Effects of Selenium Yeast on Production Performance, Egg Quality, Antioxidant Capacity, Lipid Metabolism, and Related Gene Expression in Laying Hens during the Late Laying Period: Postprint

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

Abstract

This experiment aimed to investigate the effects of yeast selenium on production performance, egg quality, antioxidant capacity, lipid metabolism, and related gene expression in laying hens during the late laying period. A total of 432 healthy 69-week-old Hy-Line Brown laying hens were selected and randomly divided into 4 groups with 6 replicates per group and 18 hens per replicate. The control group was fed a basal diet (containing 0.2 mg/kg sodium selenite, expressed as selenium), while the experimental groups were supplemented with 0.2, 0.6, and 1.0 mg/kg yeast selenium (expressed as selenium) on top of the basal diet, with a 2-week preliminary period and an 8-week formal experimental period. The results showed: 1) Compared with the control group, dietary supplementation with 0.2, 0.6, and 1.0 mg/kg yeast selenium significantly increased the laying rate ($P<0.05$) and significantly reduced the feed-to-egg ratio ($P<0.05$); dietary supplementation with 0.2 mg/kg yeast selenium significantly increased the average daily feed intake ($P<0.05$). In addition, the feed-to-egg ratio in the 0.6 mg/kg group was significantly lower than that in the 0.2 and 1.0 mg/kg groups ($P<0.05$). 2) Compared with the control, dietary supplementation with 0.2 mg/kg yeast selenium significantly increased eggshell color on day 14 ($P<0.05$); dietary supplementation with 0.6 mg/kg yeast selenium highly significantly improved eggshell strength on day 42 ($P<0.01$) and significantly increased eggshell thickness on day 42 ($P<0.05$); dietary supplementation with 1.0 mg/kg yeast selenium significantly increased albumen height on day 14 and eggshell strength on day 42, and significantly increased eggshell thickness on day 42 ($P<0.05$). With the increase of dietary yeast selenium supplementation level, yolk selenium content was highly significantly increased ($P<0.01$). 3) Compared with the control group, dietary supplementation with 0.2 mg/kg yeast selenium highly significantly increased plasma total superoxide dismutase (T-SOD) activity ($P<0.01$) and significantly decreased plasma malondialdehyde (MDA) content ($P<0.05$); dietary supplementation with 0.6 mg/kg yeast selenium highly significantly increased plasma glutathione peroxidase (GSH-Px), T-SOD activity and total antioxidant capacity (T-AOC) and liver GSH-Px activity ($P<0.01$), and significantly decreased plasma MDA content ($P<0.05$); dietary supplementation with 1.0 mg/kg yeast selenium highly significantly increased plasma GSH-Px, T-SOD and liver GSH-Px activity ($P<0.01$), significantly increased plasma T-AOC ($P<0.05$), and highly significantly decreased MDA content ($P<0.01$). 4) Compared with the control group, dietary supplementation with 0.6 and 1.0 mg/kg yeast selenium significantly decreased liver cholesterol and yolk triglyceride contents ($P<0.05$); dietary supplementation with 1.0 mg/kg yeast selenium also highly significantly decreased liver triglyceride content ($P<0.01$). The liver cholesterol and yolk triglyceride contents in the 0.6 and 1.0 mg/kg groups were significantly lower than those in the 0.2 mg/kg group ($P<0.05$). 5) Compared with the control group, dietary supplementation with 0.6 mg/kg yeast selenium highly significantly increased the relative mRNA expression levels of liver glutathione peroxidase 1 (GPx1), thioredoxin reductase 1 (Trxr1), and sterol regulatory element-binding protein-1c (SREBP-1c) ($P<0.01$), highly significantly decreased the relative mRNA expression level of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) ($P<0.01$), but had no significant effect on the relative mRNA expression level of cholesterol 7 -hydroxylase (Cyp7a1) ($P>0.05$). Thus, dietary supplementation with yeast selenium could improve production performance, eggshell strength, and yolk selenium content, and enhance antioxidant capacity and cholesterol metabolism in laying hens during the late laying period. Through regression analysis, it was concluded that supplementation with 0.60–0.77 mg/kg yeast selenium in the diet (containing 0.20 mg/kg selenium in the form of sodium selenite) yielded better results.

Full Text

Effects of Selenium Yeast Supplementation on Performance, Egg Quality, Antioxidant and Lipid Metabolism and Their Related Gene Expression in Laying Hens at Late Laying Period

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Abstract

This experiment was conducted to investigate the effects of selenium yeast supplementation on performance, egg quality, antioxidant and lipid metabolism and their related gene expression in laying hens at late laying period. A total of 432 healthy Hyline brown hens at 69 weeks of age were randomly divided into 4 groups with 6 replicates per group and 18 hens per replicate. The control group was fed a basal diet containing 0.2 mg/kg sodium selenite (as selenium), while the experimental groups were fed the basal diet supplemented with 0.2, 0.6, and 1.0 mg/kg selenium yeast (as selenium). The preliminary period lasted for 2 weeks, followed by an 8-week formal experimental period. The results showed: 1) Compared with the control group, dietary supplementation with 0.2, 0.6, and 1.0 mg/kg selenium yeast significantly increased laying rate and significantly reduced feed/egg ratio ($P<0.05$). Dietary supplementation with 0.2 mg/kg selenium yeast significantly increased average daily feed intake ($P<0.05$). Moreover, the feed/egg ratio in the 0.6 mg/kg group was significantly lower than that in the 0.2 and 1.0 mg/kg groups ($P<0.05$). 2) Compared with the control group, dietary supplementation with 0.2 mg/kg selenium yeast significantly increased eggshell color at day 14 ($P<0.05$). Supplementation with 0.6 mg/kg selenium yeast extremely significantly increased eggshell strength at day 42 ($P<0.01$) and significantly increased eggshell thickness at day 42 ($P<0.05$). Supplementation with 1.0 mg/kg selenium yeast significantly increased albumen height at day 14 and eggshell strength at day 42, and significantly increased eggshell thickness at day 42 ($P<0.05$). With increasing dietary selenium yeast supplementation, yolk selenium content increased extremely significantly ($P<0.01$). 3) Compared with the control group, dietary supplementation with 0.2 mg/kg selenium yeast extremely significantly increased plasma total superoxide dismutase (T-SOD) activity ($P<0.01$) and significantly decreased plasma malondialdehyde (MDA) content ($P<0.05$). Supplementation with 0.6 mg/kg selenium yeast extremely significantly increased plasma glutathione peroxidase (GSH-Px) and T-SOD activities, total antioxidant capacity (T-AOC), and liver GSH-Px activity ($P<0.01$), and significantly decreased plasma MDA content ($P<0.05$). Supplementation with 1.0 mg/kg selenium yeast extremely significantly increased plasma GSH-Px and T-SOD activities and liver GSH-Px activity ($P<0.01$), significantly increased plasma T-AOC ($P<0.05$), and extremely significantly decreased MDA content ($P<0.01$). 4) Compared with the control group, dietary supplementation with 0.6 and 1.0 mg/kg selenium yeast significantly decreased liver cholesterol and yolk triglyceride contents ($P<0.05$). Supplementation with 1.0 mg/kg selenium yeast also extremely significantly decreased liver triglyceride content ($P<0.01$). The liver cholesterol and yolk triglyceride contents in the 0.6 and 1.0 mg/kg groups were significantly lower than those in the 0.2 mg/kg group ($P<0.05$). 5) Compared with the control group, dietary supplementation with 0.6 mg/kg selenium yeast extremely significantly increased the mRNA relative expression levels of glutathione peroxidase 1 (GPx1), thioredoxin reductase 1 (Trxr1), and sterol regulatory element-binding protein-1c (SREBP-1c) in liver ($P<0.01$), and extremely significantly decreased the mRNA relative expression level of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) ($P<0.01$), while having no significant effect on cholesterol 7 -hydroxylase (Cyp7a1) mRNA relative expression level ($P>0.05$). In conclusion, dietary selenium yeast supplementation can improve performance, eggshell strength, and yolk selenium content, and enhance antioxidant capacity and cholesterol metabolism in laying hens at late laying period. Regression analysis indicated that better effects were achieved when adding 0.60-0.77 mg/kg selenium yeast to the diet (containing 0.20 mg/kg selenium from sodium selenite).

Keywords: selenium yeast; laying hens at late laying period; egg quality; antioxidant; lipid metabolism

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Selenium has been recognized as an essential nutrient in diets for over 40 years. The traditional method of selenium supplementation involves adding sodium selenite as a selenium source additive in diets. However, sodium selenite has several drawbacks including high toxicity, safety concerns, low bioavailability, environmental pollution, and pro-oxidative effects, leading many countries to prohibit its use in feed. Organic selenium exhibits characteristics such as high absorption rate, strong biological activity, low toxicity, and minimal environmental pollution in animals. Selenium deficiency can lead to Keshan disease, cancer, cataracts, cardiovascular diseases, and depression, making selenium-enriched eggs an important medium for selenium supplementation. Studies have shown that adding selenium-enriched probiotics can significantly improve laying rate, daily egg production, and egg weight while reducing feed/egg ratio in laying hens, whereas adding inorganic selenium has no significant effects on these parameters. Both selenium yeast and selenium-enriched alfalfa can significantly increase the laying rate of Roman laying hens, while sodium selenite has no significant effect on laying rate. Selenium source and level have significant effects on blood selenium content, glutathione peroxidase (GSH-Px) activity, and total antioxidant capacity (T-AOC) in laying hens. Organic selenium is more readily deposited in tissues such as eggs, liver, and muscle compared to inorganic selenium, significantly increasing selenium content in eggs and GSH-Px activity. After selenium supplementation, the decline in Haugh unit (an important indicator for evaluating egg freshness) is reduced, with organic selenium showing better effects than inorganic selenium in slowing this decline.

Zhao Yuxin reported that adding sodium selenite to diets had no significant effect on serum cholesterol and triglyceride contents in laying hens, although a decreasing trend was observed. Wang Zeming found that different selenium sources in diets had no significant effect on plasma cholesterol and triglyceride contents in laying hens. However, Attia et al. demonstrated that the interaction of organic and inorganic selenium supplementation could significantly reduce plasma cholesterol content and increase yolk selenium content, with the 0.4 mg/kg organic selenium group showing the best results. While studies on the effects of selenium on production performance, antioxidant function, and plasma cholesterol content in chickens have been reported, the results have been inconsistent and research on molecular mechanisms is scarce. Therefore, this experiment was conducted to investigate the effects of different levels of selenium yeast supplementation on performance, egg quality, antioxidant and lipid metabolism, and related gene expression in laying hens at late laying period, with preliminary exploration of the molecular mechanisms, aiming to determine the appropriate supplementation level and provide a theoretical basis for the rational use of selenium yeast and increasing egg selenium content.

1 Materials and Methods

1.1 Experimental Material

The selenium yeast (selenium yeast, SY) used in this experiment was purchased from Alltech Bio-Science Co., Ltd., with a selenium content of 2,000 mg/kg.

1.2 Experimental Time and Location

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

1.3 Experimental Design

A total of 432 healthy Hyline brown hens at 69 weeks of age were randomly divided into 4 groups with 6 replicates per group and 18 hens per replicate. The control group was fed a basal diet containing 0.2 mg/kg sodium selenite (as selenium), while the experimental groups were fed the basal diet supplemented with 0.2, 0.6, and 1.0 mg/kg selenium yeast (as selenium). The preliminary period lasted for 2 weeks, during which no significant differences in laying rate and egg weight were observed between the control and experimental groups ($P>0.05$), after which the formal experiment began and lasted for 8 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 (Air-Dry Basis) %

Items	Content
Ingredients	
Corn	
Soybean oil	
Soybean meal	
Rapeseed meal	
CaHPO	
Limestone	
NaCl	
Met	
Vitamin premix[1]	
VE powder	
Choline	
Trace mineral premix[2]	

Items	Content
NaHCO	
Shell red pigment	
Total	
Nutrient Levels	
ME/(MJ/kg)	
Crude protein	
Lys	
Met	
Calcium	
Phosphorus	

[1] Each kilogram of vitamin premix contained: retinol acetate 51,000,000 IU, VD 13,500,000 IU, DL- -tocopheryl acetate 50,000 mg, VK 5,000 mg, VB 5,000 mg, VB 25,000 mg, VB 14,000 mg, VB 65 mg, folic acid 4,000 mg, nicotinamide 90,000 mg, D-calcium pantothenate 32,000 mg, D-biotin 100 mg.

[2] Each kilogram of trace mineral premix contained: Mn (as manganese sulfate) 60 g, Fe (as ferrous sulfate) 60 g, Cu (as copper sulfate) 8 g, Se (as sodium selenite) 100 mg, Zn (as zinc sulfate) 60 g, I (as potassium iodide) 130 mg.

1.4 Management

The experimental hens were raised in three-tier cage systems with 3 hens per cage. They had free access to feed and water, with natural light supplemented by artificial light to 16 h. The temperature was maintained at (20±2) °C, relative humidity at 50%-60%, with natural ventilation combined with longitudinal negative pressure ventilation. Other management practices followed the routine procedures of the breeding farm.

1.5 Measurement Items and Methods

1.5.1 Performance During the experimental period, daily records were kept for the number of eggs laid and total egg weight per replicate. Feed consumption was recorded every 7 days to calculate average daily feed intake. Laying rate, average egg weight, and feed/egg ratio were then calculated on a replicate basis.

1.5.2 Egg Quality On days 14, 28, 42, and 56 of the experiment, 3 eggs were randomly selected from each replicate to determine egg weight, egg shape index, eggshell strength, albumen height, Haugh unit, yolk color, and eggshell thickness, with average values calculated.

1.5.3 Plasma and Tissue Related Indexes At the end of the experiment, one hen with good health and 接近 average body weight was randomly selected from each replicate. Blood (5 mL) was collected from the wing vein after fasting and centrifuged at 3,500 r/min for 10 min to prepare plasma, which was aliquoted and stored at -20 °C. The hens were then euthanized by jugular venipuncture, and the liver was rapidly removed under aseptic conditions, placed in liquid nitrogen, and stored at -80 °C for later analysis. Plasma GSH-Px activity, T-AOC, T-SOD activity, MDA, cholesterol, and triglyceride contents, as well as liver GSH-Px activity and cholesterol and triglyceride contents were measured using corresponding kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.5.4 Yolk Related Indexes At the end of the experiment, 2 eggs were selected from each replicate and weighed. After breaking the shell, the albumen and yolk were separated. Yolk selenium content was determined using hydride generation atomic fluorescence analysis. Yolk cholesterol and triglyceride contents were measured using corresponding kits from Nanjing Jiancheng Bioengineering Institute.

1.5.5 Liver RNA Extraction and Reverse Transcription Total RNA was extracted from liver tissue using the Trizol method. RNA integrity, concentration, and purity were determined using 1% agarose gel electrophoresis and a nucleic acid-protein analyzer. cDNA was synthesized according to the reverse transcription kit instructions and stored at -20 °C for later use.

1.5.6 Primer Design Primers were designed based on sequences of glutathione peroxidase 1 (GPx1), cholesterol 7 -hydroxylase (Cyp7a1), sterol regulatory element-binding protein-1c (SREBP-1c), thioredoxin reductase 1 (Trxr1), and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) from sequencing databases. Primers were synthesized by BGI (Beijing). Primer sequences are shown in Table 2 .

Table 2 Primer Sequences

Genes	GenBank Accession	Primer Sequence (5´-3´)	Product Length/bp
Glutathione peroxidase 1	NM_001277853	F: ACGGCGCATCTTCCAAAGR: TGTTCCCCCAACCATTTCTC	
Cholesterol 7 -hydroxylase Cyp7a1	NM_001001753	F: CCGAGTTGCTAAGGAGGATTR: CGTTGCGGTAGAAGTCAGTC	

Genes	GenBank Accession	Primer Sequence (5´-3´)	Product Length/bp
Sterol regulatory element-binding protein-1c SREBP-1c	NM_204126	F: GCCCTCTGTGCCTTTGTCTTCR: ACTCAGCCATGATGCTTCTTCC	
Thioredoxin reductase 1 Trxr1	NM_001030762	F: TACGCCTCTGGGAAATTCGTR: CTTGCAAGGCTTGTCCCAGTA	
3-hydroxy-3-methylglutaryl coenzyme A HMGCR	NM_204485	F: CTGCAGGAAACAATCTGGACR: GCTTTGGTTGATGACTCTGCT	
-actin	X00182	F: TGCGTGACATCAAGGAGAAGR: TGCCAGGGTACATTGTGGTA	

1.5.7 Real-Time Fluorescent Quantitative PCR Using the synthesized cDNA as template, PCR was performed on a real-time fluorescent quantitative PCR instrument according to the SYBR® Premix Ex Taq™ II kit (TaKaRa Co., Ltd., Dalian) instructions. Reaction conditions were: pre-denaturation at 95 °C for 30 s, followed by 45 cycles of 95 °C for 5 s and 60 °C for 34 s. Melting curve analysis was performed at 95 °C for 10 s, 60 °C for 1 min, then temperature was increased to 95 °C at a rate of 0.5 °C per 10 s. Using the relative quantitative analysis method with -actin as the internal reference gene, the relative mRNA expression levels of target genes were calculated using the $2^{(-\Delta\Delta Ct)}$ method.

1.6 Data Processing and Statistical Analysis Data were analyzed using one-way ANOVA with SPSS 19.0 statistical software. All data were expressed as "mean ± standard deviation." Duncan's multiple range test was used to compare significant differences between groups, and LSD test was used for extremely significant difference comparisons. Linear and quadratic curve regression analyses were also performed on corresponding data.

2 Results

2.1 Effects of Selenium Yeast Supplementation on Performance of Laying Hens at Late Laying Period

As shown in Table 3 , compared with the control group, dietary supplementation with 0.2, 0.6, and 1.0 mg/kg selenium yeast significantly increased laying rate and significantly reduced feed/egg ratio (P<0.05), but had no significant effect on average egg weight (P>0.05). Dietary supplementation with 0.2 mg/kg selenium yeast significantly increased average daily feed intake (P<0.05). There were no significant differences in laying rate, average egg weight, and average daily feed intake among the experimental groups (P>0.05), but the feed/egg ratio in the 0.6 mg/kg group was significantly lower than that in the 0.2 and 1.0 mg/kg groups (P<0.05).

Table 3 Effects of Selenium Yeast Supplementation on Performance of Laying Hens at Late Laying Period

Selenium yeast additive amount/(mg/kg)	Laying rate/%	Average egg weight/g	ADFI/g	Feed/egg
0 (Control)	71.27±5.46^a	65.88±2.66	119.83±0.69^a	2.49±0.01^a
0.2	77.19±2.33^b	64.61±3.69	121.17±0.55^b	2.45±0.01^b
0.6	77.72±1.65^b	65.62±4.30	120.39±0.58^ab	2.40±0.01^c
1.0	77.11±2.05^b	63.90±3.58	120.72±0.56^ab	2.64±0.01^b

In the same column, values with different small letter superscripts mean significant difference (P<0.05), and with different capital letter superscripts mean extremely significant difference (P<0.01), while with no or the same letter superscripts mean no significant difference (P>0.05). The same as Table 5 , Table 6 and Table 7 .

2.2 Effects of Selenium Yeast Supplementation on Egg Quality of Laying Hens at Late Laying Period

As shown in Table 4 , compared with the control group, dietary supplementation with 1.0 mg/kg selenium yeast significantly increased albumen height at day 14 (P<0.05). Supplementation with 0.2 mg/kg selenium yeast significantly increased eggshell color at day 14 (P<0.05). Supplementation with 0.6 mg/kg selenium yeast extremely significantly increased eggshell strength at day 42 (P<0.01), and significantly increased eggshell thickness at day 42 (P<0.05). Supplementation with 1.0 mg/kg selenium yeast significantly increased eggshell strength at day 42 (P<0.05) and significantly increased eggshell thickness at day 42 (P<0.05). With increasing dietary selenium yeast supplementation, yolk selenium content increased extremely significantly (P<0.01).

Table 4 Effects of Selenium Yeast Supplementation on Egg Quality of Laying Hens at Late Laying Period

Items	Day 14	Day 28	Day 42	Day 56
Albumen height/mm				
0 (Control)	4.54±1.01^b	5.78±1.33	5.55±0.81	4.80±1.70
0.2	5.56±0.68^ab	5.36±1.16	5.34±0.84	4.50±0.80
0.6	5.52±0.81^ab	5.43±1.16	5.88±1.18	5.20±1.20
1.0	5.80±0.24^a	4.98±0.58	5.56±1.24	4.70±2.00
Haugh unit				
0 (Control)	58.72±11.40	71.53±11.50	70.47±6.51	61.60±10.90
0.2	68.36±6.07	70.04±8.70	69.76±7.16	59.66±6.86
0.6	70.00±8.19	69.28±11.20	73.62±9.62	67.47±9.68
1.0	69.74±3.91	66.52±4.57	71.38±10.71	60.13±21.70
Egg shape index				
0 (Control)	1.31±0.04	1.32±0.02	1.35±0.06	1.33±0.08^ab
0.2	1.32±0.06	1.35±0.04	1.36±0.05	1.33±0.04^ab
0.6	1.34±0.02	1.33±0.03	1.35±0.03	1.27±0.03^b
1.0	1.33±0.03	1.34±0.06	1.35±0.05	1.36±0.07^a
Yolk color				
0 (Control)	4.83±0.41^b	6.00±1.41	6.00±1.41	5.66±1.15
0.2	6.00±1.09^a	5.60±1.34	5.6±1.34	6.16±1.33
0.6	4.67±0.51^b	4.83±1.17	6.17±1.33	6.80±2.17
1.0	5.33±0.82^ab	5.83±1.33	6.33±1.51	6.00±1.15
Eggshell strength/(kg/m²)				
0 (Control)	2.92±0.37	2.89±0.35	2.28±0.74^Bb	2.43±0.77
0.2	3.13±0.59	3.06±0.57	2.97±0.78^ABab	2.77±0.86
0.6	2.97±0.16	3.16±0.40	3.58±0.79^Aa	2.97±0.77
1.0	2.93±0.46	3.16±0.81	3.40±0.32^ABa	3.16±0.86
Eggshell thickness/mm				
0 (Control)	0.36±0.02	0.34±0.03	0.34±0.02^b	0.37±0.03
0.2	0.37±0.04	0.36±0.04	0.36±0.03^ab	0.35±0.05
0.6	0.37±0.02	0.35±0.02	0.38±0.02^a	0.38±0.03
1.0	0.37±0.03	0.35±0.03	0.38±0.02^a	0.38±0.02
Yolk selenium content/($\times 10^2$ g/g)				
0 (Control)	-	-	-	28.74±0.96^A
0.2	-	-	-	36.66±1.62^B
0.6	-	-	-	49.59±1.40^C
1.0	-	-	-	60.08±1.40^D

In the same row, values with different small letter superscripts mean significant difference ($P<0.05$), and with different capital letter superscripts mean extremely significant difference ($P<0.01$), while with no or the same letter superscripts mean no significant difference ($P>0.05$).

2.3 Effects of Selenium Yeast Supplementation on Antioxidant Indexes of Laying Hens at Late Laying Period

As shown in Table 5 , compared with the control group, dietary supplementation with 0.2 mg/kg selenium yeast extremely significantly increased plasma T-SOD activity (P<0.01) and significantly decreased plasma MDA content (P<0.05), but had no significant effect on plasma GSH-Px activity, T-AOC, or liver GSH-Px activity (P>0.05). Supplementation with 0.6 mg/kg selenium yeast extremely significantly increased plasma GSH-Px and T-SOD activities, T-AOC, and liver GSH-Px activity (P<0.01), and significantly decreased plasma MDA content (P<0.05). Supplementation with 1.0 mg/kg selenium yeast extremely significantly increased plasma GSH-Px and T-SOD activities and liver GSH-Px activity (P<0.01), significantly increased plasma T-AOC (P<0.05), and extremely significantly decreased MDA content (P<0.01).

Table 5 Effects of Selenium Yeast Supplementation on Antioxidant Indexes of Laying Hens at Late Laying Period

Selenium yeast additive amount/(mg/kg)	Plasma GSH-Px/(U/mL)	Plasma T-AOC/(U/mL)	Plasma T-SOD/(U/mL)	Plasma MDA/(nmol/mL)	Liver GSH-Px/(U/mL)
0 (Control)	79.62±1.94^A	17.07±0.33^Aa	117.66±1.55^A	5.92±0.61^Aa	9.67±0.47^A
0.2	80.66±2.05^A	17.42±0.36^Aab	121.28±0.23^B	3.73±1.42^Ab	9.94±0.11^A
0.6	97.49±2.00^C	18.99±1.16^Bb	156.21±1.84^C	3.97±0.98^ABb	16.33±0.48^C
1.0	89.17±2.06^B	18.21±1.03^ABb	146.57±1.14^C	2.89±0.90^Bb	14.15±1.64^B

2.4 Effects of Selenium Yeast Supplementation on Lipid Metabolism Indexes of Laying Hens at Late Laying Period

As shown in Table 6 , compared with the control group, dietary supplementation with 0.2, 0.6, and 1.0 mg/kg selenium yeast had no significant effect on plasma cholesterol and triglyceride contents (P>0.05). Supplementation with 1.0 mg/kg selenium yeast extremely significantly decreased liver triglyceride content (P<0.01) and significantly decreased liver cholesterol content (P<0.05). Supplementation with 0.6 mg/kg selenium yeast significantly decreased liver cholesterol content (P<0.05). Supplementation with 0.2 mg/kg selenium yeast had no significant effect on liver cholesterol and triglyceride contents (P>0.05). Supplementation with 0.6 and 1.0 mg/kg selenium yeast significantly decreased yolk triglyceride content (P<0.05), but had no significant effect on yolk cholesterol content (P>0.05). Among the three experimental groups, liver cholesterol and yolk triglyceride contents in the 0.6 and 1.0 mg/kg groups were significantly lower than those in the 0.2 mg/kg group (P<0.05), and the triglyceride content in the 1.0 mg/kg group was also extremely significantly lower than that in the 0.2 and 0.6 mg/kg groups (P<0.01).

Table 6 Effects of Selenium Yeast Supplementation on Lipid Metabolism Indexes of Laying Hens at Late Laying Period

Selenium yeast additive amount/(mg/kg)	Plasma/(mmol/L)	Liver/(mg/g)	Yolk/(mg/g)
	Cholesterol	Triglyceride	Cholesterol
0 (Control)	3.16±0.06	16.64±1.59	11.86±0.49^a
0.2	3.02±0.18	16.96±2.83	11.63±0.57^a
0.6	3.15±0.23	16.69±3.26	9.78±0.69^b
1.0	3.01±0.14	17.32±3.20	9.99±0.59^b

2.5 Effects of Selenium Yeast Supplementation on mRNA Relative Expression Levels of Antioxidant and Lipid Metabolism Related Genes in Liver of Laying Hens at Late Laying Period

As shown in Table 7 , compared with the control group, dietary supplementation with 0.6 mg/kg selenium yeast extremely significantly increased the mRNA relative expression levels of GPx1, Trxr1, and SREBP-1c in liver ($P<0.01$), and extremely significantly decreased the mRNA relative expression level of HMGCR ($P<0.01$), while having no significant effect on Cyp7a1 mRNA relative expression level ($P>0.05$).

Table 7 Effects of Selenium Yeast Supplementation on mRNA Relative Expression Levels of Antioxidant and Lipid Metabolism Related Genes in Liver of Laying Hens at Late Laying Period

Selenium yeast additive amount/(mg/kg)	GPx1	Trxr1	Cyp7a1	SREBP-1c	HMGCR
0 (Control)	1.00±0.16^A	1.00±0.16^A	1.00±0.27	1.00±0.11^A	1.00±0.09^A
0.6	2.54±0.34^B	3.18±0.51^B	1.07±0.12	2.46±0.55^B	0.70±0.04^B

2.6 Model Estimation of Selenium Yeast Requirement for Laying Hens at Late Laying Period

Regression analysis showed that yolk selenium content, plasma GSH-Px activity, T-AOC, T-SOD activity, MDA content, and liver GSH-Px activity all showed significant quadratic curve changes with increasing selenium yeast supplementation (Table 8). In terms of performance, the appropriate selenium yeast supplementation level was 0.60–0.65 mg/kg. For yolk selenium content, the appropriate level was 2.25 mg/kg. For plasma GSH-Px activity, T-AOC, T-SOD activity, and liver GSH-Px activity, the appropriate level was 0.67–0.77 mg/kg.

Table 8 Regression Model for Related Indexes of Laying Hens and Selenium Yeast Requirement

Items	Regression model	Coefficient (R^2)	P-value	Selenium yeast additive amount/(mg/kg)
Laying rate/%	y=0.046x+0.738			
Average daily feed intake/g	$y=-0.159x^2+0.207x+0.721$			
Feed/egg	y=0.404x+120.346			
Yolk selenium content/(×10² g/g)	$y=-1.65x^2+2.07x+120.18$			2.25
Plasma GSH-Px activity/(U/mL)	y=-0.115x+2.622			
Plasma T-AOC/(U/mL)	$y=0.59x^2-0.70x+2.68$			0.67–0.77
Plasma T-SOD activity/(U/mL)	y=31.16x+29.745			
Plasma MDA content/(nmol/mL)	$y=-8.91x^2+40.13x+28.83$			
Liver GSH-Px activity/(U/mL)	y=13.005x+80.883			
	$y=-38.48x^2+51.74x+76.92$			0.67–0.77
	y=1.403x+17.291			

Items	Regression model	Coefficient (R^2)	P-value	Selenium yeast additive amount/(mg/kg)
	$y=-4.20x^2+5.63x+16.86$			0.60–0.65
	$y=-2.392x+5.204$			
	$y=2.57x^2-4.98x+5.47$			
	$y=5.755x+9.933$			
	$y=-12.13x^2+17.97x+8.68$			0.67–0.77

3 Discussion

3.1 Effects of Selenium Yeast Supplementation on Performance of Laying Hens at Late Laying Period

Research results on the effects of selenium on laying hen performance have been variable. Hu et al. found that adding selenium yeast and selenium-enriched alfalfa could significantly improve the laying rate of Roman laying hens, while sodium selenite had no significant effect on laying rate. Gjorgovska et al. reported that dietary selenium yeast supplementation could improve the performance of laying hens. Pavlovic et al. found that selenium yeast could significantly increase egg production in laying hens. However, Sun et al. and Delezie et al. showed that different selenium sources and levels had no significant effect on laying hen performance. The results of this experiment showed that dietary supplementation with different levels of selenium yeast significantly increased laying rate and reduced feed/egg ratio, which is consistent with the results of Gjorgovska et al. using 80-week-old laying hens, indicating that selenium yeast supplementation can improve selenium metabolism in laying hens at late laying period, affect gonadotropin release, and increase laying rate.

3.2 Effects of Selenium Yeast Supplementation on Egg Quality of Laying Hens at Late Laying Period

Egg quality is comprehensively evaluated through indicators including eggshell thickness, eggshell strength, egg shape index, albumen height, Haugh unit, and yolk color. Eggshell strength and thickness directly affect egg breakage rate, while Haugh unit directly reflects egg freshness and albumen quality. Studies have shown that dietary selenium yeast supplementation has no significant effect on eggshell thickness. However, adding selenium yeast to diets during summer significantly improved eggshell thickness, albumen height, and Haugh unit. Selenium yeast supplementation can increase eggshell weight and strength. In contrast, Pavlovic et al. reported that dietary selenium yeast supplementation had no significant effect on eggshell quality, thickness, strength, or egg shape index. The results of this experiment showed that dietary supplementation with 0.6 mg/kg selenium yeast significantly improved eggshell strength and thickness at week 6, consistent with the findings of Invernizzi et al. The reason may be that selenium from selenium yeast is easily deposited in the eggshell membrane and shell after consumption by laying hens, though the specific mechanism requires further investigation.

3.3 Effects of Selenium Yeast Supplementation on Yolk Selenium, Cholesterol, and Triglyceride Contents

Han et al. found that selenium supplementation significantly increased selenium content in eggs. Bennett et al. also found that within a certain range of dietary selenium supplementation, egg selenium content increased with increasing selenium supplementation, with organic selenium being more effective than inorganic selenium. Paton reported that dietary selenium content was positively correlated with egg selenium content. In this experiment, yolk selenium content increased with increasing dietary selenium yeast supplementation, consistent with Paton' s findings.

Research on the effects of selenium yeast on yolk cholesterol and triglyceride contents is limited. In this experiment, dietary selenium yeast supplementation showed a trend toward reducing yolk cholesterol content, but the effect was not significant. Supplementation with 0.6 and 1.0 mg/kg selenium yeast significantly reduced yolk triglyceride content, which differs from the findings of Li et al. They reported that dietary selenium yeast supplementation reduced yolk cholesterol content in laying hens, mainly by increasing serum high-density lipoprotein cholesterol (HDL-C) content and reducing very low-density lipoprotein cholesterol (VLDL-C) content. The specific mechanism requires further investigation.

3.4 Effects of Selenium Yeast Supplementation on Antioxidant Capacity and Related Gene Expression

GSH-Px and T-SOD are important enzymes in the antioxidant system and play crucial roles in antioxidant processes. The active center of GSH-Px is selenocysteine, composed of 4 selenocysteine-containing subunits, so selenium levels in the body directly reflect GSH-Px activity. Studies have shown that dietary selenium supplementation can significantly increase serum GSH-Px activity in hens. Dietary supplementation with 0.3 mg/kg selenium yeast significantly increased GSH-Px and T-SOD activities in goose serum while significantly reducing MDA content. Adding selenium yeast prepared from brewer' s waste yeast to diets increased broiler liver GSH-Px activity with increasing supplementation levels. This experiment found that appropriate dietary selenium yeast supplementation significantly or extremely significantly increased plasma GSH-Px and SOD activities, T-AOC, and liver GSH-Px activity, and significantly or extremely significantly reduced plasma MDA content, consistent with the above studies. Moreover, antioxidant capacity showed a trend of increasing first and then decreasing with increasing selenium yeast supplementation, peaking at 0.6 mg/kg supplementation for plasma GSH-Px, T-SOD activities, T-AOC, and liver GSH-Px activity. Although antioxidant capacity was slightly lower at 1.0 mg/kg than at 0.6 mg/kg, the 1.0 mg/kg group still showed significantly or extremely significantly higher values than the control group, indicating that selenium yeast supplementation up to 1.0 mg/kg can improve antioxidant capacity, with 0.6 mg/kg being optimal.

Among GSH-Px isoforms, GPx-1 has the highest activity in liver and is the most abundant selenoprotein in cells. Trxr1 is also an important indicator of antioxidant function, and its activity is mainly affected by selenium status. However, reports on the effects of dietary selenium sources and levels on GPx1 and Trxr1 expression are inconsistent. To further explore the molecular mechanism of selenium yeast antioxidant activity, this experiment measured the effects of 0.6 mg/kg selenium yeast supplementation on liver GPx1 and Trxr1 mRNA expression in laying hens. The results showed that 0.6 mg/kg supplementation extremely significantly increased liver GPx1 and Trxr1 mRNA expression. Gao et al. found that adding sodium selenite and selenium-enriched probiotics to piglet diets had no significant effect on liver GPx1 mRNA expression. Selenium deficiency reduced liver GPx1 mRNA expression in rats, while selenium supplementation increased it. Lu et al. reported that increasing selenium status increased Trxr1 and Trxr2 protein expression, with selenium deficiency reducing liver Trxr1 mRNA expression to 15% of normal rats, while GSH-Px mRNA expression only decreased to 2% of normal rats. In this experiment, adding 0.6 mg/kg selenium yeast to a normal diet increased liver Trxr1 mRNA expression more than GPx1 mRNA, suggesting that Trxr1 mRNA is more responsive to selenium status than GPx1 mRNA.

3.5 Effects of Selenium Yeast Supplementation on Lipid Metabolism

The liver is the main organ for cholesterol synthesis in laying hens. When cholesterol content is high, SREBP-1c can promote fatty acid generation and synthesize cholesterol esters with cholesterol, thereby consuming cholesterol. HMGCR is the rate-limiting enzyme in cholesterol synthesis.

Research on the effects of selenium on blood, liver cholesterol, and triglyceride contents in laying hens is limited. Zhao Yuxin reported that adding sodium selenite to diets had no significant effect on serum cholesterol and triglyceride contents in laying hens compared with the control group, although a decreasing trend was observed. Li et al. found that adding 0.5 mg/kg selenium yeast to diets had no significant effect on serum cholesterol and triglyceride contents in laying hens. Wang Zeming also reported that different selenium sources in diets had no significant effect on plasma cholesterol and triglyceride contents in laying hens. The results of this experiment showed that dietary selenium yeast supplementation had no significant effect on plasma cholesterol and triglyceride contents, consistent with the above studies. However, supplementation with 0.6 and 1.0 mg/kg selenium yeast significantly reduced liver cholesterol content, and 1.0 mg/kg supplementation extremely significantly reduced liver triglyceride content. Supplementation with 0.6 mg/kg selenium yeast extremely significantly increased liver SREBP-1c mRNA expression and extremely significantly reduced liver HMGCR mRNA expression, which aligns with the observed reduction in liver cholesterol and triglyceride contents. This demonstrates that selenium yeast can affect lipid metabolism by influencing the expression of SREBP-1c and HMGCR mRNA.

4 Conclusion

Dietary selenium yeast supplementation can improve performance, eggshell strength, and yolk selenium content, and enhance antioxidant capacity and cholesterol metabolism in laying hens at late laying period. Regression analysis indicated that better effects were achieved when adding 0.60–0.77 mg/kg selenium yeast to the diet (containing 0.20 mg/kg selenium from sodium selenite).

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