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Effect of Dietary Selenium Nanoparticles Supplementation on Hematological, Serum Biochemical, Oxidant-Antioxidant Biomarkers, and Proinflammatory Cytokines in Broilers Challenged with *Salmonella* Typhimurium

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Abstract

The current study evaluated the efficacy of Selenium Nanoparticles (Se-NPs) on hemato-biochemical, antioxidant biomarkers, and immunological responses induced by *S.* Typhimurium in broiler chickens. Chicks (N=120) were divided into six groups. Group 1: received no treatment and set as a control group. Group 2: fed Se-NPs enriched diet (0.5 mg/kg diet). Group 3: subjected to oral challenge with $3.5x10^8$ CFU/mL/1 ml/bird of *S.* Typhimurium. Group 4: administrated Se-NPs (0.5 mg/kg diet) then on day 21 was subjected to $3.5x10^8$ CFU/mL/1 ml/bird of *S.* Typhimurium. Group 5: vaccinated by a SERVAC Tri Sal. 0.1ml subcutaneous (s/c) injection on day 3 then subjected to $3.5x10^8$ CFU/mL/1 ml/bird of *S.* Typhimurium on day 21. Group 6: treated from day 1 with Se-NPs (0.5 mg/kg diet) till the end of the experiment and vaccinated by a SERVAC Tri Sal. 0.1ml (s/c) on day 3 and then subjected to $3.5x10^8$ CFU/mL/1 ml/bird of *S.* Typhimurium on day 21. The results showed that *S.* Typhimurium significantly decreased erythrogram, lymphocytes count, total protein, albumin, A/G ratio, glucose, cholesterol, triglyceride, serum iron, and TIBC, GPX, SOD, TAC, and IL-10 expression compared to the control. Meanwhile, *S.* Typhimurium significantly increased TLC, heterophils, monocytes, serum ferritin, liver enzymes (ALT, AST), renal products (creatinine, uric acid), MDA, IL6 expression. Conversely, the dietary Se-NPs supplementation and/or *Salmonella* vaccine to the infected broiler induced, to various degrees, improvement in hemato-biochemical, antioxidant biomarkers, and proinflammatory responses compared to challenged group. In conclusion, dietary Se-NPs supplementation offered a direct protection against *S.* Typhimurium infection for sustaining poultry production and correspondingly protecting human health.

KEYWORDS
Selenium Nanoparticles, *Salmonella* Typhimurium, Broilers chickens, Proinflammatory Cytokines, Oxidative stress.

INTRODUCTION

Salmonellosis is an acute and chronic disease in avian species caused by the bacterium *Salmonella*, which has more than 2500 serotypes (Calenge *et al*., 2010). Salmonellosis is a serious economic problem in the poultry industry as poultry is considered a major reservoir for many *Salmonella* serotypes (Forkus *et al*., 2017). It can cause gastrointestinal illness with a high mortality rate in 1 to 3 days old chicks, and it can colonize the gastrointestinal tract of older chicks without any visible signs (Humphrey *et al*., 1989).

Salmonella Typhimurium is a major zoonotic pathogen in the world, especially in developing countries (Knap *et al*., 2011). *Salmonella* can infect humans through the consumption of poultry products such as eggs and meat, making it a foodborne disease that causes food poisoning (Jones *et al*., 2021). Maintaining a *Salmonella*-free status in poultry is a challenge due to its rapid expansion in the poultry industry, as indicated by the number of *Salmonella* outbreaks reported worldwide (Meeusen *et al*., 2007).

Broad-spectrum antibiotics are used to prevent the colonization of intestinal *Salmonella* pathogens. However, the misuse of these drugs has led to the creation of bacterial strains that are resistant to the drugs (CDC, 2010). The world has recently directed its efforts to encourage the use of vaccination (Nandre *et al*., 2012) and organic antibiotic alternatives such as natural probiotics, prebiotics, synbiotics, organic acids, and synthesized nanoparticles (Saad *et al*., 2021; Arif *et al*., 2022).

The use of *Salmonella* vaccination in chickens against serovars with significant public health implications has gained more attention in the poultry industry. When chickens are vaccinated, their resistance to *Salmonella* infection increases. Live attenuated vaccine, inactivated vaccine (killed vaccine), and subunit vaccines are some of the vaccines used against *Salmonella* in poultry (Jawale and Lee, 2016; Jia *et al*., 2020).

The field of nanotechnology is rapidly growing and involves various areas of academic research. Its potential for significant advances in human and animal health is notable, particularly in areas such as pathogen resistance, antioxidant use, toxin degradation, and nutrient efficiency (Reda *et al*., 2020; 2021). Selenium nanoparticles (Se-NPs) are considered one of the most effective forms of selenium, such as inorganic selenium and selenomethionine, which are used to maintain physiological functions, growth, and health in birds. Furthermore, they have antioxidant,

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anticancer, antibacterial, and antiprotozoal properties (El-Deep *et al*., 2017; Zadeh *et al*., 2018).

The aim of this study was to investigate the potential effects of selenium nanoparticles and/or *Salmonella* vaccine in reducing hematologic, biochemical, oxidative, and immunological changes induced by *Salmonella* typhimurium to evaluate the possibility of using this treatment method to control and treat the disease.

MATERIALS AND METHODS

Synthesis and characterization of selenium nanoparticles (Se-NPs)

Selenium nanoparticles were prepared at Nanomaterials Research and Synthesis Unit, Animal Health Research Institute, Agriculture Research Center, Giza, Egypt via a reduction of sodium selenite by ascorbic acid and stabilized by polysorbate 20. Briefly, 30 mg of $Na_2SeO_3.5H_2O$ was added to 90 mL of distilled water. Ascorbic acid (56.7 mM) was added dropwise to sodium selenite solution with vigorous stirring. 50 µL of polysorbate were added with ascorbic acid dropwise. The reactant solution from clear white to clear red. Selenium nanoparticles were then washed and collected by centrifuging the solution at 12000 rpm then freeze dried to obtain nano powder (Vahdati and Tohidi Moghadam, 2020).

The prepared Se-NPs were identified, and their shape, particles size, and morphology were measured. Size analysis was carried out by UV-visible spectrophotometer (SHIMADZU-2600i, USA). Shape analysis was carried out by High-resolution transmission electron microscopy (HRTEM), then the results were observed via a JEM 1400F HRTEM at a beam energy of 300 keV (Atul *et al*., 2010).

Experimental broiler chicks

A total of 120 one-day-old commercial Arbor Acres broiler chicks obtained from a local commercial hatchery (Masa hen hatcheries, Mansoura, Egypt) were used in this study. The chicks were floor reared under natural day light and specific hours of artificial light at night in strictly isolated experimental rooms, previously cleaned and disinfected. The birds have free access to tap water and commercial broiler starter ration. Birds had common vaccination program as the following: day 7 (Hitchner + IB + Gumboro intermediate), day 14 (Gumboro hot) and day 18 (Lacota gold). All vaccines were obtained from Abbasia, Cairo, Egypt.

Experimental design

This study was ethically approved by the International Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat City, Egypt (Approval number: VUSC-016-1-23). At 1-day old age, chicks were divided randomly into 6 groups, 20 for each. Group 1: received no treatment and was considered as control. Group 2: treated from day 1 with Se-NPs enriched diet (0.5 mg/kg ration) (Khan *et al*., 2022) till the end of the experiment. Group 3: subjected to oral challenge with $3.5x10^8$ CFU/mL/1 ml/bird with *S.* Typhimurium (Wu *et al*., 2020) on the day 21. Group 4: treated with Se-NPs enriched diet then at day 21 was subjected to oral challenge with *S.* Typhimurium. Group 5: vaccinated by a SERVAC Tri Sal. VACCINE 0.1 ml subcutaneous (s/c) injection on the day 3 then subjected to oral challenge with *S.* Typhimurium on the day 21. Group 6: treated from day 1 with Se-NPs enriched diet till the end of the experiment and vaccinated by a SERVAC Tri Sal. VACCINE on the day 3 then subjected to oral challenge with *S.* Typhimurium on the day 21.

Bacterial strain and Salmonella vaccine

S. Typhimurium local strain used in this study was obtained from the Serology and Bacteriology Bank in Animal Health Research Institute, Giza, Egypt. This strain was stored at -80 on nutrient broth plus glycerol and TSB and incubated at 37 for 24 h then plated on TSA (tryptic soya agar) and Xld to check purity and colony characteristics. The colony was confirmed by biochemical tests; TSI, urease hydrolysis, lysine decarboxylase and serotyping according to Iso 6579: 2014 part 3. Then the colonies collected from TSA on saline and matched with McFarland tube. Infection was done orally by administration of 1ml containing 3.5×10^8 adjusted by MacFarland tube no. 3 and confirmed by counting using pour plate method.

Inactivated *S.* Typhimurium vaccine (SERVAC Tri Sal. VACCINE) was purchased from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt.

Sample collection and preparation

Blood samples were collected from wing veins at weeks 1 and 2 post infection (WPI). The blood sample was divided into two portions. The first portion was collected on EDTA for hemogram. The second portion was collected without anticoagulant, centrifuged at 3000 rpm to separate serum for further serum biochemical investigations. After sacrificing birds at day 35, parts of the ceacum from each bird were collected in RNA later stabilization solution and frozen at -80o c for RNA analysis.

Hematological examination

Red blood cells (RBCs) and total leukocyte counts (TLC) were done according to Natt and Herrick (1952). Hemoglobin concentration (Hb) was measured by using Drabkin's method according to Campbell (1988). Packed cell volume (PCV) was determined by using microhematocrit method as described by Campbell and Coles (1986). Differential leukocytic counts were determined on blood films using the battlement technique according to Mulley (1979). Blood Indices, including mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV), were assessed according to the method of Gupta (1977).

Biochemical examination

All the following serum biochemical parameters were determined using spectrophotometer (Spekol 11, Germany) and commercial kits purchased from Biomed Diagnostics Co., Cairo, Egypt.

Serum total proteins (TP) and albumin (Alb) levels were carried out using methods described by Vassault *et al.* (1999) and Doumas *et al.* (1971) respectively. Serum globulin (Glob) was calculated by subtracting serum albumin from serum total protein and then A/G ratio was calculated by dividing albumin on globulin.

Serum Alanine (ALT) and aspartate (AST) aminotransferase activities were determined calorimetrically according to Schumann *et al.* (2010). Serum uric acid and creatinine levels were evaluated using the method described by Vassault *et al.* (1999) and Young (2001) respectively. Serum glucose, cholesterol and triglyceride concentrations were determined according to Young *et al.* (1972); Tietz (1976) and Vassault *et al.* (1999) respectively. Serum iron, ferritin, and total iron binding capacity (TIBC) were measured using the colorimetric method of Cook *et al.* (1974); Dreux (1977)

and Piccardi *et al*. (1972).

Serum contents of GPx, SOD, TAC, and MDA were estimated calorimetrically using Biodiagnostics commercial kits (Giza, Egypt) according to Paglia and Valentine (1967); Nishikimi *et al*. (1972); Koracevic *et al*. (2001) and Ohkawa *et al*. (1979) respectively.

Real Time PCR analysis of IL-6 and IL-10 gene expression

Briefly, IL gene expression (IL-6 and IL-10) in caecum tissue was quantified using RNA extraction used a RNeasy Mini Kit (Cat. No.74104, Qiagen). A 30 mg tissue sample was homogenized and processed as described by the manufacturer. IL-6 and IL-10 mRNA were amplified and quantified using a real-time PCR machine (Stratagene MX3005P, Agilent Technologies Company, USA). Primer sequences are listed in Table 1. The housekeeping gene 28S rRNA was used as a constitutive control for normaliza¬tion. The reaction mixture volume was 25 µL made up with 12.5 µL 2x QuantiTect Probe RT-PCR Master Mix (Cat. No.204443, Qiagen), 0.5 µL of 20 pmol solu¬tion of each primer, 0.125 µL probe (30 pmol), 0.25 µL QuantiTect RT Mix (Revert Aid Reverse Transcriptase) (Qiagen), 8.125 µL RNase-free water (Sedico, Egypt), and 3 µL template RNA. The real-time PCR cycling conditions were reverse transcription (50°C, 30 min), primary denaturation (94°C, 10 min), and then ampli¬fication (40 cycles) with secondary denaturation (94°C, 15 s), annealing, and extension (60°C, 1 min). Amplification curves and Ct values were deter¬mined using Stratagene MX3005P software, Agilent Technologies Company). The Ct value of each sample was compared with the positive control as described for the "ΔΔCT" method stated by Yuan *et al*. (2007).

Statical analysis

The data were presented as mean ± SE and were subjected to statistical analysis using one-way and two-way analysis of variance (ANOVA) according to Snedecor and Cochran (1980) followed by the least significant difference test (LSD) at 0.05 level of probability using SPSS® (Statistical Package for Social Sciences) Version 26, IBM Inc. (Chicago, IL, USA).

RESULTS

Characterization of selenium nanoparticles (Se-NPs)

The shape was performed to illustrate the morphology and shape of Se-NPs. TEM characterization revealed a spherical shape for the nanoparticles with 71 nm in size (Figure 1B), and the absorption spectra of the final product were scanned at 200-800 cm^{-1} using 1 cm quartz cells. The spectra of the formed product revealed a transition point at 265 nm with no clear maximum (Figure 1A).

Clinical signs and mortality

Along the experimental period, no clinical signs or mortalities were observed in the group infected with *S.* Typhimurium, as well as in the groups infected with *S.* Typhimurium and treated with Se-NPs and/or *Salmonella* vaccine.

Hematological profile

The effect of administration of Se-NPs and/or *Salmonella* vaccine on erythrogram indices of broiler infected with *S.* Ty-

Table 1. Primers sequences and probes used in real-time PCR.

Gene	*Primer sequence* (5'-3')	*Reference*
IL6	GCTCGCCGGCTTCGA GGTAGGTCTGAAAGGCGAACAG (FAM) AGGAGAAATGCCTGACGAAGCTCTCCA (TAMRA)	Suzuki *et al*. (2009)
28S rRNA	GGCGAAGCCAGAGGAAACT GACGACCGATTTGCACGTC (FAM) AGGACCGCTACGGACCTCCACCA (TAMRA)	
IL10	CATGCTGCTGGGCCTGAA CGTCTCCTTGATCTGCTTGATG (FAM) CGACGATGCGGCGCTGTCA (TAMRA)	Samy *et al*. (2015)

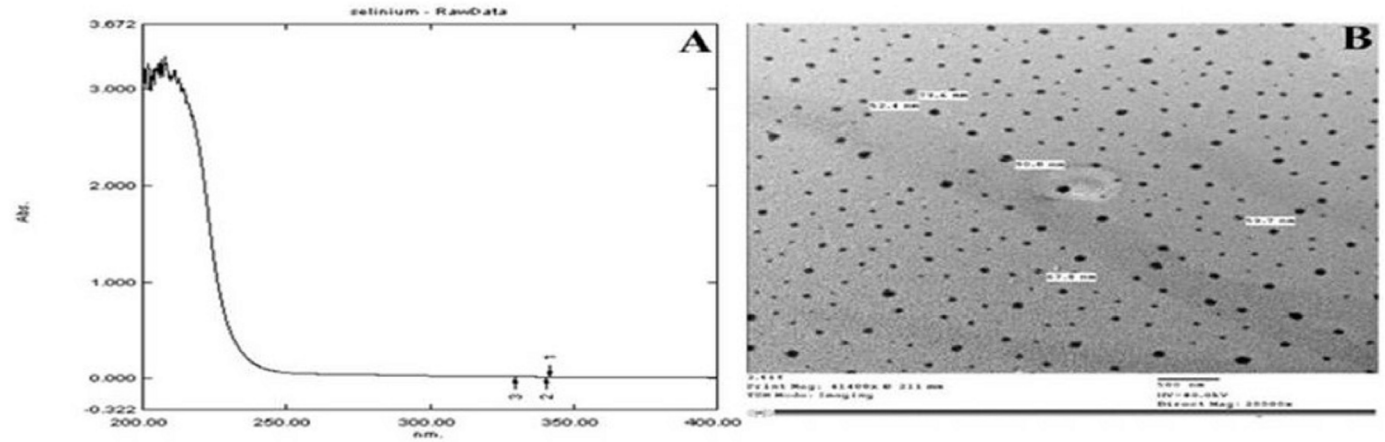

Figure 1. The XRD pattern (A) and TEM (B) Selenium Nanoparticles.

phimurium is illustrated in Table 2. No significant ($p<0.05$) differences were recorded in the mean values of RBCs, Hb, PCV, MCV, MCH, and MCHC between the control and Se-NPs groups. Conversely, the results of all erythrogram parameters implicated a significant ($p<0.05$) reduction in group challenged with *S.* Typhimurium and groups challenged with *S.* Typhimurium and treated with Se-NPs and/or *Salmonella* vaccine at weeks 1 and 2 post infection compared to the control group. Notably, there was elevation in RBCs, Hb, PCV in the groups challenged with *S.* Typhimurium and treated with Se-NPs and/or *Salmonella* vaccine at 2 weeks PI compared with the group infected with *S.* Typhimurium.

Referring to the changes in the leukogram, data presented in Table 3 showed no significant ($p<0.05$) differences between the control and Se-NPs groups. However, there was a significant ($p<0.05$) decrease in lymphocytes count and elevation in TLC, heterophils and monocytes count at weeks 1 and 2 post infection without any changes in the mean values of eosinophils and basophils in the group challenged with *S.* Typhimurium and groups challenged with *S.* Typhimurium and treated with Se-NPs and /or *Salmonella* vaccine compared to the control group. In contrary, there was a significant ($p<0.05$) improvement in heterophils in the group challenged with *S.* Typhimurium and treated with *Salmonella* vaccine or treated with Se-NPs and *Salmonella* vaccine at week 1 post infection and in the group challenged with *S.* Typhimurium and treated with Se-NPs and *Salmonella* vaccine at week 2 post infection compared with the group infected with *S.* Typhimurium.

Biochemical profile

The biochemical investigation of broiler chickens challenged with *S.* Typhimurium and treated with Se-NPs and /or *Salmonella* vaccine was presented in Table 4. No significant ($p<0.05$) differences in total protein, albumin, globulin, A/G ratio, glucose, triglycerides, and cholesterol values between the control and Se-NPs groups. However, *S.* Typhimurium infection significantly ($p<0.05$) decreased total protein, albumin, glucose, triglycerides, cholesterol, and significantly ($p<0.05$) increased globulin and A/G ratio in broiler chickens' serum at weeks 1 and 2 post infection compared to those of the control group. At the same time, treated groups with Se-NPs and/or *Salmonella* vaccine reported a marked improvement in total protein, albumin, globulin, A/G ratio values with reinstating the normal control protein value in treated groups with *Salmonella* vaccine or both Se-NPs and *Sal-*

Table 2. Effect of Se-NPs and/or *Salmonella* vaccine on erythrogram parameters of *S.* Typhimurium challenged chickens.

Parameters	Experimental Groups						
	WPI	G1	G2	G3	G4	G5	G6
RBCs (10^6/µl)	1	4.46±0.14 ^{a}	4.65±0.27 ^{a}	3.24±0.06 ^{b}	3.49± 0.03 ^{b}	3.49±0.04 ^{b}	3.56±0.30 ^{b}
	2	4.57±0.09 ^{a}	4.63±0.21 ^{a}	3.33±0.22 ^{c}	3.81± 0.04 ^{b}	3.72±0.07 ^{b}	3.85±0.02 ^{b}
Hb (g/dl)	1	13.82±0.05 ^{a}	13.41±0.03 ^{a}	7.69±0.16 ^{b}	8.37±0.29 ^{b}	8.17±0.37 ^{b}	8.41± 0.29 ^{b}
	2	13.33±0.11 ^{a}	13.36±0.11 ^{a}	7.64±0.11 ^{c}	8.57±0.29 ^{b}	8.38±0.27 ^{b}	8.56±0.32 ^{b}
PCV (%)	1	32.06±0.21 ^{a}	32.70±0.20 ^{a}	20.79±0.39 ^{c}	22.48±0.10 ^{b}	22.46±0.20 ^{b}	23.91±0.03 ^{b}
	2	32.00±0.25 ^{a}	32.7±0.21 ^{a}	20.90±0.45 ^{c}	23.77±0.45 ^{b}	23.82±0.20 ^{b}	23.46±0.22 ^{b}
MCV (fl)	1	72.03±1.90 ^{a}	70.68±3.95 ^{a}	64.12±0.15 ^{b}	64.45±0.78 ^{b}	64.25±0.91 ^{b}	63.93±1.71 ^{b}
	2	70.60±1.4 ^{a}	70.60±1.40 ^{a}	62.86±1.50 ^{b}	62.30±0.58 ^{b}	62.58±0.17 ^{b}	62.06±0.47 ^{b}
MCH (pg)	1	30.13±1.09 ^{a}	29.16±1.74 ^{a}	23.72±0.65 ^{b}	23.96±0.53 ^{b}	23.35±0.88 ^{b}	23.71±1.25 ^{b}
	2	29.15±0.33 ^{a}	28.86±0.93 ^{a}	22.98±0.58 ^{b}	22.46±0.81 ^{b}	22.55±0.82 ^{b}	22.24±0.89 ^{b}
MCHC (%)	1	41.82±0.42 ^{a}	41.23±0.15 ^{a}	37.00±1.03 ^{b}	37.21±1.24 ^{b}	36.40±1.90 ^{b}	37.08±0.95 ^{b}
	2	41.66±0.36 ^{a}	40.86±0.55 ^{a}	36.75±0.71 ^{b}	36.06±1.19 ^{b}	36.02±1.07 ^{b}	35.82±1.30 ^{b}

Values are means ± SE (n=10). Different letters (a, b, c) in the same row indicate significant differences at $p < 0.05$.G1: control; G2: treated with Se-NPs; G3: challenged with *S.* Typhimurium; G4: challenged with *S.* Typhimurium and treated with Se-NPs; G5: challenged with *S.* Typhimurium and treated with *Salmonella* vaccine; G6: challenged with *S.* Typhimurium and treated with Se-NPs and *Salmonella* vaccine. WPI: weeks post infection. RBCs: red blood cells count; Hb: hemoglobin concentration; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

Table 3. Effect of Se-NPs and/or *Salmonella* vaccine on leukogram parameters of *S.* Typhimurium challenged chickens.

Parameters	Experimental Groups						
	WPI	G1	G2	G3	G4	G5	G6
WBCs (10^3/µl)	1	10.91±0.52 ^{b}	11.15±0.38 ^{b}	14.29±0.81 ^{a}	14.65±0.66 ^{a}	13.34±0.39 ^{a}	14.04±0.38 ^{a}
	2	11.90±0.90 ^{b}	12.19±0.16 ^{b}	15.5±0.80 ^{a}	14.11±1.60 ^{a}	14.5 ±0.61 ^{a}	13.6±1.06 ^{a}
Lymphocyte (10^3/µl)	1	8.51±0.51 ^{a}	8.12±0.89 ^{ab}	6.74±0.49 ^{b}	7.30±0.25 ^{b}	6.78±0.54 ^{b}	7.11±0.32 ^{b}
	2	8.81±0.38 ^{a}	9.04±0.22 ^{a}	7.19±0.44 ^{b}	7.30±0.71 ^{b}	7.66±0.22 ^{b}	7.01±0.31 ^{b}
Heterophil (10^3/µl)	1	2.19±0.11 ^{c}	2.39±0.05 ^{c}	6.11±0.34 ^{a}	6.51±0.39 ^{a}	5.33±0.01 ^{b}	5.39±0.31 ^{b}
	2	2.75±0.35 ^{b}	2.46±0.18 ^{b}	6.01±0.27 ^{a}	5.79±0.80 ^{a}	5.80±0.37 ^{a}	5.72±0.65 ^{a}
Monocyte (10^3/µl)	1	0.43±0.03 ^{c}	0.49±0.01 ^{bc}	1.70±0.38 ^{a}	1.21±0.18 ^{a}	0.99±0.31 ^{abc}	1.30±0.31 ^{a}
	2	0.55±0.07 ^{b}	0.59±0.01 ^{b}	1.47±0.33 ^{a}	1.16±0.41 ^{a}	0.76±0.37 ^{ab}	0.69±0.65 ^{b}
Eosinophil (10^3/µl)	1	0.15±0.03 a	0.18±0.01 ^{a}	0.21±0.02 ^{a}	0.18±0.01 ^{a}	0.19±0.01 ^{a}	0.19±0.01 ^{a}
	2	0.15±0.03 ^{a}	0.16±0.01 ^{a}	0.20±0.02 ^{a}	0.18±0.01 ^{a}	0.18±0.01 ^{a}	0.18±0.01 ^{a}
Basophil (10^3/µl)	1	0.02±0.03 ^{a}	0.02±0.01 ^{a}	0.03±0.01 ^{a}	0.02±0.01 ^{a}	0.02±0.01 ^{a}	0.02±0.01 ^{a}
	2	0.03±0.01 ^{a}	0.02±0.01 ^{a}	0.03±0.01 ^{a}	0.02±0.01 ^{a}	0.03±0.01 ^{a}	0.03±0.01 ^{a}

Values are means ± SE (n=10). Different letters (a, b, c) in the same row indicate significant differences at $p < 0.05$.G1: control; G2: treated with Se-NPs; G3: challenged with *S.* Typhimurium; G4: challenged with *S.* Typhimurium and treated with Se-NPs; G5: challenged with *S.* Typhimurium and treated with *Salmonella* vaccine; G6: challenged with *S.* Typhimurium and treated with Se-NPs and *Salmonella* vaccine. WPI: weeks post infection. WBCs: white blood cells.

monella vaccine on week 1 post infection, and the normal control globulin value in treated groups with Se-NPs and/or *Salmonella* vaccine on weeks 1 and 2 post infection comparing with the challenged group with *S.* Typhimurium. Additionally, treated groups with Se-NPs and/or *Salmonella* vaccine significantly ameliorated glucose, triglycerides, cholesterol contents compared to the challenged group with restoring the normal serum glucose value at groups treated with Se-NPs or *Salmonella* vaccine at weeks 1 and 2 post infection and the normal serum triglycerides value at groups treated with Se-NPs or both Se-NPs and Se-NPs at weeks 1 and 2 post infection to the normal control values.

Serum iron panel

As displayed in Table 4, no significant ($p<0.05$) differences were recorded in the mean values of serum iron, ferritin, and TIBC between the control and Se-NPs groups. Conversely, *S.* Typhimurium challenge in chickens significantly ($p<0.05$) decreased serum iron and TIBC levels and significantly ($p<0.05$) increased serum ferritin content at weeks 1 and 2 post infection compared to the control. Regarding the mean values of challenged group, there was enhancement in serum iron, ferritin, and TIBC values in groups challenged with *S.* Typhimurium and treated with Se-NPs and/or *Salmonella* vaccine, with reinstating the normal serum iron control value in group treated with Se-NPs and *Salmonella* vaccine at week 2 post infection and serum ferritin control value in groups treated with *Salmonella* vaccine or both Se-NPs and *Salmonella* vaccine at week 1 post infection.

Liver and kidney functions

The hepatorenal functions in broiler chickens exposed to *S.* Typhimurium and treated with Se-NPs and /or *Salmonella* vaccine were presented in Table 5. Dietary Se-NPs supplementation did not significantly ($p<0.05$) affect liver and kidney functions. Meanwhile, significant ($p<0.05$) elevation in the levels of ALT,

Table 4. Effect of Se-NPs and/or *Salmonella* vaccine on blood biomarkers of *S.* Typhimurium challenged chickens.

Parameters	Experimental Groups						
	WPI	G1	G2	G3	G4	G5	G6
Protein (g/dl)	1	4.52±0.08 ^{a}	4.50±0.21 ^{a}	4.12± 0.07 ^{b}	4.12±0.05 ^{b}	4.20±0.07 ^{ab}	4.22±0.08 ^{ab}
	2	4.70±0.12 ^{a}	4.80±0.08 ^{a}	4.32±0.13 ^{b}	4.16±0.06 ^{b}	4.20±0.17 ^{b}	4.19±0.88 ^{b}
Albumin (g/dl)	1	2.38±0.05 ^{a}	2.48±0.11 ^{a}	1.71±0.03 ^{c}	1.81±0.03 ^{bc}	1.75±0.01 ^{bc}	1.91±0.03 ^{b}
	2	2.62±0.05 ^{a}	2.64±0.08 ^{a}	1.62±0.03 ^{b}	1.74±0.04 ^{b}	1.60±0.06 ^{b}	1.67±0.03 ^{b}
Globulin (g/dl)	1	2.14± 0.12 ^{ab}	2.02±0.14 ^{b}	2.40±0.09 ^{a}	2.31±0.06 ^{ab}	2.45±0.08 ^{a}	2.30±0.07 ^{ab}
	2	2.08±0.13 ^{b}	2.16±0.02 ^{b}	2.70±0.13 ^{a}	2.42±0.06 ^{ab}	2.60±0.09 ^{a}	2.43±0.09 ^{ab}
A/G ratio	1	1.13±0.08 ^{a}	1.25±0.11 ^{a}	0.71±0.04 ^{b}	0.78±0.04 ^{b}	0.72±0.03 ^{b}	0.83±0.29 ^{b}
	2	1.21±0.11 ^{a}	1.22±0.03 ^{a}	0.61±0.03 ^{b}	0.72±0.03 ^{b}	0.62±0.03 ^{b}	0.69±0.030 ^{b}
Glucose (mg/dl)	1	184.00±2.61^{ab}	191.21±1.51^{a}	151.6±3.11 ^{d}	170.42±2.61^{bc}	173.22±1.81^{bc}	167.66±1.70 ^{c}
	2	151.4±2.71 ^{ab}	157.53±1.20^{a}	143.90±1.60^{c}	147.73±0.85^{bc}	147.53±1.20^{bc}	149.9±0.77 ^{b}
Triglyceride (mg/dl)	1	133.51±0.79^{ab}	136.72±0.46^{a}	120.3±0.93 ^{d}	132.33±1.90^{bc}	129.22±1.11 ^{c}	131.62±1.43^{bc}
	2	132.81±0.42^{ab}	136.81±0.46^{a}	120.4±0.90 ^{d}	131.73±1.40^{bc}	128.91±1.11 ^{c}	132.32±1.91^{bc}
Cholesterol (mg/dl)	1	193.96±2.24 ^{a}	187.48±1.16^{a}	149.91±2.16^{e}	173.08±0.90 ^{c}	160.16±2.23 ^{d}	179.98±2.05 ^{b}
	2	191.98±1.31 ^{a}	189.52±0.96^{a}	139.90± 2.1^{d}	166.14±2.31^{bc}	159.98±2.23 ^{c}	173.08±1.68 ^{b}
Serum iron (ug/dl)	1	88.71± 1.21 ^{a}	88.22±1.80 ^{a}	62.60±0.3^{d}	68.52±1.6 ^{c}	72.41±1.7 ^{c}	83.11±2.60 ^{b}
	2	86.81±1.51 ^{a}	82.11±2.01 ^{a}	62.71±2.5^{c}	71.11±2.11 ^{b}	73.61±1.71^{b}	80.20±1.33 ^{a}
Ferritin (ng/ml)	1	0.96±0.04 ^{c}	0.87±0.01 ^{c}	1.26±0.02^{a}	1.14±0.04 ^{ab}	1.09±0.07 ^{bc}	1.07±0.05 ^{bc}
	2	0.88±0.02 ^{c}	0.76±0.03 ^{c}	1.09±0.03^{a}	1.03±0.03 ^{ab}	1.02±0.05 ^{ab}	0.98±0.03 ^{b}
TIBC (ug/dL)	1	382.8±2.11 ^{a}	381.21±3.36^{a}	291±2.21^{c}	341.4±2.31^{b}	330.4±2.80^{b}	347±2.61 ^{b}
	2	365±3.90 ^{a}	347.2±1.4 1^{ab}	227±3.13^{c}	336.6±2.50^{b}	313.6±2.52^{b}	227.4 ±4.11 ^{b}

Values are means ± SE (n=10). Different letters (a, b, c) in the same row indicate significant differences at $p < 0.05$.G1: control; G2: treated with Se-NPs; G3: challenged with *S.* Typhimurium; G4: challenged with *S.* Typhimurium and treated with Se-NPs; G5: challenged with *S.* Typhimurium and treated with *Salmonella* vaccine; G6: challenged with *S.* Typhimurium and treated with Se-NPs and *Salmonella* vaccine. WPI: weeks post infection; A/G ratio: albumin/globulin ratio; TIBC: total iron binding capacity.

Table 5. Effect of Se-NPs and/or *Salmonella* vaccine on liver and kidney function parameters of *S.* Typhimurium challenged chickens.

Parameters	Experimental Groups						
	WPI	G1	G2	G3	G4	G5	G6
ALT (U/I)	1	6.41±0.07 ^{c}	7.31±0.06 ^{c}	16.4±0.47 ^{a}	13.06±0.31 ^{b}	12.5±0.32 ^{b}	12.41±0.84 ^{b}
	2	8.49±0.09 ^{d}	9.12±0.11 ^{d}	18.22±0.40 ^{a}	15.26±0.49 ^{b}	16.06±0.25 ^{b}	13.34±0.39 ^{c}
AST (U/I)	1	119.86±2.82 ^{c}	119.86±2.77 ^{c}	159.04±4.15 ^{a}	129.78±2.69 ^{b}	125.58±2.11 ^{bc}	123.68±2.81 ^{bc}
	2	129.21±1.71 ^{c}	125.33±1.53 ^{c}	156.33±1.71 ^{a}	144±1.71 ^{b}	145±2.41 ^{b}	125.41±1.31 ^{c}
Creatinine (mg/dl)	1	0.40±0.01 ^{b}	0.41±0.01 ^{b}	0.47±0.02 ^{a}	0.46±0.01 ^{a}	0.48±0.01 ^{a}	0.45±0.01 ^{a}
	2	0.40±0.01 ^{c}	0.41±0.01 ^{c}	0.59±0.03 ^{a}	0.51±0.01 ^{b}	0.51±0.01 ^{b}	0.50±0.01 ^{b}
Uric acid (mg/dl)	1	5.66±0.21 ^{c}	5.60±0.07 ^{c}	6.74±0.48 ^{a}	6.46±0.11 ^{ab}	6.22±0.13 ^{b}	6.18±0.23 ^{b}
	2	5.86±0.11 ^{c}	5.78±0.19 ^{c}	7.00±0.12 ^{a}	6.36±0.31 ^{b}	6.37±0.21 ^{b}	6.36±0.19 ^{b}

Values are means ± SE (n=10). Different letters (a, b, c) in the same row indicate significant differences at $p < 0.05$.G1: control; G2: treated with Se-NPs; G3: challenged with *S.* Typhimurium; G4: challenged with *S.* Typhimurium and treated with Se-NPs; G5: challenged with *S.* Typhimurium and treated with *Salmonella* vaccine; G6: challenged with *S.* Typhimurium and treated with Se-NPs and *Salmonella* vaccine. WPI: weeks post infection; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

AST, creatinine, and uric acid were recorded in the group challenged with *S.* Typhimurium at weeks 1 and 2 post infection compared to those of the control group. In addition, there was a significant ($p<0.05$) reduction in serum levels of ALT, AST, creatinine, and uric acid in the groups challenged with *S.* Typhimurium and treated with Se-NPs and/or *Salmonella* vaccine compared with the challenged group with *S.* Typhimurium, with reinstating normal serum AST control values in groups treated with *Salmonella* vaccine or both Se-NPs and *Salmonella* vaccine.

Oxidant-Antioxidant profile

Results shown in Figure 2 demonstrated that the administration of Se-NPs did not induce any significant ($p<0.05$) effect on the mean values of serum oxidant/antioxidant biomarkers compared to those of the control group. However, *S.* Typhimurium challenge in chickens induced a significant decreases in the serum GPX, SOD, and TAC levels, together with a significant elevation in the MDA content, compared to the corresponding control values. Interestingly, treatment with Se-NPs and/or *Salmonella* vaccine significantly ameliorated *S.* Typhimurium alterations in the oxidant/antioxidant biomarkers, compared to the corresponding values of the EAC group, with reinstating their normal control values in all treated groups except the corresponding value of serum TAC content at week 2 post infection.

IL- 6 and IL- 10 expression levels

Data presented in Figure 3 revealed no significant ($p<0.05$) differences between the control and Se-NPs groups. In contrast, *S.* Typhimurium challenge in chickens experienced marked ($p<0.05$) upregulation in tissue IL-6 expression, together with a significant downregulation in tissue IL-10 expression, compared to the corresponding control values. Also, the group challenged with *S.* Typhimurium and treated with Se-NPs and/or *Salmonella* vaccine significantly enhanced *S.* Typhimurium changes in the proinflammatory expression, compared to the corresponding values of the EAC group, with restoring the normal IL-6 value in groups treated with Se-NPs or both Se-NPs and *Salmonella* vaccine to the normal control values.

DISCUSSION

Salmonella is a type of intracellular pathogen that infects a broad range of hosts, including poultry (Ogunleye *et al*., 2009). *S.* Typhimurium is one of the most common causes of foodborne disease in humans causing human salmonellosis due to consumption of poultry byproducts (Jones *et al*., 2021). *S.* Typhimurium infection in old birds causes colonization of the pathogen in intestine and organs with systemic dissemination (Awad and Ghareeb, 2014). Various measures have been employed to elim-

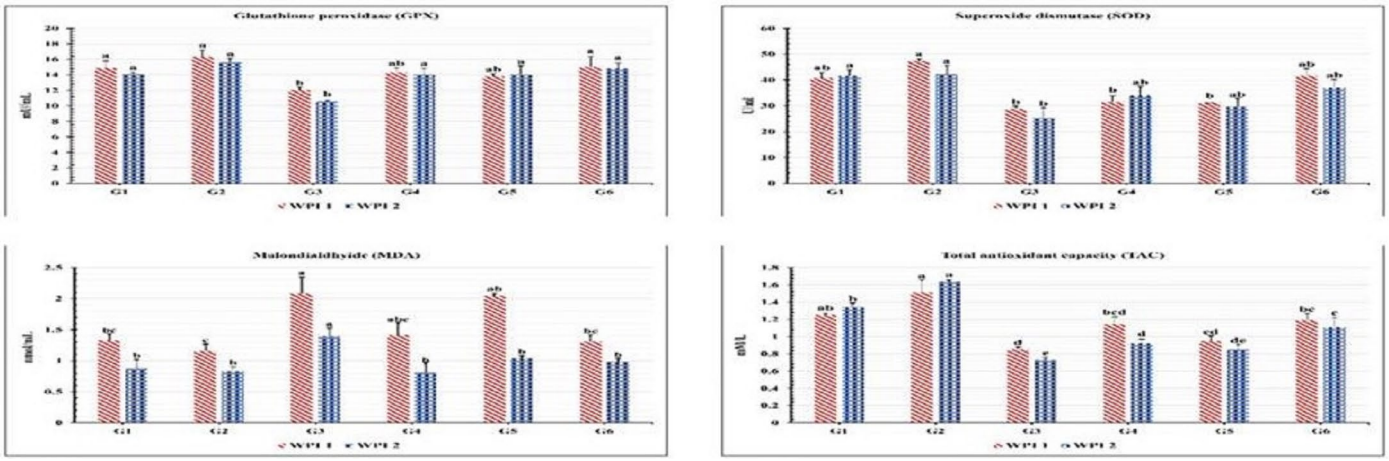

Figure 2. Effect of Se-NPs and/or *Salmonella* vaccine on serum oxidant/antioxidant status of *S.* Typhimurium challenged chickens. Different letters (a, b, c) indicate significant differences at $p < 0.05$ (n = 10). G1: control; G2: treated with Se-NPs; G3: challenged with *S.* Typhimurium; G4: challenged with *S.* Typhimurium and treated with Se-NPs; G5: challenged with *S.* Typhimurium and treated with *Salmonella* vaccine; G6: challenged with *S.* Typhimurium and treated with Se-NPs and *Salmonella* vaccine. WPI: weeks post infection.

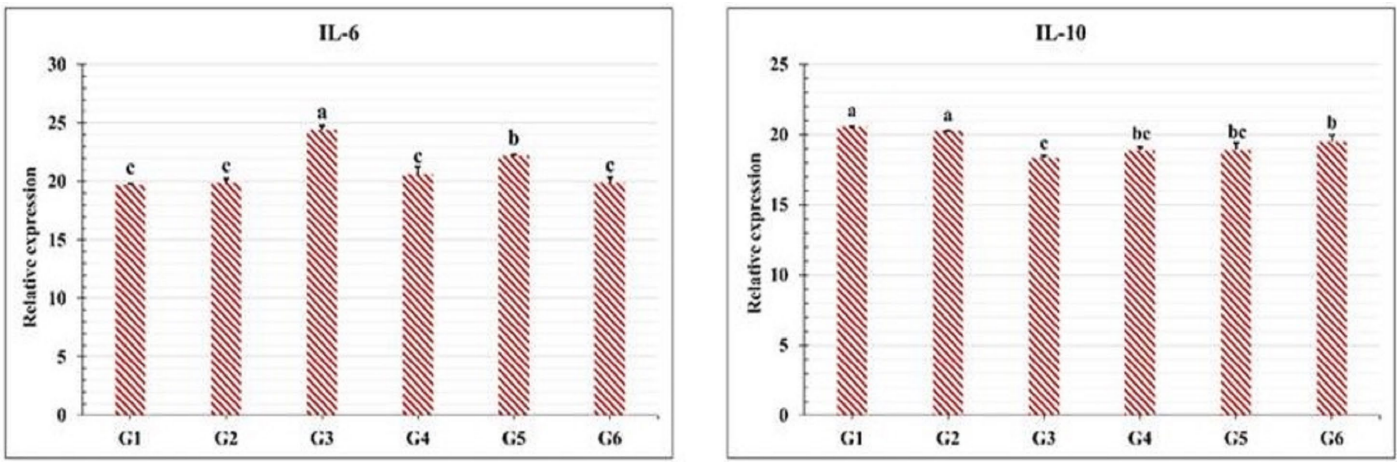

Figure 3. Effect of Se-NPs and/or *Salmonella* vaccine on the expression of pro-inflammatory cytokine (IL-6 and IL-10) in the caecum of birds challenged with *S.* Typhimurium. Different letters (a, b, c) indicate significant differences at $p < 0.05$ (n = 10). G1: control; G2: treated with Se-NPs; G3: challenged with *S.* Typhimurium; G4: challenged with *S.* Typhimurium and treated with Se-NPs; G5: challenged with *S.* Typhimurium and treated with *Salmonella* vaccine; G6: challenged with *S.* Typhimurium and treated with Se-NPs and *Salmonella* vaccine. WPI: weeks post infection.

inate *Salmonella* infection from poultry and their byproducts to protect human health (Thirabunyanon and Thongwittaya, 2012). The present study was designated to focus on the pathomechansim of the disease with particular emphasis on hemato-biochemical, antioxidant biomarkers, and proinflammatory responses of *S.* Typhimurium in broiler chickens.

The obtained results showed that no clinical signs were observed in infected birds with *S.* Typhimurium in this study, which matched well with those reported in previous studies by Wu *et al*. (2020). This finding could be attributed to the more developed immune system of adult birds (Beal *et al*., 2004).

With respect to the hematological parameter, *S.* Typhimurium-challenged birds implicated a significant decrease in RBCs, Hb and PCV values compared to the control group. This is consistent with the results of Tarabees *et al.* (2021) which may be attributed to *Salmonella* endotoxin which destructs erythrocytes intravascularly and thereby causing them to be removed from the circulation (Mdegela *et al*., 2002). Additionally, *S.* Typhimurium challenged birds showed decreased mean MCV, MCH, and MCHC values compared to the control, indicating microcytic hypochromic anemia. This condition may be caused by an inflammatory process in the intestine due to *Salmonella* infection. During the inflammatory process, interleukins are secreted, which in turn stimulate the liver secretion of hepcidin. Hepcidin acts on intestinal cells, preventing iron absorption from food, and on the mononuclear phagocytic system, sequestering circulating iron (Nairz *et al*., 2014). This interpretation is supported by the marked elevation in the proinflammatory cytokine IL-6 observed in the challenged group in this study.

Regarding the leukogram, *S.* Typhimurium-challenged birds reported a significant increase in total leukocytic count, heterophils count, and monocytes count, with a reduction in lymphocytes compared to the control group. Our results agree with those of Sharma *et al*. (2018). The occurrence of leukocytosis was due to bone marrow hyperplasia and an inflammatory response in the intestinal tract (Fotouh *et al*., 2014; Mansour *et al*., 2014). Heterophilia could be attributed to acute inflammatory and degenerative changes in internal organs. Additionally, heterophils play an important role in phagocytosis and removal of organisms (Shah *et al*., 2013). On the other hand, lymphopenia may result from the stress of *S.* Typhimurium infection, which stimulates the adrenal gland to secrete corticosteroid hormones, causing the destruction of lymphocytes (Shah *et al*., 2013).

The biochemical assessment of blood samples in this study exhibited a substantial decrease in serum total protein, albumin, and A/G ratio, together with significant hyperglobulinemia in *S.* Typhimurium-challenged birds compared to the control group. As the liver is the main site for protein synthesis, hypoproteinemia and hypoalbuminemia may be explained by impaired liver function leading to a decrease in protein synthesis (Garcia *et al*., 2010; 2013). The loss of protein through damaged kidneys, mainly albumin, could also lead to hypoalbuminemia. In addition, *Salmonella* strains produce certain enzymes, such as catalases, that induce proteolysis (Kokosharov, 2002). These views are parallel with the significant increase in liver and hepatic functions in the *S.* Typhimurium-challenged group in this study. The observed hyperglobulinemia could result from antigenic stimulation of infectious agent (Azza *et al*., 2012). Moreover, the significant decrease in A/G ratio in this study was because of the decrease in albumin concentration and increase in globulin concentration (Sharma *et al*., 2018).

In addition, the current study documented that *S.* Typhimurium-challenged birds induced a significant decrease in serum glucose, triglycerides, and cholesterol levels compared to the control group. The observed hypoglycemia may be due to increased serum glucose uptake triggered by the metabolic response to the *Salmonella* infection (Correa-Matos *et al*., 2003), or the inflammation of the intestinal tract that inhibits glucose absorption and depletes liver glycogen reserves. In severe cases, hypoglycemia may result from the suppression of liver glycogenolysis (De Freitas Neto *et al*., 2010). On the other hand, the observed changes in serum triglyceride and cholesterol levels may be attributed to hepatic insufficiency that affects lipid metabolism (Garcia *et al*., 2013). These findings are consistent with the significant increase in serum creatinine and uric acid activities detected in the *S.* Typhimurium-challenged group, further indicating compromised kidney function.

S. Typhimurium-challenged birds induced significant decrease in serum iron and TIBC values, together with a significant increment in serum ferritin level compared to the control group. Hypoferremia matched to previous results reported by Tan *et al*. (2019) who recorded decrease in serum iron during *Salmonella* infection. A common mechanism of hypoferremia of inflammation is cytokine driven increase in hepcidin (Nicolas *et al*., 2002) that down regulates ferroprotein and thereby decrease iron flow into extracellular fluid from all its source and hinder the gut from absorbing iron from the blood stream (Ganz and Nemeth, 2011). At the same time, the observed decrease in TIBC agrees with Faruqi and Mukkamalla (2023) who reported that TIBC levels decrease in multifactorial anemias or anemia of chronic inflammation. This matched by this study which showed microcytic hypochromic anemia. While the increased ferritin level is in line with Gehrer *et al*. (2022) who noted that systemic *Salmonella* infection results in increased ferritin levels in serum and tissues. Ferritin is an iron storage protein which has a role in protecting cells from iron mediated damage by reactive oxygen species (ROS) (Brissot *et al*., 2012).

Liver function enzymes are implicated in various metabolic activities inside hepatocytes and their blood elevations reflect abnormal hepatic function and/or necrosis (Mansour *et al*., 2022). The results of serum liver function tests showed a significant elevation in serum ALT and AST in the group challenged with *S.* Typhimurium compared to the control group. This is consistent with the results of Abudabos *et al*. (2017) which may be associated with hepatocellular damage with alteration in hepatic cell permeability and leakage of ALT and AST enzymes into the blood (Wu *et al*., 2020; Abd EL-Dayem *et al*., 2021).

In addition, the results revealed that the mean values of serum creatinine and uric acid were significantly increased in the group challenged with *S.* Typhimurium (Abd EL-Dayem *et al*., 2021) compared with the control group reflecting hepatotoxicity resulted from oxidative damage evident by significant increase in serum MDA and significant decrease in serum GPX, SOD, and TAC levels. This result matched partially with the results of (Ahmed *et al*., 2014; Fotouh *et al*., 2014) who reported a significant increase in serum uric acid and creatinine in experimentally infected chicks with *Salmonella*. The level of creatinine and uric acid is known to reflect the state of glomerular filtration rate and kidney function (Fotouh *et al*., 2014). Furthermore, Hyperuricemia in birds occurs with kidney tubular epithelium damage with bacterial toxins (Ahmed *et al*., 2014).

One of the immune responses that safeguards birds from oxidative stress is the antioxidant defense mechanism. The present study showed that *S.* Typhimurium-challenged birds documented a significant decrease in serum GPx, SOD, and TAC activities together with a significant increase in serum MDA compared to the control group which matched well with those reported in previous studies reported by Abd El-Dayem *et al*. (2021). Herein, the oxidative stress may be an indicator of lipid peroxidation, produced after radicals attack the unsaturated fatty acids (Sokoudjou *et al*., 2019; Taati *et al*., 2020).

In terms of proinflammatory cytokines, the current study showed marked increases in blood levels of IL-6 with a significant decrease in the anti-inflammatory cytokine IL-10 in the group challenged with *S.* Typhimurium compared to the control group. These results agreed with previously reported by Wu *et al*. (2020) and Tarabees *et al*. (2021). Cytokines are a set of molecules that cells secrete to regulate the behavior, proliferation, differentiation, and function of other cells. They have a crucial role in controlling the inflammatory response by modulating it (Saleh *et al*., 2022). Additionally, they made a significant contribution to the production of free radicals that have a crucial function in the in-

flammatory process (EL-Deeb, 2013).

Selenium is a crucial trace element for animal nutrition that has various effects on animal productivity, fertility, and the prevention of diseases (Cai *et al*., 2012). Selenium is an integral part of the enzyme glutathione peroxidase, which serves as an antioxidant enzyme that helps to control levels of hydrogen peroxide and lipid peroxides that are produced during normal metabolic activity (Cai *et al*., 2012). Additionally, virtually all branches of the immune system require dietary selenium for their activity (Surai and Dvorska, 2002).

Herein, the dietary supplementation of Se-NPs and/or *Salmonella* vaccine enhanced blood performance compared to the challenged group. This improvement might be attributed to the antioxidant effect of Se-NPs (Ahmadi *et al*., 2020), which protects the blood cell membrane from oxidative damage and decreases osmotic fragility. This, in turn, improves blood cell production (Jamima *et al*., 2020). Furthermore, the *Salmonella* vaccine might help by decreasing the number and virulence of the pathogen, which in turn improves the situation (Crouch *et al*., 2020). Furthermore, the supplementation of selenium nanoparticles as anti-stressors might prevent lipid peroxidation and form a balance between ROS production leads to reduced damage of liver and the level of lipid profile (Jamima *et al*., 2020). Inactivated *Salmonella* vaccines induce a strong production of antibodies with intestinal colonization inhibition (Methner *et al*., 1999) which might help in organs recovery.

Significant improvements were noticed in liver, kidney, and oxidative status in groups challenged with *S.* Typhimurium and treated with Se-NPs and /or *Salmonella* vaccine. This may return to the effect of Se-NPs, which could provide a beneficial effect on body cells by preventing cell disruption, apoptosis, and enhancement of antioxidant capacities (Cai *et al*., 2012; Mohapatra *et al*., 2014; Oraby *et al*., 2022). Also, *Salmonella* vaccine might help by decreasing the number and virulence of the pathogen which in turn improve the situation.

Additionally, there was a sharp enhancement in serum expression of proinflammatory cytokines IL-6 and IL-10 in the challenged groups with *S.* Typhimurium and treated Se-NPs and/or *Salmonella* vaccine Se-NPs has the capability to boost the immune response and enhance the T cell response to T cell receptor stimulation in primary porcine splenocyte. Also, this can lead to a shift from the inflammatory status to resolving the infection by attracting immune cells (Carlson *et al*., 2010).

CONCLUSION

Based on the study outcomes, *S.* Typhimurium infection induced significant adverse blood performance alterations, protein depletion with hyperglobulinemia, hypoglycemia with lower lipids, hepato-renal toxicity, immunotoxicity, and oxidative injury in broiler chickens. Dietary Se-NPs supplementation is a suitable approach to enhance broiler health status following infection with *S.* Typhimurium. Dietary Se-NPs promotes hematopoiesis, blood protein, and antioxidants synthesis; consequently, the birds display lower oxidative stress and lipid peroxidation in the infection with *S.* Typhimurium. Such an improvement in the antioxidant process inhibits oxidative damage to the bird liver and kidney. However, more research may be needed to determine the suitable doses of Se-NPs are more effective against *Salmonella* infection in broiler chickens.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abd EL-Dayem, G., Saleh, G., Abd EL-Elwahab, R., 2021. Impact of dietary Spirulina (arthrospira) platensis on growth performance, gene expression and antioxidant status of quail challenged with *Salmonella* enteritidis. Mansoura Veterinary Medical Journal 22, 38-47.

Abudabos, A.M., Alyemni, A.H., Dafalla, Y.M., Khan, R.U., 2017. Effect of organic acid blend and Bacillus subtilis alone or in combination on growth traits, blood biochemical and antioxidant status in broilers exposed to *Salmonella* typhimurium challenge during the starter phase. Journal of Applied Animal Research 45, 538-542.

Ahmadi, M., Poorghasemi, M., Seidavi, A., Hatzigiannakis, E., Milis, C., 2020. An optimum level of nano-selenium supplementation of a broiler diet according to the performance, economical parameters, plasma constituents and immunity. Journal of Elementology 25, 1187-1198.

Ahmed, M.A., Youssef, F.M., Abdel Rahman, A.G., 2014. Studies on the effect of thyme oil on *Salmonella* bacteria in broiler chicks. Assiut Veterinary Medical Journal 60, 82-95.

Arif, M., Baty, R.S., Althubaiti, E.H., Ijaz, M.T., Fayyaz, M., Shafi, M.E., Albaqami, N.M., Alagawany, M., Abd El-Hack, M.E., Taha, A.E., Salem, H.M., El-Tahan, A.M., Elnesr, S.S., 2022. The impact of betaine supplementation in quail diet on growth performance, blood chemistry, and carcass traits. Saudi J. Biol. Sci. 29, 1604-1610.

Atul, R I., Thakare, S.R., Khati, N.T., Wankhade, A.V., Burghate, D.K., 2010. Green synthesis of selenium nanoparticles under ambient condition. Chalcogenide Lett. 7, 485-489.

Awad, W. A., Ghareeb, K., 2014. Some aspects of control of *Salmonella* infection in poultry for minimising contamination in the food chain. World's poultry science journal 70, 519-530.

Azza, H., Kamel, H., Walaa, M., Amira, H., 2012. Effect of bactocell® and probiotic food supplements on growth performance, hematological, biochemical and humoral immune response of broiler chickens. World Applied Sci. J. 18, 305-316.

Beal, R.K., Wigley, P., Powers, C., Hulme, S.D., Barrow, P.A., Smith, A.L., 2004. Age at primary infection with *Salmonella enterica* serovar Typhimurium in the chicken influences persistence of infection and subsequent immunity to re-challenge. Vet. Immunol. Immunopathol. 100, 151-64.

Brissot, P., Ropert, M., Le Lan, C., Loréal, O., 2012. Non-transferrin bound iron: a key role in iron overload and iron toxicity. Biochim. Biophys. Acta. 1820, 403-410.

Cai, S.J., Wu, C.X., Gong, L.M., Song, T., Wu, H., Zhang, L.Y., 2012. Effects of nano-selenium on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers. Poult. Sci. 91, 2532–2539.

Calenge, F., Kaiser, P., Vignal, A., Beaumont, C., 2010. Genetic control of resistance to salmonellosis and to *Salmonella* carrier-state in fowl: a review. Genet. Sel. Evol. 42, 11.

Campbell, T.W., 1988. Avian hematology and cytology (No. Ed. 2). Iowa State University Press. pp. 3-17

Campbell, T.W., Coles, E.H., 1986. Avian Clinical Pathology. In Veterinary Clinical Pathology Embert H. Coles; D.V.M.; M.S.; Ph.D.4 th edition; p. 289-290.

Carlson, B.A., Yoo, M.H., Shrimali, R.K., Irons, R., Gladyshev, V.N., Hatfield, D.L., Park, J.M., 2010. Role of selenium-containing proteins in T-cell and macrophage function. Proceedings of the Nutrition Society 69, 300-310.

CDC (Centers for Disease Control and Prevention), 2010. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food - 10 states, 2009. MMWR 59, 418-422.

Cook, J.D., Lipschitz, D.A., Miles, L.E., Finch, C.A., 1974. Serum ferritin as a measure of iron stores in normal subjects. Am. J. Clin. Nutr. 27, 681-687.

Correa-Matos, N.J., Donovan, S. M., Isaacson, R.E., Gaskins, H.R., White, B.A., Tappenden, K.A., 2003. Fermentable fiber reduces recovery time and improves intestinal function in piglets following *Salmonella* typhimurium infection. J. Nutr. 133, 1845-1852.

Crouch, C. F., Nell, T., Reijnders, M., Donkers, T., Pugh, C., Patel, A., Davis, P., van Hulten, M. C.W., de Vries, S.P.W., 2020. Safety and efficacy of a novel inactivated trivalent *Salmonella enterica* vaccine in chickens. Vaccine 38, 6741-6750.

De Freitas Neto, O.C., Penha Filho, R.A.C., Barrow, P., Berchieri Junior, A., 2010. Sources of human non-typhoid salmonellosis: a review. Brazilian Journal of Poultry Science 12, 01-11.

Doumas, B. T., Watson, W. A., Biggs, H. G., 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica. Chimica. Acta. 31, 87-96.

Dreux, C., 1977. Selected method. Analysis of human serum: assay of iron II. Method using bathophenanthroline Se-Iron II (bathophenanthroline). Ann. Biol. Clin. 35, 275-277. PMID: 900604

El-Deeb, W. M., 2013. Clinicobiochemical investigations of gangrenous mastitis in does: immunological responses and oxidative stress biomarkers. J. Zhejiang Univ. Sci. B. 14, 33-39.

El-Deep, M.H., Shabaan, M., Assar, M.H., Attia, K.M., Sayed, M.A.M., 2017.

Comparative Effects of Different Dietary Selenium Sources on Productive Performance, Antioxidative Properties and Immunity in Local Laying Hens Exposed to High Ambient Temperature. J. Anim. Poult. Prod. 8, 335-343.

Faruqi, A., Mukkamalla, S.K.R., 2023. Iron Binding Capacity. In: StatPearls. StatPearls Publishing.

Forkus, B., Ritter, S., Vlysidis, M., Geldart, K., Kaznessis, Y. N., 2017. Antimicrobial Probiotics Reduce *Salmonella enterica* in Turkey Gastrointestinal Tracts. Scientific Reports 7, 40695.

Fotouh, A., Gab-Allah, M.S., Tantawy, A.A., Soufy, H., Nasr, S.M., 2014. Alterations of Blood Components in Broiler Chicks Experimentally Infected with *Salmonella* Gallinarum. Global Vet. 13, 787-793.

Ganz, T., Nemeth, E., 2011. Hepcidin and disorders of iron metabolism. Annu. Rev. Med. 62, 347-360.

Garcia, K.O., Berchieri Jr, A., Santana, A.M., Alarcon, M.F.F., Freitas Neto, O.C., Fagliari, J.J., 2013. Experimental infection of commercial layers with wild or attenuated *Salmonella* Gallinarum mutant strains: anatomic pathology, total blood cell count and serum protein levels. Brazilian Journal of Poultry Science 15, 91-104.

Garcia, K.O., Santana, A.M., Freitas Neto, O.C., Simplício, K.M.M.G., Alessi, A.C., Berchieri Jr,A., Fagliari, J.J., 2010. Experimental infection of commercial layers using a *Salmonella enterica* sorovar Gallinarum strain: blood serum components and histopathological changes. Brazilian Journal of Veterinary Pathology 3, 111-117.

Gehrer, C.M., Hoffmann, A., Hilbe, R., Grubwieser, P., Mitterstiller, A.M., Talasz, H., Fang, F.C., Meyron-Holtz, E.G., Atkinson, S.H., Weiss, G., Nairz, M., 2022. Availability of Ferritin-Bound Iron to *Enterobacteriaceae*. Int. J. Mol. Sci. 23, 13087.

Gupta, P.K., 1977. Haematological Techniques, 4th ed.; Syndicate: London, UK, p. 231.

Humphrey, T. J., Baskerville, A., Chart, H., Rowe, B., 1989. Infection of egg-laying hens with *Salmonella* Enteritidis PT4 by oral inoculation. Vet. Rec. 125, 531-532.

Jamima, J., Veeramani, P., Kumanan, K., Kanagaraju, P., 2020. Production performance, hematology and serum biochemistry of commercial broilers supplemented with nano selenium and other anti-stressors during summer. Indian Journal of Animal Research 54, 1385-1390.

Jawale, C.V., Lee, J.H., 2016. Evaluation of immunogenicity and protective efficacy of adjuvanted *Salmonella* Typhimurium ghost vaccine against salmonellosis in chickens. Vet. Q. 36, 130-136.

Jia, S., McWhorter, A.R., Andrews, D.M., Underwood, G.J., Chousalkar, K.K., 2020. Challenges in Vaccinating Layer Hens against *Salmonella* Typhimurium. Vaccines 8, 696.

Jones, M.K., Da Costa, M., Hofacre, C.L. Baxter, V.A., Cookson, K., Schaeffer, J., Barker, A., Dickson, J., Berghaus, R. D., 2021. Evaluation of a modified live *Salmonella* Typhimurium vaccination efficacy against *Salmonella enterica* serovar Infantis in broiler chickens at processing age. J. Appl. Poult. Res. 30, 100156.

Khan, I., Zaneb, H., Masood, S., Ashraf, S., Rehman, H.F., Tahir, S.K., Rehman, H.U., Khan, A., Taj, R., Rahman, S.U., Shah, M., 2022. Supplementation of Selenium Nanoparticles-Loaded Chitosan Improves Production Performance, Intestinal Morphology, and Gut Microflora in Broiler Chickens. The journal of poultry science 59, 272-281.

Knap, I., Kehlet, A.B., Bennedsen, M., Mathis, G.F., Hofacre, C.L., Lumpkins, B.S., Jensen, M. M., Raun, M., Lay, A., 2011. Bacillus subtilis (DSM17299) significantly reduces *Salmonella* in broilers. Poultry Science 90, 1690-1694.

Kokosharov, T., 2002. Clinical and hematological effects of *Salmonella* gallinarum endotoxin in cockerels. Veterinarski Arhiv 72, 269-276.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., Cosic, V., 2001. Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol. 54, 356-361.

Mansour, E.S., Allam, H.H., Shawky, N.A., El Rashidy, R., Abdalla, S.H., 2014. Studies on Effect of *Salmonella* Pullorum in Balady Chicks and its Treatment with Doxycycline. Zagazig Veterinary Journal 42, 145-156.

Mansour, A.T., Mahboub, H.H., Amen, R.M., El-Beltagy, M.A., Ramah, A., Abdelfattah, A.M., El-Beltagi, H.S., Shalaby, T.A., Ghazzawy, H.S., Ramadan, K.M.A., Alhajji, A.H.M., Hamed, H.S., 2022. Ameliorative Effect of Quercetin against Abamectin-Induced Hemato-Biochemical Alterations and Hepatorenal Oxidative Damage in Nile Tilapia, Oreochromis niloticus. Animals 12, 3429.

Mdegela, R.H., Msoffe, P.L., Waihenya, R.W., Kasanga, J.C., Mtambo, M.M., Minga, U.M., Olsen, J.E., 2002. Comparative pathogenesis of experimental infections with *Salmonella* gallinarum in local and commercial chickens. Trop. Anim. Health Prod. 34, 195-204.

Meeusen, E.N., Walker, J., Peters, A., Pastoret, P.P., Jungersen, G., 2007. Current status of veterinary vaccines. Clinical microbiology reviews 20, 489-510.

Methner, U., Barrow, P.A., Berndt, A., Steinbach, G., 1999. Combination of vaccination and competitive exclusion to prevent *Salmonella* colonization in chickens-experimental studies. Int. J. Food Microbiol. 49, 35-42.

Mohapatra, P., Swain, R.K., Mishra, S.K., Behera, T., Swain, P., Mishra, S.S., Behura, N.C., Sabat, S.C., Sethy, K., Dhama, K., Jayasankar, P., 2014. Effects of dietary nano-selenium on tissue selenium deposition, antioxidant status and immune functions in layer chicks. Int. J. Pharmacol. 10, 160-167.

Mulley, R.C., 1979. Haematology and blood chemistry of the black duck Anas superciliosa. Journal of wildlife diseases 15, 437-441.

Nairz, M., Haschka, D., Demetz, E., Weiss, G., 2014. Iron at the interface of immunity and infection. Front. Pharmacol. 5, 152.

Nandre, R.M., Matsuda, K., Chaudhari, A.A., Kim, B., Lee, J.H., 2012. A genetically engineered derivative of *Salmonella* Enteritidis as a novel live vaccine candidate for salmonellosis in chickens. Res. Vet. Sci. 93, 596-603.

Natt; M.P., Herrick; C.A., 1952. A new Blood Diluent for Counting of Erythrocytes and Leukocytes of The Chicken. Poult. Sci. 31, 735-738.

Nicolas, G., Chauvet, C., Viatte, L., Danan, J.L., Bigard, X., Devaux, I., Beaumont, C., Kahn, A., Vaulont, S., 2002. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J. Clin. Invest. 110, 1037-1044.

Nishikimi, M., Appaji, N., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 46, 849-854.

Ogunleye, A.O., Ajuwape, A.T.P., Adetosoye, A.I., 2009. Hematological changes in *Salmonella* paratyphi A infected pullets. Revue Élev. Méd. vét. Pays trop. 62, 23-26.

Ohkawa, H., Ohishi, W., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351.

Oraby, N.H., Hassan, A.A.A., Mansour, M.K., Arafa, M.M., Ibrahim, E., Talkhan, O.F., 2022. Protective and detoxificating effects of selenium nanoparticles and thyme extract on the aflatoxin B1 in rabbits. Egyptian Journal of Agricultural Research 100, 284-293.

Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70, 158-169.

Piccardi, G., Nyssen, M., Dorche, J., 1972. Determination of iron and the total capacity for binding in human serum by a method without deproteinization. Clin. Chim. Acta. 40, 219-228.

Reda, F.M., El-Saadony, M.T., Elnesr, S.S., Alagawany, M., Tufarelli, V., 2020. Effect of dietary supplementation of biological curcumin nanoparticles on growth and carcass traits, antioxidant status, immunity and caecal microbiota of Japanese quails. Animals 10, 754.

Reda, F.M., El-Saadony, M.T., El-Rayes, T.K., Attia, A.I., El-Sayed, S.A., Ahmed, S.Y., Madkour, M., Alagawany, M., 2021. Use of biological nano zinc as a feed additive in quail nutrition: biosynthesis, antimicrobial activity and its effect on growth, feed utilization, blood metabolites and intestinal microbiota. Ital. J. Anim. Sci. 20, 324-335.

Saad, A.M., El-Saadony, M.T., El-Tahan, A.M., Sayed, S., Moustafa, M.A., Taha, A.E., Taha, T.F., Ramadan, M.M., 2021. Polyphenolic extracts from pomegranate and watermelon wastes as substrate to fabricate sustainable silver nanoparticles with larvicidal effect against Spodoptera littoralis. Saudi J. Biol. Sci. 28, 5674-5683.

Saleh, N., Allam, T.S., Omran, A., Abdelfattah, A.M., 2022. Evaluation of Changes in Hemato-Biochemical, Inflammatory, and Oxidative Stress Indices as Reliable Diagnostic Biomarkers for Subclinical Mastitis in Cows. Alexandria Journal for Veterinary Sciences 72, 23-34.

Samy, A.A., El-Enbaawy, M.I., El-Sanousi, A.A., Abd El-Wanes, S.A., Ammar, A.M., Hikono, H., Saito, T., 2015. In vitro assessment of differential cytokine gene expression in response to infections with Egyptian classic and variant strains of highly pathogenic H5N1 avian influenza virus. Int. J. Vet. Sci. Med. 3, 1-8.

Schumann, G., Canalias, F., Joergensen, P. J., Kang, D., Lessinger, J. M., Klauke, R., 2010. Committee On Reference Systems for Enzymes C-Rse, International Federation of Clinical Chemistry and Laboratory Medicine Scientific Division, 2010. IFCC reference procedures for measurement of the catalytic concentrations of enzymes: corrigendum, notes and useful advice. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)-IFCC Scientific Division. Clinical Chemistry and Laboratory Medicine 48, 615-621.

Shah, S. N., Kamil, S. A., Darzi, M. M., Mir, M. S., Bhat, S. A., 2013. Haematological and some biochemical changes in experimental fowl typhoid infection in broiler chickens. Comp. Clin. Pathol. 22, 83-91.

Sharma, S., Azmi, S., Sharma, S., Rahman S., Nashiruddullah, N., 2018. Ef-

fect of Vitamin C on Experimental Inoculation with *Salmonella* Enteritidis in Broiler Chickens with Reference to Haemato-Biochemical Profile. Int. J. Curr. Microbiol. App. Sci. 7, 88-96.

Snedecore, G.W., Cochran, W.G., 1980. Statistical Methods. 7th edition, Iowa State University Press, USA.

Sokoudjou, J.B., Fodouop, S.P.C., Djoueudam, F.G., Kodjio, N., Kana, J.R., Fowa, A.B., Kamsu, G.T., Gatsing, D., 2019. AntiSalmonellal and antioxidant potential of hydroethanolic extract of Canarium schweinfurthii Engl. (Burseraceae) in *Salmonella enterica* serovar Typhimurium-infected chicks. Asian Pac. J. Trop. Biomed. 9, 474-83

Surai, P.F., Dvorska, J.E., 2002. Effect of selenium and vitamin E on lipid peroxidation in thigh muscle tissue of broiler breeder hens during storage. Arch. Geflugelkd 66, 120.

Suzuki, K., Okada, H., Itoh, T., Tada, T., Mase, M., Nakamura, K., Kubo, M., Tsukamoto, K., 2009. Association of increased pathogenicity of Asian H5N1 highly pathogenic avian influenza viruses in chickens with highly efficient viral replication accompanied by early destruction of innate immune responses. Journal of Virology 83, 7475-7486.

Taati, B., Arazi, H., Suzuki, K., 2020. Oxidative Stress and Inflammation Induced by Waterpipe Tobacco Smoking Despite Possible Protective Effects of Exercise Training: A Review of the Literature. Antioxidants 9, 777.

Tan, Z., Chekabab, S.M., Yu, H., Yin, X., Diarra, M.S., Yang, C., Gong, J., 2019. Growth and Virulence of *Salmonella* Typhimurium Mutants Deficient in Iron Uptake. ACS omega 4, 13218-13230.

Tarabees, R., Hafez, H., Shehata, A., Allam, T., Setta, A., ELsayed, M., 2021. Effects of Probiotic and/or Prebiotic Supplementations on Immune Response, Haematology, Oxidant-antioxidant Biomarkers, and Cytokine mRNA Expression Levels in the Caeca of Broilers Infected with *Salmonella*. Poultry Science Journal 9, 41-52.

Thirabunyanon, M., Thongwittaya, N., 2012. Protection activity of a novel probiotic strain of Bacillus subtilis against *Salmonella* Enteritidis infection. Res Vet Sci. 93, 74-81.

Tietz, N., 1976. Fundamentals of Clinical Chemistry. W.B. Sauders Co. Philadelphia.

Vahdati, M., Tohidi Moghadam, T., 2020. Synthesis and Characterization of Selenium Nanoparticles-Lysozyme Nanohybrid System with Synergistic Antibacterial Properties. Sci. Rep. 10, 510.

Vassault, A., Grafmeyer, D., de Graeve, J., Cohen, R., Beaudonnet, A., Bienvenu, J., 1999. Quality specifications and allowable standards for validation of methods used in clinical biochemistry. Ann. Biol. Clin. 57, 685-695.

Wu, Y.T., Yang, W.Y., Wu, Y.H.S., Chen, J.W., Chen, Y.C., 2020. Modulations of growth performance, gut microbiota, and inflammatory cytokines by trehalose on *Salmonella* Typhimurium-challenged broilers. Poultry science 99, 4034-4043.

Young D.S., 2001. Effects of disease on clinical lab tests, 4th edition AACC.

Young, D.S., Thomas, D.W., Friedman, R.B., Pestaner, L.C., 1972. Effects of drugs on clinical laboratory tests. Clin Chem. 18, 1041-1303.

Yuan, J.M., Guo, Y.M., Yang, Y., Wang, Z.H., 2007. Characterization of fatty acid digestion of Beijing fatty and arbor acres chickens. Asian. Australas. J. Anim. Sci. 20, 1222-1228.

Zadeh, M. H., Kermanshahi, H., Sanjabi, M., Golian, A., Azin, M., Majidzadeh-Heravi, R., 2018. Comparison of Different selenium Sources on Performance, Serum Attributes and Cellular Immunity in Broiler Chickens. Poultry Science Journal 6, 191-203.