Effects of Astragalus Extract on Laying Performance, Egg Quality and Cholesterol Metabolism in Laying Hens (Postprint)

Authors: Jin Na, Jiang Xiaoqian, Shi Yaran, Liu Yilin, Zou Wenshu, Liu Fenghua

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

Abstract

This experiment aimed to investigate the effects of Qicao extract on laying performance, egg quality, and cholesterol metabolism in laying hens. A total of 3,360 45-week-old Dawu Jinfeng laying hens were randomly divided into 2 groups with 12 replicates per group and 140 hens per replicate. The control group was fed a basal diet, while the experimental group was fed the basal diet supplemented with 400 mg/kg Qicao extract. The experimental period lasted for 12 weeks. The results showed: 1) At weeks 8 and 12, the laying rate and feed-to-egg ratio of hens in the experimental group were significantly higher than those in the control group ($P<0.05$). 2) At weeks 8 and 12, the albumen height and Haugh unit of eggs in the experimental group were significantly higher than those in the control group ($P<0.05$); at week 12, the eggshell strength and eggshell thickness of eggs in the experimental group were significantly higher than those in the control group ($P<0.05$). 3) At week 12, the serum total cholesterol content of hens in the experimental group was significantly lower than that in the control group ($P<0.05$), and the gene expression levels of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) in the liver and very low-density lipoprotein receptor (VLDLR) in the ovary of hens in the experimental group were significantly lower than those in the control group ($P<0.05$). 4) From weeks 4 to 12, the yolk cholesterol content of eggs in the experimental group gradually decreased; at week 12, the yolk cholesterol content of eggs in the experimental group was significantly lower than that in the control group ($P<0.05$). In conclusion, dietary supplementation with Qicao extract can improve laying performance and egg quality, and reduce egg yolk cholesterol content by regulating serum lipid metabolism indices and inhibiting HMGR and VLDLR gene expression.

Full Text

Effects of Qicao Extract on Laying Performance, Egg Quality, and Cholesterol Metabolism in Laying Hens

JIN Na, JIANG Xiaoqian, SHI Yaran, LIU Yilin, ZOU Wenshu, LIU Fenghua*

Animal Science and Technology College, Beijing University of Agriculture, Beijing 102206, China

Abstract

This experiment was conducted to investigate the effects of Qicao extract on laying performance, egg quality, and cholesterol metabolism in laying hens. A total of 3,360 healthy Dawu Jinfeng laying hens aged 45 weeks were randomly divided into 2 groups with 12 replicates per group and 140 hens per replicate. The control group was fed a basal diet, while the experimental group received the basal diet supplemented with 400 mg/kg Qicao extract. The trial lasted for 12 weeks. The results showed: 1) On weeks 8 and 12, the laying rate and feed-to-egg ratio of hens in the experimental group were significantly higher than those in the control group ($P<0.05$). 2) On weeks 8 and 12, the albumen height and Haugh unit of eggs in the experimental group were significantly higher than those in the control group ($P<0.05$); on week 12, the eggshell strength and eggshell thickness of eggs in the experimental group were significantly higher than those in the control group ($P<0.05$). 3) On week 12, the serum total cholesterol content of hens in the experimental group was significantly lower than that in the control group ($P<0.05$), and the gene expression levels of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) in the liver and very low density lipoprotein receptor (VLDLR) in the ovary were significantly lower than those in the control group ($P<0.05$). 4) From weeks 4 to 12, the yolk cholesterol content in eggs from the experimental group gradually decreased; on week 12, the yolk cholesterol content was significantly lower than that in the control group ($P<0.05$). In conclusion, dietary supplementation with Qicao extract can improve laying performance and egg quality in laying hens, and reduce yolk cholesterol content by regulating serum lipid metabolism indices and inhibiting the expression of HMGR and VLDLR genes.

Keywords: Qicao extract; laying hens; laying performance; egg quality; cholesterol metabolism

---

Introduction

With China' s rapid economic development and improving living standards, consumer demand for animal-derived foods has gradually shifted from quantity to quality. Eggs are one of the most popular livestock products in daily life. Recent studies have shown that healthy adults eating an average of one egg per day may help reduce the risk of cardiovascular and cerebrovascular diseases, but long-term excessive consumption increases disease risk. A typical egg contains approximately 250 mg of cholesterol, while daily cholesterol intake should not exceed 300 mg, causing consumers to limit egg consumption. Therefore, reducing cholesterol content in eggs to enable confident consumption has become an urgent problem in egg production.

Medicinal and edible homologous Chinese herbs are increasingly used as natural plant feed additives in animal production. Dietary supplementation with Chinese herbal additives can effectively improve intestinal function, regulate metabolic processes, and promote feed intake, thereby enhancing production performance in laying hens [1-2]. Research has found that *Leonurus japonicus* (motherwort) can improve production performance in green-shell laying hens during mid-to-late laying periods by promoting the expression of genes related to egg production, thereby increasing egg yield and improving egg quality [3]. Xu [4] reported that adding daidzein to laying hen diets significantly reduced yolk cholesterol content, with more pronounced effects over time. Guo et al. [5] found that astragalus polysaccharides could reduce cholesterol ester content in foam cells, with the lowest content observed at 100 mg/L concentration. Liu et al. [6] added *Leonurus japonicus* extract to laying hen diets and observed significant reductions in serum cholesterol and triglyceride (TG) levels.

Qicao extract is derived from natural plants including *Astragalus membranaceus* and *Leonurus japonicus* through drying and extraction. *Astragalus* can dilate coronary arteries, improve myocardial blood supply, enhance immune function, and delay cellular aging [7-8]. *Leonurus* has blood-activating and stasis-resolving properties that can improve blood circulation in the uterus and ovaries, regulate sex hormone levels, and promote hormone secretion. These natural plants (Chinese herbs) listed in the feed ingredient directory offer disease prevention and immune regulation benefits, multiple nutritional functions, and advantages including low toxicity, no drug resistance, and minimal residue [9-11]. As a novel feed additive, Qicao extract was determined through preliminary safety tests and pilot studies to have an appropriate supplementation level of 400 mg/kg. Therefore, this experiment was conducted at an expanded scale to investigate the effects of Qicao extract on laying performance, egg quality, and cholesterol metabolism in laying hens, aiming to explore lipid metabolism characteristics and its influence on cholesterol metabolism during egg formation.

Materials and Methods

1.1 Experimental Animals and Design A total of 3,360 healthy Dawu Jinfeng laying hens aged 45 weeks were selected and randomly divided into 2 groups with 12 replicates per group and 140 hens per replicate, housed in double-deck cages. The control group was fed a basal diet, while the experimental group received the basal diet supplemented with 400 mg/kg Qicao extract. The trial lasted for 12 weeks. The composition and nutrient levels of the basal diet are shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (DM basis) %

Items	Content
Ingredients	
Corn	
Soybean meal	
Limestone	
Wheat middling	
Cottonseed meal	
Soybean oil	
CaHPO	
Premix[1]	
NaCl	
Total	
Nutrient levels[2]	
ME/(MJ/kg)	
CP	
AP	
Lys	
Met	
Thr	

[1]The premix provided the following per kg of diet: VA 12,000 IU, VD 4,300 IU, VB 3 mg, VB 7 mg, VB 10 mg, VB 7 mg, VB 0.2 mg, VE 20 IU, VK 3.2 mg, biotin 0.15 mg, folic acid 1.1 mg, nicotinic acid 50 mg, Cu 9 mg, Fe 50 mg, Mn 100 mg, Zn 85 mg, Se 0.3 mg.

[2]Nutrient levels were calculated values.

1.2 Management Practices Experimental hens were housed in the same building under conventional management practices in fully-folded double-deck cages with 4 hens per cage. Hens had free access to feed and water with a lighting schedule of 16 h/d.

1.3 Measurements 1.3.1 Laying Performance

Daily health observations were conducted, and egg production was recorded by replicate. Feed consumption was measured weekly to calculate laying rate, average egg weight, average daily feed intake, and feed-to-egg ratio.

1.3.2 Egg Quality Measurements

At the end of weeks 4, 8, and 12, 6 eggs were randomly collected from each replicate for quality analysis. Albumen height and Haugh unit were measured using a multifunctional egg quality analyzer (EA-01, ORKA/Israel). Eggshell strength was determined using an eggshell force gauge (EFR-01, ORKA/Israel). Eggshell thickness and egg shape index were measured using vernier calipers.

1.3.3 Yolk Cholesterol Content

At the end of weeks 4, 8, and 12, 6 eggs were randomly collected from each replicate. Yolks were separated and weighed, and 1 g samples were taken to determine cholesterol content using a cholesterol assay kit from Beijing Beihua Kangtai Clinical Reagents Co., Ltd.

1.3.4 Serum Lipid Metabolism Indices

At the end of week 12, one hen was randomly selected from each replicate, and 5 mL blood was collected from the wing vein. Serum was separated by centrifugation at 3,000 r/min for 15 min. Serum total cholesterol (TC), TG, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using an automatic biochemical analyzer (BS-200, Mindray Medical International Co., Ltd.).

1.3.5 Tissue Cholesterol Metabolism Indices

At the end of week 12, one hen was randomly selected from each replicate, and liver and ovarian tissues were collected and immediately stored in liquid nitrogen. Total RNA was extracted from liver and ovarian tissues using Trizol reagent kit (Invitrogen, USA) according to the manufacturer' s instructions. mRNA was reverse-transcribed into cDNA using a reverse transcription kit (TaKaRa). Gene expression levels of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) in liver and very low density lipoprotein receptor (VLDLR) in ovary were determined by reverse transcription PCR (RT-PCR) using -actin as the internal reference. Primer sequences are shown in Table 2 .

Table 2 Primer sequences

Genes	Sequences (5' -3')	Product size/bp
3-hydroxy-3-methylglutaryl-CoA reductase (HMGR)	F: GGTTCAAGGTGCAAGCCAAGR: AGCTGCCTTCTTAGTGCAGG	
Very low density lipoprotein receptor (VLDLR)	F: TGGAGCTGAATTGGTCACCCR: TGTTGAATCCTCCACATCTCAG	

Genes	Sequences (5' -3')	Product size/bp
-actin	F: GAGAAATTGTGCGTGA-CATCAR: CCTGAACCTCTCATTGCCA	

1.4 Statistical Analysis Data were processed using SPSS 16.0 statistical software. One-way ANOVA and multiple comparisons were used for data analysis and significance testing. Results are expressed as mean±SD, with P<0.05 indicating significant difference.

Results

2.1 Effects of Qicao Extract on Laying Performance As shown in Table 3 , on weeks 8 and 12, the laying rate and feed-to-egg ratio of hens in the experimental group were significantly higher than those in the control group (P<0.05). On weeks 4, 8, and 12, average egg weight and average daily feed intake showed no significant differences between groups (P>0.05).

Table 3 Effects of Qicao extract on laying performance of laying hens

Items	Time/week	Control group	Experimental group	P-value
Average egg weight/g	4	56.83±1.62	56.71±1.73	
	8	57.03±1.63	57.28±1.79	
	12	57.38±1.02	58.07±1.25	
Laying rate/%	4	84.23±1.38	85.55±1.49	
	8	84.96±1.54	87.23±1.94	<0.05
	12	84.03±2.25	88.23±3.02	<0.05
Average daily feed intake/(g/d)	4	124.51±2.42	122.74±2.89	
	8	126.14±2.47	123.49±2.84	
	12	125.89±2.39	123.60±2.97	
Feed/egg ratio	4	3.09±0.40	3.51±0.47	<0.05
	8	3.27±0.37	3.67±0.43	<0.05
	12	3.22±0.38	3.65±0.45	<0.05

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

2.2 Effects of Qicao Extract on Egg Quality As shown in Table 4 , on weeks 8 and 12, the albumen height and Haugh unit of eggs in the experimental group were significantly higher than those in the control group (P<0.05). On week 12, the eggshell strength and eggshell thickness of eggs in the experimental group were significantly higher than those in the control group (P<0.05). On weeks 4, 8, and 12, egg shape index showed no significant differences between groups (P>0.05).

Table 4 Effects of Qicao extract on egg quality of laying hens

Items	Time/week	Control group	Experimental group	P-value
Egg shape index	4	1.27±0.02	1.27±0.02	
	8	1.27±0.01	1.28±0.02	
	12	1.28±0.01	1.28±0.02	
Albumen height/mm	4	6.23±0.04	6.25±0.10	
	8	6.26±0.09	6.36±0.10	<0.05
	12	6.27±0.09	6.39±0.11	<0.05
Haugh unit	4	78.54±2.63	77.60±2.45	
	8	78.47±2.32	80.64±2.34	<0.05
	12	80.98±2.98	82.11±2.63	<0.05
Eggshell strength/(N/cm^2)	4	37.58±2.16	38.21±2.09	
	8	38.32±1.99	40.04±2.05	
	12	38.12±1.88	40.06±2.18	<0.05
Eggshell thickness/×10 ^{2}mm	4	35.54±1.64	35.56±1.07	
	8	35.92±1.45	36.03±1.12	
	12	35.91±0.99	36.87±0.92	<0.05

2.3 Effects of Qicao Extract on Serum Lipid Metabolism Indices As shown in Table 5 , on week 12, the serum TC content of hens in the experimental group was significantly lower than that in the control group (P<0.05). The serum TG and LDL-C contents decreased while HDL-C content increased in the experimental group, but these differences were not significant compared with the control group (P>0.05).

Table 5 Effects of Qicao extract on serum lipid metabolism indices of laying hens (mmol/L)

Items	Control group	Experimental group	P-value
Triglycerides (TG)	13.22±2.12	12.74±2.03	
Total cholesterol (TC)	2.02±0.23	1.74±0.21	<0.05

Items	Control group	Experimental group	P-value
High-density lipoprotein cholesterol (HDL-C)	0.98±0.14	1.02±0.11	
Low-density lipoprotein cholesterol (LDL-C)	0.84±0.21	0.78±0.18	

2.4 Effects of Qicao Extract on Yolk Cholesterol Content As shown in Figure 1 [Figure 1: see original paper], on weeks 4 and 8, yolk cholesterol content showed no significant differences between groups (P>0.05). On week 12, yolk cholesterol content in the experimental group was significantly lower than that in the control group (P<0.05). With prolonged experimental duration, yolk cholesterol content in the experimental group showed a gradually decreasing trend.

Figure 1 Effects of Qicao extract on yolk cholesterol content of laying hens

Value columns with indicate significant difference (P<0.05). The same applies below.*

2.5 Effects of Qicao Extract on HMGR and VLDLR Gene Expression Levels As shown in Figure 2 [Figure 2: see original paper], the expression level of HMGR gene in liver of hens in the experimental group was significantly lower than that in the control group (P<0.05).

Figure 2 Effects of Qicao extract on expression level of HMGR gene in liver of laying hens

As shown in Figure 3 [Figure 3: see original paper], the expression level of VLDLR gene in ovary of hens in the experimental group was significantly lower than that in the control group (P<0.05).

Figure 3 Effects of Qicao extract on expression level of VLDLR gene in ovary of laying hens

Discussion

3.1 Effects of Qicao Extract on Laying Performance Qicao extract is derived from natural plants including *Astragalus membranaceus* and *Leonurus japonicus* through drying and extraction. *Astragalus* can dilate coronary arteries, improve myocardial blood supply, enhance immune function, and delay cellular aging [2,8]. Dietary supplementation with astragalus polysaccharides can significantly improve laying rate, reduce feed-to-weight ratio, and enhance production performance in late-stage laying hens [12]. Pharmacological studies have shown that *Leonurus* has blood-activating and stasis-resolving properties that improve blood circulation in the uterus and ovaries, regulate sex hormone levels, and promote hormone secretion. Single-herb *Leonurus* can significantly increase the ratio of uterine and ovarian weight to body weight in mice [13]. Adding *Leonurus* extract to laying hen diets has been shown to increase laying rate and average egg weight while reducing average daily feed intake and feed-to-egg ratio [14]. The present results demonstrate that dietary Qicao extract supplementation significantly improved laying rate and feed-to-egg ratio on weeks 8 and 12, consistent with previous reports. The *Astragalus* component in Qicao extract enhanced disease resistance and feed utilization, maintaining high metabolic and reproductive capacity. The *Leonurus* component promoted follicular development and enhanced metabolic levels, thereby increasing laying rate while improving feed digestion and absorption, intestinal function, and ultimately feed conversion efficiency.

3.2 Effects of Qicao Extract on Egg Quality Egg quality is an important indicator reflecting laying performance and indirectly indicates the health status of hens [15]. Egg quality comprises internal and external traits. Internal quality includes albumen height, Haugh unit, and yolk color that cannot be directly observed from the eggshell surface [16], while external quality includes egg shape index, eggshell strength, and egg weight. The Haugh unit evaluates the thinning degree of thick albumen, reflecting egg freshness and albumen quality [17]. Higher albumen height indicates fresher eggs, and greater Haugh values indicate better albumen viscosity [18]. In this study, dietary Qicao extract supplementation significantly increased albumen height and Haugh unit on weeks 8 and 12, suggesting that Qicao extract can influence egg protein metabolism, though the mechanism remains unclear. Wu et al. [19] investigated the effects of *Astragalus* on calcium-phosphorus metabolism in rats with chronic kidney disease and found that *Astragalus* could alleviate calcium-phosphorus metabolic abnormalities, reduce renal pathological damage, and improve calcium-phosphorus metabolism. The eggshell, as the hardest part of the egg, protects the contents from damage, prevents microbial invasion, and controls water and gas exchange during embryonic development [20]. The significant improvement in eggshell quality observed with Qicao extract may be attributed to *Astragalus* improving calcium-phosphorus metabolism, increasing calcium content in eggshells, and consequently enhancing eggshell strength and thickness, thereby improving overall egg quality.

3.3 Effects of Qicao Extract on Serum Lipid Metabolism Indices High-cholesterol diets can induce coronary heart disease and atherosclerosis, characterized by elevated TC and TG levels, high blood lipid content, increased blood viscosity, and reduced blood flow, leading to thrombosis formation. Low-density lipoprotein (LDL) is the primary carrier for endogenous cholesterol transport, while high-density lipoprotein (HDL) functions in reverse cholesterol transport, moving lipids from peripheral tissues to the liver for catabolism. HDL-C transports cholesterol esters into hepatocytes for hydrolysis, helping eliminate blood cholesterol, whereas LDL-C transports endogenous cholesterol from the liver to peripheral tissues. Elevated serum LDL-C content increases serum TC and TG levels, resulting in hyperlipidemia [21]. Therefore, high HDL-C content can prevent hyperlipidemia and facilitate lipid metabolism and transport. Astragalus polysaccharides have lipid-regulating effects, effectively reducing TC and LDL-C levels in normal human blood. Zhu et al. [22] demonstrated that astragalus polysaccharides significantly reduced serum TG content and increased HDL-C content in diabetic model rats. The guanidine structure in leonurine has significant anti-platelet aggregation effects and can increase coronary blood flow, commonly used as a quality control indicator in medicine [23]. The present results show that dietary Qicao extract supplementation reduced serum TG and LDL-C contents while increasing HDL-C content, with TC content significantly decreased. Serum TC content positively correlates with yolk cholesterol content, suggesting that Qicao extract may effectively reduce cholesterol by regulating blood lipids.

3.4 Effects of Dietary Qicao Extract on Yolk Cholesterol Content The present results demonstrate that dietary Qicao extract supplementation gradually reduced yolk cholesterol content over time, with a significant decrease observed on week 12, indicating that Qicao extract requires a certain period to exert its effects. Xu [4] reported that daidzein supplementation significantly reduced yolk cholesterol content, with more pronounced effects over longer durations. These findings are consistent with previous research, primarily because *Astragalus* and *Leonurus* are rich in alkaloid active substances that can partially bind with cholesterol [24], reducing intestinal cholesterol absorption and thereby decreasing cholesterol content. Further research is needed to determine the optimal duration of Qicao extract supplementation and whether increasing the dosage could shorten the onset time.

3.5 Effects of Qicao Extract on Cholesterol Metabolism in Liver and Ovarian Tissues Yolk cholesterol originates primarily from the liver and ovary. Cholesterol synthesis is a multi-step enzymatic reaction process, with HMGR in the liver serving as the rate-limiting enzyme that determines reaction speed and controls de novo cholesterol synthesis. Cholesterol is carried by apolipoprotein-B (Apo-B) and released from the liver with the assistance of various transport factors, eventually reaching developing oocytes. Under lysosomal action, it is degraded into free cholesterol, which accumulates in the yolk as oocytes grow, with 95% of yolk cholesterol deposited through this pathway [25-26]. Inhibition of this enzyme and gene expression has significant effects on reducing yolk cholesterol content.

In this study, dietary Qicao extract supplementation significantly decreased VLDLR gene expression in the ovary, indirectly indicating reduced cholesterol deposition in oocytes. Serum TC content positively correlated with yolk cholesterol content, both showing decreasing trends under Qicao extract influence. The mechanism by which Qicao extract affects yolk cholesterol content requires further investigation.

Conclusion

Dietary supplementation with Qicao extract can improve laying performance and egg quality in laying hens, and reduce yolk cholesterol content by regulating serum lipid metabolism indices and inhibiting the expression of HMGR and VLDLR genes.

References

[1] Chen YZ. Factors affecting cholesterol content in eggs[J]. Heilongjiang Animal Science and Veterinary Medicine, 2011(5):37–39.

[2] Liu Y. Effects of organic farming on production performance and egg quality of green-shell laying hens[D]. Master' s thesis. Nanjing: Nanjing Agricultural University, 2009.

[3] Sun XJ. Effects of motherwort on production performance, serum indices, and related gene expression in green-shell laying hens[D]. Master' s thesis. Harbin: Northeast Agricultural University, 2013.

[4] Xu GY. Study on the effects of dietary isoflavone supplementation on production performance and its endocrine mechanisms in broilers and laying hens[D]. Master' s thesis. Nanjing: Nanjing Agricultural University, 2007.

[5] Guo J, Wang ZP, Gao XH, et al. Analysis of the relationship between astragalus polysaccharides upregulating ABCA1 expression and cholesterol content in foam cells[J]. Journal of Changzhi Medical College, 2017, 31(4):244–246.

[6] Liu PP, Zang SM, Wang J, et al. Effects of spirulina powder, rehmannia polysaccharide, and motherwort extract on egg quality and some biochemical indices in laying hens[J]. China Poultry, 2016, 38(12):25–30.

[7] Qiu Y, Hu YL, Cui BA, et al. Immunopotentiating effects of four Chinese herbal polysaccharides administered to vaccinated chickens[J]. Poultry Science, 2007, 86:2530-2535.

[8] Si CD, Min YH. Effects of astragalus polysaccharides on immune function in broiler chickens[J]. Chinese Journal of Preventive Veterinary Medicine, 2008, 30(12):978-980.

[9] Zhang GZ, Luo YH, Jin SX, et al. Effects of Chinese herbal Lianbanqi mixture on production performance and immune function in broiler chickens[J]. Chinese Journal of Animal Science, 2012, 48(15):55-58.

[10] Xiao MH, Shi DY, Mo GF, et al. Effects of Chinese herbal compound on production performance and HSP70 expression in heart tissue of chronically heat-stressed laying hens[J]. Journal of Traditional Chinese Veterinary Medicine, 2011, 30(1):23-25.

[11] Song YX, Jin B, Wang XL, et al. Effects of Chinese herbal additives on egg cholesterol content[J]. Acta Agriculturae Boreali-occidentalis Sinica, 2002, 11(3):4-7.

[12] Guan LH, Zhang LY, Liu HB, et al. Effects of astragalus polysaccharides on production performance, reproductive hormones, and blood physiological and biochemical indices in laying hens[J]. Journal of the Chinese Cereals and Oils Association, 2015, 30(7):70-76.

[13] Fu HQ. Study on treating dairy cow ovarian quiescence with traditional Chinese medicine[D]. Master' s thesis. Harbin: Northeast Agricultural University, 2008.

[14] Liu PP, Zang SM, Yang LY, et al. Effects of motherwort extract on production performance and egg quality in laying hens[J]. Heilongjiang Animal Science and Veterinary Medicine, 2017(8):178-180.

[15] Batkowska J, Brodacki A, Knaga S. Quality of laying hen eggs during storage depending on egg weight and type of cage system (conventional vs. furnished cages)[J]. Annals of Animal Science, 2014, 14(3):707-719.

[16] Onyimonyi AE, Ugwu SOC. Performance of laying hens fed varying dietary levels of Bambara (*Voandzeia subterrenea* Thouars) offals[J]. International Journal of Poultry Science, 2007, 6(3):223-226.

[17] Momoh OM, Ani AO, Ugwuowo LC. Part-period egg production and egg quality characteristics of ecotypes of Nigerian local chickens and their crosses[J]. International Journal of Poultry Science, 2010, 9(8):744-748.

[18] Ying Y, Han CY, Tabassum CM, et al. Effect of quercetin on egg quality and components in laying hens of different weeks[J]. Journal of Northeast Agricultural University, 2015, 22(4):23-32.

[19] Wu YQ, Gao YL, Tao J, et al. Effects of astragalus on calcium-phosphorus metabolism and FGF23-Klotho axis in rats with chronic kidney disease[J]. Journal of Nanjing University of Traditional Chinese Medicine, 2018, 34(2):118-122.

[20] Zhang Y. Study on effects of tea polyphenol extract supplementation in laying hen diets on egg quality[D]. Master' s thesis. Ya' an: Sichuan Agricultural

University, 2016.

[21] Arai H, Teramoto T, Daida H, et al. Efficacy and safety of the cholesteryl ester transfer protein inhibitor anacetrapib in Japanese patients with heterozygous familial hypercholesterolemia[J]. Atherosclerosis, 2016, 249:215-223.

[22] Zhu XY, Xie JJ, Wang CS. Effects of polygonatum polysaccharide on glucose-lipid metabolism and lipid peroxidation in diabetic model rats[J]. Jiangsu Journal of Traditional Chinese Medicine, 2008, 40(10):114-116.

[23] Zhou YP, Liu WH, Shao GX. Anti-platelet aggregation activity of leonurine, syringylguanidine alcohol, and syringic acid amino alcohol esters and their structure-activity relationships[J]. Chinese Pharmaceutical Journal, 1996(5):271-274.

[24] Zhu YZ, Ge JS, Yao BQ, et al. Effects of fermented plant protein feed replacing soybean meal on laying performance and egg quality in laying hens[J]. Feed Review, 2010(10):1-5.

[25] Schriewer H, Nolte W, Schulte H, et al. VLDL cholesterol and VLDL apolipoprotein B. Preliminary cross-sectional data of the prospective epidemiological study of company employees in Westphalia[J]. Journal of Clinical Chemistry and Clinical Biochemistry, 1987, 25(5):293-298.

[26] Lu CW, Lo YH, Chen CH, et al. VLDL and LDL, but not HDL, promote breast cancer cell proliferation, metastasis and angiogenesis[J]. Cancer Letters, 2017, 388:130-138.

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