Effects of Grape Proanthocyanidins on Production Performance and Antioxidant Capacity of Laying Hens during the Late Laying Period (Postprint)

Authors: Zhang Yu, Yupeng Gao, Wu Shugeng, Wang Jing, Hongyuan Yue, Qi Guanghai, Zhang Haijun

Date: 2017-10-10T00:00:00+00:00

Abstract

This study aimed to investigate the effects of grape procyanidins (GPC) on production performance, egg quality, and antioxidant capacity in plasma, liver, and egg yolk of late-phase laying hens. Two hundred eighty-eight 64-week-old Lohmann laying hens with similar laying rates and body weight, and in good health condition, were selected and randomly allocated into 4 groups, each consisting of 6 replicates with 12 hens per replicate. The control group received a basal diet; the GPC50 and GPC100 groups received the basal diet supplemented with 50 and 100 mg/kg GPC, respectively; the tert-butyl hydroquinone (TBHQ) group received the basal diet + 200 mg/kg TBHQ. The preliminary period lasted 7 days, followed by a 56-day formal experimental period. The results showed: 1) GPC significantly inhibited the decline in laying rate and egg Haugh unit ($P<0.05$) and significantly increased eggshell thickness ($P<0.05$). 2) In the GPC50 group, total superoxide dismutase (T-SOD) activity and total antioxidant capacity (T-AOC) in plasma and liver were significantly increased ($P<0.05$), while malondialdehyde (MDA) contents in plasma and liver were significantly decreased ($P<0.05$); TBHQ significantly increased plasma T-SOD activity ($P<0.05$) and significantly decreased liver MDA content ($P<0.05$). 3) GPC significantly increased T-SOD activity in egg yolk ($P<0.05$) and significantly decreased MDA and cholesterol contents in egg yolk ($P<0.05$); TBHQ significantly decreased MDA content in egg yolk ($P<0.05$). In summary, dietary GPC supplementation improved laying rate and Haugh unit, increased eggshell thickness, and enhanced systemic antioxidant capacity in late-phase laying hens, with 50 mg/kg GPC showing superior effects compared to 200 mg/kg TBHQ.

Full Text

Effects of Grape Procyanidins on Production Performance and Antioxidant Capacity of Old Laying Hens

ZHANG Yu[1], WU Shugeng[2], WANG Jing[2], YUE Hongyuan[2], QI Guanghai[2], GAO Yupeng[1], *ZHANG Haijun*[2]

[1]College of Animal Science and Technology, Northwest A&F University, Yangling 712100, China
[2]Key Laboratory of Feed Biotechnology of Ministry of Agriculture, National Engineering Research Center of Biological Feed, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

Abstract

This experiment was conducted to investigate the effects of grape procyanidins (GPC) on production performance, egg quality, and antioxidant capacity in plasma, liver, and egg yolk of old laying hens. Two hundred eighty-eight healthy 64-week-old Roman laying hens with similar laying rates and body weights were randomly assigned to four groups with six replicates of twelve hens each. The control group received a basal diet, while the GPC50 and GPC100 groups received the basal diet supplemented with 50 and 100 mg/kg GPC, respectively, and the tert-butyl hydroquinone (TBHQ) group received the basal diet plus 200 mg/kg TBHQ. The experiment included a 7-day adaptation period followed by a 56-day measurement period. The results showed that: (1) GPC significantly inhibited the decline in laying rate and Haugh unit of eggs with age and increased eggshell thickness ($P<0.05$). (2) The GPC50 group exhibited significantly increased total superoxide dismutase (T-SOD) activity and total antioxidant capacity (T-AOC) in plasma and liver, along with significantly reduced malondialdehyde (MDA) content in these tissues ($P<0.05$). TBHQ supplementation significantly increased plasma T-SOD activity ($P<0.05$) and decreased liver MDA content ($P<0.05$). (3) Dietary GPC significantly increased yolk T-SOD activity and decreased yolk MDA and cholesterol contents ($P<0.05$), whereas TBHQ only significantly decreased yolk MDA content ($P<0.05$). In conclusion, dietary GPC can alleviate the decline in laying rate and Haugh unit, increase eggshell thickness, and improve the antioxidant capacity of old laying hens. Supplementation at 50 mg/kg GPC demonstrated superior effects compared to 200 mg/kg TBHQ.

Key words: grape procyanidins; old laying hens; performance; egg quality; antioxidant capacity

---

Oxidative stress in animals represents a fundamental cause of disease and abnormal production performance. In recent years, the livestock industry has focused on eliminating drug-dependent approaches to ensuring animal welfare and

achieving healthy animal production from a sustainable development perspective through source control, with the development and application of animal-specific antioxidants representing one important pathway. Research and application of animal antioxidants generally fall into two categories. The first comprises synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ). While these are inexpensive, readily available, and demonstrate good antioxidant effects, they all carry potential toxic side effects that can harm animal health with long-term use. This "emergency response" characteristic of non-sustainable application fails to effectively address the persistent nature of oxidative stress in animal bodies, making it difficult to fundamentally resolve the hazards of oxidation. The second category involves the exploration of new, non-toxic, sustainable natural plant antioxidants, an area that has attracted increasing research attention.

Grape procyanidins (GPC), the plant polyphenol selected for this study, are present in grape skins and seeds and consist of polymers with varying degrees of polymerization formed from catechin, epicatechin, and epicatechin gallate. In vitro studies have demonstrated that GPC possesses strong anti-superoxide anion and free radical scavenging capabilities, with free radical scavenging capacity superior to vitamin C, vitamin E, and β-carotene. Administration of GPC can significantly increase plasma total antioxidant capacity (T-AOC) in rats, significantly reduce serum malondialdehyde (MDA) content and increase total superoxide dismutase (T-SOD) activity in mice, and significantly increase blood T-SOD and glutathione peroxidase (GSH-Px) activities while decreasing blood MDA content in humans. GPC can also serve as an antioxidant and immunomodulator in broiler chickens and significantly reduce egg yolk cholesterol content.

Modern laying hens are high-producing animals with extremely vigorous metabolism and relatively short utilization cycles. Following the high-load, high-intensity metabolic period of peak egg production, the metabolic and physiological functions of laying hens gradually deteriorate, particularly the weakening of endogenous antioxidant capacity, which leads to reduced disease resistance and contributes to various metabolic diseases as well as severe infectious diseases. Production practice has demonstrated that the most effective operational measure to reduce this risk is to address the source of antioxidant function, thereby delaying the decline in production performance, as changes in laying performance represent the most sensitive indicator of hen health. Previous research on GPC has primarily focused on in vitro studies, mice, and broiler chickens with physiological functions different from those of old laying hens, with limited research conducted on laying hens. Therefore, this study used the synthetic antioxidant TBHQ as a reference to investigate the effects of dietary supplementation with 50 and 100 mg/kg GPC on production performance, egg quality, and antioxidant capacity of old laying hens, aiming to provide a basis for developing natural antioxidant feed additives for improving the health of old laying hens and egg quality.

1. Materials and Methods

1.1 Experimental Materials GPC was provided by Tianjin Jianfeng Natural Product R&D Co., Ltd., with a procyanidin content of 99.47%, comprising 65.19% oligomeric procyanidins, 9.88% monomeric procyanidins, and 24.93% polymeric procyanidins. TBHQ was provided by Yancheng Jieyang Fine Chemical Co., Ltd., with a purity of 99%.

1.2 Experimental Design and Diets Two hundred eighty-eight healthy 64-week-old Roman laying hens with similar laying rates and body weights were randomly divided into four groups with six replicates of twelve hens each. The control (CON) group was fed a basal diet; the GPC50 and GPC100 groups received the basal diet supplemented with 50 and 100 mg/kg GPC, respectively; and the TBHQ group received the basal diet plus 200 mg/kg TBHQ. The experiment included a 7-day adaptation period and a 56-day measurement period.

A corn-soybean meal powdered basal diet was formulated according to NRC (2004), Chinese Feeding Standard of Chickens (NY/T 33-2004), and the Roman laying hen management manual. The composition and nutrient levels of the basal diet are presented in Table 1 .

1.3 Animal Management The hens were housed in a windowed poultry house with three-tier step cages, three hens per cage, with equal numbers of hens from each group on each tier. Natural lighting was supplemented with artificial lighting to achieve 16 h/d of light. The average temperature in the poultry house during the experimental period was 20.5°C, with relative humidity of 45%-65% and negative-pressure longitudinal ventilation. Feed was provided three times daily (08:00, 11:30, and 16:00), with egg collection and manure removal performed once daily and disinfection every seven days. Hens had free access to feed and water, with other routine management practices followed.

1.4 Sample Collection and Measurements

1.4.1 Production Performance The health status of hens in each group was monitored. Daily feed intake, egg number, and egg weight were recorded per replicate, with feed consumption calculated every seven days. At the end of weeks 4 and 8 of the experimental period, laying rate (LR), average egg weight (AEW), average daily feed intake (ADFI), and feed-to-egg ratio (F/E) were calculated for each replicate.

1.4.2 Egg Quality On days 28, 42, and 56 of the experimental period, five eggs were randomly selected from each replicate to determine egg shape index using a Japanese Fuji Tsubo egg shape index meter. Albumen height, Haugh unit, and yolk color were measured using a SONOVA Egg Analyzer™ (Orka Technology Ltd.). Eggshell strength was determined using an Egg Force Reader

(Orka Technology Ltd.), and eggshell thickness was measured using an Egg Shell Thickness Gauge (Orka Technology Ltd.).

1.4.3 Antioxidant Capacity of Laying Hens 1.4.3.1 Sample Collection and Preparation

At the end of the experiment, three hens with body weights similar to the replicate average were randomly selected from each replicate and weighed. Blood samples were collected from the wing vein using heparin sodium as an anticoagulant, centrifuged at 3,600 r/min for 10 minutes to prepare plasma, which was stored at -20°C for subsequent analysis. After blood collection, the left lobe of the liver was excised from each hen, wrapped in aluminum foil, snap-frozen in liquid nitrogen, and stored at -80°C. For tissue homogenate preparation, 5 g of liver sample stored at -80°C was thawed and mixed with nine volumes of physiological saline to prepare a 10% tissue homogenate, which was centrifuged at 3,000 r/min for 10 minutes. The supernatant was collected and aliquoted for liver antioxidant index determination (all homogenization operations were performed on ice).

On day 56 of the experiment, three eggs were randomly collected from each replicate for determination of yolk antioxidant indices and cholesterol content.

1.4.3.2 Index Determination

Plasma, liver, and yolk MDA content and T-SOD activity, plasma and liver T-AOC, plasma GSH-Px activity, and yolk cholesterol content were all determined using assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis Experimental data are expressed as "mean ± standard deviation." All data were processed using Excel 2003 and analyzed using one-way ANOVA procedure in SPSS 16.0. When $P<0.05$, Duncan' s multiple range test was used for post-hoc comparisons.

2. Results

2.1 Effects of Dietary GPC on Alleviating Production Performance Decline in Old Laying Hens As shown in Table 2 , no significant differences were observed in average egg weight, average daily feed intake, or feed-to-egg ratio among the GPC groups and the control or TBHQ groups ($P>0.05$). During weeks 1-4, the laying rate of the GPC50 group tended to be higher than the other three groups ($P>0.05$). During weeks 5-8, the GPC50 group exhibited significantly higher laying rate compared to the control and TBHQ groups ($P<0.05$), with no significant differences among the other groups ($P>0.05$). Over the entire 8-week experimental period, the GPC50 group showed significantly higher laying rate than the control group ($P<0.05$), with no significant differences among the other three groups ($P>0.05$). Laying rates declined in all groups from the start through weeks 1-4 and weeks 5-8. Comparing the start and weeks 5-8, laying rate decreased by 5.2% in the control group, 3.3% in the TBHQ group,

0.2% in the GPC50 group, and 2.1% in the GPC100 group. Therefore, GPC can inhibit the decline in laying rate of old hens, with effects becoming more pronounced as the experimental period progressed.

2.2 Effects of Dietary GPC on Egg Quality of Old Laying Hens As shown in Table 3 , compared with the control and TBHQ groups, GPC supplementation had no significant effect on albumen height, yolk color, egg shape index, or eggshell strength during the experimental period (P>0.05). However, GPC significantly increased eggshell thickness and Haugh unit at week 8 (P<0.05). Over time, Haugh unit of eggs from old hens gradually decreased, but supplementation with TBHQ and GPC alleviated this decline. Comparing week 8 to week 4, Haugh unit decreased by 4.0% in the control group, 2.8% in the TBHQ group, 1.1% in the GPC50 group, and 1.9% in the GPC100 group. Thus, GPC can inhibit the decline in Haugh unit of eggs from old hens, with 50 mg/kg showing better effects than 100 mg/kg, and 100 mg/kg performing better than 200 mg/kg TBHQ.

2.3 Effects of Dietary GPC on Plasma Antioxidant Capacity of Laying Hens As shown in Table 4 , plasma MDA content in the GPC50 group was significantly lower than in the control, TBHQ, and GPC100 groups (P<0.05), with no significant differences among the latter three groups (P>0.05). Plasma T-AOC level in the GPC50 group was significantly higher than in the control group (P<0.05), but did not differ significantly from the TBHQ and GPC100 groups (P>0.05). Plasma T-SOD activity in both the TBHQ and GPC50 groups was significantly higher than in the control and GPC100 groups (P<0.05), with the GPC50 group showing greater improvement than the TBHQ group (P<0.05). No significant differences in plasma GSH-Px activity were observed among the four groups (P>0.05).

2.4 Effects of Dietary GPC on Liver Antioxidant Capacity of Old Laying Hens As shown in Table 5 , liver MDA content in the TBHQ and GPC50 groups was significantly lower than in the control and GPC100 groups (P<0.05), with no significant difference between the TBHQ and GPC50 groups (P>0.05). Liver T-AOC level in the GPC groups was significantly higher than in the control and TBHQ groups (P<0.05). Liver T-SOD activity in the GPC50 group was significantly higher than in the control and GPC100 groups (P<0.05), but did not differ significantly from the TBHQ group (P>0.05).

2.5 Effects of Dietary GPC on Egg Yolk Antioxidant Capacity and Cholesterol Content As shown in Table 6 , egg yolk T-SOD activity in the GPC groups was significantly higher than in the control group (P<0.05), while no significant difference was observed between the TBHQ and control groups (P>0.05). Egg yolk MDA content in all three treatment groups was significantly lower than in the control group (P<0.05), with no significant differences among the treatment groups (P>0.05). Egg yolk cholesterol content in the GPC groups

was significantly lower than in the control group ($P<0.05$), while no significant difference was observed between the TBHQ and control groups ($P>0.05$).

Aging is a physiological phenomenon characterized by the gradual decline of organ functions. However, oxidative stress can accelerate aging, while antioxidant activity can delay it. As high-producing animals, laying hens generate large amounts of reactive oxygen species through vigorous metabolism. Under normal conditions, the production and elimination of free radicals maintain relative equilibrium. As hens age, the activity of endogenous antioxidant enzymes declines, as does the ability to absorb or synthesize antioxidant vitamins and trace elements, resulting in reduced free radical scavenging capacity. Accumulation of excessive free radicals can cause lipid peroxidation, damage cell membranes, affect cell function, and lead to metabolic disorders that impact health and production. In this study, MDA content in plasma, liver, and egg yolk of the control group was significantly higher than in GPC-supplemented groups, consistent with Kara et al. who reported that dietary supplementation with 4% and 6% grape pomace significantly reduced plasma MDA content in 80-week-old laying hens. These findings indicate that oxidative-antioxidant imbalance occurs in old laying hens, with excess free radicals causing lipid peroxidation, making antioxidant research highly relevant.

The GPC structure contains multiple active phenolic hydroxyl groups that can donate protons to neutralize free radicals and regulate antioxidant response elements through the Nrf2 signaling pathway, thereby increasing antioxidant enzyme expression and reducing free radical regeneration. Zhao et al. reported that dietary supplementation with 100 mg/kg GPC significantly increased T-AOC in serum and liver, enhanced serum superoxide dismutase (SOD) activity and hydroxyl radical ($\cdot OH$) scavenging capacity, and improved serum GSH-Px activity in stressed piglets. Peng et al. found that grape seed powder significantly increased SOD and GSH-Px activities and decreased MDA content in aged rats. The present study demonstrated that dietary supplementation with 50 mg/kg GPC significantly increased T-SOD activity in plasma, liver, and egg yolk and improved plasma GSH-Px activity in old laying hens, consistent with our laboratory' s previous findings that low-dose GPC could restore T-SOD and GSH-Px activities and reduce MDA content in broiler chickens infected with coccidia.

TBHQ is a newly synthesized antioxidant with good antioxidant effects, demonstrating superior performance to BHA and BHT when applied in edible oils. Zhou et al. reported that dietary supplementation with 1% TBHQ significantly improved liver antioxidant levels in heat-stressed mice. The present study also showed that 200 mg/kg TBHQ significantly increased plasma T-SOD activity and decreased liver and egg yolk MDA content in old laying hens, with a trend toward increasing plasma GSH-Px activity and liver and egg yolk T-SOD activity. However, the overall antioxidant effect was not as pronounced as that of 50 mg/kg GPC. This may be attributed to the multiple active phenolic hydroxyl groups in GPC providing superior antioxidant effects compared to TBHQ, and

possibly because plant-derived GPC is more readily absorbed and utilized by animals, though the specific mechanisms require further investigation.

In addition to its antioxidant effects, GPC has been reported to inhibit egg cholesterol synthesis enzyme activity and increase cholesterol catabolism in laying hens, thereby reducing egg cholesterol content. The present study confirmed that dietary GPC supplementation significantly reduced egg yolk cholesterol content.

Egg quality affects not only hatching value but also table and market value. Albumen height and Haugh unit are important indicators of egg white quality and freshness, with higher Haugh units indicating better albumen viscosity and quality. Hen age and environmental stress can affect Haugh unit, which decreases with advancing hen age. Research indicates that protein denaturation or functional loss results from free radical damage to proteins. In old laying hens, accumulation of free radicals from prolonged oxidative metabolism may damage albumen formation and reduce Haugh unit. Under identical environmental conditions and hen ages, targeted exogenous supplementation represents the primary approach for improving Haugh unit. In this study, Haugh unit of eggs from the control group decreased gradually with hen age, while TBHQ showed some improvement and GPC demonstrated superior effects, with the GPC50 group performing best and showing significantly higher Haugh unit than the control group at week 8. Kaya et al. reported that grape seed and grape seed extract had no significant effects on egg breakage rate or feed conversion ratio but linearly increased Haugh unit. Eggshell thickness is a critical indicator of egg breakability, with thinner shells being more fragile. Calcium and phosphorus absorption capacity declines in old laying hens, resulting in poorer shell quality. The present study demonstrated that GPC supplementation significantly increased eggshell thickness.

Egg production is a complex physiological process regulated by the neuroendocrine system. GPC contains plant flavonoids that can interact with estrogen receptors in laying hens, altering endogenous estrogen composition and improving physiological status in old hens, thereby increasing laying rate. This study showed that dietary supplementation with 50 mg/kg GPC significantly inhibited the decline in laying rate of 64-week-old hens, consistent with findings by Hu et al. that low-dose GPC supplementation significantly improved laying rate. However, Kara et al. reported that dietary grape pomace had no significant effects on average daily feed intake, laying rate, or feed conversion ratio in laying hens. These discrepancies may be related to GPC feeding form, supplementation level, hen age, management practices, and environmental conditions.

High concentrations of high-polymerization-degree GPC have been reported to affect nutrient digestion and utilization. The GPC used in this study was a mixture of polymers, which may explain why supplementation at 50 mg/kg produced better effects on antioxidant capacity, egg quality, and production performance than 100 mg/kg throughout the experimental period.

For old laying hens, dietary supplementation with 50 mg/kg GPC provided superior antioxidant effects compared to 200 mg/kg TBHQ, slowed the decline in laying rate, improved Haugh unit and eggshell thickness, and reduced egg yolk cholesterol content.

References

[1] JAYAPRAKASHA G K, SINGH R P, SAKARIAH K K. Antioxidant activity of grape seed (Vitis vinifera) extracts peroxidation models vitro[J]. Food Chemistry, 2001, 73(3): 285-290.

[2] Song Zhenshuai, Zhuang Xueqin. Research progress on the application of natural plant antioxidants[C]//Proceedings of the 3rd Symposium on Poultry Diseases of the Poultry Disease Professional Committee of Shandong Animal Husbandry and Veterinary Association. Weifang: Shandong Animal Husbandry and Veterinary Association, 2013.

[3] Wang Ruojin, Yuan Baojing, Jin Lizhi. Review on the biological and comprehensive functions of natural plant extract additives[J]. Chinese Journal of Animal Science, 2015, 51(8): 72-78.

[4] BI S, HASLAM E, HE X Q. Gelatin-polyphenol interaction[J]. Journal of American Leather Chemists Association, 1995, 89(4): 98-104.

[5] MERFORT I, HEILMANN J, WEISS M, et al. Radical scavenger activity of three flavonoid metabolites studied by inhibition of chemiluminescence in human PMNs[J]. Planta Medica, 1996, 62(4): 289-292.

[6] BAGCHI D, GARG A, KROHN R L, et al. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro[J]. Research Communications in Molecular Pathology and Pharmacology, 1997, 95(2): 179-189.

[7] BUSSEROLLES J, GUEUX E, BALASIŃSKA B, et al. In vivo antioxidant activity of procyanidin-rich extracts from grape seed and pine (Pinus maritima) bark rats[J]. International Journal for Vitamin and Nutrition Research, 2006, 76(1): 22-27.

[8] Yin Jin, Hu Yixiu, Hu Yuming, et al. Effects of grape seed procyanidin extract on MDA, SOD and GSH-Px in mice[J]. China Tropical Medicine, 2009, 7(8): 1285-1286.

[9] Feng Dianjuan, Xu Guifa. Effects of grape seed extract on antioxidant capacity in humans[J]. Journal of Shandong University: Health Sciences, 2007, 45(10): 985-987.

[10] BRENES A, VIVEROS A, GOÑI I, et al. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens[J]. Poultry Science, 2008, 87(2): 307-316.

[11] Yang Jinyu, Wang Jing, Wu Shugeng, et al. Combined effects of grape procyanidins and wheat-based diets on growth performance and immune function of broiler chickens[J]. Chinese Journal of Animal Nutrition, 2014, 26(8): 2270-2280.

[12] Hu Rujiu, Wang Ying, Wang Xiao, et al. Effects of grape seed extract on production performance and egg yolk cholesterol content in laying hens[J]. Chinese Journal of Animal Nutrition, 2013, 25(9): 2074-2081.

[13] HARMAN D. Aging: a theory based on free radical and radiation chemistry[J]. Journal of Gerontology, 1956, 11(3): 298-300.

[14] KARA K, GÜCLÜ B K, BAYTOK E, et al. Effects of grape pomace supplementation to laying hen diet on performance, egg quality, egg lipid peroxidation and some biochemical parameters[J]. Journal of Applied Animal Research, 2016, 44(1): 303-310.

[15] Zhao Jiao, Zhou Zhaohong, Liang Xiaofang, et al. Effects of grape seed procyanidins and vitamin E on growth performance, serum redox status and liver oxidative damage in stressed piglets[J]. Scientia Agricultura Sinica, 2013, 46(19): 4157-4164.

[16] Peng Liang, Fu Weizhong, Yao Siyu, et al. Study on antioxidant effects of grape seed powder on naturally aging rats[J]. Chinese Journal of Health Laboratory Technology, 2012, 22(10): 2376-2378.

[17] Yang Jinyu. Regulatory effects and mechanisms of grape procyanidins on coccidia-infected broiler chickens[D]. Master's thesis. Beijing: Chinese Academy of Agricultural Sciences, 2015.

[18] Zhou Xin, Huang Yi, Li Yansen, et al. Alleviating effects of tert-butyl hydroquinone on liver oxidative damage in heat-stressed mice[J]. Chinese Journal of Animal Nutrition, 2014, 26(9): 2779-2788.

[19] DEL BAS J M, FERNÁNDEZ-LARREA J, BLAY M, et al. Grape seed procyanidins improve atherosclerotic risk index and induce liver CYP7A1 and SHP expression in healthy rats[J]. The FASEB Journal, 2005, 19(3): 479-481.

[20] HENRIQUE M R, ANTONIO G R, DELLA TORRE DA S J, et al. Effect of supplementation of diets for quails with vitamins A, D and E on performance of the birds and quality and enrichment of eggs[J]. Revista Brasileira de Zootecnia, 2011, 40(6): 1222-1230.

[21] SILVERSIDES F G, SCOTT T A. Effect of storage and layer age on quality of eggs from two lines of hens[J]. Poultry Science, 2001, 80(8): 1240-1245.

[22] Shao Hong, Wang Shiliang, You Zhongyi, et al. Oxygen free radicals and protein metabolism[J]. Foreign Medical Sciences: Biochemistry, 1990, 12(1): 42-44.

[23] KAYA A, YILDIRIM B A, KAYA H, et al. The effects of diets supplemented with crushed and extracted grape seed on performance, egg quality parameters,

yolk peroxidation and serum traits in laying hens[J]. European Poultry Science, 2014, 78: 1612-9199.

[24] ROSS J A, KASUM C M. Dietary flavonoids: bioavailability, metabolic effects, and safety[J]. Annual Review of Nutrition, 2002, 22: 19-34.

[25] GONTHIER M P, DONOVAN J L, TEXIER O, et al. Metabolism of dietary procyanidins in rats[J]. Free Radical Biology and Medicine, 2003, 35(8): 837-844.

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