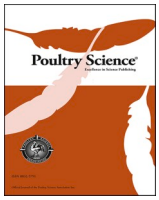
104 (2025) 104584

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj



Optimizing selenium-enriched yeast supplementation in laying hens: Enhancing egg quality, selenium concentration in eggs, antioxidant defense, and liver health

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ARTICLE INFO

Keywords:
Antioxidant function
Laying hens
Liver health
Selenium-enriched yeast
Selenium-enriched eggs

ABSTRACT

This study evaluated the effects of selenium-enriched yeast (SY) supplementation at various levels on health and production parameters in laying hens, including egg production, egg quality, selenium (Se) concentrations in eggs, liver health, serum biochemical markers, antioxidant function, and immune responses. A total of 360 Hy-Line Brown hens (28 weeks old) were randomly assigned to four dietary groups with six replicates of 15 birds each, monitored over a 12-week feeding trial after a two-week acclimatization period. The dietary groups included a control (basal diet without selenium) and three SY-supplemented groups with Se levels of 0.3 mg/kg (SY03), 1.5 mg/kg (SY15), and 6.0 mg/kg (SY60). The results showed no significant effects of dietary SY on laying performance or feed efficiency ($P > 0.05$). However, the SY15 group showed significant improvements in egg quality, particularly in albumen height, Haugh Unit and yolk color ($P < 0.05$). Selenium concentrations in eggs, albumen, and yolk increased dose-dependently, with significant differences in the SY-supplemented groups ($P < 0.001$). Increased activities of liver enzymes including alanine transaminase, alkaline phosphatase, and aspartate transaminase, alongside elevated levels of uric acid were notable in the SY60 group ($P < 0.05$). In addition, histological analysis revealed significant hepatocyte degeneration and a higher liver organ index ($P < 0.05$), in the SY60 group. All of which suggests potential liver toxicity at higher selenium levels. Antioxidant capacity of the birds were significantly enhanced due to dietary supplementation of SY as indicated by increased serum levels of total antioxidant capacity, and activities of catalase, glutathione peroxidase, and superoxide dismutase ($P < 0.05$). Analysis of hepatic genes expression revealed that SY15 supplementation significantly upregulated key antioxidant-related genes (*Nrf2, HO-1, CAT*, and *NQO1)* and downregulated *Keap1* expression ($P < 0.05$), suggesting strong activation of the antioxidant defense system. In conclusion, SY supplementation at 1.5 mg/kg improved egg quality, increased Se concentrations in eggs, and enhanced antioxidant capacity without affecting laying performance or liver health. This makes it a balanced approach to improving egg quality and poultry health. However, higher supplementation levels (6.0 mg/kg) resulted in liver damage, underscoring the importance of careful dosage consideration.

Introduction

Selenium (Se) is an essential trace element that vital for maintaining health and physiological functions in humans and animals. As an integral component of selenoproteins, selenium participates in biochemical processes, including antioxidant defense, immune modulation, and metabolic regulation (Wang et al., 2020; Razaghi et al., 2021). Its incorporation into enzymes such as glutathione peroxidase (GSH-Px) and thioredoxin reductase, improve cellular protection against oxidative damage by neutralizing reactive oxygen species (ROS) and maintaining redox homeostasis (Zhang et al., 2023). The European Food Safety Authority (EFSA) recommends selenium levels of 0.15 to 0.3 mg/kg feed

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https://doi.org/10.1016/j.psj.2024.104584
Received 23 September 2024; Accepted 21 November 2024
Available online 22 November 2024

for poultry nutrition (EFSA, 2024), highlighting its importance in animal nutrition.

Despite its natural presence in soil and water, selenium distribution varies significantly across regions, leading to deficiencies in areas such as China, parts of Europe, and the United States: this poses a risk to human and animal health (Rayman, 2020; Kieliszek and Serrano Sandoval, 2023). In selenium-deficient animals, including poultry, there is an increased risk of oxidative stress, metabolic disorders, and immune dysfunction, which can result in severe health issues such as erythrocyte hemolysis (Zheng et al., 2019). Given that the recommended daily intake for adults ranges from 30 to 40 μg, with an upper limit of 40 μg to prevent toxicity (Lee and Jeong, 2012; Marchetti et al., 2014), optimizing selenium levels in poultry diets becomes crucial for enhancing health, productivity, and the production of functional foods.

Selenium supplementation in poultry has gained prominence for its dual benefits: enhancing animal health and producing selenium-enriched eggs, valued as functional foods with human health benefits. Selenium is commonly supplemented as inorganic (e.g., sodium selenite) or organic forms, with organic selenium gaining preference due to its superior bioavailability, efficacy, and safety (Surai et al., 2018). Additionally, organic selenium sources have exhibited rapid and efficient deposition of Selenium into eggs at higher concentrations (Lu et al., 2018; Lu et al., 2020; Liu et al., 2020), attributable to its absorption via specific amino acid transport pathways in the small intestine (Xin and Gao, 2022). Our previous research work demonstrated the higher efficacy of organic selenium in enhancing antioxidant function, immune response, and production of selenium-enriched eggs in hens (Qiu et al., 2021a, 2021b). Furthermore, organic selenium also supports animal health and product quality by improving gut morphology and microbial composition (Muhammad et al., 2021), mitigating the effects of heat stress (Wang et al., 2022), and extending the shelf life of eggs (Li et al., 2024).

Selenium-enriched yeast (SY), an organic selenium supplement primarily composed of selenomethionine, provides high bioavailability and low toxicity, efficiently mimicking methionine in metabolic pathways to enhance absorption and deposition in tissues and eggs (Suhajda et al., 2000; Hachemi et al., 2023). Previous studies have shown that SY supplementation at various dosage levels exert varying effects on animal performance and health (Meng et al., 2019; Muhammad et al., 2021; Li et al., 2024; Chen et al., 2024). The study by Meng et al. (2019) and Muhammad et al. (2021), reported that SY supplementation at 0.3 mg/kg was found to improve laying rate, egg weight, and feed efficiency. Conversely, research suggests that a lower dose of 0.15 mg/kg is more effective than 0.3 mg/kg when substituting for inorganic selenium, particularly in enhancing performance in laying hens (Li et al., 2024). Meanwhile, a dosage of 2.0 mg/kg, enhanced laying performance but not antioxidant function, without negative health consequences (Chen et al., 2024). However, determining the optimal SY dosage remains a critical challenge due to potential toxicity risk at excessive levels.

Furthermore, despite the established benefits, the effects of SY on liver health and antioxidant gene expression remain underexplored. This study seeks to address this gap by investigating the impact of dietary SY supplementation on hepatic gene expression (antioxidant genes: *Nrf2, HO-1, CAT, Keap1* and *NQO1*), which play crucial roles in oxidative stress response and liver health. Therefore, the study investigated the impact of dietary SY supplementation at dosages of 0.3, 1.5, and 6.0 mg/kg on egg quality, selenium deposition, serum antioxidant enzyme activities, liver histology, and hepatic gene expression in Hy-Line Brown laying hens. We hypothesize that SY supplementation will enhance selenium deposition, upregulate hepatic antioxidant genes, and bolster antioxidant defenses, thereby improving overall health and performance. Identifying the optimal dosage of SY will provide valuable insights into producing functional foods while ensuring the welfare of poultry.

Materials and methods

Animal ethics statement

All experimental protocols were approved by the Animal Care and Use Committee of the Institute of Feed Research, Chinese Academy of Agricultural Sciences (ACE-CAAS-20230628), and all animal experiments were conducted following the ARRIVE guidelines (Kilkenny et al., 2010).

Birds, diets and study design

A total of 360 healthy Hy-Line Brown laying hens, aged 28 weeks, were procured from a commercial poultry farm (Hebei Shengxuan Agricultural Technology Development Co., Ltd). The selection of hens for the experiment was based on similar body weight and laying rate. The hens were randomly assigned to four experimental groups, each containing 90 hens (six replicates of 15 hens each). The groups were designated as follows: Control (0 mg/kg Se), basal diets supplemented with SY with each diet containing Se at: 0.3 mg/kg (SY03), 1.5 mg/kg (SY15), and 6.0 mg/kg (SY60). The basal diets were formulated devoid of selenium, according to the Chicken Feeding Standards (NY/T 33-2004). The nutrient composition of the basal diet is presented in Tables 1 and 2. The experiment period lasted for 12 weeks (age of birds: 28 weeks old to 39-week-old). The birds were kept in battery cages (3 tiers: 40 cm × 40 cm × 35 cm), fed *ad libitum*, and the environmental conditions (temperature range of 22-24°C and a relative humidity of 60-70%) were maintained throughout the feeding trial. The animals were healthy throughout the feeding trial.

Sample collection and analytical determination

Laying performance

Daily egg production and egg weight were monitored and recorded on a replicate basis. The laying rate is expressed as the average hen-day production, calculated from the total number of eggs divided by the total

Table 1
Composition and nutrient levels of the basal diet (as-fed basis, %).

Ingredient	Content (%)	Nutrient level[2]	Content (%)
Corn	64.67	Metabolizable energy (MJ/kg)	11.33
Soybean meal (44.8% CP)	23.50	Crude protein	16.07 (16.45)
Soybean oil	0.60	Calcium	3.50 (4.35)
Limestone	9.00	Total phosphorus	0.53 (0.45)
Dicalcium phosphorus	0.84	Non-phytate phosphorus	0.32
Sodium chloride	0.15	Lysine	0.75 (0.751)
Sodium bicarbonate	0.65	Methionine	0.39 (0.405)
DL-Methionine (98%)	0.17	Methionine + cysteine	0.65 (0.685)
L-Lysine-HCl (78%)	0.02	Threonine	0.55 (0.613)
L-Threonine (98%)	0.04	Selenium	0 (0.040)
Choline chloride (50%)	0.20		
Premix[1]	0.13		
Phytase	0.03		
Total	100		

[1] Premix supplied per kilogram of diet: vitamin A, 12,500 IU; vitamin D3, 4,125 IU; vitamin E, 15 IU; vitamin K3, 2 mg; thiamine, 1 mg; riboflavin, 8.5 mg; pyridoxine 8 mg vitamin B12, 0.04 mg; biotin, 0.1 mg; folic acid, 1.25 mg; Ca-pantothenate, 50 mg; niacin, 32.5 mg; Cu, 8 mg; Zn, 65 mg; Fe, 60 mg; Mn, 65 mg; I, 1 mg.

[2] The values in parenthesis indicate analyzed values. Others are calculated values.

CP (GB/T6432-2018), Ca (GB/T6436-2018) and TP (GB/T6437-2018) were measured values, while the other nutrient levels were calculated values referred to NY/T33-2004.

Table 2
The Se level of experimental diets.

Item		Experimental treatment[1]		
	CON	SY03	SY15	SY60
Measured value, mg/kg	0.040	0.204	1.870	5.703

[1] Abbreviations: CON, control; SY03, 0.3 mg/kg; SY15, 1.5 mg/kg; SY60, 6.0 mg/kg.

number of days multiplied by 100. Whereas, egg weight was expressed as average egg weight (AEW), calculated from total egg weight in grams per number of eggs produced. Feed intake (FI) was recorded on a replicate basis at weekly intervals and expressed as average daily feed intake (ADFI). Feed to egg ratio was calculated as grams of feed consumed per grams of eggs produced.

Egg quality assessment

A total of 144 eggs (six eggs per replicate = 36 eggs per group) were collected at the end of weeks 4 (31 weeks-old), 8 (35 weeks-old), and 12 (39 weeks-old), for egg quality assessment. The collected eggs were kept at room temperature, and all egg quality indicators were assessed with various instruments, within 24 h of collection.

The egg shape index was measured using an egg-shaped index apparatus (Egg Index Reader, Fujihira Industry Co., Tokyo, Japan). The eggshell strength was obtained using an eggshell strength analyzer (Egg Force Reader™, Model EFR-01, Orka Food Technology Ltd., Ramat Hasharon, Israel). The eggshell thickness was determined at three specific points (the air cell, equator, and sharp end) with an Egg Shell Thickness Gauge (Orka Technology Ltd., Ramat Hasharon, Israel). Furthermore, the albumen height, Haugh Unit (HU), and yolk color were precisely measured using an egg quality auto-analyzer (Egg Analyzer™, Orka Technology Ltd., Ramat Hasharon, Israel).

Se assay

A total of 288 eggs (12 eggs per replicate = 72 eggs per group) were collected at the end of feeding trial (Week 12). The eggs were utilized for analyzing selenium deposition or content in the whole egg, as well as in the albumen and yolk, respectively. The eggs were divided into two sets; In the first set, the eggs (n = 144 eggs, six eggs per replicate = 36 eggs per group) were broken, and an egg separator was used to separate the egg yolk and albumen. Whereas, in the second set, eggs (n = 144 eggs, six eggs per replicate = 36 eggs per group) were broken, and albumen and yolk were homogenized to obtain whole egg sample. The respective sample: whole egg, albumen and yolk, were dried, ground, and digested with concentrated HNO_3. The selenium content in both egg yolks and albumen was precisely determined using a method specifically designed for inductively coupled plasma mass spectrometry (ICP-MS), a highly sensitive and accurate analytical technique. We adopted the procedure described by Mohammadsadeghi et al. (2023).

It is worth mentioning that based on our findings for egg quality and selenium deposition in eggs, both the 1.5 mg/kg and 6.0 mg/kg groups showed comparable effects on egg quality and selenium concentration, thus, we excluded the SY03 group during the biochemical analysis using blood parameters and histological examination of the liver. This ensures focus on dosage-dependent effects, allowing for optimal dosage investigation.

Clinical blood parameters

At the end of the 12th week (age: 39 weeks old), 24 birds (1 per replicate) were selected and deprived of feed for about eight hours before slaughter. About 5 ml of blood was collected from the jugular vein into a collection tube, kept in a slant position to stand for about 2 h. Then, centrifuged at 2500 rpm for 10 min, the harvested serum samples were transferred into Eppendorf tubes and stored at -20°C for serum biochemical analysis. Serum levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), globulin (GLB), and uric acid (UA) were measured using an automatic biochemical analyzer (Zhuoyue 300, Kehua Bio. Co., Ltd. Shanghai, China). The GLB content was mathematically derived by subtracting the albumin content from the TP content. The enzymatic activities of total antioxidant capacity (T-AOC), catalase (CAT), glutathione peroxidase, and superoxide dismutase (SOD) in serum were measured using commercial assay kits provided by the (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Serum immunoglobulin (Ig) indices, specifically IgA, IgM, and IgG, were determined using assay kits specifically for birds (Shanghai Meilian Bio. Co., Ltd. Shanghai, China). All the protocols used were strictly based on the manufacturer's instructions.

Organ index determination

After blood collection, the hens were euthanized and dissected to obtain the following organs: liver, heart, spleen, lung, and kidney, which were then weighed and measured using an electrical scale (quantitative analysis at 0.01 g level). The organ index was calculated by the formula as follows: $Organ\ index = organ\ weight/body\ weight \times 100\%$

Histological examination of the liver tissue

A portion of liver tissue from each bird was cut and fixed in 4% paraformaldehyde for 24 h, for histological examination, according to that described by Peng et al. (2019). The essence of the fixation is to preserve tissue morphology and cellular integrity, while enhancing the penetration of staining reagent in subsequent processing steps, improving the visibility and staining quality. Following fixation, the tissue was processed for paraffin embedding, and serial sections of 5-7 μm thickness were subsequently cut using a microtome. These sections were then de-paraffinized through solvents, stained with hematoxylin and eosin (H & E) for histological analysis, and finally mounted on glass slides. The stained slides were examined under an optical microscope (Nikon Eclipse E600, Japan), with magnification (40 x), for detailed histological assessment. Procedures described by Bancroft et al. (1990) was used.

Owing to the fact that SY supplementation at 1.5 mg/kg have shown optimal performance for egg quality and physiological responses, with almost a zero score for histopathology of the liver. Also, there was a marked significance between the control and the dietary groups for most parameters evaluated, suggesting distinct dietary influence. We therefore, selected only the control and SY15 group for analysis of hepatic antioxidant gene expression, to further highlight the molecular mechanisms underlying the dietary influence.

Hepatic gene expression analysis

A portion of liver tissue from each bird was cut and placed into a freezing tube, then stored at -80°C for RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis (gene expression analysis). Briefly, the frozen liver sample was grounded under liquid nitrogen condition, and the total RNA was extracted the samples using the TransZol Up Plus RNA kit (Alltech Jinsheng Biotech Co., Ltd. Beijing, China). The concentration and purity of the extracted RNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and stored at -80°C until further processing. The RNA was reverse transcribed into cDNA using the FastQuant RT Kit (Tiangen Biochemical Technology Co., Ltd. Beijing, China), ensuring accurate and efficient conversion. The resulting cDNA was then carefully stored at -20°C to preserve its integrity for subsequent experiments. The RT-PCR amplification was performed on a Bio-Rad C1000 Thermal Cycler (Bio-Rad Laboratories, Inc., California, USA), equipped with a CFX96 Touch Real-Time PCR Detection System using SuperReal PreMix Plus (SYBR Green, FP205, Tengen Biotech, Beijing, China), to quantify the mRNA expression levels of *Nrf2, Keap1, HO-1, NQO1*, and *CAT* genes. The procedures described in Wang et al. (2019) was adopted. The primer sequences used for amplification are shown in Table 3. The PCR reaction conditions were set as follows:

Table 3
Gene-specific primers for real-time quantitative reverse transcription PCR.

Genes	Primers (5′-3′)	Gene number
Nrf2	Forward: GGTGACACAGGAACAACA Reverse: AAGTCTTATCTCCACAGGTAG	NM_205117.2
Keap1	Forward: ATCACCTCTTCTGCACCGAA Reverse: GGTTCGGTTACCGTCCTGC	XM_015274015.1
HO-1	Forward: CTGAAGGAAGCCACCAAG Reverse: CCAGAGCAGAGTAGATGAAG	NM_205344.2
NQO1	Forward: CACCATCTCTGACCTCTAC Reverse: CCGCTTCAATCTTCTTCTG	NM_001277620.2
CAT	Forward: CACTGTTGCTGGAGAATCT Reverse: GGCTATGGATGAAGGATGG	NM_001031215.2
β-actin	Forward: TATGTGCAAGGCCGGTTTC Reverse: TGTCTTTCTGGCCCATACCAA	NM_205518.2

Abbreviations: *Nrf2*, Nuclear factor erythroid 2-related factor 2; *Keap1*, Kelch-like ECH-associated protein 1; *HO-1*, Heme oxygenase-1; *NQO1*, NAD(P)H: quinone oxidoreductase 1; *CAT*, Catalase; *β-actin*, Beta-actin.

initial denaturation at 95°C for 15 min, denaturation at 95°C for 10 s, annealing at 60°C for 30 s, and a final extension for 40 cycles (as required by the protocol). Amplification was stopped at the end of the cycle, and measurements were repeated for each sample to ensure reproducibility. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), and β-actin was used as the reference gene. This allows comparative analysis of gene expression across samples.

Statistical analysis

All data were analyzed using SPSS statistical software (version 27.0). A one-way analysis of variance (ANOVA) was followed by Duncan's multiple comparison test, which was performed to compare the means among different treatments. A t-test was used to analyze the hepatic expression of antioxidant genes between the control and SY15 group. Differences were considered statistically significant at $P < 0.05$. Data are presented as means ± pooled SEM.

Results

Effect of dietary SY on laying performance

The effects of dietary SY supplementation on laying performance are shown in Table 4. There were no significant effects of dietary SY on egg production (EP), average egg weight, average daily feed intake, or feed conversion ratio (FCR) between the control and treatment groups ($P > 0.05$) throughout the 12-week experimental period.

Effect of dietary SY on egg quality

As presented in Table 5, no significant differences in egg quality parameters, including egg shape index, eggshell thickness, eggshell strength, albumen height, Haugh unit and yolk colour, were observed across the groups during weeks 4 and 8, stages of the experiment ($P > 0.05$). However, by the end of the week 12, the SY15 group showed a significant improvement in albumen height, Haugh unit and yolk color compared to the control ($P < 0.05$), while egg shape index, eggshell thickness, and eggshell strength were not significantly influenced by diets ($P > 0.05$).

Effect of dietary SY on Se concentration in whole eggs, albumen and yolk

The impact of dietary SY supplementation on selenium concentrations in egg, albumen and yolk were presented in Table 6. There was a significant increase in the concentrations of selenium in whole egg, albumen and yolk in a dose-dependent manner ($P < 0.001$). The highest selenium concentrations were found in the eggs of SY60 group, followed by that in the SY15 and SY03 groups, with significant differences across all groups ($P < 0.001$). Notably, within each SY supplementation group, the selenium concentration in the yolk was consistently higher than that in the albumen ($P < 0.001$).

Effect of dietary SY on serum biochemical parameters

The effect of dietary SY supplementation on serum biochemical indices in are presented in Fig. 1. There were significant changes in serum biochemical indices of laying hens due to dietary supplementation of SY ($P < 0.05$). The SY60 group exhibited significantly higher activities of liver enzymes such as ALT, ALP, AST, and level of protein

Table 4
Effect of dietary selenium-enriched yeast on performance of laying hens.

Item	Experimental treatment				SEM	*P* value		
	CON	SY03	SY15	SY60		ANOVA	Linear	Quadratic
28 to 31 wk								
EP, %	87.24	89.74	89.05	87.69	0.756	0.648	0.678	0.737
AEW, g	56.06	56.42	57.36	57.31	0.281	0.270	0.156	0.134
ADFI, g	102.88	103.68	104.39	104.69	0.687	0.817	0.434	0.637
FCR	2.11	2.05	2.05	2.09	0.023	0.798	0.840	0.736
32 to 35 wk								
EP, %	80.22	80.22	81.04	78.65	0.639	0.638	0.284	0.424
AEW, g	58.71	58.77	57.25	58.62	0.482	0.665	0.971	0.473
ADFI, g	107.00	104.24	105.98	107.95	0.598	0.149	0.126	0.278
F/E	2.28	2.21	2.29	2.35	0.024	0.247	0.081	0.225
36 to 39 wk								
EP, %	81.01	80.95	82.34	77.78	0.714	0.131	0.045	0.058
AEW, g	56.59	58.00	58.56	58.26	0.346	0.191	0.295	0.161
ADFI, g	111.84	109.21	111.05	112.05	0.898	0.699	0.534	0.817
F/E	2.29	2.19	2.17	2.32	0.025	0.088	0.148	0.069
28 to39 wk								
EP, %	83.24	84.13	84.51	82.15	0.456	0.274	0.145	0.154
AEW, g	57.12	57.73	57.72	58.06	0.277	0.709	0.353	0.607
ADFI, g	106.26	105.04	106.39	107.44	0.460	0.347	0.130	0.326
F/E	2.21	2.14	2.16	2.23	0.016	0.182	0.138	0.196

Abbreviations: CON, control; SY03, 0.3 mg/kg; SY15, 1.5 mg/kg, SY60, 6.0 mg/kg; EP, Egg production; AEW, Average egg weight; ADFI, Average daily feed intake; F/E, feed-to-egg mass ratio. $n = 6$ replicates per treatment.

Table 5
Effect of dietary selenium-enriched yeast on egg quality of laying hens.

Item	Experimental treatment				SEM	*P* value		
	CON	SY03	SY15	SY60		ANOVA	Linear	Quadratic
27 wk								
Egg shape index	1.40	1.39	1.38	1.38	0.006	0.428	0.274	0.266
Eggshell thickness, × 0.01 mm	41.42	41.94	40.37	40.33	0.939	0.921	0.606	0.817
Eggshell strength, N	41.25	40.79	40.68	39.36	0.887	0.905	0.450	0.757
Albumen height, mm	8.35	7.81	7.97	7.62	0.157	0.420	0.217	0.455
Haugh unit	91.55	88.37	88.32	86.54	0.955	0.326	0.142	0.285
Yolk color	4.92	5.39	5.06	5.39	0.089	0.142	0.221	0.463
33 wk								
Egg shape index	1.39	1.38	1.39	1.38	0.005	0.840	0.968	0.844
Eggshell thickness, × 0.01 mm	37.37	38.43	37.67	37.39	0.585	0.923	0.757	0.952
Eggshell strength, N	36.74	37.62	37.80	35.59	0.801	0.779	0.404	0.595
Albumen height, mm	8.19	8.60	8.75	8.48	0.103	0.274	0.813	0.240
Haugh unit	89.70	92.20	93.06	92.18	0.533	0.130	0.402	0.132
Yolk color	5.14	5.19	4.75	5.14	0.117	0.536	0.931	0.373
39 wk								
Egg shape index	1.33	1.35	1.35	1.33	0.004	0.212	0.507	0.266
Eggshell thickness, × 0.01 mm	32.89	32.55	32.40	32.42	0.123	0.483	0.347	0.373
Eggshell strength, N	42.13	40.46	43.79	42.09	0.727	0.478	0.801	0.526
Albumen height, mm	7.65[c]	8.04[b]	8.50[a]	8.21[b]	0.118	0.045	0.287	0.031
Haugh unit	86.21[c]	88.91[b]	91.68[a]	89.07[b]	0.686	0.033	0.489	0.017
Yolk color	4.86[c]	5.07[b]	5.65[a]	5.22[b]	0.090	0.004	0.389	0.002

Abbreviations: CON, control; SY03, 0.3 mg/kg; SY15, 1.5 mg/kg; SY60, 6.0 mg/kg.
[a-c] Within a row, means with no common superscript differ significantly ($P < 0.05$). $n = 6$ replicates per treatment.

Table 6
Effect of dietary selenium-enriched yeast on the concentration of selenium in egg, albumen and yolk.

Item	Experimental treatment				SEM	*P* value		
	CON	SY03	SY15	SY60		ANOVA	Linear	Quadratic
Egg Se, μg/100g	12.00[d]	37.10[c]	96.12[b]	151.30[a]	11.397	<0.001	<0.001	<0.001
Albumen Se, μg/100g	5.04[d]	21.20[c]	72.34[b]	104.71[a]	8.453	<0.001	<0.001	<0.001
Yolk Se, μg/100g	30.00[d]	77.57[c]	158.49[b]	273.90[a]	19.532	<0.001	<0.001	<0.001

Abbreviations: CON, control; SY03, 0.3 mg/kg; SY15, 1.5 mg/kg; SY60, 6.0 mg/kg.
[a-d] Within a row, means with no common superscript differ significantly ($P < 0.05$). $n = 6$ replicates per treatment.

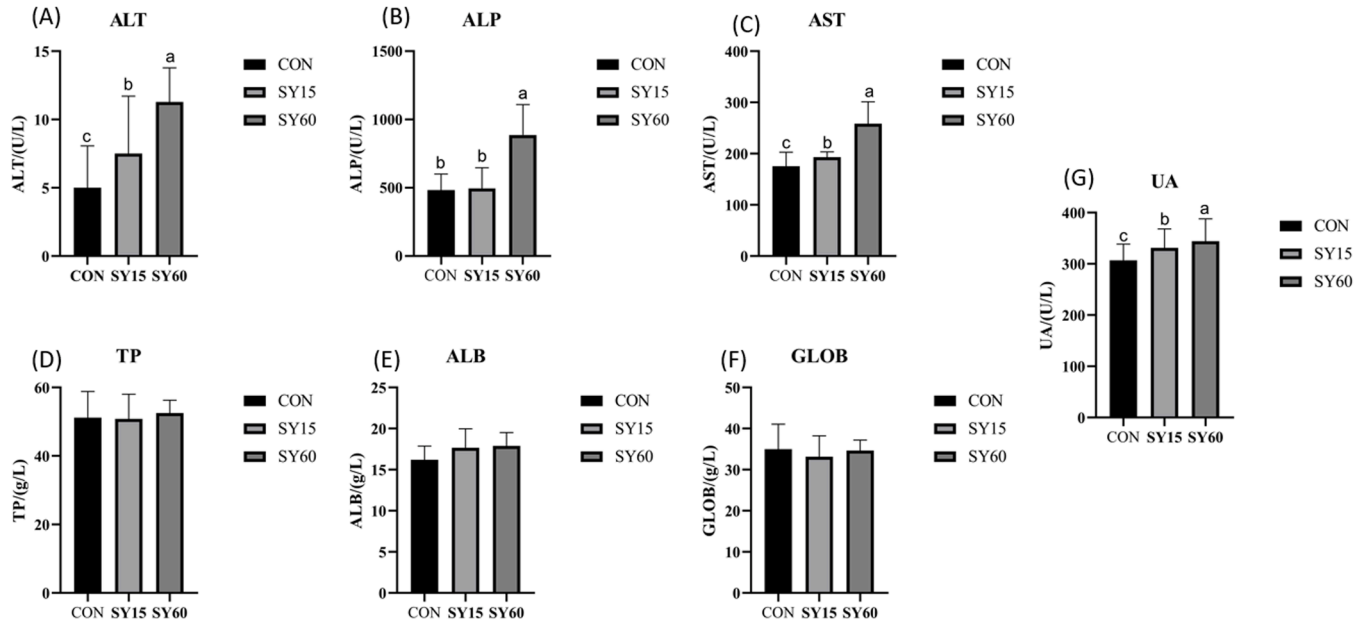

Fig. 1. Effect of dietary selenium-enriched yeast on the serum biochemical of laying hens. CON, control; SY15, 1.5 mg/kg; SY60, 6.0 mg/kg. (A) ALT, alanine transaminase. (B) ALP, alkaline phosphatase. (C) AST, aspartate transaminase. (D) TP, total protein. (E) ALB, albumin. (F) GLB, globulin. (G) UA, uric acid. [a-c] Bars with no common superscript differ significantly ($P < 0.05$). $n = 6$ replicates per treatment.

metabolic indicator like UA, while the control group showed the lowest values ($P < 0.05$). There was no significant influence of dietary SY on other protein metabolic indicators such as total protein, albumin, or globulin levels ($P > 0.05$), as they were comparable to the control group.

The effects of SY supplementation on the immunoglobulin levels and activities of antioxidant enzymes are presented in Fig. 2.

Dietary SY significantly influenced IgM levels ($P < 0.05$). The SY60 group was significantly higher ($P < 0.05$) than the SY15 group. However, no significant dietary influences were observed for IgA and IgG levels ($P > 0.05$).

There was a marked significant effect of SY on the activities of antioxidant enzymes CAT, GSH-Px, and SOD as well as levels of T-AOC ($P < 0.05$). The activities of T-AOC, and CAT were comparable between SY15 and SY60 ($P > 0.05$), while the activity of SOD was significantly higher in the SY60 group compared to the S15 group ($P < 0.05$). Moreover, the activity of GSH-Px was higher in SY15 group ($P < 0.05$), compared to that in SY60 group.

Effect of dietary SY on organ index

The effects of dietary SY on the organ index of the heart, lung, kidney, liver and spleen of laying hens are presented in Fig. 3. The dietary addition of SY60 significantly increased the liver index compared to the control group ($P < 0.001$), with SY group recording the highest value ($P < 0.05$), while the values for SY15 and control group were comparable ($P > 0.05$). However, dietary SY had no significant effect on the heart, spleen, lung, and kidney index ($P > 0.05$), and was comparable to the control group.

Effect of dietary SY on histomorphology of the liver

Effect of dietary selenium-enriched yeast on the histomorphology and histopathology scores of the liver in laying hens, in control and experimental groups were illustrated in Fig. 4 A and B, respectively. There were significant effects of dietary SY on histopathology scores of the liver ($P < 0.05$). The effect of SY on histopathology scores of the liver was highly significant for SY60 group ($P < 0.001$), compared to SY15 and control groups which recorded much lower values nearest to zero level. There were significant effects of dietary SY on histomorphology of the liver ($P < 0.05$). The hepatocytes in the SY60 group, displayed signs of eosinophilic degeneration in hepatocytes, manifested by abundant eosinophilic cytoplasm and rounded nuclei, indicative of oxidative cellular stress or damage. In contrast, the hepatocytes in control group, exhibited normal morphology characterized by distinct cellular boundaries, clear cytoplasmic detail, and moderately stained cytoplasm.

Effect of dietary SY on expression of antioxidant genes in the liver

The influence of SY supplementation on the hepatic expression of key antioxidant genes and pathways are shown in Fig. 5. There was significant influence of SY supplementation on the relative expression of antioxidant genes in the liver ($P < 0.05$). The relative expression levels of *Nrf2, HO-1*, and *NQO1* were significantly upregulated, while the *Keap1* was downregulated in the SY15 group compared to the control ($P < 0.05$). Contrarily, no significant effect of dietary treatment was observable in the expression of *CAT* genes ($P > 0.05$).

Discussion

As per nutritional guidelines, the Se requirement for laying hens ranges from 0.05 to 0.08 mg/kg, with toxic effects observed at doses of tenfold higher (Surai, 2002). In this study, all dietary treatments exceeded this range, ensuring sufficient Se intake. The observed toxicity at 6.0 mg/kg is consistent with selenium's narrow safety margin, highlighting the need for precise dosage management. Our results confirm that SY effectively accumulates selenium in eggs due to its high bioavailability and efficiency (Utterback et al., 2005; Pavlovic et al., 2009). These findings support our hypothesis that varying SY dosages affect laying hens differently, possibly due to their impact on liver health and antioxidant function.

Laying performance

The study revealed no significant effects of SY supplementation on laying performance parameters such as EP, AEW, ADFI, or F/E. These findings align with previous studies reporting no substantial impact of

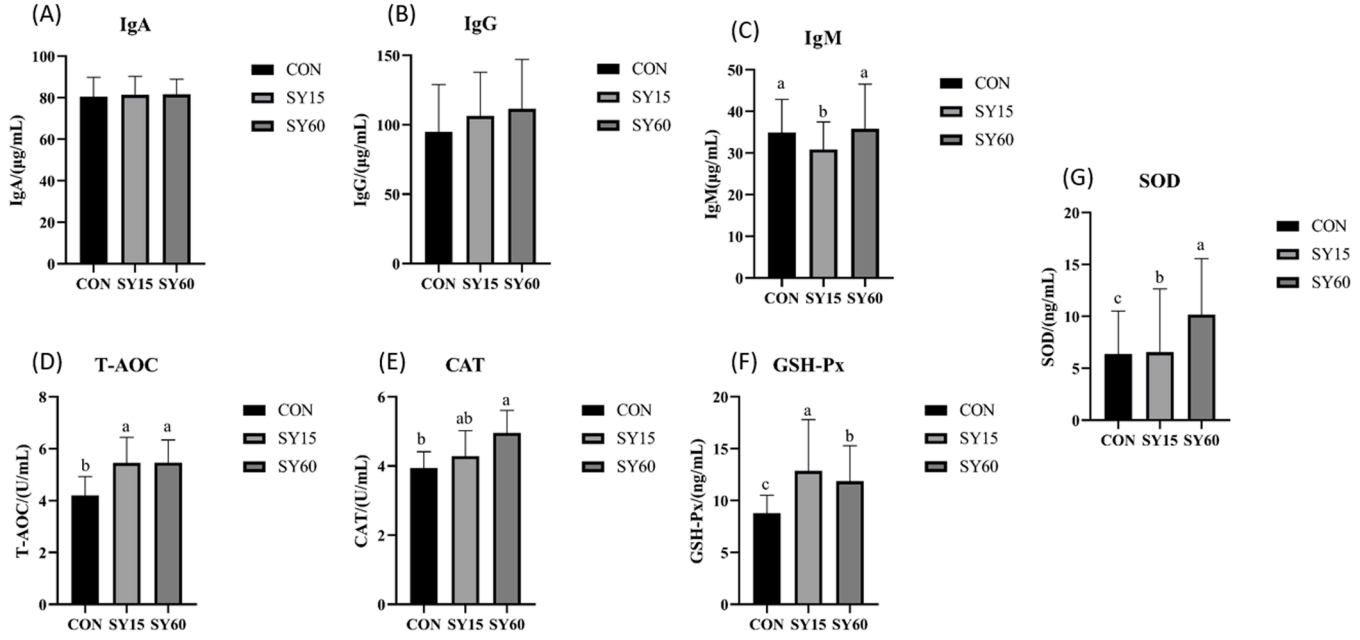

Fig. 2. Effect of dietary selenium-enriched yeast on the serum immunological and antioxidant parameters of laying hens. CON, control; SY15, 1.5 mg/kg; SY60, 6.0 mg/kg. (A) IgA, immunoglobulin A. (B) IgG, immunoglobulin G. (C) IgM, immunoglobulin M. (D) T-AOC, total antioxidant capacity. (E) CAT, catalase. (F) GSH-Px, glutathione peroxidase. (G) SOD, superoxide dismutase. [a-c] Bars with no common superscript differ significantly ($P < 0.05$). $n = 6$ replicates per treatment.

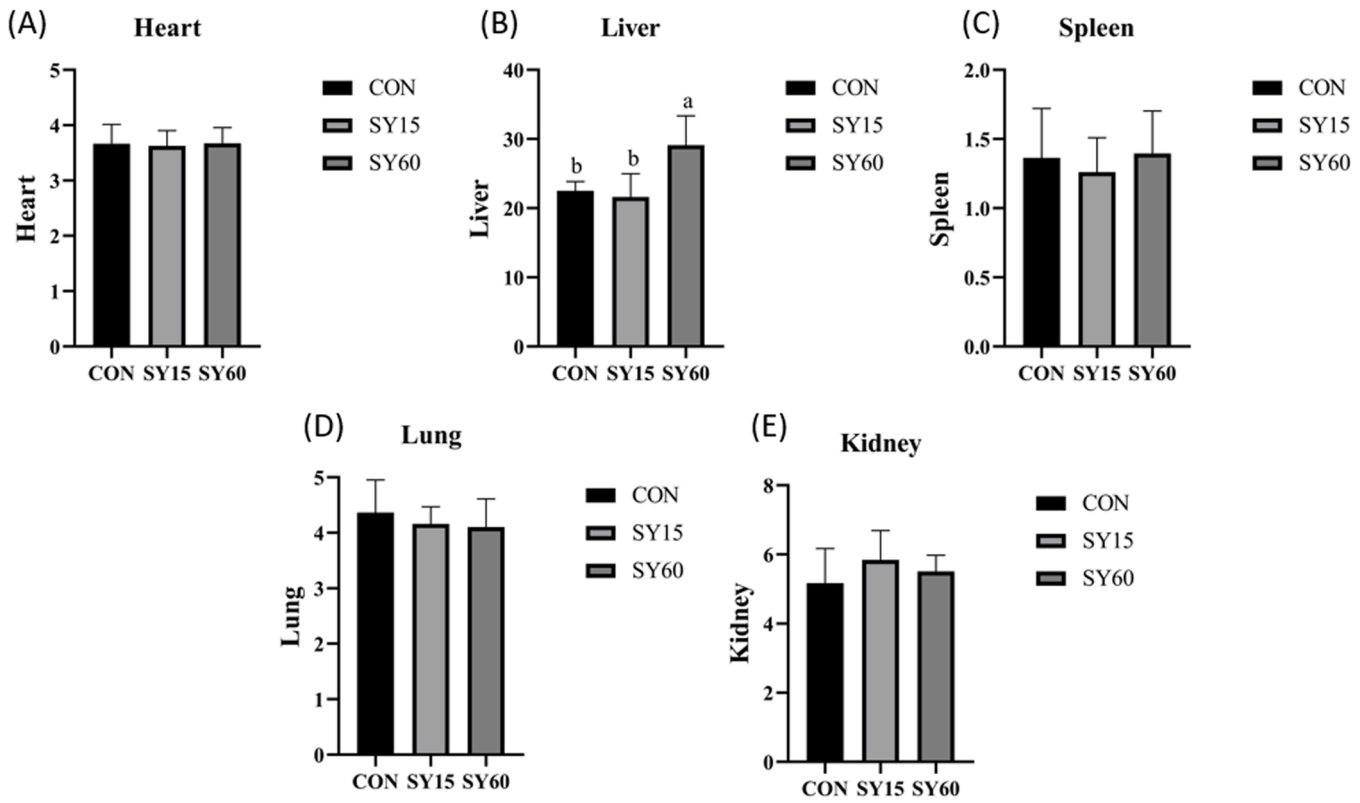

Fig. 3. Effect of dietary selenium-enriched yeast on the organs index of laying hens. CON, control; SY15, 1.5 mg/kg; SY60, 6.0 mg/kg. [a,b] Bars with no common superscript differ significantly ($P < 0.05$). $n = 6$ replicates per treatment.

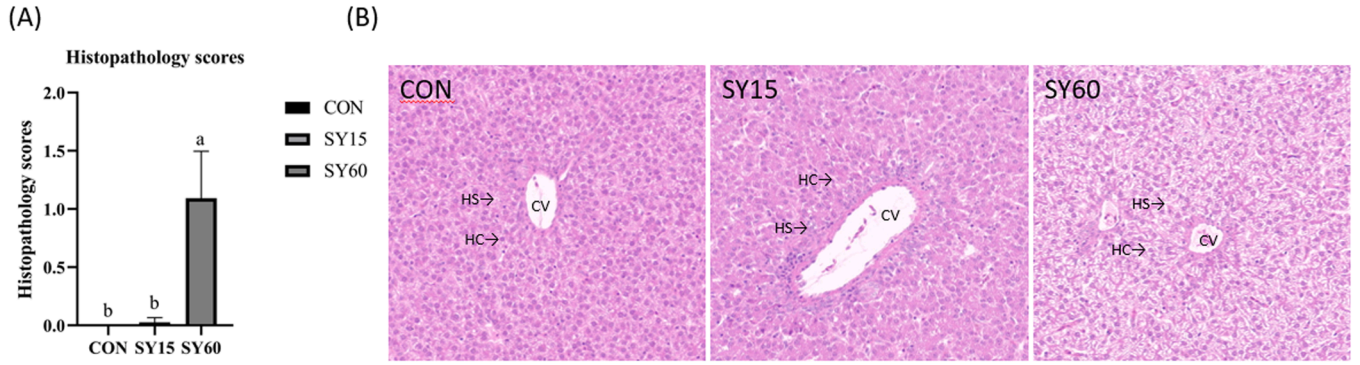

Fig. 4. Effect of dietary selenium-enriched yeast on the histomorphology and histopathology scores of the liver in laying hens. (H&E, 40 ×) staining of liver sections, scale bar: 100 μm. CV, central vein; HC, hepatic cell; HS, hepatic sinusoid; CON, control; SY15, 1.5 mg/kg; SY60, 6.0 mg/kg. [a,b] Bars with no common superscript differ significantly ($P < 0.05$). $n = 6$ replicates per treatment.

SY on production performance metrics (Lu et al., 2018; Lu et al., 2019; Lu et al., 2020). This suggests that the inclusion of selenium in its organic form at the tested doses does not directly enhance the fundamental production efficiency of laying hens, particularly during shorter feeding periods. Selenium's primary role as an antioxidant likely supports physiological processes that maintain overall health but may not immediately translate to measurable improvements in production metrics. Interestingly, studies involving long-term feeding of SY at 0.4–0.8 mg/kg (Pavlovic et al., 2009) and 2 mg/kg (Chen et al., 2024) reported significant improvements in laying performance and feed efficiency over a 16 weeks feeding trial. This implies that prolonged selenium exposure promotes tissue selenium accumulation, enhancing its utilization efficiency and potentially contributing to improved production outcomes. However, discrepancies in findings across studies, where SY has been reported to enhance both laying rate and feed efficiency (Zia et al., 2016; Meng et al., 2019; Muhammad et al., 2021), improve laying rate without affecting feed efficiency (Liu et al., 2020), or increase feed efficiency without impacting laying rate (Meng et al., 2021), may stem from variations in factors such as bird age, feeding trial duration, and SY dosages.

Our findings on the slight reduction in laying rates in the 6.0 mg/kg SY group during weeks 37–40 suggest that higher doses of SY may contribute to long-term physiological stress, highlighting the need for dosage specificity. This finding underscores the need for additional research to investigate the long-term impacts of high-dose SY supplementation on both performance metrics and oxidative stress levels. Understanding the optimal dosage and duration for selenium supplementation is critical for maximizing performance while safeguarding the health of laying hens. Additionally, exploring combined selenium products may offer insights for enhancing laying performance. For example, Han et al. (2017) demonstrated that combining sodium

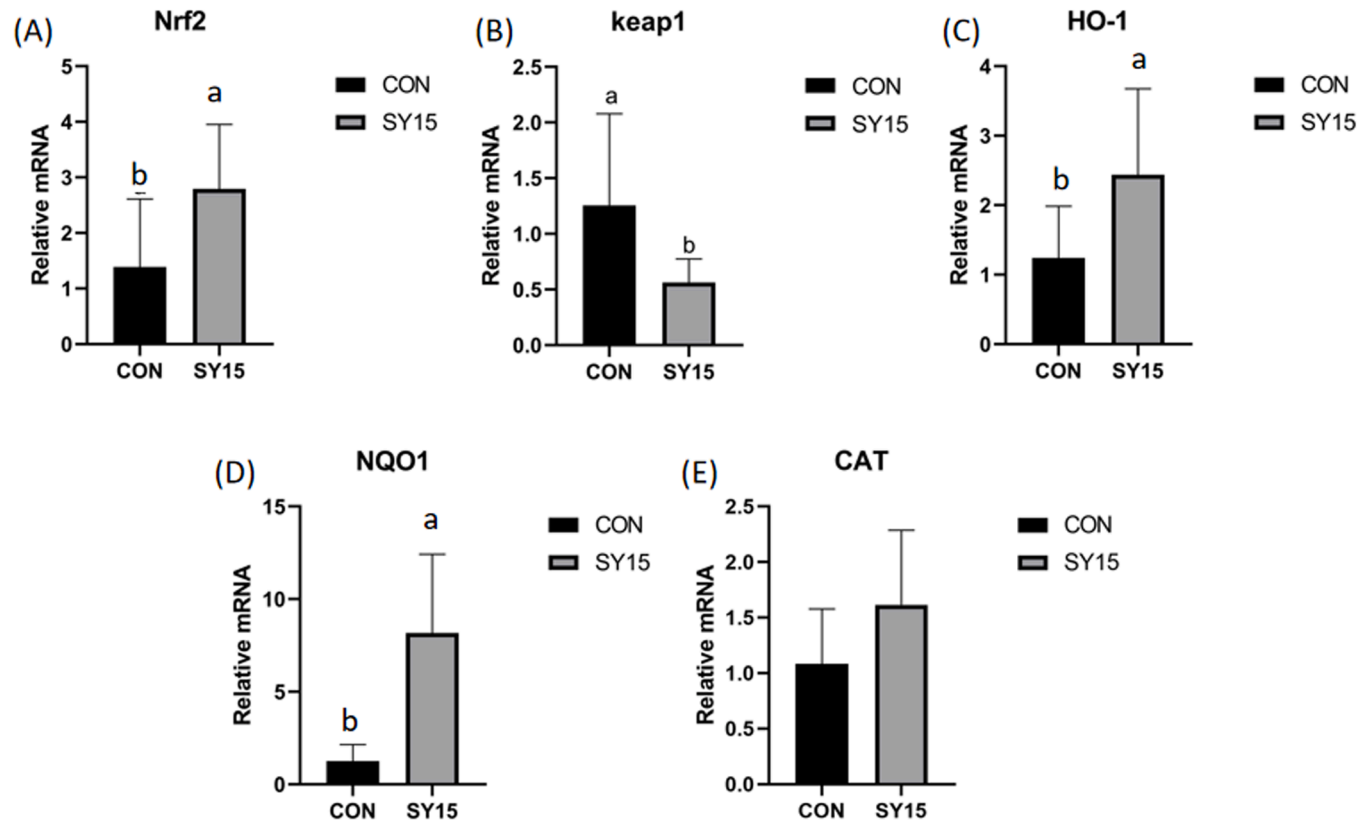

Fig. 5. Effect of dietary selenium-enriched yeast on hepatic gene expression (relative to β-actin) in laying hens. CON, control; SY15, 1.5 mg/kg. (A) *Nrf2* mRNA expression. (B) *Keap1* mRNA expression. (C) *HO-1* mRNA expression. (D) *NQO1* mRNA expression. (E) *CAT* mRNA expression. Primer pairs used for these analyses are listed in Table 2. [a,b] Bars with no common superscript differ significantly ($P < 0.05$). $n = 6$ replicates per treatment.

selenite with SY significantly improved laying rates compared to using either component alone, likely due to enhanced absorption efficiency. Such synergistic interactions between selenium compounds merit further investigation to elucidate their mechanisms and contributions to both short-term and long-term production outcomes. Although SY supplementation did not markedly improve laying performance in this study, examining its effects on egg quality may provide a clearer understanding of the relationship between selenium absorption efficiency and its benefits in poultry production

Egg quality

Egg quality parameters, such as albumen height, HU, yolk color, and eggshell strength, serve as critical economic indicators in the poultry industry, reflecting internal freshness and consumer preferences. In our study, supplementation with 1.5 mg/kg of SY resulted in significant improvements in albumen height, HU, and yolk color, all of which are essential for both consumer appeal and functional properties in food processing. The results align with previous reports which demonstrated that SY supplementation at 0.3 mg/kg (Muhammad et al., 2021) and 2 mg/kg (Chen et al., 2024), respectively, enhanced HU and eggshell strength, and albumen height over a 16-weeks feeding trial. These enhancements can be attributed to selenium's antioxidant properties, which help preserve the integrity of egg proteins and support efficient protein metabolism. Improved albumen quality is particularly advantageous for the food industry due to its desirable technological properties, including gelling, foaming, and emulsification. Contrarily, supplementation of SY at various dosages of 0.2 mg/kg (Li et al., 2024), 0.3-3.0 mg/kg (Lu et al., 2019), and 0.3-0.5 mg/kg (Liu et al., 2020), 0.1-0.4 mg/kg (Lu et al., 2020) had no significant effect on egg quality traits. Additionally, the study by Han et al. (2017) demonstrated that supplementation of SY in the diet of laying hens for 11 weeks had no significant impact on egg quality traits. Discrepancies among studies may not be solely attributed to dosage levels and the duration of feeding trials; factors such as the age of the animals and environmental conditions may also contribute to the variability in results. Notably, supplementation at 6.0 mg/kg led to increased selenium deposition in eggs but did not yield additional improvements in egg quality metrics when compared to the 1.5 mg/kg level. This suggests a plateau effect and potential metabolic strain at higher doses. These findings highlight the importance of optimizing selenium supplementation levels to achieve desired egg quality outcomes while avoiding undue physiological burdens on the hens. Future research should focus on elucidating the molecular mechanisms underlying selenium's role in protein preservation and its broader implications for egg quality.

Selenium deposition in eggs

Selenium-enriched eggs are increasingly becoming consumers utmost preference due to their considerable health benefits, due to their antioxidant and immune boosting properties (Davil-Vega et al., 2023). As a mineral absorption and retention model, avian eggs are particularly valuable for assessing selenium's bioavailability.

Our findings demonstrated the dose-dependent increase in selenium deposition in whole eggs, albumen, and yolk, affirming the high bioavailability and efficiency of SY as a selenium source. Previous studies have shown similar trends, with SY outperforming other selenium sources (Lu et al., 2018; Słupczyńska et al., 2018; Lu et al., 2020; Li et al., 2024), and non-selenium supplemented diets (Lu et al., 2019), in bioavailability and deposition efficiency. The primary component of SY, selenomethionine, mimics methionine, enabling rapid absorption through specific amino acid transport pathways in the small intestine. This facilitates its efficient incorporation into proteins, resulting in superior selenium retention compared to inorganic sources (Sunde et al.,

2016; Hariharan and Dharmaraj, 2020). Also, there were reports that SY supplementation increased Se deposition in yolk compared to albumen (Li et al., 2024). The elevated concentrations of selenium in the yolk relative to the albumen correspond with the yolk's function as the primary nutrient reservoir, reinforcing its critical role as the primary nutrient reservoir (Chen et al., 2024). The enhanced retention of selenium in whole eggs and individual components, further substantiates the superior bioavailability of organic selenium in terms of its incorporation into egg matrices. Future research should focus on understanding selenium deposition dynamics across egg components, particularly the yolk, to refine supplementation strategies for producing selenium-enriched functional foods. The efficiency of selenium deposition in eggs is closely tied to liver function, as the liver plays a central role in selenium metabolism and the synthesis of selenoproteins, which are critical for antioxidant defenses and the transport of selenium to target tissues like the yolk.

Liver health and toxicity at high Se levels

Liver health and function are critical indicators of systemic wellbeing and are often assessed by measuring enzymatic activities of ALT, ALP, and AST (Guerrini et al., 2022). Elevated levels of these enzymes indicate hepatocellular damage or dysfunction, commonly associated with oxidative stress or selenium toxicity at high doses (Zhang et al., 2023). In this study, the SY60 group exhibited significantly elevated enzyme activities alongside a higher liver index, suggesting liver damage, increased intrahepatic fat, or liver dysfunction, likely linked to selenium metabolism in the liver. These findings underscore the need for further investigation into the dose-dependent effects of selenium and the factors contributing to its toxicity.

Histopathological analysis provided additional insight into the specific structural effects of SY on the liver cell morphology, assessing any signs of inflammation, fibrosis, or other pathological changes using microscopy (Malyar et al., 2021; Li et al., 2023). The liver, a vital organ located in the upper right quadrant of the abdomen, is organized into hepatic lobules composed of hepatocyte cords and sinusoids surrounding the central vein of each lobule (Michalczuk et al., 2021). Examination of liver samples revealed eosinophilic degeneration of hepatocytes in the SY60 group, a hallmark of hepatocyte injury associated with diminished cellular function and impaired liver metabolism (Hora and Wuestefeld, 2023). This degeneration is indicative of hepatocellular stress and early signs of liver damage caused by excessive selenium intake. Selenium toxicity is linked to the generation of free radicals, which induce oxidative stress, cause DNA damage, and disrupt protein functions due to selenium's high affinity for thiol groups (Letavayova et al., 2008).

In contrast, the SY15 group exhibited relatively healthy hepatocytes, suggesting that moderate SY supplementation (1.5 mg/kg) supports liver health and avoids hepatocellular damage. Similarly, lower selenium dosages, such as 0.4 mg/kg, have been shown to protect hepatocytes against oxidative stress, highlighting the importance of appropriate dosage management to prevent liver damage and metabolic disturbances (Abbas et al., 2022).

The findings suggest that while selenium plays a crucial role in antioxidant defense and overall health, excessive doses can overwhelm the liver's metabolic capacity, leading to toxicity and structural damage. It is essential to monitor the progression of hepatic degeneration in high-dose groups like SY60 to evaluate potential long-term health risks. Further research is needed to elucidate the molecular mechanisms underlying selenium-induced liver damage, particularly at higher dosages, to optimize supplementation strategies and ensure safety in poultry production.

Effect of SY on various organ indices

In addition to the observed effects on liver health, SY supplementation had no significant impact on the indices of non-hepatic organs such as the heart, spleen, lung, and kidney. This indicates that SY supplementation within the tested range does not induce systemic toxicity or abnormal organ development in tissues not directly involved in selenium metabolism. These findings support the overall safety of SY supplementation in poultry, emphasizing that observed hepatotoxicity at higher doses is likely confined to selenium's metabolism in the liver. This reinforces the importance of dosage optimization to maximize health benefits while minimizing adverse effects.

Serum biochemical indices

Serum biochemical indicators can provide insights into animals' metabolic and health status; Total protein, albumin, and uric acid are key indicators of protein metabolism, while globulin is closely tied to immune function (Geng et al., 2021). Activities or levels of protein metabolism indices, immunoglobulins, and antioxidant enzymes often encompass the serum biochemical indices used for birds' metabolic assessment in response to dietary treatments. This stability in organ indices aligns with the unchanged serum biochemical markers observed in this study, further supporting the systemic safety of SY supplementation at the tested dosages.

Protein metabolism indices

Serum biochemical markers, including TP, ALB, and GLB, remained unchanged at both 1.5 mg/kg and 6.0 mg/kg SY supplementation, indicating no adverse effects on protein metabolism. Previous studies reported similar trends at lower dosages, such as 0.3-3 mg/kg (Lu et al., 2019) and 0.3-0.5 mg/kg (Lu et al., 2020). These findings suggest that SY supplementation, within the tested range, supports protein balance through regulatory mechanisms. Although, supplementation of SY at 6.0 mg/kg, had no effect on protein metabolism indicators, it caused a negative impact on liver heath and function particularly affecting fat metabolism and energy balance, indicating that SY supports protein balance through regulatory mechanisms. This suggests that protein metabolism markers are less sensitive to selenium-induced liver dysfunction than liver-specific enzymes, and excessive selenium may primarily affect liver health without altering serum protein levels.

Immune function and antioxidant activity

Immune function

Immunoglobulins, such as IgA, IgG, and IgM, are critical markers of humoral immunity in avian species, playing essential roles in infection defense (Schroeder and Cavacini, 2010). In this study, SY supplementation did not significantly affect IgA or IgG levels but did influence IgM concentrations, suggesting that the 84-day feeding regimen supported immune function without compromising immunity. The IgM, a key marker of humoral immunity (Liu et al., 2019) was significantly influenced, aligning with previous findings that selenium-enriched diets benefit immune function in poultry (Li et al., 2024). However, the inconsistent effects of SY on IgA and IgG highlight the need for further research to clarify its specific impact on these immunoglobulins and optimize supplementation strategies for immune enhancement in laying hens.

Antioxidant activity

The antioxidant system, comprising key enzymes such as SOD, GSH-Px, CAT, and T-AOC, plays a critical role in neutralizing oxidative stress and maintaining cellular health and productivity in animals (Ozgocmen et al., 2007). The GSH-Px, a selenium-dependent enzyme, reduces harmful peroxides to protect cells, while SOD and CAT work to detoxify reactive oxygen species (Delwing-Dal et al., 2016; Muhammad et al.,

2022). T-AOC reflects the combined antioxidant activity of enzymatic and non-enzymatic compounds. These components work synergistically to protect cells from oxidative stress, ensuring animals' overall health and well-being by enhancing their resilience against environmental and metabolic challenges.

In this study, SY supplementation significantly increased SOD and CAT activities in the SY60 group, with both SY15 and SY60 groups showing elevated T-AOC levels, while GSH-Px activity were notably higher in the SY15 group, highlighting selenium's critical role in redox homeostasis (Liu et al., 2023). These findings align with previous studies demonstrating SY's positive impact on antioxidant enzymes, such as increased activities of GSH-Px (Han et al., 2017), GSH-Px, SOD, and T-AOC levels (Li et al., 2024). Another study reported that Se supplementation enhanced the T-AOC levels in the serum, while SY specifically enhanced activities of CAT and SOD, although both are not Se-dependent enzymes (Meng et al., 2021). The positive effect of selenium (Se) on the antioxidant defense system is attributed to its role in forming selenocysteine, a crucial component of glutathione peroxidase (GSH-Px), which reduces harmful peroxides and mitigates oxidative damage (Yang et al., 2016). This underscores selenium's importance as a vital nutrient for enhancing the body's resilience against oxidative stress. However, conflicting results have been reported, with some studies finding no significant effect (Delezie et al., 2014) or lower effect (Meng et al., 2019) of SY on GSH-Px compared to other selenium sources such as sodium selenite, probably due to absorption efficiency. In another study, supplementation of SY at 2 mg/kg had no significant effect on T-AOC and GSH-Px but increased SOD (Chen et al., 2024). Variations may stem from differences in selenium sources, bioavailability, and dosages.

Interestingly, the SY60 group did not provide additional antioxidant benefits, indicating that 1.5 mg/kg is optimal for enhancing antioxidant capacity without toxicity risks. While higher doses (6 mg/kg) increased antioxidant enzyme activity, they also posed liver damage risks, as evidenced by elevated liver enzymes and histopathological changes. These findings emphasize the importance of optimal dosage selection/utilization to make the most of selenium's benefits without compromising health or productivity.

To further understand selenium's antioxidant effects, mRNA expression of key antioxidant genes (*Nrf2, Keap1, HO-1, NQO1*, and *CAT*) in the liver was analyzed. These insights could clarify the molecular mechanisms by which selenium modulates antioxidant defenses, contributing to optimized supplementation strategies in poultry.

Hepatic gene expression

This study utilized molecular techniques, such as qPCR to analyze liver gene expression in hens treated with selenium yeast (SY) at varying dosages. The focus was on key antioxidant pathway genes: *Nrf2, Keap1, HO-1, NQO1*, and *CAT*, to elucidate SY's regulatory effects on liver function at the transcriptional level (Seehofer et al., 2008). Selenium supplementation has been shown to enhance the expression of antioxidant genes, likely through direct regulation of enzyme activity (Meng et al., 2019; Chen et al., 2016).

Our findings demonstrated that SY15 supplementation significantly upregulated *Nrf2, HO-1*, and *NQO1* expression while downregulating *Keap1*, suggesting enhanced hepatic antioxidant capacity via *Nrf2* pathway activation. The *Nrf2*, a transcription factor critical for cellular protection against oxidative stress, is regulated by *Keap1*, whose reduced expression may promote *Nrf2* activation and downstream antioxidant responses (Ngo and Duennwald, 2022). These results support the hypothesis that SY modulates liver antioxidant defenses by influencing *Nrf2* signaling pathways. Particularly, *CAT* mRNA expression was unaffected, likely due to its unique regulatory mechanisms and role within the antioxidant system.

The observed transcriptional changes align with selenium's known role in bolstering antioxidant defenses, particularly through selenoprotein functions. A study by Lin et al. (2020) found that dietary SY increased the activity of *GPX1* compared to Nano selenium. However, Meng et al. (2021) reported no significant differences in *GPX1* activity or the expression of other antioxidant genes in the liver when comparing different selenium sources. These findings align with the broader understanding of selenium's role in modulating gene expression to bolster the body's defense against oxidative damage (Alshammari et al., 2022).

We could deduce that supplementation of SY 1.5 mg/kg is optimal for meeting physiological needs and improving performance without inducing toxicity. These findings clarify the specific effects of SY on antioxidant gene expression and offer valuable insights for optimizing selenium supplementation strategies in poultry.

SY enhances selenium retention in eggs by boosting antioxidant defenses and supporting liver health, with optimal dosing (1.5 mg/kg) ensuring efficient deposition in the yolk. Excessive doses (6.0 mg/kg), however, cause oxidative stress and reduce retention efficiency. Balanced dosing is key to producing selenium-enriched functional foods while maintaining hen health.

Conclusion

This study demonstrated that selenium-enriched yeast (SY) supplementation at 1.5 mg/kg optimally enhances egg quality, selenium deposition, antioxidant capacity, and hepatic gene expression while maintaining protein metabolism and immune function without adverse effects on liver health. Supplementation of SY significantly upregulated key antioxidant genes (*Nrf2, HO-1, NQO1*) while downregulating *Keap1*, and improved activities serum antioxidant enzyme (GSH-Px, SOD) and levels of T-AOC, supporting enhanced oxidative stress defense. Conversely, 6.0 mg/kg SY induced hepatocellular damage, as indicated by elevated liver enzymes and histopathological changes, despite increased selenium deposition. These findings emphasize the importance of balanced SY dosing to optimize functional benefits while safeguarding hen health and productivity. Future research should explore the long-term effects of selenium supplementation on physiological and molecular responses to refine dietary strategies for poultry.

Disclosures

All authors approve the submission of this manuscript and declare no conflict of interest.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Shugeng Wu reports financial support was provided by Beijing Municipal Poultry Innovation Team (BAIC06-2024), the National Natural Science Foundation of China (32272907), the Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (ASTIP). The Authors they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by Beijing Municipal Poultry Innovation Team (BAIC06-2024), the National Natural Science Foundation of China (32272907), the Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (ASTIP).

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