Effects of Combined Selenium Yeast and Alfalfa Extract on Egg Quality, Antioxidant Capacity, and Cholesterol Metabolism in Late-Phase Laying Hens: Postprint

Authors: Yang Yu, Sun Yu, Sun Baosheng, Lu Yingjie, Li Jianhui, Zhang Junzhen

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Abstract

The present study aimed to investigate the effects of combined supplementation of different levels of selenium yeast and alfalfa extract on egg quality, antioxidant capacity, and cholesterol metabolism in laying hens during the late laying period. A total of 756 69-week-old Hy-Line Brown laying hens were selected and randomly divided into 7 groups with 6 replicates per group and 18 hens per replicate. The control group was fed the basal diet, while groups 2-7 were fed the basal diet supplemented with 0.2, 0.6, and 1.0 mg/kg selenium yeast in combination with 500 and 1,000 mg/kg alfalfa extract. The experiment consisted of a 2-week pre-trial period and an 8-week formal trial period. The results showed that: 1) Dietary supplementation with selenium yeast and alfalfa extract significantly increased laying rate ($P<0.05$), significantly decreased feed-to-egg ratio ($P<0.05$), and tended to improve eggshell strength, albumen height, and Haugh unit. 2) Dietary supplementation with selenium yeast and alfalfa extract extremely significantly increased plasma glutathione peroxidase (GSH-Px) and total superoxide dismutase (T-SOD) activities, liver GSH-Px activity, and hepatic GSH-Px1 and thioredoxin reductase 1 mRNA expression levels ($P<0.01$); extremely significantly decreased liver triglyceride content and 3-hydroxy-3-methylglutaryl-coenzyme A reductase mRNA expression level ($P<0.01$), extremely significantly increased hepatic cholesterol 7 - hydroxylase and sterol regulatory element-binding protein-1c mRNA expression levels ($P<0.01$); and extremely significantly increased yolk selenium content ($P<0.01$). 3) Supplementation with 0.6 mg/kg selenium yeast and 1,000 mg/kg alfalfa extract extremely significantly increased plasma total antioxidant capacity ($P<0.01$), extremely significantly decreased plasma MDA content ($P<0.01$), and extremely significantly decreased yolk cholesterol and triglyceride contents

as well as liver cholesterol content (P<0.01). In conclusion, dietary supplementation with selenium yeast and alfalfa extract can improve production performance, egg quality, antioxidant capacity, and lipid metabolism function in laying hens during the late laying period, with the combination of 0.6 mg/kg selenium yeast and 1,000 mg/kg alfalfa extract showing the best effect.

Full Text

Effects of Combined Selenium Yeast and Polysavone Supplementation on Egg Quality, Antioxidant Capacity, and Cholesterol Metabolism in Laying Hens During the Late Laying Period

YANG Yu, SUN Yu, SUN Baosheng, LU Yingjie, LI Jianhui, ZHANG Junzhen

(College of Animal Science and Technology, Shanxi Agricultural University, Taigu 030801, China)

Abstract

This experiment investigated the effects of dietary supplementation with different levels of selenium yeast and polysavone on egg quality, antioxidant capacity, and cholesterol metabolism in laying hens during the late laying period. A total of 756 healthy 69-week-old Hyline Brown laying hens with approximately 80% laying rate were randomly allocated into 7 groups, each consisting of 6 replicates of 18 hens. The control group received a basal diet, while groups 2–7 received the basal diet supplemented with 0.2, 0.6, or 1.0 mg/kg selenium yeast combined with 500 or 1000 mg/kg polysavone. The experiment included a 2-week preliminary period followed by an 8-week formal trial period.

The results demonstrated three key findings. First, dietary supplementation with selenium yeast and polysavone significantly increased laying rate and decreased feed-to-egg ratio ($P < 0.05$), while showing a trend toward improved eggshell strength, albumen height, and Haugh unit. Second, supplementation dramatically enhanced plasma glutathione peroxidase (GSH-Px) and total superoxide dismutase (T-SOD) activities, liver GSH-Px activity, and hepatic mRNA expression of GSH-Px1 and thioredoxin reductase 1 ($P < 0.01$). It also significantly reduced liver triglyceride content and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) mRNA expression ($P < 0.01$), while markedly increasing hepatic cholesterol 7 -hydroxylase (CYP7A1) and sterol regulatory element-binding protein-1c (SREBP-1c) mRNA expression ($P < 0.01$), and substantially elevating yolk selenium content ($P < 0.01$). Third, the combination of 0.6 mg/kg selenium yeast and 1000 mg/kg polysavone proved particularly effective, significantly increasing total antioxidant capacity (T-AOC) in plasma ($P < 0.01$), reducing plasma malondialdehyde (MDA) content ($P < 0.01$), and decreasing yolk cholesterol and triglyceride levels as well as liver cholesterol content ($P <$

0.01).

In conclusion, dietary supplementation with selenium yeast and polysavone improved production performance, egg quality, antioxidant capacity, and lipid metabolism in late-period laying hens, with the optimal combination being 0.6 mg/kg selenium yeast and 1000 mg/kg polysavone.

Keywords: selenium yeast; polysavone; laying hens; egg quality; antioxidant capacity; cholesterol metabolism

Introduction

Selenium is an essential trace element for both humans and animals. Selenium deficiency can lead to various diseases including white muscle disease, Keshan disease, liver cirrhosis, cancer, cataracts, cardiovascular disorders, and depression. Currently, over 40 countries worldwide suffer from low or deficient selenium status, with China being among the most severely affected. Since selenium in enriched eggs exists in organic forms that are readily absorbed and utilized by humans, developed countries have commercially produced selenium-enriched eggs using selenium-enriched yeast as an organic selenium source. Polysavone, a natural plant extract, possesses both antioxidant and immune-enhancing properties, suggesting its potential for applications in long production cycles or situations with serious disease risks.

Previous research has demonstrated the individual benefits of these supplements. Wang et al. reported that various selenium sources significantly increased serum and liver GSH-Px and T-SOD activities and total antioxidant capacity while reducing MDA and hydrogen peroxide content in geese, with selenium yeast showing superior antioxidant effects compared to sodium selenite and nano-selenium. Studies in laying hens have shown that selenium source and level significantly affect blood selenium content, GSH-Px activity, and T-AOC, with organic selenium being more readily deposited in eggs, liver, and muscle tissues than inorganic forms, thereby increasing egg selenium content and GSH-Px activity. Alfalfa-derived active compounds have been shown to enhance serum antioxidant capacity, reduce serum MDA content, and prevent cardiovascular disease. Dietary alfalfa saponins can increase GSH-Px activity in liver and serum while decreasing MDA content, protecting animals from lipid peroxide damage. Deng et al. found that aqueous alfalfa extract significantly reduced serum and liver cholesterol and lipid content in yolk, serum, and liver without affecting low-density lipoprotein cholesterol (LDL-C) or high-density lipoprotein cholesterol (HDL-C). Zhou et al. further analyzed the cholesterol-lowering effects of alfalfa saponin extract using digital gene expression profiling, identifying 120 mg/kg as the optimal supplementation level.

Our previous research demonstrated that dietary polysavone supplementation reduced total yolk cholesterol and triglyceride content while improving pro-

duction performance, eggshell strength, antioxidant capacity, and cholesterol metabolism in late-period laying hens, with 1000 mg/kg identified as the appropriate level. However, while both organic selenium and polysavone possess antioxidant properties, few studies have investigated their combined effects on egg quality, antioxidant capacity, and lipid metabolism. Therefore, this experiment was designed to evaluate the effects of combined selenium yeast and polysavone supplementation on production performance, selenium deposition, antioxidant capacity, and cholesterol metabolism-related gene expression in late-period laying hens, aiming to determine optimal supplementation levels and provide a theoretical basis for improving egg quality and sustainable development of the laying hen industry.

Materials and Methods

1.1 Experimental Period and Location

The experiment was conducted from June 11 to August 21, 2015, at Jianxin Breeding Farm in Datong County, Datong City, Shanxi Province, with laboratory analyses performed at the Provincial Key Laboratory of Animal Genetics, Breeding, and Nutrition at Shanxi Agricultural University.

1.2 Experimental Materials and Design

Polysavone, a natural plant extract from alfalfa containing 15% polysaccharides, 5% flavonoids, and 5% triterpenoid saponins, was purchased from Sichuan Deyang Sanfengyuan Technology Co., Ltd. Selenium yeast (containing 2000 mg/kg selenium) was obtained from Alltech Bio-Science Co., Ltd.

A total of 756 healthy 69-week-old Hyline Brown laying hens with approximately 80% laying rate were randomly divided into 7 groups with 6 replicates of 18 hens each. Group 1 served as the control, receiving a basal diet containing 0.20 mg/kg selenium. The basal diet was formulated according to the Hyline Brown Management Guide (2014) with modifications based on farm conditions; its composition and nutrient levels are presented in . Groups 2–7 received the basal diet supplemented with combinations of selenium yeast (0.2, 0.6, or 1.0 mg/kg) and polysavone (500 or 1000 mg/kg) as shown in . The experiment included a 2-week preliminary period during which all hens received the basal diet, followed by an 8-week formal experimental period.

1.3 Management Practices

Hens were housed in three-tier battery cages with 3 hens per cage, provided ad libitum access to feed and water, and exposed to 16 hours of daily light (natural plus artificial lighting). The environmental temperature was maintained at (20 ± 2)°C with relative humidity of 50–60%. Ventilation combined natural

air exchange with longitudinal negative pressure systems. Other management practices followed conventional farm procedures.

1.4 Sampling and Measurements

1.4.1 Production Performance Daily records were maintained for egg number and total egg weight per replicate. Feed consumption was measured weekly to calculate average daily feed intake (ADFI). Laying rate and feed-to-egg ratio were calculated for each group.

1.4.2 Egg Quality Assessment On days 14, 28, 42, and 56 of the experiment, 3 eggs were randomly selected from each replicate for quality analysis. Egg weight, albumen height, Haugh unit, and yolk color were measured using a multifunctional egg quality analyzer (ORKA, Israel). Eggshell strength was determined using an eggshell force gauge (ORKA, Israel), and eggshell thickness was measured with an eggshell thickness gauge (NFN380, Japan).

1.4.3 Plasma and Liver Biochemical Indices At the end of the experiment, one healthy hen with body weight close to the replicate average was selected from each replicate. Blood samples (5 mL) were collected from the wing vein after overnight fasting and centrifuged at 3,500 rpm for 10 minutes to obtain plasma, which was stored at -20°C. Liver samples were collected from the same anatomical location. Plasma GSH-Px and T-SOD activities, T-AOC, MDA, cholesterol, and triglyceride contents, as well as liver GSH-Px activity, cholesterol, and triglyceride contents were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute).

1.4.4 Yolk Composition Analysis At the end of the experiment, 2 eggs from each replicate were weighed, and yolks were separated from albumen. Yolk selenium content was determined by hydride generation atomic fluorescence spectrometry. Yolk cholesterol and triglyceride contents were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute).

1.4.5 Liver Total RNA Extraction and Reverse Transcription After blood collection, hens were euthanized by cervical dislocation. Liver tissue samples from the same anatomical location were rapidly collected under sterile conditions, snap-frozen in liquid nitrogen, and stored at -80°C until analysis. Total RNA was extracted using the Trizol method. RNA integrity, concentration, and purity were assessed by 1% agarose gel electrophoresis and spectrophotometry. Complementary DNA (cDNA) was synthesized using a reverse transcription kit according to manufacturer instructions and stored at -20°C.

1.4.6 Primer Design Primers for GSH-Px1, cholesterol 7 -hydroxylase (CYP7A1), sterol regulatory element-binding protein-1c (SREBP-1c), thioredoxin reductase 1 (TrxR1), and 3-hydroxy-3-methylglutaryl-CoA reductase

(HMGCR) were designed based on sequences from the GenBank database and synthesized by BGI (Beijing). Primer sequences are listed in .

1.4.7 Real-Time Quantitative PCR Real-time quantitative PCR was performed using SYBR® Premix Ex Taq™ II kit (TaKaRa, Dalian) with the synthesized cDNA as template. The reaction conditions were: initial denaturation at 95°C for 30 s, followed by 45 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 34 s. Melting curve analysis was performed from 60°C to 95°C at a rate of 0.5°C per 10 s. Relative mRNA expression levels were calculated using the 2^(-ΔΔCt) method with -actin as the internal reference gene.

1.5 Statistical Analysis

Data were analyzed using SPSS 19.0 software and expressed as "mean ± standard deviation." One-way ANOVA was used for group comparisons, with Duncan' s multiple range test for post-hoc analysis. Least significant difference (LSD) test was applied for extremely significant differences. mRNA expression data were analyzed by one-way ANOVA. $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered extremely significant.

Results

2.1 Effects on Production Performance

As shown in , average egg weight and ADFI did not differ significantly among groups ($P > 0.05$). Laying rate was significantly higher in all treatment groups except groups 3 and 6 compared to the control ($P < 0.05$). Feed-to-egg ratio was significantly lower in all treatment groups than in the control ($P < 0.05$), with group 5 showing the highest laying rate and lowest feed-to-egg ratio. The interaction between selenium yeast and polysavone significantly reduced feed-to-egg ratio ($P < 0.01$) and showed a trend toward increasing laying rate, though this difference was not statistically significant ($P > 0.05$).

2.2 Effects on Egg Quality and Yolk Selenium, Cholesterol, and Triglyceride Content

As presented in , dietary supplementation with selenium yeast and polysavone showed a trend toward increasing Haugh unit, with group 4 exhibiting significantly higher values than the control ($P < 0.05$). Eggshell strength was significantly higher in groups 2, 4, and 5 compared to the control ($P < 0.05$), while other egg quality parameters did not differ among groups ($P > 0.05$). Yolk selenium content increased with selenium yeast supplementation level and was significantly higher in all treatment groups than in the control ($P < 0.01$). Yolk cholesterol and triglyceride contents were lower in all treatment groups compared to the control, with groups 3 and 5 showing extremely significant

reductions in cholesterol ($P < 0.01$) and groups 3, 5, and 7 showing extremely significant reductions in triglycerides ($P < 0.01$). The interaction between selenium yeast and polysavone did not significantly affect egg quality parameters or yolk selenium, cholesterol, and triglyceride contents ($P > 0.05$).

2.3 Effects on Plasma and Liver Antioxidant Capacity

As shown in , plasma and liver GSH-Px activities were significantly higher in all treatment groups compared to the control ($P < 0.01$). Plasma T-SOD activity was also significantly elevated in all treatment groups ($P < 0.01$). Plasma T-AOC was significantly higher in all treatment groups except groups 2 and 3 compared to the control ($P < 0.01$). Plasma MDA content was significantly lower in groups 2, 4, and 5 compared to the control ($P < 0.05$), with group 5 showing an extremely significant reduction ($P < 0.01$). The interaction between selenium yeast and polysavone significantly enhanced plasma and liver GSH-Px activities and plasma T-SOD activity ($P < 0.01$), but did not significantly affect plasma T-AOC or MDA content ($P > 0.05$).

2.4 Effects on Lipid Metabolism

As presented in , plasma cholesterol content showed a decreasing trend in treatment groups, though differences were not statistically significant ($P > 0.05$). Liver cholesterol and triglyceride contents were lower in all treatment groups compared to the control, with groups 5, 6, and 7 showing extremely significant reductions in liver cholesterol ($P < 0.01$) and all treatment groups showing extremely significant reductions in liver triglycerides ($P < 0.01$). The interaction between selenium yeast and polysavone significantly affected liver cholesterol content ($P < 0.05$) and extremely significantly affected liver triglyceride content ($P < 0.01$).

2.5 Effects on Hepatic mRNA Expression of Antioxidant and Lipid Metabolism-Related Genes

As shown in , dietary supplementation with selenium yeast and polysavone significantly increased hepatic mRNA expression of TrxR1, CYP7A1, and SREBP-1c ($P < 0.01$), and significantly enhanced GSH-Px1 mRNA expression ($P < 0.05$), which decreased significantly with increasing selenium yeast levels ($P < 0.01$). Supplementation also significantly reduced HMGCR mRNA expression ($P < 0.01$), with group 5 showing the lowest expression that was significantly lower than group 7 ($P < 0.05$).

Discussion

3.1 Effects on Production Performance

Previous studies by Zhu et al. and Pavlović et al. demonstrated that organic selenium supplementation improved laying rate and daily egg production while reducing feed-to-egg ratio. Similarly, Yang et al. and Xie reported that polysavone significantly increased laying rate and decreased feed-to-egg ratio. Research on the combined effects of selenium yeast and polysavone remains limited. Our results indicate that combined supplementation significantly improved laying rate and reduced feed-to-egg ratio, slowing the decline in production performance after 71 weeks of age more effectively than either supplement alone. This suggests synergistic effects between selenium yeast and polysavone in improving metabolic status.

3.2 Effects on Egg Quality

Conventional egg quality indicators include eggshell thickness, eggshell strength, egg shape index, albumen height, Haugh unit, and yolk color. Eggshell strength and thickness directly affect resistance to breakage, while albumen height and Haugh unit reflect egg freshness—higher values indicate superior albumen quality and viscosity. Yolk color primarily depends on the types and quantities of carotenoids consumed.

Guo et al. reported that selenium yeast supplementation during summer significantly improved egg quality, including eggshell thickness, albumen height, and Haugh unit. However, Pavlović et al. found no significant effects of selenium yeast on eggshell quality, thickness, strength, or shape index. Polysavone has been shown to significantly increase eggshell strength without affecting thickness, while alfalfa saponins had no significant impact on conventional egg quality parameters.

In our study, combined supplementation showed a trend toward increasing albumen height and Haugh unit, with significant improvements observed at 0.6 mg/kg selenium yeast and 500 mg/kg polysavone. This suggests that enhanced antioxidant activity improved egg quality. Although eggshell strength increased in several groups, eggshell thickness showed no consistent pattern, possibly due to active components in selenium yeast and polysavone affecting calcium and phosphorus absorption, thereby increasing eggshell density and strength—a finding consistent with Invernizzi et al.

3.3 Effects on Antioxidant Capacity

Antioxidant function involves clearing lipid peroxidation products (MDA, hydroperoxides, hydrogen peroxide) to protect against free radical damage. Key indicators include GSH-Px, T-SOD, T-AOC, MDA, and TrxR. Jing et al. reported that selenium supplementation increased plasma GSH-Px and T-SOD activities while reducing MDA content in laying hens. Lu et al. demonstrated

that selenium increased thioredoxin 1 and 2 protein expression. Shi et al. found that alfalfa saponins enhanced GSH-Px and T-SOD activities while decreasing MDA content in piglets.

Our results confirm these findings, showing that the interaction between selenium yeast and polysavone significantly increased plasma and liver GSH-Px and plasma T-SOD activities while reducing plasma MDA content. Consistent patterns in hepatic GSH-Px1 and TrxR1 mRNA expression suggest that combined supplementation affects these enzymes at the transcriptional level, synergistically enhancing antioxidant enzyme activities and overall antioxidant capacity.

3.4 Effects on Cholesterol and Triglyceride Content

The liver plays a central role in lipid metabolism, with cholesterol conversion to bile acids representing a major metabolic pathway. Key regulatory enzymes include CYP7A1 (the rate-limiting enzyme in bile acid synthesis), SREBP-1c (which promotes fatty acid synthesis and cholesterol esterification), and HMGCR (the rate-limiting enzyme in endogenous cholesterol synthesis). Previous research indicated that selenium supplementation had no significant effect on blood cholesterol and triglyceride levels, while polysavone reduced cholesterol and triglyceride content in blood, liver, and eggs. Zhou et al. proposed that alfalfa saponins reduce cholesterol by promoting hepatic cholesterol efflux and decreasing ovarian deposition.

Our study confirms that combined supplementation significantly reduced liver cholesterol and triglyceride contents, consequently decreasing yolk cholesterol and triglyceride levels. The observed increase in hepatic CYP7A1 and SREBP-1c mRNA expression and decrease in HMGCR mRNA expression suggests multiple mechanisms: reduced HMGCR expression decreases cholesterol synthesis, increased SREBP-1c expression promotes fatty acid synthesis and cholesterol esterification, and elevated CYP7A1 expression accelerates cholesterol conversion to bile acid, thereby reducing ovarian cholesterol deposition. Thus, combined supplementation affects both cholesterol synthesis and metabolism, ultimately reducing cholesterol and triglyceride accumulation in liver and yolk.

3.5 Effects on Yolk Selenium Content

Previous studies demonstrate that egg selenium content increases with dietary selenium level, with organic selenium being more effective than inorganic forms. Limited research exists on the combined effects of polysavone and selenium yeast, though polysavone is believed to enhance trace element absorption through gastrointestinal metabolism. Our results show that combined supplementation significantly increased yolk selenium content in a dose-dependent manner without reaching a plateau, indicating that selenium levels did not exceed tolerance limits. Although the combination of 1.0 mg/kg selenium yeast with polysavone showed lower antioxidant performance than the 0.6 mg/kg selenium yeast and 1000 mg/kg polysavone combination, all treatment groups maintained signif-

icantly higher antioxidant indices and gene expression than the control, confirming that 0.6 mg/kg selenium yeast with 1000 mg/kg polysavone provides optimal results.

Conclusion

Dietary supplementation with selenium yeast and polysavone in late-period laying hens effectively increased yolk selenium content, reduced yolk cholesterol and triglyceride levels, improved eggshell strength, enhanced production performance, and boosted antioxidant capacity and cholesterol metabolism, with a trend toward improved albumen height and Haugh unit. The optimal combination was 0.6 mg/kg selenium yeast and 1000 mg/kg polysavone.

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