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Unlocking the Benefits of Organic Selenium: How Different Levels Affect Laying Hen Health and Egg Quality

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Abstract

This study evaluated the effects of organic selenium supplementation at different concentrations on laying hen performance, egg quality, and antioxidant status. We hypothesized that increasing levels of organic selenium would improve egg quality, antioxidant capacity, and Se deposition, outperforming inorganic selenium. This study provides novel insights by using a unique yeast strain (PTCC5052) and evaluating nonlinear Se dose–response patterns. A total of 360 Super Nick hens (38 weeks old) were randomly assigned to six groups and fed either a control diet (no selenium), a diet with 0.3 mg/kg Se from an inorganic source, or 0.1, 0.2, 0.3, or 0.4 mg/kg Se from an organic source derived from *Saccharomyces cerevisiae PTCC5052*—a unique yeast strain. Over 10 weeks, egg production, feed conversion ratio, egg quality, and Se deposition in eggs were assessed. Production performance was not significantly affected by selenium supplementation. However, hens receiving 0.3 and 0.4 mg/kg of organic Se showed significantly thicker eggshells and higher egg selenium content ($P < 0.05$). Antioxidant enzyme activities (glutathione peroxidase, superoxide dismutase) were significantly enhanced in organic Se groups, while malondialdehyde levels were reduced ($P < 0.05$). The strongest antioxidant capacity was observed at 0.1–0.2 mg/kg Se, suggesting a nonlinear dose–response pattern. Serum protein, uric acid, AST, and ALT remained unchanged. These results demonstrate that organic selenium from *S. cerevisiae PTCC5052* improves egg quality and antioxidant capacity without affecting production performance, highlighting the importance of selenium source and dose–response dynamics.

Introduction

Selenium (Se) is an essential trace element in animal physiology, participating in various critical biological functions (Bai ShiPing *et al*., 2017; Bodnar *et al*., 2012). Selenium is absorbed mainly in the small intestine. Organic compounds, including selenomethionine and selenocysteine, show higher bioavailability and tissue retention than inorganic forms like selenite and selenate (Abdelqader *et al*., 2013; Agus *et al*., 2018; Alagawany *et al*., 2021; Lu *et al*., 2018). After absorption, Se binds to proteins and contributes to selenoprotein synthesis, including glutathione peroxidases and thioredoxin reductases, which are central to antioxidant defense, thyroid metabolism, and immune regulation (Attia *et al*., 2020; Bao *et al*., 2010; Baylan *et al*., 2011; Kieliszek & Błażejak, 2013; Novoselec *et al*., 2022; Thiry *et al*., 2012). The antioxidant properties of organic Se can alleviate oxidative stress, which negatively affects animal welfare and productivity (Elnesr *et al*., 2024). Selenium is also crucial for enzymatic activity, immune function, and detoxification, emphasizing its

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role in maintaining health and preventing oxidative damage. Notably, certain microorganisms, particularly yeast species, can incorporate substantial amounts of Se into proteins, mainly as selenomethionine, considered the most effective organic Se form.

Yeast strains of the Saccharomyces genus are widely used in mineral biobinding studies due to rapid growth, high biomass yield, cost-effectiveness, and safety. Their cell walls contain phosphomannan and surface proteins with functional groups such as carboxyl, hydroxyl, amine, phosphate, and hydrosulfide. These structures facilitate efficient absorption and accumulation of microminerals at high concentrations (De Nicola *et al*., 2009; Yuan *et al*., 2011). Se-enriched yeast offers enhanced bioavailability and safety compared to inorganic Se supplements (Elnesr *et al*., 2024; Han *et al*., 2017; Surai & Kochish, 2019; Zhang *et al*., 2020). Selenium also plays a key role in reproductive health, fertility, and embryonic development (Zhang *et al*., 2021). Dietary selenium improves laying performance and egg quality, supporting animal health and productivity (Abou–Ashour *et al*., 2023; Zhang *et al*., 2021). However, previous studies often did not evaluate multiple inclusion levels of organic Se or directly compare organic versus inorganic Se. This study aims to fill this gap by testing a range of organic Se doses and comparing them with an inorganic control.

Specifically, we investigated the effects of dietary supplementation with organic Se derived from *S. cerevisiae PTCC5052* on production parameters, egg quality, antioxidant status, and selected blood parameters in laying hens. By assessing dose-response relationships, this study addresses the novelty gap and identifies optimal Se supplementation strategies.

Materials and Methods

Se-enriched Yeast

Initially, *Saccharomyces cerevisiae* was employed to produce organic Se. Yeast cells were gradually adapted to sodium selenite (Na_2SeO_3) to enable Se incorporation into amino acids, particularly methionine and cysteine, forming selenomethionine and selenocysteine. The culture medium was optimized using Design Expert software, fermentation was performed in a fermentor, and the final product was spray-dried and mixed with maltodextrin before inclusion in poultry diets (Gong *et al*., 2023; Tan *et al*., 2025). The Se concentration of the final yeast preparation was verified using atomic absorption spectroscopy to ensure consistent dosing.

Experimental Design

A total of 360 Super Nick laying hens (38 weeks old) were randomly assigned to six treatment groups (10 replicates per group, 6 hens per replicate, 60 hens per treatment). Treatments were as follows (See Table 1): Group 1: Negative control (Se-deficient basal diet), Group 2: Positive control (0.3 mg/kg inorganic Se as sodium selenite), Group 3: Basal diet + 0.1 mg/kg organic Se, Group 4: Basal diet + 0.2 mg/kg organic Se, Group 5: Basal diet + 0.3 mg/kg organic Se, Group 6: Basal diet + 0.4 mg/kg organic Se. A 2-week adaptation period with a basal diet was conducted before the trial. Daily egg production and weekly feed intake were recorded to establish baseline performance. This design allowed evaluation of the dose-response effects of organic Se.

Table 1: Dietary treatments and selenium levels

Group	Treatment description	Selenium level (mg/kg) (Se in the premix)	Source
1	Negative control	0.0	Basal diet
2	Positive control (inorganic)	0.3	Sodium selenite
3	Organic Se (low)	0.1	Se-enriched yeast
4	Organic Se (medium-low)	0.2	Se-enriched yeast
5	Organic Se (medium-high)	0.3	Se-enriched yeast
6	Organic Se (high)	0.4	Se-enriched yeast

Summary of the six experimental treatments used in the study. Se levels indicate the amount of selenium supplemented per kilogram of diet. Inorganic Se was provided as sodium selenite(Na_2SeO_3) and organic Se as Se-enriched yeast.

Diet and Feeding

A corn–soybean meal basal diet met or exceeded Supernick nutrient recommendations (See Table 2). The basal diet served as the 0 ppm Se control. Selenium was added as Se-enriched yeast (0.1–0.4 ppm) or sodium selenite (0.3 ppm) for respective groups. Feed and egg collection schedules were standardized. Lighting: 16 h light, 8 h dark.

Table 2: Ingredients and nutrient levels of the basal diet

Ingredients (%)	Composition
Corn	63
Soybean meal	25.41
Limestone	9
Mono Calcium Phosphate	1.20
DL-Methionine	0.19
NaCl	0.24
$NaHCO_3$	0.19
Vitamin and mineral premix*	0.50
Choline-HCl	0.07
Permix	0.20
Nutrients (%)	
Metabolizable energy (Kcal/Kg)	2730
Crude protein	16
Calcium	3.70
Available phosphorus	0.37
Sodium	0.17
Digestible methionine	0.44
Digestible methionine + cystin	0.66
Digestible lysine	0.74
Digestible threonine	0.51

*Premix includes (per kilogram of diet): Vitamin A: 10,000 IU; Vitamin D_3:2,500 IU; Vitamin E: 20 IU; Cobalamine: 0.015mg; Riboflavin: 4 mg; Niacin: 30 mg; Pantothenic acid: 10 mg; Menadione: 3 mg; Folic acid: 0.5 mg; Pyridoxine: 3 mg; Thiamine: 1 mg; Biotin: 0.05 mg; Manganese: 100 mg; Zinc: 60 mg; Iron: 25 mg; Copper: 5 mg; and Iodine: 0.5 mg.

Laying Hens' Performance

Daily egg production was recorded. Weekly egg production (%) was calculated per replicate: weekly eggs ÷ hen-days × 100. Hen-days = total viable birds per replicate × days. Weekly FCR = feed intake ÷ total egg mass. Composite FCR = average of weekly FCR over 70-day trial. Average daily feed intake = total feed ÷ number of birds ÷ days. Egg weight and mass were recorded daily. Body weight gain = final – initial body mass.

Egg Quality

Egg quality was evaluated using 360 eggs (60 per treatment). Measurements included egg weight, Yolk and albumen Weight, and eggshell thickness (measured at three points with a micrometer). Se concentration in eggs was determined by microwave digestion (HNO_3–H_2O_2) and atomic absorption spectroscopy (Lipiec *et al*., 2010).

Hematological and Biochemical Analysis

Ten hens were randomly selected per treatment (n = 60). Blood samples (3 mL) were collected from the alar vein of one bird per replicate. Samples were divided for hematological (EDTA tubes) and serum biochemical analyses (clot activator tubes). After centrifugation (5,000 g, 15 min), serum was stored at −20 °C. Analyses included uric acid, total protein, AST, ALT, total antioxidant capacity (TAC) (Fingerova *et al*., 2007), glutathione peroxidase (GPx), superoxide dismutase (SOD) (Madi *et al*., 2016), and malondialdehyde (MDA), following standard protocols and commercial kits (Darman Faraz Kave, Randox Iran, Isfahan).

Statistical Analysis

The experiment was conducted using a completely randomized design (CRD). Hens were randomly allocated to six dietary treatments to minimize bias. Data were analyzed using SPSS software (version 2022). Assumptions of normality and homogeneity of variances were checked prior to analysis. One-way ANOVA was performed to detect treatment effects. When significant differences were found, Tukey's HSD post hoc test was applied for multiple comparisons. Statistical significance was declared at $P < 0.05$. The cage served as the experimental unit for performance and egg quality traits, while individual birds were considered for hematological and biochemical measurements.

Results and Discussion

Laying Hens' Performance

Table 3 summarizes the performance outcomes of laying hens across the six treatment groups. Overall, no statistically significant differences were observed among treatments for egg production, feed conversion ratio (FCR), average daily feed intake, egg weight, egg mass, or hen body weight gain ($P > 0.05$ for all parameters). Although no significant differences were detected, a numerical trend toward improved FCR and egg mass was observed with increasing levels of organic Se, suggesting a potential dose-dependent improvement in feed utilization

efficiency. These findings align with previous studies, for example, Pavlović *et al*. (2009) in a 16-week study evaluating 0.4 mg/kg and 0.8 mg/kg of sodium selenite and Se-enriched yeast, reported no significant influence of dietary Se form or concentration on FCR ($P > 0.05$). Likewise, C Chinrasri *et al*. (2009) showed that FCR was not affected by Se source or supplementation levels of 0.3, 1, or 3 mg/kg. Although the absence of statistical significance aligns with the literature, the consistent numerical improvement in FCR at higher organic Se levels suggests a possible dose-dependent trend that may become more pronounced with a longer supplementation period.

Table 3: Effects of different selenium sources on laying performance

Treatment	Egg production (%)	FCR (g of feed/g of egg)	Average daily feed intake (grams /bird /day)	Egg weight(g)	Egg mass (g/hen/day)	Hen weight gain (g)
Negative Control	93.72±1.23	1.63±0.18	98.49±1.01	64.37±0.94	60.30±1.82	75.70±2.56
Positive Control	95.04±1.85	1.59±0.21	98.92±1.16	65.18±1.42	61.90±2.23	76.50±2.68
0.1 mg/kg organic Se	94.67±2.01	1.58±0.52	96.94±2.18	64.79±0.99	61.30±2.25	85.70±3.85
0.2 mg/kg organic Se	96.57±2.36	1.56±0.58	99.36±2.64	65.52±1.40	63.30±2.34	80.70±3.25
0.3 mg/kg organic Se	95.39±2.17	1.58±1.08	98.70±2.53	65.36±1.09	62.30±2.28	78.30±3.16
0.4 mg/kg organic Se	96.64±2.39	1.55±1.18	98.32±2.49	65.39±1.36	63.20±2.26	77.80±2.84
P-value	0.11	0.35	0.07	0.21	0.90	0.49

The results are presented as mean ± standard deviation (SD).

Egg Quality Parameters

Table 4 displays the measurements of egg quality characteristics observed across the experimental groups. Statistical analysis revealed significant differences ($P < 0.05$) between the control and inorganic Se groups compared to organic Se-supplemented treatments. Notably, yolk and albumen weights increased in response to higher levels of organic Se, with the 0.4 mg/kg group exhibiting the highest values for both parameters.

Among prior studies, Stibilj *et al.* (2004) also reported a significant increase in albumen weight following dietary inclusion of 0.3 mg/kg of Se-enriched yeast in laying hens. In the present study, dietary supplementation with organic selenium significantly enhanced eggshell thickness ($P < 0.05$) and increased selenium deposition in eggs. These results corroborate the findings of Arpášová *et al.* (2009), who demonstrated similar improvements using Se-enriched yeast. However, other studies reported inconsistent results. Utterback *et al*. (2005) and Skrivan *et al*. (2006) found no significant differences ($P > 0.05$) in eggshell thickness between hens fed 0.3 mg/kg of Se-enriched yeast and those receiving sodium selenite. Similarly, Qiu *et al.* (2021) concluded that eggshell thickness was not affected by Se source at supplementation levels of 0.3, 1, and 3 mg/kg.

Table 4: Effects of different selenium sources on egg quality parameters

Treatment	Yolk and albumen weight (g)	% of total egg weight	Eggshell thickness (mm)	Selenium concentration (ppm)
Negative Control	35.51±0.90^{b}	55.17 %	0.44±0.03^{b}	0.07±0.05^{c}
Positive Control	36.92±1.19^{b}	56.64 %	0.44±0.02^{b}	0.15±0.08^{c}
0.1 mg/kg organic Se	40.41±2.45^{a}	62.37 %	0.45±0.00^{b}	0.22±0.32^{c}
0.2 mg/kg organic Se	40.18±1.76^{a}	61.32 %	0.47±0.01^{ab}	0.28±0.41^{bc}
0.3 mg/kg organic Se	41.19±1.84^{a}	63.02 %	0.49±0.01^{a}	0.42±0.46^{a}
0.4 mg/kg organic Se	41.84±1.08^{a}	63.99 %	0.49±0.01^{a}	0.35±0.32^{ab}
P-value	<0.001	<0.001	<0.001	<0.001

The results are presented as mean ± standard deviation (SD). Significance level of $P<0.05$. Means with different letters in column (a, b, c) indicate significant differences between treatments.

Importantly, dietary inclusion of organic selenium resulted in a significant elevation ($P<0.01$) of total selenium content in eggs. The highest Se concentration was observed in Treatment 5 (0.3 mg/kg organic Se). The decline in Se deposition at 0.4 mg/kg compared to 0.3 mg/kg may be attributed to a saturation of intestinal Se absorption or homeostatic regulation mechanisms, which limit

excessive Se accumulation. Interestingly, Se deposition in eggs followed a nonlinear pattern, showing a plateau beyond 0.3 mg/kg, suggesting a saturation effect in Se incorporation into egg components. This nonlinear trend is consistent with previous reports indicating that Se deposition efficiency decreases at higher dietary Se levels, likely due to metabolic regulation of Se absorption and incorporation (Powers & Jackson, 2008).

These findings align with multiple studies demonstrating that Se-enriched yeast is more efficient than inorganic sources (e.g., sodium selenite) in increasing egg Se content. This enhanced bioavailability is mainly attributed to the presence of selenomethionine in Se-enriched yeast, which is actively absorbed and incorporated into egg proteins. Wang *et al.* (2022) demonstrated that selenomethionine is effectively integrated into eggs, mimicking methionine utilization pathways. Moreover, selenomethionine can be converted to selenocysteine and subsequently to selenite (Chen *et al*., 2024).

While animals can synthesize selenomethionine from inorganic Se, the underlying metabolic pathways are not fully elucidated (Khan *et al*., 2025). Nevertheless, evidence supports that organic Se is more effective in supporting selenoprotein synthesis than inorganic sources (Piray *et al*., 2025). Consistently, eggs from hens receiving Se-enriched yeast exhibited higher yolk Se content than those from sodium selenite or selenocysteine supplementation (Fisinin *et al*., 2008; Hachemi *et al*., 2023; Vlaicu & Untea, 2025). This highlights Se-enriched *Saccharomyces cerevisiae* as a superior source for producing Se-enriched functional eggs.

Hematological Parameters

Table 5 provides a comparative overview of blood biochemical parameters and antioxidant status across treatment groups. Significant differences ($P<0.05$) were observed in antioxidant enzyme activity among treatments. All organic Se treatments (0.1, 0.2, 0.3, and 0.4 mg/kg) differed significantly from both the control and inorganic Se groups. Hens supplemented with organic Se exhibited lower MDA levels ($P<0.01$) and higher GPx and SOD activities ($P<0.05$) compared with the control group. The observed reduction in SOD activity at the highest Se level, despite increased GPx activity, may reflect an adaptive antioxidant response or inhibitory feedback at supranutritional Se levels. Notably, MDA levels were significantly reduced in the 0.3 and 0.4 mg/kg groups, reflecting the potent antioxidant capacity of organic Se. Conversely, MDA was highest in the inorganic Se treatment. GPx and SOD activities were markedly elevated in the 0.4 mg/kg group, suggesting that higher doses of organic Se may enhance oxidative stress defense mechanisms.

Table 5:| Effects of different selenium sources on hematological parameters

Treatment	MDA (nmol/ml)	SOD (u/g hb)	GPx (u/g hb)	TAC (mmol/L)	Uric acid (mg/dl)	AST (u/l)	ALT (u/l)	Pro (g/dl)
Negative Control	2.57±0.29^{a}	749.62±23.39^{a}	46.50±3.93^{c}	1.10±0.09^{d}	4.70±1.14	160±13.33	16.20±3.15	5.79±0.60
Positive Control	3.25±0.35^{b}	677.18±14.58^{b}	44.20±4.60^{c}	1.30±0.16^{cd}	5.30±0.90	157±14.94	17±3.09	6.03±0.59
0.1 mg/kg organic Se	2.70±0.20^{b}	773.40±14.58^{a}	75.30±7.12^{b}	1.70±0.16^{ab}	4.90±1.18	155±21.21	14.90±2.88	6.07±0.74
0.2 mg/kg organic Se	1.61±0.37^{c}	762.82±59.68^{a}	75.00±5.84^{b}	1.80±0.29^{a}	4.70±0.38	156±11.73	14.70±2.40	6.01±0.35
0.3 mg/kg organic Se	1.27±0.34^{cd}	765.68±36.36^{a}	76.20±4.71^{b}	1.40±0.14^{bc}	5.10±0.79	154±20.65	14.60±2.27	5.65±0.38
0.4 mg/kg organic Se	1.17±0.27^{d}	633.90±12.07^{c}	83.60±5.32^{a}	1.40±0.11^{bc}	5.50±0.87	155±12.69	15.40±2.83	5.81±0.28
P-value	<0.001	<0.001	<0.001	<0.001	0.14	0.33	0.33	0.25

The results are presented as mean ± standard deviation (SD). Significance level of $P< 0.05$. Means with different letters in column (a, b, c) indicate significant differences between treatments.

Similarly, TAC was significantly improved ($P<0.05$) in the 0.1 and 0.2 mg/kg organic Se groups compared with both control and inorganic groups, with progressive increases observed at higher supplementation levels. Literature indicates that Se influences GPx activity through pre-translational regulation of GPx gene expression and mRNA stability. The inverse association between MDA levels and GPx activity observed in this study is well established (Ahmad *et al*., 2012), confirming that enhanced antioxidant enzyme activity corresponds with reduced oxidative damage. SOD, a major enzymatic antioxidant, catalyzes the conversion of superoxide radicals to hydrogen peroxide and

oxygen, thereby mitigating reactive oxygen species. Organic Se enhances antioxidant defenses not only through enzymatic activity but also via non-enzymatic pathways involving molecules such as glutathione and vitamin E (Lu *et al*., 2020).

Uric acid levels did not differ significantly ($P > 0.05$) among groups, nor were there significant changes in AST or ALT levels, indicating that Se supplementation—particularly organic Se—did not adversely affect liver function (Islam *et al*., 2024). Likewise, total protein levels remained unchanged, consistent with previous studies showing no significant impact of Se on protein metabolism or serum protein concentrations (Abdel Magied *et al*., 2020). Unchanged AST and ALT levels indicate no hepatotoxic effect of Se supplementation within the tested range.

Considerations on Intervention Duration

It is important to acknowledge that the intervention duration (70 days) may have influenced the extent of observable effects. This timeframe was chosen for practical and ethical reasons, including adherence, logistical feasibility, and animal welfare. However, it is possible that this relatively short duration was insufficient for certain physiological responses to fully manifest, which may explain why some results, despite showing trends, did not reach statistical significance. Future studies with extended intervention periods may provide a more comprehensive understanding of the long-term and cumulative effects of organic Se supplementation.

Conclusion

This study demonstrates that dietary supplementation with organic selenium, particularly Se-enriched yeast, improves egg quality and enhances antioxidant capacity in laying hens. These improvements can help maintain bird health and reduce disease risk, especially under environmental stress conditions. Beyond production benefits, Se-enriched eggs offer added nutritional value for consumers, supporting the development of functional food products. This provides poultry producers with opportunities to differentiate their products and enhance market value. Overall, organic selenium supplementation represents a practical and sustainable strategy to improve both animal health and product quality in commercial egg production.

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Ethical Approval

This investigation was performed following review and official sanction by the Institutional Animal Research Ethics Board (Approval Identifier: [IR.IAU.CTB.REC.1403.081]), confirming compliance with animal protection protocols.

Conflict of Interest Rights

The research team affirms the absence of any personal or financial interests that could be construed as influencing this study.

CRediT authorship contribution statement

Tara Mirzaei: Data collection and organization, experimental investigation, statistical analysis, research design and execution, provision of materials and tools, and manuscript drafting. Behin Omidi and Seyed Naser Mousavi: Conceptualization, Supervision, Validation, Writing – review & editing. Maryam Tajabadi Ebrahimi and Mehrdad Azin: Supervision, Project administration, Funding acquisition.

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