C levels, and elevated plasma total cholesterol and LDL-C levels were risk factors for cardiovascular disease. Azeke and Ekpo (2008) added 1% and 2% of black tea powder to the diet of hens, the results showed that the egg yolk cholesterol decreased by 29% and 66%, respectively. Uuganbayar *et al.* (2005) compared the effect of Japanese green tea powder, Korean green tea powder and Chinese green tea powder on the cholesterol content of egg yolk, and found that only 1% and 2% of Chinese green tea powder could significantly decrease the cholesterol of egg yolk. Kaya *et al.* (2014) reported that cholesterol content of egg yolk was significantly affected by adding 2%-10% of the waste of black tea processed by factories to the diet of laying hens. This study suggests that diet addition of TP significantly reduces the cholesterol content of egg yolk, which was similar to the above results, and the 300 mg/kg and 350 mg/kg TP groups decreased by 18.06% and 21.61%, respectively. It could be seen that the regulation of TP on the cholesterol content of egg yolks was not only related to the concentration, but also to the difference in additive of tea products.

Serum Cholesterol and Triglycerides

As shown in Table 5, the contents of TG, TC and LDL-C in the plasma decreased with the addition of TP, the higher concentration of the TP added, the lower contents of the TG, TC and LDL-C were in the plasma. Compared with the control group, there was a significant decrease in the contents of TG in the plasma in the 250 mg/kg and 300 mg/kg TP groups (p<0.05), but the content of TG in the plasma in 350 mg/kg TP group was higher than that in 250 mg/kg and 300 mg/kg TP groups (p<0.05). The group fed 300 mg/kg TP in their diet had 21.6% lower plasma TC content compared with the control group. There was no significant difference of HDL-C among six groups (p>0.05). The contents of LDL-C in the plasma in 200-350 mg/kg TP groups was significantly lower than that in the control group and 150 mg/kg TP group (p<0.05), and that in the group which was supplied with 300 mg/kg TP decreased mostly by 33.64%.

Laying hens' endogenous cholesterol mainly synthesized in the liver and transported to the extrahepatic tissues by LDL-C, while HDL-C was mainly responsible for transporting the cholesterol from the peripheral tissues to the liver and excreting it through bile pathway. Lipid and cholesterol deposition in eggs were closely related to plasma TG, TC and LDL-C levels (Qiu e*t al.*, 2018). This study showed that diet addition of 300 mg/kg TP significantly decreased the content of TG, TC and LDL-C in plasma, which was consistent with the result of yolk cholesterol content. Tian *et al.* (2013) fed a high-fat diet to male rats and found that TG, TC and LDL-C/HDL-C in the TP group were significantly lower than those in the high-fat group. Ding *et al.* (2017) supplementing green tea water to atherosclerosis model mice found that green tea polyphenols can significantly improve lipid metabolism of mice, reduce plasma oxidized low-density lipoprotein levels, and inhibit the development of atherosclerosis. Huang *et al.* (2015) reported that feeding broilers with high Epigallocatechin gallate (EGCG) for 4 weeks significantly reduced the levels of TG and LDL-C in the blood. LDL-C is mainly cleared by Low density lipoprotein receptor (LDLR) located in liver cells. A large number of in vitro and in vivo experiments reported that TP could enhance LDLR expression in liver cells or liver (Goto e*t al.*, 2012; Hirsova e*t al.*, 2012; Liu e*t al.*, 2015) . Therefore, LDL-C might be the target of TP, and the effect of TP on lowering lipids and cholesterol is closely related to the decrease of LDL-C in plasma. HDL-C is known as the "good cholesterol" because it carries cholesterol from peripheral tissues back to the liver for clearance, and enables the reverse transport of cholesterol (RCT). Several investigators have reported that plasma HDL-C levels reflected RCT levels. High-fat model animal experiments also showed that TP could improve the level of HDL-C in plasma (Kim e*t al.*, 2009; Moreno e*t al.*, 2014). When male rats were fed with a high-fat diet and added different concentrations TP into the high-fat diet, the plasma HDL-C content in the high-fat diet with TP group was significantly higher than that in the high-fat control group, and HDL-C was significantly lower than that in the normal diet group (Li and wu, 2018). The above results were obtained under the high-fat model, for the body with different lipid metabolism levels, the effect of TP on HDL-C may be different. It has been reported that HDL-C receptor scavenger receptor BI (*SR-BI*) increased expression in the liver for the addition of Epigallocatechin gallate (Hirsova e*t al.*, 2012). *SR-BI* mainly mediated two-way flow of cholesterol and other lipids between HDL and cells, the decrease of HDL-C level might be related to the increase of *SR-BI* expression.

Serum Antioxidant Capacity of Laying Hens

As shown in Table 6, compared with the control group, the level of plasma GSH-Px was significantly increased by increasing TP concentration (p<0.01) and the 300 mg/kg TP group increased mostly by 83.14%, and there was no significant difference in the level of

Table 6 – Effect of TP addition on serum antioxidative index of laying hens.

Items	TP supplemented level (mg/kg)						
	0	150	200	250	300	350	*p*
GSH-Px (U/ml)	166.53±11.32^{a}	178.54±7.30^{ab}	186.85±10.88^{ab}	210.14±15.71^{b}	304.99±13.57^{c}	254.67±5.14^{c}	<0.001
T-AOC (mmol/L)	0.91±0.07	0.94±0.07	0.90±0.05	1.05±0.04	1.04±0.08	1.04±0.07	0.582
SOD (U/ml)	1230.23±61.01	1345.38±46.64	1281.35±60.54	1170.03±54.51	1257.75±42.68	1250.22±62.09	0.420
MDA (nmol/ml)	7.97±0.48^{d}	5.78±0.54^{bc}	5.61±0.22^{bc}	5.39±0.60^{abc}	4.00±0.27^{a}	4.14±0.45^{ab}	<0.001

Legend: Different shoulder marks (a, b) in the same row of data indicates significant differences (p<0.05).

Tea polyphenol (TP), serum glutathione peroxidase (GSH-Px), total antioxidant capacity (GSH-Px), superoxide dismutase (SOD), methane dicarboxylic aldehyde (MDA).

plasma GSH-Px between the 300 mg/kg group and the 350 mg/kg TP group (p>0.05). The level of serum MDA decreased by increasing TP concentration (p<0.01), in the 300 mg/kg TP group it decreased the most, by 49.81%, and there was no significant difference in the level of serum MDA between the 300 mg/kg TP group and the 350 mg/kg TP group (p>0.05). The level of T-AOC increased in treatment groups although there was no significant difference among six groups (p>0.05).

Antioxidant indicators in the serum reflect the antioxidant capacity of body. Serum GSH-Px, T-SOD, T-AOC, and MDA are antioxidant indicators in general. Reactive oxygen species (ROS), such as H_2O_2 、OH•、$^1O_2$•, are by-products of aerobic metabolism, excessive production of ROS can cause damage to lipids, proteins and DNA, leading to cell death, namely oxidative stress. T-SOD enzyme can rapidly transform the superoxide ion $O_2^{\bullet-}$ produced by mitochondria into H_2O_2, while GSH-Px can catalyze the decomposition of H_2O_2 into water, and at the same time catalyze the transformation of GSH into GSSG, H_2O_2 can react with Fe^{2+} to generate hydroxyl radicals (OH•), and further react with membrane lipids to produce lipid peroxides, MDA is the final product of lipid peroxidation. T-AOC is a comprehensive indicator to measure the antioxidant system (Schieber e*t al.*, 2014). The stimulation of the external environment, such as high temperature, usually leads to oxidative stress in the body and decreases the production performance of animals (Akbarian e*t al.*, 2016). Therefore, these indicators can reflect the body's ability to resist stress in a certain extent. The chemical structure of tea polyphenols usually contains more than two hydroxyl groups, so that tea polyphenols can provide active hydrogen and effectively remove the excess reactive oxygen radicals in the body. Therefore, tea polyphenols have a strong antioxidant effect, and its beneficial effects such as enhancing anti-atherosclerosis, anti-tumor and immune activity might be closely related to its antioxidant activity (Lambert e*t al.*, 2010; Hayakawa e*t al.*, 2016). Catechin, the main component of tea polyphenols, is a powerful antioxidant that scavenges free radicals and prevents the formation of ROS by chelating metal ions (Sang *et al.,* 2011). Among tea catechins, EGCG has the strongest antioxidant activity (Chen & Yang, 2020). Experiments *in vitro* have showed that low concentration of EGCG could inhibit intracellular ROS production (Kucera e*t al.*, 2015). In vivo, the effects of EGCG (and other catechins) are more complex, depending on the dose used and the physiological conditions (Li *et al.* 2010, Yang & Zhang 2019). It has been reported that diet addition of TP could improve the decrease of albumen height caused by vanadium, reduce oxidative stress in the liver, and shorten the recovery time after injury (Azeke & Ekpo, 2008). The above reports showed that TP could enhance the antioxidant and anti-stress capacity of laying hens. This study suggests that adding TP to the diet significantly increased the content of GSH-Px in plasma and decreased the content of MDA, and the best influence was in group supplied with 300 mg/kg TP.

In conclusion, tea polyphenol as an additive can decrease the cholesterol content, and the cholesterol content of egg yolk would further decrease with the increase of tea polyphenol concentration within the range of this experiment. The cholesterol content of egg yolk was mostly decreased when the additive amount of TP was 300 mg/kg and 350 mg/kg. Adding 300 mg/kg TP to the diet had no negative effect on the production performance of laying hens, yet decreased the cholesterol content of egg yolk and enhanced the antioxidant capacity of laying hens at the same time. So the best additive amount of TP is 300mg/kg in this study.

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