Effects of Dietary High-Dose Tea Polyphenol Supplementation on Production Performance, Egg Quality, and Lipid Metabolism in Late-Laying Hens (Postprint)

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Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary supplementation with high-dose tea polyphenols on production performance, egg quality, serum lipid metabolism indices, and hepatic lipid metabolism-related gene expression in late-phase laying hens. A total of 480 65-week-old Roman Pink-shell laying hens were selected as experimental animals and randomly divided into 4 groups with 8 replicates per group and 15 hens per replicate. The control group was fed a basal diet, while three experimental groups were fed the basal diet supplemented with 666, 1,333, and 2,666 mg/kg tea polyphenols (purity 63.58%, with catechin content 40.20%), respectively. The experiment consisted of a 2-week preliminary period followed by a 9-week formal experimental period. The results showed: 1) Compared with the control group, supplementation with 2,666 mg/kg tea polyphenols significantly decreased laying rate (weeks 1–3, weeks 4–6, and weeks 1–9) and average daily feed intake (weeks 1–3, weeks 4–6, weeks 7–9, and weeks 1–9), and significantly increased feed-to-egg ratio (weeks 1–3 and weeks 4–6). The average egg weight in the 1,333 and 2,666 mg/kg tea polyphenol groups was significantly lower than that in the control group during weeks 1–3, weeks 4–6, and weeks 1–9 ($P < 0.05$). 2) Compared with the control group, dietary supplementation with 2,666 mg/kg tea polyphenols significantly increased Haugh unit at week 2 ($P < 0.05$) and significantly decreased eggshell ratio at week 2 ($P < 0.05$). Dietary supplementation with 1,333 mg/kg tea polyphenols significantly increased Haugh unit at week 4 ($P < 0.05$) and significantly decreased eggshell ratio at week 2 ($P < 0.05$). Dietary supplementation with 666, 1,333, and 2,666 mg/kg tea polyphenols all significantly decreased yolk cholesterol content at week 9 ($P < 0.05$). Dietary supplementation with 666, 1,333, and 2,666 mg/kg tea polyphenols had no significant effects on eggshell

strength, yolk color, yolk ratio, albumen ratio, or yolk fatty acid composition ($P > 0.05$). 3) Compared with the control group, dietary supplementation with 666, 1,333, and 2,666 mg/kg tea polyphenols significantly decreased serum total cholesterol and low-density lipoprotein cholesterol ($P < 0.05$), while dietary supplementation with 1,333 and 2,666 mg/kg tea polyphenols significantly decreased serum triglyceride content ($P < 0.05$). 4) Compared with the control group, dietary supplementation with 666, 1,333, and 2,666 mg/kg tea polyphenols significantly increased the relative mRNA expression level of hepatic adenosine monophosphate-activated protein kinase (AMPK) ($P < 0.05$). In conclusion, dietary supplementation with high doses (1,333 and 2,666 mg/kg) of tea polyphenols can decrease production performance in late-phase laying hens, but can improve egg Haugh unit, reduce serum total cholesterol and low-density lipoprotein cholesterol contents and yolk cholesterol content, and upregulate hepatic AMPK gene expression.

Full Text

Effects of Adding High Doses of Tea Polyphenols into Diets on Performance, Egg Quality and Lipid Metabolism of Laying Hens during Later Laying Period

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Abstract

This experiment was conducted to study the effects of adding high doses of tea polyphenols (TP) into diets on performance, egg quality, serum lipid metabolism parameters, and hepatic lipid metabolism-related gene expression in laying hens during the later laying period. A total of 480 Lohmann pink-shell laying hens aged 65 weeks were selected as experimental animals and randomly divided into 4 groups with 8 replicates per group and 15 hens per replicate. The control group was fed a basal diet, while the three experimental groups were fed the basal diet supplemented with 666, 1,333, and 2,666 mg/kg TP (purity of 63.58%, with catechin content of 40.20%), respectively. There was a 2-week pre-trial period followed by a 9-week formal trial period. The results showed: 1) Compared with the control group, adding 2,666 mg/kg TP significantly decreased laying rate (weeks 1-3, 4-6, and 1-9), average daily feed intake (weeks 1-3, 4-6, 7-9, and 1-9), and significantly increased feed-to-egg ratio (weeks 1-3 and 4-6); the average egg weight in the 1,333 and 2,666 mg/kg TP groups was significantly lower than the control group in weeks 1-3, 4-6, and 1-9 ($P<0.05$). 2) Compared with

the control group, dietary supplementation with 2,666 mg/kg TP significantly increased Haugh unit in week 2 ($P<0.05$) and significantly decreased eggshell ratio in week 2 ($P<0.05$); dietary supplementation with 1,333 mg/kg TP significantly increased Haugh unit in week 4 ($P<0.05$) and significantly decreased eggshell ratio in week 2 ($P<0.05$); dietary supplementation with 666, 1,333, and 2,666 mg/kg TP all significantly decreased egg yolk cholesterol content in week 9 ($P<0.05$); dietary supplementation with 666, 1,333, and 2,666 mg/kg TP had no significant effects on eggshell strength, yolk color, yolk ratio, albumen ratio, or yolk fatty acid composition ($P>0.05$). 3) Compared with the control group, dietary supplementation with 666, 1,333, and 2,666 mg/kg TP significantly decreased serum total cholesterol and low-density lipoprotein cholesterol ($P<0.05$), and dietary supplementation with 1,333 and 2,666 mg/kg TP significantly decreased serum triglyceride content ($P<0.05$). 4) Compared with the control group, dietary supplementation with 666, 1,333, and 2,666 mg/kg TP significantly increased the relative mRNA expression level of AMP-activated protein kinase (AMPK) in the liver ($P<0.05$). In conclusion, adding high doses (1,333 and 2,666 mg/kg) of TP to diets decreases the performance of laying hens during the later laying period, but can increase the Haugh unit of eggs, reduce serum total cholesterol and low-density lipoprotein cholesterol contents and egg yolk cholesterol content, and up-regulate the expression of the AMPK gene in the liver.

Key words: tea polyphenols; laying hens; performance; egg quality; lipid metabolism

Introduction

China is the world' s largest egg producer and consumer, accounting for approximately 45% of global egg production, with a trend of yearly increase. Currently, the laying rate of high-producing laying hens declines to about 88% at 45 weeks of age during the later laying period, and the rate of decline gradually accelerates, dropping to approximately 60% at 80 weeks of age. Meanwhile, accumulated stress from long-term high egg production leads to decreased egg quality, including shell quality and albumen quality. Therefore, improving egg quality, particularly during the later laying period, is of paramount importance. Previous studies have confirmed that oxidative stress-induced reproductive performance decline may be the primary cause of reduced laying performance in older hens and during stress periods[1].

Tea polyphenols (TP) are the general term for polyphenolic compounds in tea, primarily catechins (accounting for 60%-80%), and represent one of the main health-promoting components in tea. TP possess strong antioxidant capacity, with studies indicating their antioxidant activity is 25 times that of vitamin C and 80 times that of vitamin E[2-3]. TP exhibit antibacterial, disease-resistant, gut microflora-improving, and lipid-lowering effects, which can safeguard animal health, but their effects on laying hen performance have been inconsistent[4]. Additionally, research has reported that TP can improve egg quality

to some extent and reduce egg cholesterol content[4]. Lou et al.[5] found that dietary supplementation with 250, 550, and 1,000 mg/kg TP (purity 40%) improved laying rate to some degree, but no increasing trend was observed with higher supplementation levels; supplementation with 400 mg/kg TP reduced serum total cholesterol, triglyceride, and low-density lipoprotein contents while increasing high-density lipoprotein content in 46-week-old laying hens. Zhang et al.[6] reported that dietary supplementation with 100 mg/kg TP (purity 98%) significantly increased average egg weight and significantly reduced feed-to-egg ratio and egg yolk cholesterol content. Biswas et al.[7] found that dietary supplementation with 300 mg/kg green tea powder significantly decreased feed intake and average egg weight in laying hens. These inconsistent reports on the effects of TP on laying hen performance and egg quality may be related to differences in TP purity and supplementation dosage. Currently, research on the effects of TP on lipid metabolism in laying hens is limited and requires further investigation. Therefore, this experiment examined the effects of high-dose TP on performance, egg quality, and lipid metabolism in laying hens during the later laying period to explore nutritional strategies for improving laying performance and egg quality in older hens.

Materials and Methods

1.1 Experimental Material

The tea polyphenols product used in this experiment was provided by Sichuan Cruni Tea Biotechnology Co., Ltd., with a purity of 63.58%, catechin content of 40.20%, epigallocatechin gallate (EGCG) content of 21.97%, and caffeine content of 6.84%.

1.2 Experimental Animals and Diets

A total of 480 healthy Lohmann pink-shell laying hens aged 65 weeks were selected for this experiment. A single-factor completely randomized design was employed, dividing the hens into 4 groups with 8 replicates per group and 15 hens per replicate. There was a 2-week pre-trial period followed by a 9-week formal trial period.

The basal diet used in this experiment was a corn-soybean meal type diet formulated at the experimental base of the Institute of Animal Nutrition, Sichuan Agricultural University, according to the nutrient recommendations for laying hens in the Chinese Feeding Standard for Chickens (NY/T 33-2004). The composition and nutrient levels of the basal diet are shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Items	Content
Ingredients	
Corn	

Items	Content
Soybean meal	
Soybean oil	
CaCO3	
CaHPO4	
NaCl	
Choline chloride	
DL-Met	
Vitamin premix1)	
Mineral premix2)	
Total	
Nutrient levels3)	
ME/(MJ/kg)	
CP	
AP	
Met	
Lys	

1) Vitamin premix provided the following per kilogram of the diet: VA 8,000 IU, VD3 1,600 IU, VE 5 IU, VB1 0.8 mg, VB2 2.5 mg, VB6 1.5 mg, VB12 0.004 mg, D-pantothenic acid 2.2 mg, folic acid 0.25 mg, nicotinic acid 20 mg, biotin 0.1 mg.

2) Mineral premix provided the following per kilogram of the diet: Mn (MnO2) 60 mg, Zn (ZnSO4 · 7H2O) 80 mg, Cu (CuSO4 · 5H2O) 8 mg, Fe (FeSO4 · 7H2O) 60 mg, I (KI) 0.35 mg, Se (Na2SeO3 · 5H2O) 0.3 mg.

3) ME was a calculated value, while the others were measured values.

1.3 Experimental Design and Management

The 4 groups of laying hens were randomly divided into 1 control group and 3 experimental groups. Hens in the control group were fed the basal diet, while those in the experimental groups were fed the basal diet supplemented with 666, 1,333, and 2,666 mg/kg TP, respectively.

The feeding trial was conducted at the experimental farm of the Institute of Animal Nutrition, Sichuan Agricultural University. All experimental hens were housed in 3-tier step cages with 3 hens per cage; 5 consecutive cages constituted 1 replicate, with 8 replicates per group. Powdered feed was used throughout the trial. The lighting schedule was 16 h, house temperature was maintained at approximately 22 °C, and water was provided ad libitum. Feed was provided twice daily (09:00 and 15:00), and hens were observed for any abnormal behavior. Eggs were collected and weighed daily at 16:00. House sanitation was cleaned regularly, and routine immunization and disinfection procedures were followed.

1.4.1 Performance Measurements

During the formal trial period, the number of eggs laid and egg weight were recorded daily by replicate to calculate laying rate and average egg weight. Average daily feed intake was determined by recording feed provision and wastage daily, with total feed consumption calculated weekly. Feed-to-egg ratio was calculated based on total egg weight and total feed consumption.

1.4.2 Egg Quality Measurements

At the end of week 9 of the experiment, 2 intact eggs with weights close to the average egg weight were collected from each replicate (collected at 16:00, egg quality measured at 19:00). An automatic egg quality analyzer (EMT-7300, Japan) was used to measure eggshell strength, yolk color, and Haugh unit. After carefully removing the albumen, yolk weight and eggshell weight were measured to calculate eggshell ratio, yolk ratio, and albumen ratio.

Eggshell ratio (%) = 100 × eggshell weight / egg weight
Yolk ratio (%) = 100 × yolk weight / egg weight
Albumen ratio (%) = 100 × (egg weight –yolk weight –eggshell weight) / egg weight

Egg yolk cholesterol content was determined using high-performance liquid chromatography according to GB/T 22220-2008. Egg yolk fatty acid composition was determined using gas chromatography according to NY/T 2068-2011.

1.4.3 Serum Lipid Metabolism Measurements

At the end of week 9 of the experiment, 1 healthy laying hen was randomly selected from each replicate, and 10 mL of blood was collected from the wing vein. Serum was separated and stored at -20 °C. Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents were measured using an automatic biochemical analyzer (HITACHI 7020, Japan).

1.4.4 Hepatic Lipid Metabolism-Related Gene mRNA Expression Measurements

After blood collection, hens were slaughtered by jugular vein exsanguination. Approximately 1.5 g of the liver left lobe tip was isolated, placed in a 2 mL sterile EP tube, immediately stored in liquid nitrogen, and then transferred to a -80 °C freezer for determination of relative mRNA expression levels of hepatic lipid metabolism-related genes.

Primers were designed based on gene sequences for fatty acid synthase (FAS), low-density lipoprotein receptor (LDLR), very low-density lipoprotein receptor (VLDLR), AMP-activated protein kinase (AMPK), and -actin published in NCBI, and primer specificity was verified. -actin was used as the reference

gene. Primers were synthesized by Invitrogen, and primer sequences for target genes and the reference gene -actin are shown in Table 2 .

RNA extraction from liver tissue was performed using a two-step method according to the instructions. Fluorescence quantitative PCR was conducted using the SYBR® Premix Ex Taq™ Kit (TaKaRa Bio Inc., Japan). PCR amplification was performed using the Applied Biosystems 7900HT Real-Time PCR System (Applied Biosystems, CA) with the following program: pre-denaturation at 95 °C for 30 s; 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 34 s; melting curve conditions: 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s. Relative mRNA expression levels of target genes were calculated using the 2-ΔΔCt method with -actin as the reference gene[8], and expressed as fold change relative to the control group.

Table 2 Primer sequences (5' -3')

Genes	Primer sequences	Accession number
-actin	Forward: TCAGGGTGT-GATGGTTGGTATGReverse: TGTTCAATGGGGTACTTCAGGG	NM_205518.1
FAS	Forward: ACTGTGGGCTC-CAAATCTTCAReverse: ACCGGTGTTGGTTTG-CAAA	J04485
VLDLR	Forward: AGTGTGCACCTC-CAACATGTReverse: AGCATCATCATCACACAC-CCA	NM_205229.1
LDLR	Forward: CACTCCCTCAGCAGTGT-GTCReverse: GAGTCCTCGAAGACGGT-GAC	NM_204452.1
AMPK	Forward: TATGCGCA-GACTCAGCTGTTReverse: GTAATGCC-CAATCTTCACTCGC	NM_001039603.1

1.5 Statistical Analysis

Experimental data were analyzed using SPSS 17.0 software for one-way ANOVA, and Duncan' s multiple comparison test was used for inter-group comparisons. P 0.05 was considered statistically significant.

Results

2.1 Effects of Adding High Doses of TP into Diets on Performance of Laying Hens

As shown in Table 3 , compared with the control group, the 2,666 mg/kg TP group exhibited significantly decreased laying rate in weeks 1-3, 4-6, and 1-9 ($P<0.05$). Compared with the control group, the average daily feed intake of all TP supplementation groups showed varying degrees of reduction; however, as the experiment progressed, the average daily feed intake of TP supplementation groups gradually increased, narrowing the gap with the control group. The average daily feed intake of the 2,666 mg/kg TP group was significantly lower than the control group in weeks 1-3, 4-6, 7-9, and 1-9 ($P<0.05$); the 1,333 mg/kg TP group was significantly lower than the control group in weeks 1-3 and 4-6 ($P<0.05$) but significantly higher than the 2,666 mg/kg TP group ($P<0.05$); and the 666 mg/kg TP group was significantly lower than the control group in weeks 1-3 ($P<0.05$) but significantly higher than the 2,666 mg/kg TP group ($P<0.05$). The average egg weight of the 2,666 mg/kg TP group was significantly lower than both the control group and the 666 mg/kg TP group in weeks 1-3 and 1-9 ($P<0.05$), while the 1,333 and 2,666 mg/kg TP groups were significantly lower than the control group in weeks 4-6 ($P<0.05$). The feed-to-egg ratio of the 2,666 mg/kg TP group was significantly higher than all other groups in weeks 1-3 and 4-6 ($P<0.05$).

Table 3 Effects of adding high doses of TP into diets on performance of laying hens

Items	TP supplemental dose/(mg/kg)	SEM	P-value
Laying rate/%	0	666	1,333
Week 1-3	84.56a	81.37a	83.21a
Week 4-6	80.72a	79.01a	66.83b
Week 7-9	81.82a	80.57a	79.45a
Week 1-9			
Average daily feed intake/g			
Week 1-3	114.3a	115.5a	116.7a
Week 4-6	64.4a	109.3b	106.4b
Week 7-9	114.8a	114.0a	107.7b
Week 1-9	115.5a	115.7a	106.8b
Average egg weight/g			
Week 1-3	113.0ab	111.9b	103.6c
Week 4-6	63.5ab	62.6bc	61.8c
Week 7-9	63.7ab	63.4b	63.1b
Week 1-9	64.0a	63.4ab	62.9bc
Feed/egg			
Week 1-3	2.11b	2.22b	2.07b
Week 4-6	2.27a	2.24b	2.28b

Items	TP supplemental dose/(mg/kg)	SEM	P-value
Week 7-9			
Week 1-9			

In the same row, values with different small letter superscripts mean significant difference (P 0.05), while with the same letter or no letter superscripts mean no significant difference (P>0.05). The same as below.

2.2 Effects of Adding High Doses of TP into Diets on Egg Quality of Laying Hens

As shown in Table 4 , compared with the control group, dietary supplementation with different doses of TP had no significant effects on eggshell strength, yolk color, yolk ratio, or albumen ratio (P>0.05). The Haugh unit of the 2,666 mg/kg TP group was significantly higher than the control group in week 2 (P<0.05), and the Haugh unit of the 1,333 mg/kg TP group was significantly higher than all other groups in week 4 (P<0.05). The eggshell ratio of the 2,666 mg/kg TP group was significantly lower than both the control group and the 1,333 mg/kg TP group in week 2 (P<0.05), and the eggshell ratio of the 1,333 mg/kg TP group was also significantly lower than the control group in week 2 (P<0.05).

Table 4 Effects of adding high doses of TP into diets on egg quality of laying hens

Items	TP supplemental dose/(mg/kg)	P-value
Eggshell strength/(kg/cm2)	0	666
Week 2		
Week 4		
Week 6		
Week 9		
Yolk color		
Week 2		
Week 4		
Week 6		
Week 9		
Haugh unit		
Week 2	83.63b	86.98ab
Week 4	83.18b	86.21b
Week 6		
Week 9		
Egg yolk ratio/%		
Week 2		
Week 4		
Week 6		

Items	TP supplemental dose/(mg/kg)	P-value
Week 9		
Eggshell ratio/%		
Week 2	11.60a	10.84bc
Week 4		
Week 6		
Week 9		
Albumen ratio/%		
Week 2		
Week 4		
Week 6		
Week 9		

2.3 Effects of Adding High Doses of TP into Diets on Egg Yolk Cholesterol Content and Fatty Acid Composition of Laying Hens

As shown in Table 5 , compared with the control group, dietary supplementation with different doses of TP significantly affected egg yolk cholesterol content ($P<0.05$), with a decreasing trend in yolk cholesterol content as TP supplementation level increased. The 666, 1,333, and 2,666 mg/kg TP groups showed significantly lower yolk cholesterol content than the control group ($P<0.05$), and the 2,666 mg/kg TP group also had significantly lower yolk cholesterol content than the 666 mg/kg TP group ($P<0.05$). Dietary supplementation with different doses of TP had no significant effect on yolk fatty acid composition ($P>0.05$).

Table 5 Effects of adding high doses of TP into diets on egg yolk cholesterol content and fatty acid composition of laying hens

Items	TP supplemental dose/(mg/kg)	P-value
	0	666
Cholesterol/(mg/g)	13.49a	12.68b
Fatty acids/%		
C14:0		
C16:0		
C16:1		
C18:0		
C18:1		
C18:2		
C18:3		
C20:1		
C22:6		

2.4 Effects of Adding High Doses of TP into Diets on Serum Lipometabolism Parameters of Laying Hens

As shown in Table 6 , compared with the control group, dietary supplementation with different doses of TP had no significant effect on serum high-density lipoprotein cholesterol content in laying hens (P>0.05). Compared with the control group, the 666, 1,333, and 2,666 mg/kg TP groups showed significantly reduced serum total cholesterol and low-density lipoprotein cholesterol contents (P<0.05), and the 1,333 and 2,666 mg/kg TP groups showed significantly reduced serum triglyceride content (P<0.05).

Table 6 Effects of adding high doses of TP into diets on serum lipometabolism parameters of laying hens

Items	TP supplemental dose/(mg/kg)	P-value
	0	666
TC	3.91a	2.86b
TG/(mmol/L)	20.02a	15.45ab
HDL-C		
LDL-C	0.14a	0.06b

2.5 Effects of Adding High Doses of TP into Diets on Expression of Hepatic Lipometabolism-Related Genes of Laying Hens

As shown in Figure 1 [Figure 1: see original paper], the relative mRNA expression level of hepatic AMPK in the 666, 1,333, and 2,666 mg/kg TP groups was significantly higher than in the control group (P<0.05), and the relative mRNA expression level of hepatic AMPK in the 2,666 mg/kg TP group was also significantly higher than in the 666 and 1,333 mg/kg TP groups (P<0.05). There were no significant differences among groups in the relative mRNA expression levels of other hepatic lipid metabolism-related genes (FAS, LDLR, and VLDLR) (P>0.05).

Discussion

3.1 Effects of Adding High Doses of TP into Diets on Performance of Laying Hens

The 9-week TP feeding trial in laying hens showed that supplementation with 666 mg/kg TP had no significant effect on laying rate, while supplementation with 2,666 mg/kg TP significantly decreased laying rate. This may be related to the reduced feed intake observed with TP supplementation in this experiment. These findings differ from previous studies. Lou et al.[5] found that dietary supplementation with 250, 550, and 666 mg/kg TP (purity 40%) improved laying rate to some extent, but no increasing trend was observed with higher supplementation levels. The discrepancy between the results of Lou et al.[5] and this

experiment may be attributed to differences in TP source and supplementation dosage. The TP product used in this experiment contained some caffeine (6.84%), which may be associated with reduced feed intake in laying hens, and the TP dosage in this experiment was much higher than that used by Lou et al.[5].

In this experiment, dietary supplementation with different doses of TP reduced average egg weight in laying hens, which is consistent with related reports. For example, dietary supplementation with 0.67% tea extract slightly reduced average egg weight[9], and dietary supplementation with 5% and 10% green tea powder significantly reduced egg weight[10]. In addition to reduced feed intake affecting egg weight, catechins inhibit intestinal fat absorption and lipase activity, hindering yolk lipid formation[9-10], which also affects egg weight.

In this experiment, all TP supplementation groups had higher feed-to-egg ratios than the control group, which differs from previous research findings. Wang[11] demonstrated that TP significantly reduced feed-to-egg ratio; Wang et al.[12] found that 400 mg/kg TP significantly reduced average egg weight during the first 4 weeks of the trial period, while 200 mg/kg TP significantly reduced feed-to-egg ratio during the last 4 weeks. The differences between these research results and this experiment may be due to variations in TP source and supplementation dosage.

The average daily feed intake of laying hens decreased after dietary supplementation with different doses of TP, and the reduced laying rate and average egg weight along with increased feed-to-egg ratio were all closely related to decreased feed intake. According to Yin et al.[13], feed intake in laying hens increased with increasing green tea powder supplementation, which differs from the results of this experiment. Biswas et al.[7] reported that dietary supplementation with 0.3% green tea powder (TP content approximately 600 mg/kg) significantly decreased feed intake in laying hens, which is similar to the results of this experiment. Possible reasons for these different results include: laying hens may not be adapted to the bitter taste of TP itself or caffeine, and hens may have a gradual adaptation process to TP, with feed intake dropping sharply in the early stage of the experiment and then gradually increasing; additionally, TP contains antinutritional factors such as phenolic acids, caffeine, and condensed tannins[14], which damage or hinder the digestion and absorption of nutrients in laying hens, resulting in decreased protein digestibility and symptoms such as loss of appetite[15].

3.2 Effects of Adding High Doses of TP into Diets on Egg Quality of Laying Hens

In week 2 of the experiment, the eggshell ratio of the 2,666 mg/kg TP group was significantly lower than the control group, which may be related to the caffeine content (6.84%) in the TP product used in this experiment. Previous studies have shown that caffeine intake can lead to calcium loss, osteoporosis, and other

adverse effects, significantly inhibiting calcium absorption in the digestive tract and causing calcium deficiency in the body, thereby affecting eggshell quality and resulting in decreased eggshell ratio[16-17].

Dietary TP supplementation can increase the Haugh unit of eggs, which reflects egg freshness[18]. Factors affecting Haugh unit include storage time and temperature, hen age, stress, nutrition, nutrient supplementation, and disease[19-20]. Under identical environmental and conditional circumstances, nutrition is the key factor influencing Haugh unit. Free radicals can damage proteins, leading to protein denaturation or loss of function[21], and TP possess strong free radical scavenging capacity. The primary reason for increased Haugh unit with dietary TP supplementation may be related to their antioxidant function.

Catechins may inhibit intestinal fat absorption and fatty acid synthase activity, thereby hindering yolk lipid formation[22]. This experiment found that TP had no significant effect on yolk ratio, suggesting that the reduced egg weight after TP supplementation was not caused by TP inhibiting intestinal fat absorption and lipase activity, but rather due to antinutritional factors in TP affecting laying hen performance.

In this experiment, dietary TP supplementation reduced egg yolk cholesterol content, consistent with the trend reported by Qi et al.[23] that yolk cholesterol content gradually decreased with increasing dietary TP supplementation. Most cholesterol in eggs originates from lipoproteins synthesized in the hen' s liver, primarily very low-density lipoprotein (VLDL), followed by vitellogenin. The liver and ovary are the main sites of cholesterol biosynthesis in laying hens. The liver can synthesize cholesterol at high rates and rapidly transport cholesterol to the blood in lipoprotein form, with most blood cholesterol transferred to the yolk via VLDL and excreted through egg production[24].

3.3 Effects of Adding High Doses of TP into Diets on Serum Lipid Metabolism of Laying Hens

In this experiment, dietary supplementation with different doses of TP reduced serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol contents in laying hens, which is similar to the results of Lou et al.[5] showing that dietary supplementation with 0.4% TP reduced serum cholesterol, triglyceride, and low-density lipoprotein contents in 46-week-old laying hens. As TP supplementation dosage increased, serum triglyceride content showed a decreasing trend, indicating enhanced fat decomposition. The lipid-lowering mechanism of TP may be related to estrogen. TP can regulate estrogen and weaken estrogen' s role in maintaining high blood lipids, thereby exhibiting lipid-lowering effects. Additionally, TP can inhibit lipogenic enzyme activity and gene expression in the body, thus suppressing lipid synthesis in the liver.

3.4 Effects of Adding High Doses of TP into Diets on Expression of Hepatic Lipid Metabolism-Related Genes

The liver plays an important role in lipid metabolism in poultry and is the primary site for de novo fatty acid synthesis[25], accounting for over 90% of fatty acid synthesis. The regulation of fat tissue synthesis and decomposition in animals is complex and mainly accomplished by key enzymes in lipid metabolism. The abundance and activity of enzyme proteins are determined by the expression levels of key enzyme genes. AMPK is a key substance for maintaining energy balance in the body[26]. When AMPK is activated, metabolic direction shifts toward inhibiting energy-consuming anabolism and promoting energy-producing catabolism. After AMPK activation, lipid metabolism can be regulated in many tissues. In liver tissue, AMPK can promote fatty acid oxidation, reduce cholesterol and triglyceride synthesis, and inhibit fatty acid production. This experiment found that compared with the control group, dietary supplementation with 666, 1,333, and 2,666 mg/kg TP significantly increased the relative mRNA expression level of hepatic AMPK, with no significant effects on the relative mRNA expression levels of other hepatic lipid metabolism-related genes. Numerous recent studies have shown that after AMPK gene activation, fatty acid and cholesterol synthesis in animal cells and the body can be reduced[27]. AMPK can inhibit sterol regulatory element sequences, and sterol regulatory element-binding proteins can transcriptionally activate LDLR gene expression by binding to sterol regulatory elements, thereby accelerating cholesterol synthesis and metabolism. AMPK has achieved considerable research results in animal experiments, providing new ideas for reducing cholesterol content in eggs. Based on the important role of AMPK in lipid metabolism, we can speculate that TP may affect cholesterol metabolism in laying hens by regulating AMPK gene expression.

Conclusion

Dietary supplementation with high doses (1,333 and 2,666 mg/kg) of TP decreases laying hen performance, but can increase egg Haugh unit, reduce egg yolk cholesterol content, and decrease serum total cholesterol, low-density lipoprotein cholesterol, and triglyceride contents.

Dietary supplementation with high doses (1,333 and 2,666 mg/kg) of TP upregulates AMPK gene expression in the liver of laying hens, with no significant effects on the expression of other hepatic lipid metabolism-related genes.

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Note: Figure translations are in progress. See original paper for figures.