

Effects of Bacterial Organic Selenium, Selenium Yeast and Sodium Selenite on Antioxidant Enzymes Activity, Serum Biochemical Parameters, and Selenium Concentration in Lohman Brown-Classic Hens

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Abstract

This study compares the effects of sodium selenite, selenium yeast, and enriched bacterial organic selenium protein on antioxidant enzyme activity, serum biochemical profiles, and egg yolk, serum, and tissue selenium concentration in laying hens. In a 112-d experiment, 144 Lohman Brown Classic hens, 23-wks old were divided into four equal groups, each has six replicates. They were assigned to 4 treatments: 1) a basal diet (Con), 2) Con plus 0.3 mg/kg feed sodium selenite (SS); 3) Con plus 0.3 mg/kg feed Se-yeast (SY); 4) Con plus 0.3 mg/kg feed bacterial enriched organic Se protein (ADS18) from *Stenotrophomonas maltophilia* bacteria. On d 116, hens were euthanized (slaughtered) to obtain blood (serum), liver organ, and breast tissue to measure antioxidant enzyme activity, biochemical profiles, and selenium concentration. The results show that antioxidant enzyme activity of hens was increased when fed bacterial organic Se (ADS18), resulting in a significant ($P < 0.05$) increase in serum GSH-Px, SOD, and CAT activity compared to other treatment groups. However, ADS18 and SY supplementation increase ($P < 0.05$) hepatic TAC, GSH-Px, and CAT activity, unlike the SS and Con group. Similarly, dietary Se treatment reduced total cholesterol and serum triglycerides concentrations significantly ($P < 0.05$) compared to the Con group. At 16 and 18 weeks, selenium concentration in hen egg yolks supplemented with dietary Se was higher ($P < 0.05$) than in Con, with similar patterns in breast tissue and serum. Supplementation with bacterial organic Se (ADS18) improved antioxidant enzyme activity, decreased total serum cholesterol and serum lipids, and increased Se deposition in egg yolk, tissue, and serum. Hence, organic Se may be considered a viable source of Se in laying hens.

Introduction

In natural sciences, the term “antioxidant” is increasingly common as it gains attention because of its health advantages (Huang et al. 2005). Synthetic or natural substances applied to products to retard or avert their degradation by the action of oxygen in the air are a more bio-logically applicable concept of antioxidants (Sugiharto 2019; Cimrin et al. 2020). Dietary antioxidants are substances in food that, as described by the Institute of Medicine (Meyers 2000), significantly scavenge and reduce or inhibit the unfavorable effects of reactive species (oxidants), like oxygen or nitrogen species (ROS or RNS), prevent certain diseases, and promote normal physiological functions in living being (Salehi et al. 2018; Aziz et al. 2019). Dietary antioxidants primarily consist of free radicals, reactive oxygen species, metal chelators, enzyme inhibitors, and antioxidant enzyme cofactors (Huang et al. 2005). In a biological system, oxidation is promoted mainly by a host of redox enzymes. Nonenzymatic lipid oxidation, however, can still occur and ultimately result in cell oxidative stress (Kurutas 2016). Biological antioxidants, therefore, include enzymatic antioxidants (like glutathione peroxidase, catalase, and superoxide dismutase) and nonenzymatic antioxidants such as Vitamin E (Aksoz et al. 2020; Gouta et al. 2021) and Vitamin C (Chiaiese et al. 2019; Giuffrè 2019; Saracila et al. 2020), oxidative enzyme inhibitors (aspirin, cyclooxygenase, ibuprofen), antioxidant enzyme cofactors (Se, Coenzyme Q₁₀), meta chelators (EDTA) and scavenge reactive oxygen/nitrogen species (ROS/RNS) (Huang et al. 2005; Kurutas 2016). Biological antioxidants, according to (Halliwell 1990), are substances that “protect, prevent, or minimize the level of

oxidative damage of biomolecules when present at minute concentrations compared to the biomolecules they protect. To produce high-quality livestock products, it is therefore important to use dietary antioxidants as they are capable of reducing lipid peroxidation in serum lipid profile, increases the antioxidant status and its concentration in the animal products, and providing benefits to both animals and humans health (Surai and Dvorska 2002).

Dietary selenium is important in animal nutrition especially the organic form which is highly available in animal tissues compared to inorganic sources for different physiological functions. Selenium required for various physiological functions in animals is a cofactor of selenoproteins (e.g., glutathione peroxidase) that reduces peroxides to alcohol and water (Dalia et al., 2017). Selenium is commonly supplemented in animal nutrition in an inorganic salt or organic form. Because of its biochemical and physiological functions in animals, organic Se is strongly retained in animal tissues relative to inorganic Se sources (Surai and Dvorska 2002; Canoğullari et al. 2010). Different sources of Se in animal tissues have different metabolic effects, according to studies published in the literature (Yuan et al. 2012; Boiago et al. 2014). Dietary supplementation of an antioxidant such as selenium can have a positive effect on the blood (biochemical or haematological) profile of animals. Blood is one of the most accurate markers of an animal's health status, and it can be affected by several factors including nutrition, disease, animal status, environmental, and climatic (Shi et al. 2018).

Blood biochemical index, in particular, may provide details about the animal's nutritional conditions (Mu et al. 2019) and health status (Reda et al. 2020), with aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and bilirubin, creatinine, uric acid, gamma-glutamyl transferase (GGT) as markers of liver and kidney oxidative injury (Abdel-Daim et al. 2020). Lower concentrations of these biochemical indices are related to enhanced antioxidant status and, as a result, the safer condition of kidneys and liver. The administration of biologically or chemically synthesized nano-Se to growing rabbits and laying hens decreased serum urea, triglycerides (TG), glutamyl transferase (GGT), albumin (ALB), and glutamate pyruvate transaminase (GPT), and enhanced the antioxidant markers (Sheiha et al. 2020; Zhou et al. 2021). Similarly, plasma creatinine, the activity of AST enzyme, and plasma total cholesterol, and plasma LDL concentrations all decreased in organic Se supplemented rabbits (Ayyat et al. 2018; Abdel-Azeem et al. 2019). Furthermore, when breeder quails were fed 0.5 to 2.5 g/kg DL-methionine, lipid profile markers, and lactate dehydrogenase (LDH) activity were reduced (Reda et al. 2020). Broiler birds fed 0.1 to 0.5 mg/kg of feed nano-Se reduces blood ALB concentrations (Ahmadi et al. 2018), influenced oxidation resistance (Chen et al. 2013). Se supplementation raises total protein, albumin, total cholesterol, and TG in laying hens during the hot season while decreasing liver enzymes (ALT) and thyroid hormone (thyroxin) (Abd El-Hack et al. 2017).

The mechanisms through which Se functions are implemented exclusively by Se-containing proteins (Mangiapane et al. 2014; Wrobel et al. 2016). Selenocysteine is the major part of which, after absorption into selenoproteins, Se exercises its biological function in a system (Mahima et al. 2014). As a result, selenoprotein concentrations and selenoprotein mRNA yield are influenced by Se supply. In chicken, more identified, all of which play important roles in the catalytic

action of the enzyme. The synthesis of selenoproteins is influenced by nutritional levels of Se supplementation in the diet (Zhang et al. 2013; Dalia et al., 2017). A large number of studies have found a connection between dietary Se supplementation and selenoprotein expression in animal tissues. Furthermore, laying hens with a Se supplemented diet had a significant elevation in antioxidant capacity (Han et al. 2017). Many studies have shown that supplementing the diet of laying hens with Se improves their antioxidant capacity (glutathione peroxidase, superoxide dismutase, and catalase) (Han et al. 2017; Sun et al. 2020). Supplementation with Se improves immune and antioxidant status (Sun et al. 2020), increases the content of selenium in eggs (Liu et al. 2020b), and prevents clinical problems due to deficiency of Se (Nabi et al. 2020). Mineral utilization is primarily determined by their bioaccumulation and retention (Li et al. 2018). The quantity and form of ingested Se determine how it is absorbed and stored in the body (Payne and Southern 2005).

Dietary Se supplementation increased Se deposition and concentration in eggs (Pan et al. 2011; Meng et al. 2019; Liu et al. 2020b). Selenium-fortified eggs can therefore be produced by supplementing the diet of hens with selenium. Compared to the widely used inorganic sodium selenite, organic Se has been reported to increase Se deposition in eggs and improve the quality of eggs (Liu et al. 2020a; Nabi et al. 2020). The efficacy of the organic form of selenium was due to its greater utilization and absorption compared to other selenite sources (Utterback et al. 2005; Delezie et al. 2014; Han et al. 2017). Liu et al. (2020c) reported that the addition of 0.5 mg/kg SY increases egg yolk Se compared to 0.3 and 0.5 mg/kg SS and 0.3 mg/kg SY. Furthermore, hens fed nano-Se and Se-yeast had a significant Se deposition in their egg, liver, and kidney (Meng et al. 2020).

To produce organic Se, different strains of microorganisms can be used in the microbial reduction pathway. *Stenotrophomonas maltophilia* (ADS18) has been linked to organic Se-containing proteins that can be used as Se sources in poultry (Dalia et al., 2017; Dalia et al. 2018). In laying hens, dietary Se (yeast or bacteria) improved antioxidant capacity, increased serum biochemical markers, and boosted Se deposition efficiency (meat, eggs, and blood) (Mohapatra et al., 2014; Han et al., 2017; Nasiri et al., 2019; Wang et al., 2019; Lu et al., 2020; Timur and Utlu 2020; Muhammad et al., 2021). Although Se may help the antioxidant system, there is little scientific evidence on the effect of this new organic Se source on layers. No published research on the effect of bacterial organic Se from the ADS18 source (Dalia et al. 2017), on antioxidant enzyme activity, blood biochemical parameters, and selenium concentration in layers has been recorded. In this study, the antioxidant enzyme activity, blood biochemical parameters, and selenium concentration in laying hens were examined utilizing bacterial organic Se as an alternative organic Se source with other selenium sources.

Materials And Methods

Animal ethics

This study was reviewed and approved by the Institutional Animal Care and Use Committee of University

Loading [MathJax]/jax/output/CommonHTML/jax.js 8). All procedures were performed under the guidelines and

regulations for the administration affairs concerning experimental animals as stipulated.

Animals Experimental Design And Diets

A total of 144 23-wk-old Lohman Brown classic hens (1702 ± 183 g) were divided into four equal groups, each 36 hens reared in a ventilated henhouse and two-tier stainless-steel cages with one hen per cage at Ladang 15, Universiti Putra Malaysia. The cage measured 30 cm in width, 50 cm in depth, and 40 cm in height. Except for Se, which was supplemented as 0.3 mg/kg feed according to (Surai 2006), a basal diet for laying hens was prepared according to NRC (National Research Council) (NRC 1994) guidelines (Table 1). Three supplemented diets were designated as control, basal diet + 0.3 mg/kg feed sodium selenite (SS), Se-yeast (SY), and bacterial organic Se (ADS18), respectively. The production and extraction of the bacterial Se content are described by (Dalia et al., 2017). The experimental diets were formulated with FeedLIVE software and adhered to the nutrient requirements of the Lohman management guide (2018), with hens limited to 120 grams per day. During the experimental process, the hens were fed a day (07:00–08:00) and had *ad libitum* access to water and treatment diets at an ambient temperature of approximately $30 \pm 5^\circ\text{C}$ during the experimental phase. With the light beginning at 17:00 local time and following the Lohman Brown-Classic, (2018), a sixteen-hour light and eight-h dark lightning schedule were exercised. The feeding trial lasted for 16 weeks, with a four-week adaptation period.

Table 1

Ingredient composition and analyzed nutrient concentration of the basal diet (on a dry matter basis).

Ingredients	%
Corn	44.00
Soybean Meal 48%	29.00
Wheat Pollard	11.00
Crude Palm Oil	3.50
L-Lysine	0.10
DL-Methionine	0.25
Dicalcium Phosphate (18%)	2.00
Calcium Carbonate	7.70
Choline Chloride	0.10
Salt	0.35
Mineral Mix ^a	0.60
Vitamin Mix ^b	0.60
Antioxidant ^c	0.40
Toxin Binder ^d	0.40
Total	100.00
Analysed composition	
Metabolizable energy Kcal/Kg	2761.24
Crude protein (%)	17.66
Crude fat (%)	5.30
Fibre (%)	3.98
Calcium (%)	3.65
Total phosphorus (%)	0.88
Available phosphorus for poultry (%)	0.48
^a Mineral premix supplied (per kg of diet): Cu ²⁺ 15 mg, Zn ²⁺ 120 mg, Fe ²⁺ 120 mg, Mn ²⁺ 150 mg, iodine 1.5 mg, and cobalt 0.4 mg.	

Ingredients	%
^b Vitamin premix supplied (per kg of diet): Vitamin A (retinyl acetate) 10.32 mg, vitamin E (DL-tocopherol acetate) 90 mg, cholecalciferol 0.250 mg, vitamin K 6 mg, cobalamin 0.07 mg, thiamine 7 mg, riboflavin 22 mg, niacin 120 mg, folic acid 3 mg, biotin 0.04 mg, pantothenic acid 35 mg and pyridoxine 12 mg.	
^c Antioxidant contains butylated hydroxyanisole (BHA).	
^d Toxin binder contains natural hydrated sodium calcium aluminum silicates to reduce the exposure of feed to mycotoxins.	
^e The Se content measured using ICP:MS.	
^f Feed live International Software (Nonthaburi, Thailand) was used to formulate the diets.	

Slaughtering, Blood, And Tissue Collection

To collect blood and tissue samples, twenty-four hens were randomly selected from the four treatments (one from each replicate) and slaughtered according to Halal procedures, as defined in the Malaysian Standard (Malaysia 2009). Blood samples (10 ml) were taken from each hen's jugular vein and collected in BD Vacutainer® Plus Plastic Serum Tubes (Becton Dickinson, New Jersey, USA) during slaughtering. Blood samples were centrifuged at 3,000 x g at 4°C for 10 min, and the resultant supernatant (serum) was separated and stored at - 80°C for biochemical serum and antioxidant capacity analysis (Humam et al. 2021). For the antioxidant activity assay, a portion of liver tissue was sliced and snapped frozen in liquid nitrogen before being stored at - 80°C. A portion of the breast muscle sample was snapped frozen in liquid nitrogen and stored at - 80°C for further assays.

Determination Of Serum And Tissue Antioxidant Enzymes Activity

The serum and liver were tested for total antioxidant capacity (T-AOC), glutathione peroxidase activity (GSH-Px), and total superoxide dismutase activity (T-SOD), catalase activity (CAT). Phosphate-buffered saline (PBS) was used to homogenize liver tissue on ice, then centrifuged at 3,000 x g for 10 min at 4°C to extract supernatant for enzyme assays (Dalia et al., 2017; Humam et al. 2021).

Total antioxidant capacity (T-AOC) was measured from serum and liver using the QuantiChrom™ Antioxidant Assay Kit (DTAC-100, BioAssay Systems, Hayward, USA), following the manufacturer's instructions. The assay measures the total antioxidant capacity of the sample's antioxidant in which Cu^{2+} is reduced by antioxidant to Cu^+ , a colored complex with a dye is formed with a resulted Cu^+ , and the intensity of the color was proportional with the total antioxidant capacity present in the sample. The

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100 µM Trolox equivalents. Briefly, 5 µL of the standard with

245 μL distilled water (1 mM Trolox) was prepared, and 20 μL each of standards and samples was transferred into 96-well plate following the serial concentration and separate wells for samples, respectively. Working reagent for sample and standard was mixed, for each assay well, containing 100 μL Reagent A and 8 μL of Reagent B. 100 μL working reagent was added to all assay wells, gently tap to mix and incubate at room temperature for 10 min. Finally, the absorbance of the TAC was read at 570 nm using a microplate reader (Multiskan Go, Thermo Scientific, Waltham, Massachusetts, USA) (Dalia et al., 2017; Humam et al. 2021). The standard curve was used to calculate the TAC activity in the serum and liver samples.

2.3.2. Glutathione peroxidase (GPx) activity was performed in serum and liver samples using EnzyChrom™ Glutathione Peroxidase Assay Kit (EGPX-100, BioAssay Systems, Hayward, USA) following the manufacturer's instructions. The assay directly measures NADPH consumption in the enzyme-coupled reactions. The measured reduction in optical density at 340 nm is directly proportional to the enzyme activity present in the sample. The detection range of the kit was 40 to 800 U/L GPx. Briefly, 10 μL of the samples added with 90 μL of working reagent (80 μL assay buffer, 5 μL glutathione, 3 μL HADPH (35 mM), and 2 μL GR enzyme) were transferred into the 96-microplate well and gently tap the plate for the mixture. A 100 μL of the substrate solution was added immediately to each sample including control wells. The optical density of the samples and standards absorbance measurement was immediately measured at time zero (OD0), and at 4 min (OD4) using a microplate reader (Multiskan Go, Thermo Scientific, Waltham, Massachusetts, USA) at 340 nm (Dalia et al., 2017; Humam et al. 2021). The standard curve was a plot using NADPH standards. The standard curve was used to calculate the GPx activity in the serum and liver samples.

$$GSH - PxActivity(U/L) = \frac{\Delta ODS - \Delta ODB}{Slope(mM - 1) * 4(min)} * 1000 * n$$

where;

- $\Delta ODS = (OD0 - OD4)$ for the samples.
- $\Delta ODB = (OD0 - OD4)$ for the background control.
- The factor 1000 converts mmoles to μmoles .
- N is the sample dilution factor.

Superoxide dismutase (SOD) activity was performed for serum and liver using EnzyChrom™ Superoxide Dismutase Assay Kit (ESOD-100, BioAssay Systems, Hayward, USA) following the manufacturer's instructions. The assay relies on the addition of xanthine oxidase (XO) to the samples as a source of superoxide (O_2^-). The O_2^- forms a colored product as it interacts with a specific (WST-1) dye. The sample's SOD activity, which acts as a superoxide scavenger, scavenges the O_2^- , thus lowering the color intensity. The kit has a detection range of 0.05 to 3 U/mL SOD. A microplate reader (Multiskan Go, Thermo Scientific, Waltham, Massachusetts, USA) set to 440 nm was used to calculate the color intensity

indicating SOD activity in a sample. The concentration of SOD in the samples was calculated using the standard curve.

Catalase (CAT) activity was measured from serum and liver using the EnzyChrom™ Catalase Assay Kit (ECAT-100, BioAssay Systems, Hayward, USA), following the manufacturer's instructions. The assay measures the degradation of H₂O₂ using a redox dye, and the detection range of the kit was 0.2 to 5 U/L CAT. As described, 10 µL of the sample, positive control, and assay buffer as blank plus 90 µL of substrate buffer (50 µM) were loaded into 96 micro-plate wells, gently shaken, and incubated at room temperature for 30 min. While waiting for incubation time, the standard curve was prepared by mixing 40 µL of the 4.8 mM H₂O₂ reagent with 440 µL of distilled water in the serial concentration, then 10 µL of the standard solution with 90 µL of assay buffer were placed into standard wells. At the end of incubation time, 100 µL of detection reagent was mixed in each well and incubated for 10 min at room temperature. A microplate reader (Multiskan Go, Thermo Scientific, Waltham, Massachusetts, USA) was used to read the optical density of CAT at 570 nm (Dalia et al., 2017; Humam et al. 2021). The CAT activity in the serum and liver samples was calculated using the standard curve.

Blood Biochemical Assay

The biochemical parameters measured were activities of enzymes (AST, ALP, ALT, and GGT), and concentration of metabolites creatinine, cholesterol, triglycerides, LDL, high-density lipoprotein (HDL), very low-density lipoprotein (VLDL) and lactate dehydrogenase (LDH), total protein, total bilirubin, ALB, globulin, and urea. All constituents were measured using an Auto-blood biochemical analyzer (Automatic Analyser 902, Hitachi, Germany), except serum globulin and albumin/globulin ratio (A/G), which were extrapolated as follows: G = total protein – albumin, A/G = albumin/globulin (Abdel-Azeem et al. 2019). All samples were run in replicates.

Determination of egg yolk, breast tissue, and serum selenium concentration

Eggs were collected, broken, and fractionated into albumen and yolk. They were capped in a container, frozen (> 24 h) at -80°C, and lyophilized at -50°C for 72 h (Labconco FreeZone plus 6, USA), and stored in -20°C until further analysis (Lipiec et al. 2010; Tufarelli et al. 2016). The lyophilized samples were ground to powder using porcelain mortar and pestle and stored at 4°C. All chemicals/reagents were of analytical grade purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) and were used throughout the analysis unless stated differently with the dilutions prepared daily. The procedure of total selenium determination of egg yolk as described (Lipiec et al. 2010), tissues (Jagtap and Maher 2016) was followed with modifications. Briefly, approximately 0.5 mL serum and lyophilized 0.5 g egg yolk and breast tissue each were weighed into 10 ml Teflon digestion vessels (A. I. Scientific, Australia) and 5 ml of concentrated HNO₃ (Sigma-Aldrich, USA) and 3 mL H₂O₂ (Emsure® ISO, Merck) added. Digestion was carried out in DigiPrep (SCP Science, Courtaboeuf, France) to heat the samples for 4 h at 100°C starting at a lower temperature (65°C) for 30 min (approximately) and raises gradually. The vessel was allowed to

cool for 60 min at room temperature (25°C) after digestion and then diluted with distilled or deionized water in a polyethylene vial to a final volume of 10 ml. Total Se concentration in diluted digest was determined with a Perkin Elmer DRC-e ICP-MS with calibrations performed every 20 samples. The quantification (external calibration) was carried out by preparing standard of five different concentrations (0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/L). The ICP MS collision was pressurized with hydrogen. All the samples were injected via a Micromist nebulizer fitted with a Scott double-pass spray chamber for determination (Lipiec et al. 2010).

Statistical analysis

All data analyses were performed using the Statistical Analysis System (SAS) 9.4 Version (SAS Institute, Cary, North Carolina, USA) in a completely randomized design. The data were analyzed by the General Linear Model (GLM) procedure of SAS and Duncan Multiple Range Test was used to separate means. The significant differences between the treatments each with six replicates were established at a P -value < 0.05 level. In all figures and tables, the results were presented as mean \pm SEM.

Results

Antioxidant enzyme activity in serum and liver

The effects of different dietary Se supplementation on the antioxidant enzyme activities of TAC, GSH-Px, SOD, and CAT in laying hen serum and liver (Fig. 1) were summarized. The Se-yeast group had significantly ($P < 0.05$) higher serum TAC activity than did the ADS18, SS, and basal diet groups. Furthermore, supplementation with bacterial organic Se of ADS18 resulted in a significant ($P < 0.05$) increase in serum SOD, and CAT activity when compared to other groups, but GSH-Px activity was similar to the Se-yeast group. The results of GSH-Px activity in SS and non-supplemented hens were not significantly ($P > 0.05$) different. The Se-yeast, SS, and control groups did not differ ($P > 0.05$) significantly in terms of CAT activity. When compared to basal diets, bacterial organic Se (ADS18), Se-yeast and SS supplementation result in a significant ($P < 0.05$) increase in hepatic TAC, GSH-Px, and CAT activity. Furthermore, hepatic GSH-Px activity was decreased in the Con group, and it differed significantly ($P < 0.05$) from the ADS18 and Se-yeast groups, but was similar to the SS group. ADS18 and Se-yeast-fed hens had increased ($P < 0.05$) liver CAT activity than sodium selenite or control treatments. Dietary Se had no effect ($P > 0.05$) on laying hens' hepatic SOD activity in any of the treatment groups. Despite the lack of a regular trend, Se supplementation in any form (inorganic or organic) was associated with a significant ($P < 0.05$) increase in liver antioxidant indicators when compared to the basal diet.

Serum Biochemical Parameters

Table 2 summarizes the effect of dietary Se treatments on serum biochemical parameters in 39-weeks-old laying hens. There were no significant ($P > 0.05$) differences in plasma proteins (total protein, serum

albumin, globulin, albumin globulin ratio), kidney markers (gamma-glutamyl transpeptidase, total bilirubin, creatinine, urea), or uric acid between treatment groups. Hens fed ADS18, Se-yeast, or SS diets showed a decrease ($P < 0.05$) serum AST and ALP activities. There was no significant difference ($p > 0.05$) in serum ALT activities, as the dietary treatment groups values were all less than four units per liter (U/L).

The ADS18, Se-yeast, or SS-supplemented groups had significantly ($P < 0.05$) reduced serum total cholesterol concentrations than did the control group. Serum triglycerides and VLDL were significantly ($P < 0.05$) lower in the ADS18 and Se-yeast dietary Se treatment groups than in the SS and control groups, while the SS and SY groups differ significantly ($P < 0.05$) in HDL. However, there was no significant ($P > 0.05$) differences in LDL and LDH concentrations in either of the dietary treatment groups. There was no significant difference ($P > 0.05$) in serum uric acid concentrations between the dietary treatment groups.

Table 2
Serum biochemical profiles of laying hens fed different selenium sources

Parameters	Experimental diets				<i>P</i> -value	Ref
	Con	SS	SY	ADS18		
Plasma proteins						
Total Protein (g/L)	51.10 ± 2.10	53.92 ± 0.48	51.10 ± 2.01	54.52 ± 1.84	0.375	17–49
Albumin (g/L)	16.45 ± 0.53	17.68 ± 0.38	16.83 ± 0.46	16.75 ± 0.69	0.407	8–28
Globulin (g/L)	34.65 ± 1.98	36.23 ± 0.63	34.27 ± 1.81	37.15 ± 1.99	0.604	4–21
Albumin Globulin Ratio	0.480 ± 0.025	0.489 ± 0.023	0.496 ± 0.022	0.493 ± 0.027	0.968	2–5.2
Kidneys functions						
GGT (U/L)	16.333 ± 3.92	15.333 ± 3.68	13.000 ± 4.58	12.667 ± 3.46	0.891	NA
Total bilirubin (umol/L)	1.767 ± 0.19	1.850 ± 0.14	1.533 ± 0.14	1.72 ± 0.12	0.501	NA
Creatinine (umol/L)	18.00 ± 0.73	18.00 ± 0.93	18.17 ± 1.33	16.50 ± 1.57	0.730	9–71
Urea (mmol/L)	0.233 ± 0.03	0.267 ± 0.05	0.283 ± 0.05	0.317 ± 0.03	0.557	0.41–2.14
Liver functions						
AST (U/L)	270.33 ± 16.12 ^a	254.67 ± 13.12 ^{ab}	225.00 ± 13.34 ^b	219.00 ± 7.74 ^b	0.033	115–290
ALP (U/L)	356.00 ± 33.87 ^{ab}	419.33 ± 48.47 ^a	270.83 ± 40.58 ^b	268.50 ± 28.32 ^b	0.032	
ALT (U/L)	< 4	< 4	< 4	< 4	NA	< 8
Plasma lipids						
Cholesterol (mmol/L)	3.517 ± 0.201 ^a	3.167 ± 0.145 ^{ab}	2.833 ± 0.233 ^{bc}	2.617 ± 0.075 ^c	0.009	NA

The enzyme unit (μmol/min) is a measure of enzyme catalytic activity. GGT gamma-glutamyl transpeptidase, AST aspartate aminotransferase, ALP alkaline phosphatase, ALT alanine aminotransferase, LDL low-density lipoprotein cholesterol, HDL high-density lipoprotein cholesterol, VLDL very-low-density lipoprotein cholesterol, LDH lactate dehydrogenase. Data represent mean ± SD of six replicates of six hens. ^{a-c} Means vary significantly within a row with different superscripts (*P* < 0.05). ^{a-c} Means vary significantly within a row with different superscripts (*P* < 0.05). Loading [MathJax]/jax/output/CommonHTML/jax.js

Parameters	Experimental diets				<i>P</i> -value	Ref
	Con	SS	SY	ADS18		
Triglyceride (mmol/L)	14.685 ± 1.175 ^a	12.332 ± 1.133 ^a	7.863 ± 1.325 ^b	8.875 ± 0.612 ^b	0.001	NA
LDL (mmol/L)	1.235 ± 0.185	0.995 ± 0.055	1.478 ± 0.408	1.165 ± 0.093	0.531	NA
HDL (mmol/L)	0.158 ± 0.008 ^{ab}	0.140 ± 0.003 ^b	0.193 ± 0.003 ^a	0.165 ± 0.011 ^{ab}	0.022	NA
VLDL (mmol/L)	2.94 ± 0.23 ^a	2.47 ± 0.23 ^a	1.57 ± 0.27 ^b	1.78 ± 0.12 ^b	0.001	NA
LDH (U/L)	448.17 ± 33.73	409.83 ± 29.79	418.50 ± 69.72	326.67 ± 29.49	0.273	1010–2489
Antioxidative status						
Uric Acid (umol/L)	153.0 ± 22.89	199.83 ± 32.32	216.0 ± 53.61	165.0 ± 30.85	0.6061	NA
<p>The enzyme unit (μmol/min) is a measure of enzyme catalytic activity. GGT gamma-glutamyl transpeptidase, AST aspartate aminotransferase, ALP alkaline phosphatase, ALT alanine aminotransferase, LDL low-density lipoprotein cholesterol, HDL high-density lipoprotein cholesterol, VLDL very-low-density lipoprotein cholesterol, LDH lactate dehydrogenase. Data represent mean ± SD of six replicates of six hens. ^{a-c} Means vary significantly within a row with different superscripts (<i>P</i> < 0.05). NA is not statistically analyzed or not available.</p>						

Egg Yolk, Breast Tissue, And Serum Selenium Concentration

As a study baseline, the egg yolk Se concentration in each treatment was measured three days after commencing the treatment diets and found to be comparable (*P* > 0.05) in all groups. However, selenium concentrations in egg yolks of hens supplemented with dietary Se were higher (*P* < 0.05) than in hens fed a basal diet at the end (16-wks) of the experimental period (Fig. 2a) and 14 days post-storage (4°C ± 2) after the experiment (18-wks) (Fig. 2b). For fresh and stored egg yolk, organic Se supplemented hens showed greater yolk Se contents than inorganic SS and the basal diet. Except for the SS group, which had significantly higher (*P* < 0.05) yolk Se concentrations than did hens fed the control at the end of the experiment (16-wks) and 14-days post stored eggs (18-wks), no significant (*P* < 0.05) differences were found between the ADS18 and Se-yeast egg yolk Se concentrations at 16-wks (fresh) and 18-wks (stored). Between 16 and 18 weeks, the concentrations of egg yolk Se in all treatment groups were statistically identical, indicating that storage had no effect on egg yolk Se concentration and that there was a significant (*P* < 0.05) difference over the experimental period (Fig. 2b).

Se concentrations in breast tissue and serum increased (*P* < 0.05) in hens given dietary Se compared to the non-supplemented group (Table 3). Organic Se (ADS18 or Se-yeast) treatment groups had no Loading [MathJax]/jax/output/CommonHTML/jax.js at Se concentrations, but they were significantly higher (*P* <

0.05) than those for inorganic and non-supplemented hens. Similarly, hens provided bacterial organic supplementation had the highest ($P < 0.05$) serum Se concentration compared to non-supplemented hens. The serum Se concentration of the hens was ADS18 > Se-yeast > SS and control or basal diet, in that order.

Table 3

Breast tissue and serum selenium concentration of laying hens fed organic and inorganic Se sources

Parameters	Experimental diets*				<i>P</i> -value
	Con	SS	SY	ADS18	
Breast muscle (µg/g)	0.052 ± 0.001 ^c	0.056 ± 0.001 ^b	0.087 ± 0.001 ^a	0.086 ± 0.002 ^a	< .0001
Serum (µg/ml)	0.044 ± 0.000 ^d	0.052 ± 0.001 ^c	0.054 ± 0.001 ^b	0.059 ± 0.001 ^a	< .0001

*Con = control, SS = Sodium selenite; SY = Selenium yeast; ADS18 = Bacterial enriched organic Se. ^{a - c} Values within the same column with different superscript letters differ ($P < 0.05$) significantly.

Discussion

Animal wellbeing may be achieved by enhanced antioxidant capacity (Li et al. 2018). CAT, SOD, and GSH-Px, and lactoferrin, carotene, vitamin C, glutathione (GSH) as non-enzymatic constituents, are antioxidant enzymes metabolites in physiological antioxidant systems (Eşrefoğlu 2009). The main selenium-dependent enzymes are; glutathione peroxidases (GSH-Px) which catalyze H₂O₂ and ROS to water (Behne and Kyriakopoulos 2001), superoxide dismutase (SOD) catalyzes superoxide anion to H₂O₂ and molecular O₂ (Okado-Matsumoto and Fridovich 2001), and catalase (CAT) catalyze hydrogen peroxide decomposition to yield water and oxygen, thus, protecting cells from oxidative damage (Nandi et al. 2019). Hence, dietary Se supplementation enhanced antioxidant capacity in animals (Surai and Dvorska 2002).

In general, the efficacy of organic Se over bioavailability and tissue retention is superior to that of inorganic Se. Minerals' utilization is dependent on their bioaccumulation and retention (Li et al. 2018). Similarly, compared to inorganic, dietary organic, and Nano-Se supplementation could improve the concentration of breast muscles, liver, and serum Se (Mohapatra et al. 2014; Mohamed et al. 2020), possibly resulting in greater activity of GSH-Px. Additive supplementation (e.g. Se) improves the activity of antioxidant enzymes in chickens through antioxidant capacity (Mohapatra et al. 2014; Markovic et al. 2018).

In the present study, organic Se bacteria (ADS18) and yeast (Se-yeast) have demonstrated stronger antioxidant activity in laying hens serum and liver compared to inorganic (sodium selenite) Se and non-supplemented hens, in line with previous findings. The serum TAC value was significantly higher in the control group, and Se-yeast supplementation also

enhanced serum CAT and SOD activity in Brown Hy-line hens (Meng et al. 2020). Moreover, compared to positive control groups of local Chinese yellow male chickens infected with *Eimeria tenella*, (Mengistu et al. 2020) reported higher serum SOD and GSH-Px1 activities with Se-enriched probiotics. Xia et al. (2020) observed a linear and quadratic increase in liver GSH-Px1 and SOD activity in breeder ducks with increased dietary Se levels. In T-2 toxin (T-2) or HT-2 toxin (HT-2)-induced cytotoxicity and oxidative stress broiler hepatocytes, Yang et al. (2019) observed a significant increase in hepatic GSH-Px, SOD, and CAT activity that was activated by toxins with 1 μ M DL-Selenomethionine. Also, relative to those laying hens fed with the basal diet, Meng et al. (2019) reported an improvement in serum GSH-Px, T-AOC, and CAT activities in the nano-Se or sodium selenite group. Dalia et al. (2017) found the highest serum GSH-Px activity and CAT liver with bacterial organic Se supplementation of ADS18, respectively. Li et al. (2017) reported increased serum and breast GSH-Px activity with Se-yeast, Met-Se, and Nano-Se dietary supplementation compared with the SS group. Selenium is an indispensable constituent of the GSH-Px enzyme, actively involved in oxidative damage defense (Rotruck et al., 1973; Fernández-Lázaro et al., 2020).

In this study, the enzyme's activity was significantly enhanced by Se supplementation (organic) in serum and liver. The response of external stimuli and free radicals' metabolism capacity in organisms can be assayed by T-AOC (Huma et al., 2019). The body's total antioxidant capacity can be measured through TAC values (Zhang et al. 2011), with low or higher T-AOC suggesting oxidative stress or susceptibility to oxidative damage, respectively (Meng et al. 2020). Consequently, dietary supplementation with organic Se of ADS18 bacteria or Se-yeast could promote the antioxidant capacity of laying hens, thereby ensuring that egg-laying efficiency is preserved. The potential reason was that ADS18 or Se-yeast contains organic Se, which is much less harmful and more bioavailable and effectively preserved in the tissues of the body.

Furthermore, hens supplemented with Se (regardless of Se form) improves all the measured antioxidant indexes except SOD which was not affected by dietary Se treatments in the liver. In summary, with the addition of bacterial organic Se, the serum and liver TAC, GSH-Px, CAT, and SOD activities produced by the cells to prevent the occurrence of oxidative damage (Xu et al. 2016; Yang et al. 2019), were further enhanced, indicating that the bacterial organic Se of ADS18 could partially reduce oxidative damage by regulating the activities of enzymes (antioxidases). Although catalase and superoxide dismutase are not Se-dependent enzymes for their functions, the presence of Se in animal rations can influence their activities via thyroid hormone metabolism (Meng et al. 2020; Mohamed et al. 2020).

Markers of nutritional conditions in growing animals may be serum biochemical parameters (Mu et al. 2019). The maintenance of plasma osmotic pressure, provision of energy, repairing the worn-out tissue, carrier, and transporter of nutrients to sustain body tissue protein, active balance of cells is the role of albumin protein, which is synthesized in the liver (Surai 2002). Liu et al. (2020a) found no major variations in albumin, total protein, or blood urea nitrogen after adding 0.3 and 0.5 mg/kg addition of sodium selenite and selenium yeast, respectively. Similarly, Hossein Zadeh et al. (2018) did not note any

Loading [MathJax]/jax/output/CommonHTML/jax.js organic or inorganic forms of Se.

Different Se sources supplementation did not affect blood albumin, total protein, globulin, or the albumin globulin ratio in this study. Besides, as kidney function makers, gamma-glutamyl transpeptidase, total bilirubin, creatinine, and urea have not been affected and are per the previous reports (Kumar et al. 2008; Alimohamady et al. 2013; Sethy et al. 2015). Se has a greater impact on serum biochemical parameters, with a significant impact on lipid metabolism and a lesser impact on liver functions. The decreased total cholesterol, triglycerides, and VLDL observed as a result of supplementation with Se-yeast or ADS18 showed that the organic form of Se could play an anabolic role in fat deposition than the inorganic source of Se (Jeyanthi 2010; Sheoran 2017). Besides, the composition of fatty acids in the whole body could be modulated by supplementation Se via organic forms in yeast or bacteria. In a research study conducted by Dhingra and Bansal (2006) and Yang et al. (2010), they reported dietary Se supplementation plays a role in increasing the activity of LDL receptor, but, reduces the expression of 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase in rat and also invariably decrease serum LDL and cholesterol.

The results of this research were inconsistent with the study by Abdel-Azeem et al. (2019) and Amer et al. (2018), which showed the hypolipidemic effect of organic selenium (Se-yeast) in wean male rabbits by significantly reducing serum total cholesterol and LDL-cholesterol. In an in vitro study with Wistar rats, Urbankova et al. (2021) reported that Se deficiency tends to result in increased total cholesterol, LDL, and a significant decrease in HDL concentrations. Antioxidants' hypocholesterolemic activity may be due to oxysterols' inhibition of sterol biosynthesis (Revilla et al. 2009; Hozzein et al. 2020). Consequently, the antioxidant effect is principally attributed to selenoenzymes, glutathione peroxidase (GPX's), and thioredoxin reductase. In studies with growing pullets, Jegede et al. (2012) showed that, compared to CuSO₄, supplementary trace (Cu-P) minerals reduced plasma cholesterol, LDL, and triglycerides. Kim et al. (1992) showed that the mechanism of Cu is to control cholesterol biosynthesis by reducing hepatic glutathione concentration and changes the hepatic GSH: GSSG (oxidized glutathione) ratio, thereby, increased the activity of 3-hydroxy-3-methylglutaryl Co-(HMG-CoA) reductase. Glutathione plays an important role in regulating cholesterol biosynthesis via HMG-CoA reductase stimulation (Konjufca et al. 1997), which is the primary enzyme of cholesterol biosynthesis, and in turn, decreases plasma cholesterol concentration. The above-mentioned pathway can explain the reduction in plasma cholesterol by supplementing the organic form of Se.

Organic Se supplementation (Se-yeast or ADS18) has demonstrated a significant decrease in triglyceride concentration relative to inorganic and non-supplemented hens in the present study. These findings were consistent with the results of Jegede et al. (2012), who reported a significant decrease in triglyceride concentration in growing pullets supplemented with Cu-P compared to CuSO₄. Moreover, dietary Se appears to have a major effect on aspartate aminotransferase (AST), alkaline phosphatase (ALP), but no effect on alanine aminotransferase (ALT) in the present study. Sizova et al. (2021) observed a substantial increase in ALT activity in broilers fed organic zinc on days 35 and 42, compared to control, though AST did not change significantly. Broiler chickens fed 0.3 ppm organic Se (Perić et al. 2009), 0.5 and 1.0 mg Se per Kg had significantly lower ALT and AST enzyme activity (Biswas et al., 2011).

In contrast, none of the blood constituents ALT, AST, TP, albumin, urea, and creatinine are affected by either inorganic (0.5 and 0.15 mg Se) or organic (0.35 mg Se) (Okunlola et al. 2015). Blood enzymes (ALT, AST, ALP, LDH) cause oxidative damage to the liver and kidneys, which can be reduced, imparted, and enhanced through redox status to protect against oxidative damage (Zhang et al. 2018).

The concentration of Se in egg yolk, breast muscle, and serum increased after dietary Se supplementation with Se, according to this study. Avian eggs are ideal vectors used to study the absorption and retention of microminerals, including Se at varying dosages and forms (Pan et al. 2007; Delezie et al. 2014). Dietary supplementation with Se increased egg yolk Se in the current study, which is consistent with previous studies (Liu et al. 2020b; Zhang et al. 2020). Lu et al. (2020) found higher Se concentrations in eggs and breast tissue of laying hens fed 0.1 to 0.4 mg/kg of Se from Se-enriched yeast than in eggs and breast tissue of hens fed SS or basal diet. Also, Liu et al. (2020a) found that 0.5 mg/kg of Se-yeast resulted in higher Se deposition in egg yolk than did sodium selenite in laying hens. According to Zhang et al. (2020), adding Se-yeast to the diets of laying hens helps to increase Se deposition in eggs. Likewise, hens fed with hydroxy selenomethionine and Se-yeast had higher concentration yolk Se than when fed the SS and basal diets (Moslehi et al. 2019).

Dietary Se supplementation with Vitamin E, Se, and their blend significantly increased the concentration of Se in breast tissue and certain laying hens' organs (Çelebi 2019). Similar results were found in eggs and breast meat Se concentration in laying hens (Lu et al. 2019; Lv et al. 2019), serum, liver, and muscle Se in growing lambs fed different concentrations (0.2 to 1.4 mg/kg DM) and Se sources (Se-met or Se-yeast and SS) (Paiva et al. 2019). Hens fed organic Se showed higher egg Se content compared to inorganic Se in response to the effectiveness of organic Se over inorganic form (Skřivan et al. 2006; Chantiratikul et al. 2008).

The difference in Se deposition in egg yolk between inorganic and organic Se sources may be due to their dissimilar metabolizable pathways, as SS cannot be completely metabolized to SeMet in poultry, which could explain the current findings (Sunde et al. 2016). Sodium selenite has a lower absorption rate and a higher excretion than organic Se (Mahan and Parrett 1996). Organic Se is actively absorbed and is dependent on the metabolization and integration of mainly organic selenomethionine with methionine into egg proteins and tissues (Čobanová et al. 2011; Surai and Kochish 2019). Selenoproteins from the liver are incorporated as part of egg yolk synthesis, whereas the uterine tubes incorporate selenomethionine as a part of egg white synthesis (Mahan and Kim 1996; Lv et al. 2019). The higher Se deposition in hens' eggs fed Se-yeast might perhaps be connected to the upregulation of methionine (Met) metabolism gene glycine N-methyltransferase (GNMT) in the liver (Meng et al. 2019). With different Se levels and time, there is a positive increase in egg Se concentration (Lu et al. 2019).

As hypothesized, with dietary Se supplementation, the concentration of Se in breast tissue and serum of laying hens was increased, and organic Se (ADS18 or Se-yeast) was more efficient compared to inorganic Se. Paiva et al. (2019) relate serum Se concentration to time and dose-dependent regardless of Se form. Application of 0.30 mg/kg selenomethionine in broiler breeder diets results in higher serum and tissue Se

deposition than other sources of Se (Li et al. 2018). Also, Han et al. (2017) reported a higher Se concentration from different Se sources in serum and organs of layers fed 0.3 mg/kg Se. Moreover, layer chicks fed a 0.3 mg/kg diet of both nano-Se and sodium selenite showed a significant increase in Se concentrations in tissues, organs, and serum (Mohapatra et al., 2014). Because SeMet is available in an organic form of Se and is closely linked to its bioavailability and assimilation (Briens et al. 2013; Mohapatra et al., 2014), the present findings are justified.

The significant differences found between the treatment groups in serum and breast tissue may be due to dissimilar metabolic pathways, as Se can be incorporated into selenoprotein as selenocysteine from inorganic and organic sources, while SeMet is incorporated in a nonspecific direction as methionine (Surai and Kochish 2019). Inorganic Se compounds are mainly used to synthesize selenoproteins and not replenish Se deposits in tissues in the Se metabolism pathway (Moslehi et al. 2019). The addition of organic Se (Se-yeast or SeMet) into the diet is connected with a significant increase of Se level in laying hens tissue (Invernizzi et al. 2013; Jing et al. 2015). Therefore, the quantity of Se uptake and its absorption in egg, tissue, and blood of laying hens is determined by the Se chemical form in organic sources (Jing et al. 2015). However, more research is required to explore the complete metabolic pathway of organic (Se-yeast or ADS18) sources.

Conclusion

In conclusion, dietary Se supplementation, particularly organic forms (ADS18 or Se-Yeast), enhances hen serum and hepatic antioxidant enzyme activity while lowering total serum cholesterol and serum triglycerides concentrations. Moreover, hens given dietary Se treatments exhibited greater selenium concentration in their egg yolks, serum, and breast tissue. In comparison to inorganic (sodium selenite) Se, 0.3 mg/kg of enriched bacterial protein from ADS18 Se improves antioxidant enzymes activity, serum biochemical parameters, and Se concentrations in laying hens, making it a significant alternative source of Se.

Declarations

Authors' contributions

A.I.M designed and conduct the animal experiments and all the laboratory analyses, analyzed and interpreted data, and drafted the manuscript. A.A.S. designed, supervised, and administrated the overall research project, A.M.D, T.C.L, and H. A participated in the whole preparation of the manuscript. All authors read and approved the final manuscript.

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Data availability

Not applicable.

Code availability

Not applicable.

Ethics approval

All experiential steps were implemented according to the Local Experimental Animal Care Panel and permitted by the Institutional Animal Care and Use Committee of University Putra Malaysia (UPM/IACUC/AUP-R063/2018).

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Competing interests

The authors declare that they have no competing interests.

Consent to participate

Not applicable.

Consent to publish

All authors give consent for publication.

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Figures

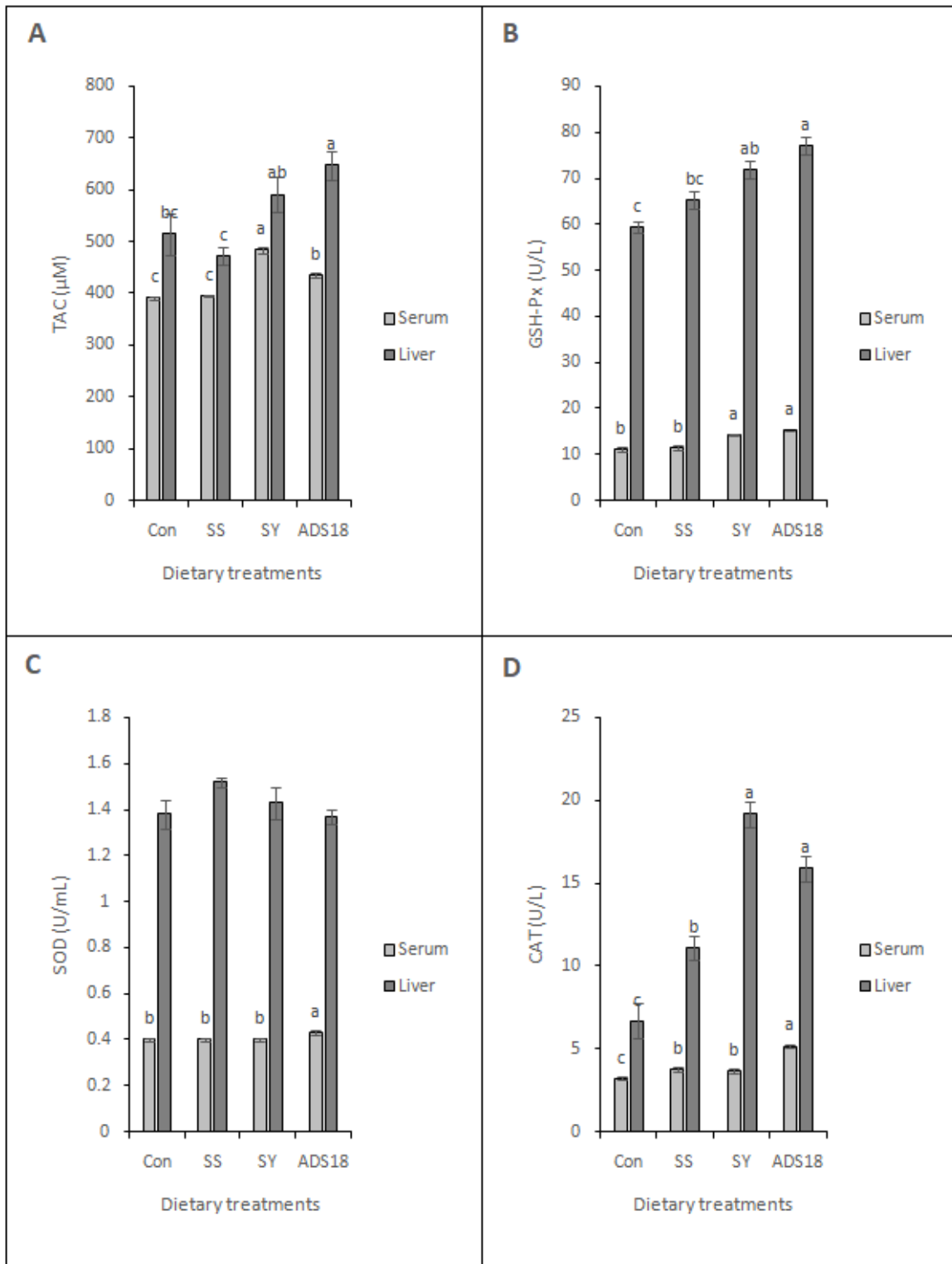


Figure 1

Serum and hepatic antioxidant enzyme activities in laying hens fed different dietary selenium sources. *Con = Control, SS = Sodium selenite; SY = Selenium yeast; ADS18 = Bacterial enriched organic Se. a - c Values within the same column with different superscript letters differ ($P < 0.05$). TAC expressed as μM Trolox Equivalents. Glutathione peroxidase activity is expressed as U/L (one unit is the amount of GSH-Loading [MathJax]/jax/output/CommonHTML/jax.js). SOD one unit corresponds to the amount of enzyme needed

to scavenges the dismutation of the superoxide radical. CAT activity is expressed as U/L (one unit is the amount of CAT that decomposes 1 μ mole of H₂O₂ per min).

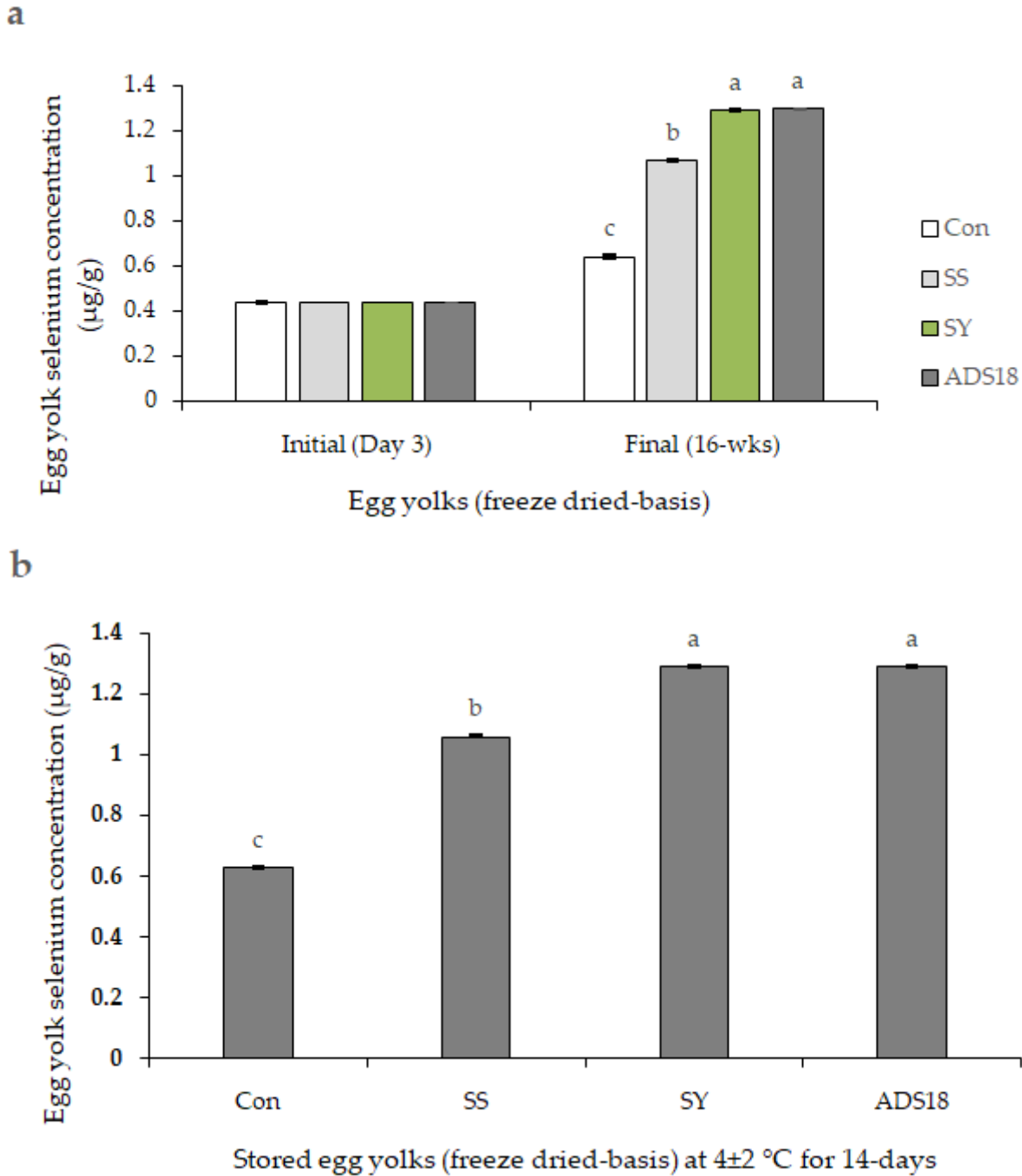


Figure 2

Egg yolk Se concentration (μ g/g freeze-dried basis). a. Egg yolk Se concentrations of hens at initial (3-d) and final (16 wks). b. Egg yolk Se concentrations of post stored (14-days at 4 ± 2 °C) eggs at 18 wks. Experimental diets: Con = control, SS = Sodium selenite; SY = Selenium yeast; ADS18 = Bacterial enriched organic Se. Bars with different superscripts (a, b, c) are significantly different at $P < 0.05$. Egg yolk samples were initial, final (16 wks), and stored (18 wks) at 4 ± 2 °C for 14 days. Data are means of 6 sample).