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Immunological and Nutritional Effects of Selenium and Nano-selenium on Broiler Chickens Exposed to Heat Stress

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Abstract

This study was undertaken to investigate the ameliorative effect of sodium selenite and nano-selenium on chicks exposed to heat stress. Ninety one day-old broiler chicks were divided into six groups each of 15 birds. Birds in group one and four were fed on basal diet and served as a control group, while birds in group two and five were fed on basal diet treated with 0.15 mg/Kg diet prepared selenium nanoparticles and birds in group three and six were fed on basal diet treated with 0.15 mg/Kg sodium selenite. On day 37 of experiment birds in 4th, 5th and 6th group were exposed to artificial heat stress at 40°C for 12 hours. Based on the results of total body weight gain and FCR lower parameters of body weight, body weight gain, feed conversion rate and higher feed intake ratio were observed in heat stressed chicks. Nano-selenium and Na selenite supplementation decreased the serum AST, ALT, urea, creatinine and MDA while the serum total protein, SOD, LZM, IgG, IgM and C3 were increased, also nano-selenium has ameliorative effect on hematological and histopathological findings. In conclusion, both nano-selenium and sodium selenite have an essential role in treatment of adverse effect of heat stress on chicks, with superiority of nano-selenium in improving anti- oxidative status and boosting immune response.

KEYWORDS
Antioxidant, Chicks, Immunology, Nano–selenium

INTRODUCTION

Global warming has been caused by rising ambient temperatures, which is still trending upward. In order to improve broiler chicken production in hot climates, it is necessary to increase the thermo-tolerance of broiler chickens without sacrificing their productivity (Leinonen and Kyriazakis 2016; Vandana *et al*. 2020). Modern broiler breeds are more susceptible to heat stress than earlier genotypes. Each year, heat stress that affects chicken production results in a significant loss of capital investment (Melesse *et al*., 2011). Baziz *et al*. (1996) calculated that the feed intake and growth of broilers would decline by roughly 3.6 and 1.5%, respectively, for every degree over 22°C. According to Mujahid *et al*. (2005), hyperthermia may increase the generation of reactive oxygen species (ROS), which in turn increases lipid peroxidation in the tissues, resulting in the buildup of free radicals and an increase in oxidative stress. The need to improve broilers breeds' thermo-tolerance exists since contemporary broilers breeds are more susceptible to heat stress than earlier genotypes. The body's antioxidant capacity is exceeded during extended or intensely combative stress, which affects cellular processes and reduces production performance (Maini *et al*., 2007). Research has shown that including antioxidants in broilers meals will lessen the metabolic changes and lipid peroxidation brought on by heat exposure (Sahin *et al*., 2002). Animals benefit from the antioxidant effects of the micronutrient selenium (Se), which is a necessary micromineral. Selenium has a key role in the enzyme glutathione peroxidase, which protects cells and cell membranes from oxidation (Oliveira *et al*., 2014). The most popular type of selenite used in poultry diets to meet the Se requirement (Perić *et al*., 2009). Due to the impact of free radical sequestration, antioxidant minerals like selenium can decrease the effects of stress brought on by high temperatures and thereby enhance the growth performance and physiological behaviors of birds (Habibian *et al*., 2014). As nanotechnology developed, attention was drawn to nano-selenium (Nano-Se), which has peculiar qualities such a large surface area, high surface activity, high catalytic efficiency, great adsorption capacity, and low toxicity. According to Mohapatra *et al*. (2014), the physiological effects of sodium selenite and nano-Se may differ due to their different metabolism and absorption mechanisms. Additionally, the hepatic and serum GSH-Px activities of chicken were enhanced by the addition of Nano- Se to the diet. Dietary Nano-Se may improve antioxidant activity and oxidative stability (Cai *et al*., 2012). With a broilers' supplementation limit of 1.0 ppm, the ideal dose of Nano-Se supplementation was between 0.3 and 0.5 ppm. Therefore, the major goal of this study was to examine the impact of

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dietary supplementation of sodium-selenite and nano-selenium on the physiological and productive abilities of chickens exposed to heat stress.

MATERIALS AND METHODS

Selenium and selenium nanoparticles

From the German company MERCK, sodium selenite ($Na_2SeO_3.5H_2O$) was purchased. According to a modified procedure used by Malhotra *et al*. (2014), selenite was chemically reduced with ascorbic acid to produce selenium nanoparticles, which were then stabilized by being coated with dextrin. Particle spectroscopy, a scanning electron microscope (SEM), and X-ray diffraction (XRD) were used in the analysis of prepared selenium nanoparticles to determine their size, shape, morphology, and crystallinity. This investigation used produced nanoparticles that were 60 nm in size. In Giza, Egypt's Naqaa Foundation for Scientific Research, Technology and Development, selenium nanoparticles were created.

Preparation of experimental meals including nano- and sodium selenite

Each of the generated selenium nanoparticles and sodium selenite were utilized at a concentration of 0.15 mg/kg diet, dispersed in 5 ml of distilled water, misted into poultry feed, rotated in a mixer, and then allowed to air dry for 24 hours.

Experimental approach

According to the regulations issued by the Zagazig University's Animal Care and Use Committee, all institutional and national criteria for the care and use of birds were adhered to. The ZU-IACUC/2/F/50/2023 ethical approval number was given to this project.

A commercial hatchery in the Egyptian province of Sharkia provided 90 day-old broiler chicks (Cobb, Al-Watania Poultry Company), which were raised there over the course of 38 days. The chicks were allocated into six groups (Gps) of fifteen each at random. As control groups, the first and fourth groups of birds were fed a basal diet without any additional supplements, while the second and fifth groups of birds received a basal diet supplemented with 0.15 mg/kg of prepared selenium nanoparticles, and the third and sixth groups of birds received a basal diet supplemented with 0.15 mg/kg of sodium selenite [Na_2SeO_3]. Birds in the fourth, fifth, and sixth groups underwent a 12-hour period of artificial heat stress at 40°C on the experiment's 37th day. The birds were raised in a room with controlled environmental conditions and had unrestricted access to food and water. According to NRC (1994), all the diets in Table 1 were designed to meet the needs of broilers, and chemical analysis of the diets was carried out by the Food Analysis Centre of the Faculty of Veterinary Medicine at Zagazig University. The same environmental controls (temperature, moisture, ventilation, and light) applied to all birds.

Growth Performance

Every six days, body weight, weight growth, and the feed-to-gain ratio (feed/gain) were assessed separately; during the study period, feed intake was recoded every day.

Blood Samples

On days 37 and 38, the wing veins of five birds from each group were the site of two blood samples being taken. Sera were collected for biochemical studies and estimation of some biochemical, immunological, and antioxidant parameters from the second sample, which was collected in a clean, dry centrifuge tube without anticoagulant and allowed to clot at room.

Hematological parameters

A Sysmex XN series differential hematology autoanalyzer was used to collect blood samples while using EDTA as an anticoagulant in order to prepare for the study of the complete blood

Table 1. Composition of the basal diets.

Ingredient %	Starter up to 21 days	Grower from 22 to 38 days
Corn grain	55.3	63.73
Soya bean 44% protein	33.3	17.4
Concentrate	10	10
Corn gluten	2.2	6
Vegetable oil	3	2.5
Di-calcium phosphate	0.65	0.5
DL-Methionine	0.22	0.03
L-Lysine	0.15	0.3
Threonine	0.14	0.09
Sodium chloride	0.3	0.3
Limestone	0.05	0.05
Chemical analysis of basal diets	Starter	Grower
Crude protein	22.48	20.04
ME (Kcal/Kg diet)	3051.5	3105.9
Calcium	1.04	1.01
Phosphorus (%) (available)	0.5	0.45
Lysine (%)	1.38	1.15
Methionine (%)	0.7	0.5
Methionine + Cysteine (%)	0.73	0.84

count, according to Münster (2013). White blood cells (WBCs) count, hemoglobin concentration, hematocrit value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and WBC differential count were all recorded and analyzed as part of the hematological profiles.

Biochemical studies

According to Drupt (1974), albumin and globulin were estimated, and Doumas *et al*. (1981) method was used to estimate serum total protein. According to Murray (1984), the activity of the liver transferases (alanine aminotransferase, or ALT, and aspartate aminotransferase, or AST) were estimated. Serum urea (Fawcet and Scott. 1960), and serum creatinine (Henry. 1974) were estimated. The Placer *et al*. (1966) method was used to measure the generation of malondialdehyde (MDA). The method developed by Marklund and Marklund (1974) was used to measure SOD levels. The Engstad *et al*. (1992)-described turbid metric method was used to gauge the activity of serum lysosomes. According to Bianchi *et al*. (1995), sandwich ELISA was used to determine IgG and IgM levels. A sandwich enzyme-linked immunosorbent assay (ELISA) kit from CAT was used to measure the serum. Complement 3 levels in the meantime (Life Span Biosciences, Inc., Seattle, WA, USA). LS-F9287), following Lie *et al*. (1986).

Histopathological analysis

Samples were taken from the liver, heart, brain, and kidneys of dead and sacrificed chicken from groups (four, five, and six). They were fixed in 10% neutral buffered formalin, processed as 4 m paraffin sections, stained with hematoxylin and eosin (H&E), and ready for microscopic analysis (Bancroft and Stevens 1990).

Statistical analysis

Analysis of variance (ANOVA) was used for the statistical analysis. Differences in treatment mean were determined using Duncan's Multiple Range (Duncan, 1955) at a significance level of 0.05. Using the computer and the SPSS program SPSS (2004), all statistics were conducted.

RESULTS

Performance characteristics

Table 2 shows the impact of dietary supplementation with Nano-Se and SSE in various environmental settings on body weight gains (BWG), feed intake (FI), and feed conversion ratio (FCR). Compared to groups one, two and three significantly increased their body weight, gained weight, and reduced their feed intake and feed conversion rate. But when compared to the other groups, gr4 showed a substantial drop in body weight, body weight gain, and feed conversion rate with a much higher feed intake.

In addition, heat stress caused large increases in body weight and body fat in groups 5 and 6, compared to group 4, and non-significant changes in feed intake and feed conversion rate in groups 4, 5, and 6, except for groups 5, which saw a significant decline in FCR compared to group 4.

Hematological parameters

As shown in Table 3, hematological analyses of chicks from various groups before and after acute heat stress exposure revealed that Gp. 2 revealed a significant increase ($P<0.05$) in all RBC counts, Hb concentration, PCV value, WBCs, and heterophil

Table 2. Effect of different selenium forms on body Weight gains (BWG), feed intake (FI) and feed conversion ratio (FCR) during the experimental period (n=5).

Item	Gp. 1	Gp. 2	Gp. 3	Gp. 4	Gp. 5	Gp. 6
Initial body weight	38.9±3.0	39.5±2.0	39.0±3.0	39.0±3.0	39.5±2.0	39.0±2.0
Final weight /gram	2000.0±20.0^{b}	2250.0±300.0^{a}	2100.0±20.0^{a}	1700.0±25.0^{c}	1900.0±30.0^{b}	1850.0±30.0^{b}
Body weight gain/gram	1961.0±70.0^{b}	2210.5±100.0^{a}	2061.0±60.0^{a}	1661.0±70.0^{c}	1860.5±65.0^{b}	1811.0±85.0^{b}
Feed intake /gram	3173.9±30.0^{a}	3094.7±50.0^{b}	2988.45±60.0^{b}	2740.6±60.0^{c}	2697.7±70.0^{c}	2807.05±55.0^{c}
Feed conversion rate (FCR)	1.54±0.03^{a}	1.40±0.02^{c}	1.45±0.04^{b}	1.60±0.05^{a}	1.50±0.06^{b}	1.56±0.03^{a}

Values are presented as means±SEM. Different letters indicate significant differences ($P<0.05$).

Table 3. Effect of different selenium forms on blood profile of treated chickens during the experimental period (n=5).

Groups Parameter	Gp.1	Gp.2	Gp.3	Gp.4	Gp.5	Gp.6
RBCs (x10^{6}/cmm)	3.31±0.08^{b}	4.21±0.12^{a}	3.17±0.04^{b}	2.9±0.08^{c}	4.15±0.08^{a}	2.85±0.05^{c}
Hb (g/dl)	10.2±0.13^{b}	12.35±0.14^{a}	10±0.12^{bc}	9±0.07^{d}	12.66±0.09^{a}	9.8±0.17^{c}
PCV (%)	31.8±0.3^{b}	34.15±0.15^{a}	31.5±0.14^{b}	29.2±0.11^{c}	34.5±0.07^{a}	28.84±0.27^{c}
MCV (fl)	96.16±1.6^{a}	81.44±2.4^{b}	98.7±1.8^{a}	101±3.1^{a}	83.2±1.7^{b}	98.2±1.9^{a}
MCH (pg)	30.8±0.5^{b}	29.4±0.97^{b}	31.47±0.8^{b}	31.05±0.9^{b}	30.5±0.8^{b}	34.3±0.62^{a}
MCHC (g/dl)	32.1±0.3^{c}	36.14±0.3^{a}	31.7±0.4^{cd}	30.8±0.3^{d}	36.7±0.35^{a}	34.9±0.3^{b}
WBCs (x10^{3}/cmm)	12.9±0.2^{d}	14.03±0.12^{b}	13.64±0.20^{bc}	15.24±0.22^{a}	13.24±0.09^{cd}	13.92±0.19^{b}
Heterophil (%)	24.88±0.17^{e}	27.44±0.16^{d}	27.37±0.14^{d}	32.30±0.26^{a}	30.55±0.28^{c}	31.58±0.20^{b}
Lymphocyte (%)	70.10±0.21^{a}	67.81±0.14^{b}	67.25±0.16^{c}	63.55±0.23^{f}	65.45±0.14^{d}	64.89±0.16^{e}
Monocyte (%)	4.58±0.11^{b}	4.75±0.07^{b}	5.05±0.03^{a}	3.59±0.07^{d}	4.04±0.06^{c}	3.69±0.05^{d}
Eosinophil (%)	0.26±0.02^{a}	0.24±0.01^{ab}	0.28±0.02^{a}	0.26±0.01^{a}	0.27±0.01^{a}	0.21±0.01^{b}
Basophil (%)	0.17±0.01^{c}	0.14±0.00^{d}	0.15±0.01^{cd}	0.19±0.01^{b}	0.23±0.01^{a}	0.13±0.01^{d}

Means with different letters at the same row are significantly different ($P<0.05$)

counts while also revealing a significant decrease (P<0.05) in lymphocyte percentage and a non-significant change (P>0.05) in monocyte percentage when compared to the control-ve group (Gp. 1). In contrast, Gp. 3 showed a non-significant change (P>0.05) in RBC count, Hb concentration, and PCV value compared to Gp. 1, a significant decrease (P<0.05) in those parameters compared to Gp. 2, and significant increases in WBC count, heterophils, and monocyte% compared to Gp. 1 as well as a significant decrease (P<0.05) in lymphocyte% compared to both Gps. 1 and 2. Furthermore, after heat exposure, control +ve (Gp. 4) showed significant decrease (P<0.05) in each of RBCs count, Hb concentration PCV value and lymphocyte and monocyte % with significant increase (P<0.05) in both WBCs counts and heterophils % compared to Gp. 1. Gp. 5, revealed significant increase (P<0.05) in RBCs count, Hb conc., PCV value, lymphocytes and monocytes percentage with significant decrease (P<0.05) in both WBCs counts and heterophils % compared to both Gp. 4 and Gp. 6.

Biochemical parameters

As demonstrated in Table 4, heat stress resulted in a significant (P< 0.05) decrease in the levels of plasma total protein albumin and globulin compared with those of control (Gp. 1), while heat stressed chickens and treated with Na selenite and Nano-selenium (Gps. 5 and 6) resulted in increase in the levels of plasma total protein and albumin, globulin compared with those heat stressed non treated chickens (Gp. 4). In the present study chickens in Gp. 4) evoked a significant (P< 0.05) increase in the levels of AST, ALT compared with those of Gp. 1. Treatment of heat stressed chickens with Na selenite and Nano-selenium evoked a significant (P< 0.05) decrease in AST, ALT levels in Gps 5 and 6 compared with Gp. 4. In the present study chickens in Gp. 4 evoked a significant (P< 0.05) increase urea and creatinine compared with Gp. 1, while treated chickens in Gps. 5 and 6 evoked a significant decrease (P< 0.05) in the four mentioned parameters compared with (gp 4). Our results recorded the highest value of MDA in (gp4) while the lowest values were observed in Gps. 2, 3 and 5. The highest values of SOD, LZM, IgG, IgM and C3 were founded in Gps. 2 and 5, a significant increase in SOD showed in Gps. 3 and 6 and non-significantly change in other parameters except IgM which recorded a significant increase comparing with the control.

Histopathological results

Liver of Groups 4 and 6 showed diffuse Yellowish and pale livers and distended gallbladders (Figure 1A.). Microscopically, the liver of broilers in Gp. 2 revealed severe congestion of blood vessels and sinusoids, and fatty degeneration represented by vacuolation in the hepatic cytoplasm (Figure 1B and C). Meanwhile, broilers supplied with nanoselenium and exposed to heat stress (Gp. 5) exhibited mild leucocytic infiltration around the central vein with their hepatic cells, vacuolar degeneration, and mild congestion (Figure 1 D). Supplementation with selenium in (Gp. 6) broilers showed severe congestion of the central vein with fibrosis, focal coagulative necrosis of hepatic cells, and few leukocytic infiltrations of the liver parenchyma (Figure 1 E and F). Kidneys of broilers in Gp. 4, which are exposed to heat stress showed generalized edema and severely congested blood vessels. The renal tubules showed diffuse coagulative necrosis, hyaline casts, and damaged glomeruli (Figure 2A and B). The Kidneys of broilers supplied with nanoselenium and exposed to heat stress (Gp. 5) showed focal vacuolar and hydropic degeneration of some renal tubules (Figure 2B). The kidneys of broilers which were supplied with selenium and exposed to heat stress (Gp. 6) revealed interstitial hemorrhages, cloudy swelling of the renal tubules, edema, coagulative necrosis, and glomerular collapse (Figure 2C). The heart of broilers in Gps. 4 and 6, which were exposed to heat stress revealed heart enlargement and severely congested blood vessels (Figure 3A.) Microscopically, the heart showed massive myofibrillar degenerations (Figure 3B.). Vacuolation of myofibers and diffuse edema were recognized. The heart of broilers in Gp. 5 which was supplied with nanoselenium and exposed to heat stress showed focal leukocytic infiltration, and mild congestion (Figure 3C) compared with the heart tissue of Gp. 6 showed mild edema, mild myocardial degeneration, and mild congestion (Figure 3D). The brain of broilers in Gp. 4 showed congested meningeal blood vessels macroscopically. Microscopically, Gp. 2 showed congested blood vessels, neuronal degeneration represented by vacuolation of the cytoplasm, and shrunken deeply stained nuclei. Focal liquefactive necrosis of the cerebrum was observed. Submenengial leucocytic infiltrations (mainly lymphocytes) and areas of gliosis, and neuronophagia between the degenerated neurons were observed (Figure 4A and B.). The brain of broilers in Gp. 5 which was exposed to heat stress showed normal architecture. The brain of broilers in Gp. 6 which was supplied

Table 4. Effect of different selenium forms on biochemical and immunological parameters of treated chickens during the experimental period (n=5).

Item	Gp 1	Gp2	Gp3	Gp4	Gp5	Gp6
Total protein (g/dl)	4.67±0.20^{b}	5.27 ±0.19^{a}	5.62±0.25^{a}	2.64 ±0.09^{c}	4.33±0.23^{b}	4.24 ±0.12^{b}
Albumin (g/dl)	2.68±0.11^{ab}	2.83 ±0.17^{a}	3.05±0.33^{a}	1.72±0.08^{c}	2.31±0.05^{b}	2.36 ±0.09^{b}
globulin (g/dl)	1.98 ±0.13^{bc}	2.4 ±0.06^{b}	2.57±0.08^{a}	0.92±0.08^{d}	2 ±0.07^{bc}	1.88 ±0.06^{c}
ALT (U/I)	16.7±1.3^{b}	17.74 ±1.7^{b}	16.2±2.08^{b}	23.59±1.4^{a}	14.38 ±2.1^{b}	15.22 ±1.8^{b}
AST (U/I)	27.37±1.19^{b}	27.15±1.2^{b}	26.47±1.09^{b}	42.5±1.45^{a}	29.6±0.78^{b}	30.45±1.3^{b}
Creatinine (mg/dL)	0.42±0.02^{c}	0.40±0.01^{c}	0.38±0.03^{c}	0.64±0.02^{a}	0.50±0.015^{b}	0.51±0.02^{b}
Urea (mg/dl)	15.38±1.2^{b}	15.61±1.9^{b}	14.67±1.9^{b}	28.53±1.1^{a}	16.64±1.7^{b}	17.3±1.3^{b}
MDA (nmol/ml)	3.6±0.49^{b}	2.11±0.37^{c}	2.34±0.35^{c}	5.83±0.58^{a}	2.7±0.4^{bc}	3.53±0.30^{b}
SOD (U/ml)	93.18±7.8^{c}	212.6±25.34^{a}	165.9±16.4^{ab}	90.07±16.41^{c}	192±9.93^{a}	142±8.84^{b}
LZM (ng/ml)	2.19±0.40^{bc}	4.95±0.55^{a}	2.54±0.41^{b}	1.16±0.18^{d}	4.25±0.54^{a}	1.74±0.18^{c}
IgG (ng/ml)	260±26.82^{cd}	537±65.87^{a}	362.0 ± 46.93^{bc}	156±18.93^{d}	458±39.81^{ab}	369±31.46^{bc}
IgM (ng/ml)	259±28.2^{cd}	565±51.18^{a}	441.0±44.45^{b}	164.0±21.05^{d}	442 ± 42.36^{b}	342 ±41.17^{bc}
C3 (mg/dl)	31.35±4.68^{b}	54.84±7.8^{a}	36.67±3.31^{b}	27.75±3.48^{b}	62.41±7.72^{a}	45.31± 6.52^{ab}

Means with different letters at the same row are significantly different (P <0.05) ALT: alanine aminotransferase; AST: aspartate aminotransferase MDA: malondialdehyde, SOD: superoxide dismutase; LZM: lysosme; IgG: Immunoglobulin G; IgM: immunoglobulin M; C3: complement 3

with selenium and exposed to heat stress showed congested blood vessels and submenengial leucocytic infiltration with few focal encephalomalacias represented by shrunken deeply stained nuclei surrounded by gliosis (Figure 4C).

DISCUSSION

In many parts of the world, high and low ambient temperatures are significant stressors that have an impact on chicken growth performance, and considerable economic losses typically result from decreased output and increased mortality (Altan *et al*., 2000). Recently, there has been a lot of scientific interest in the application of chemical nanominerals, mostly because of the possible advantages for poultry. One of the nutrients that can be included in diets to maintain growth performance is selenium (Zhang *et al*., 2008). Due to its low toxicity, high bioavailability, potent adsorption capacity, and high catalytic effectiveness,

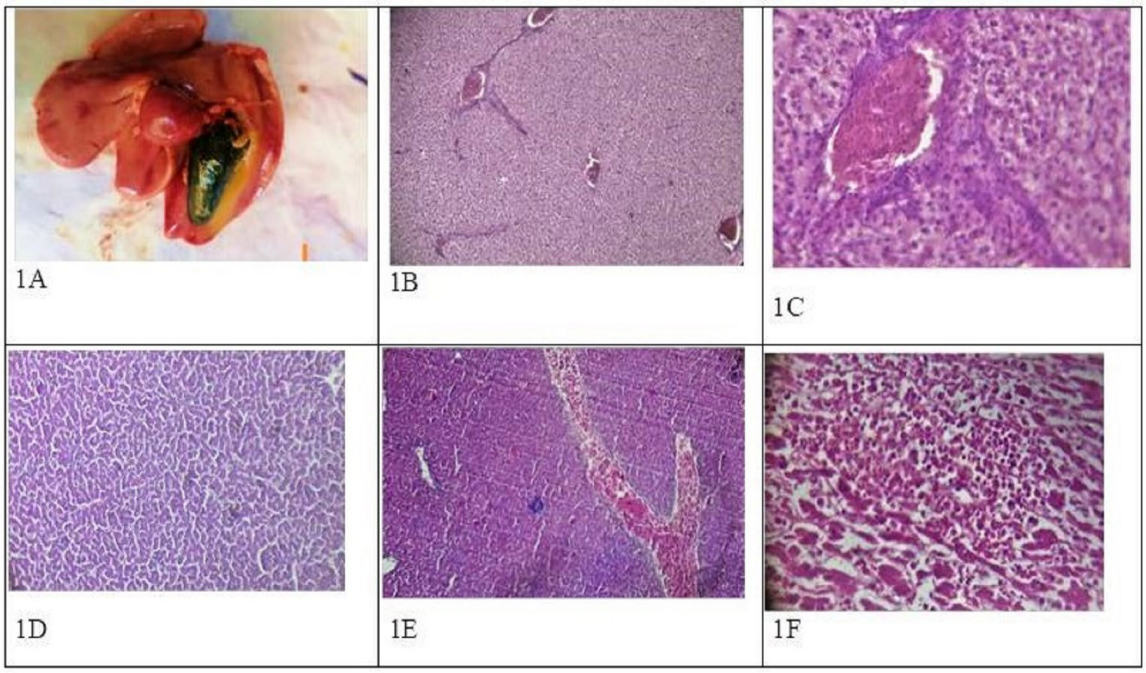

Fig. 1. 1A) Liver of broilers showing diffuse pale liver and distended gallbladders. 1B & 1C) The liver of broilers in Gp. 2 showing severe congestion of blood vessels and sinusoids, and fatty degeneration represented by vacuolation in the hepatic cytoplasm. 1D) Broilers in Gp. 5 showing mild leukocytic infiltration around the central vein with their hepatic cells. 1E & 1F) Broilers I Gp. 6 showing severe congestion of the central vein with fibrosis, focal coagulative necrosis of hepatic cells, and few leukocytic infiltrations of the hepatic parenchyma.

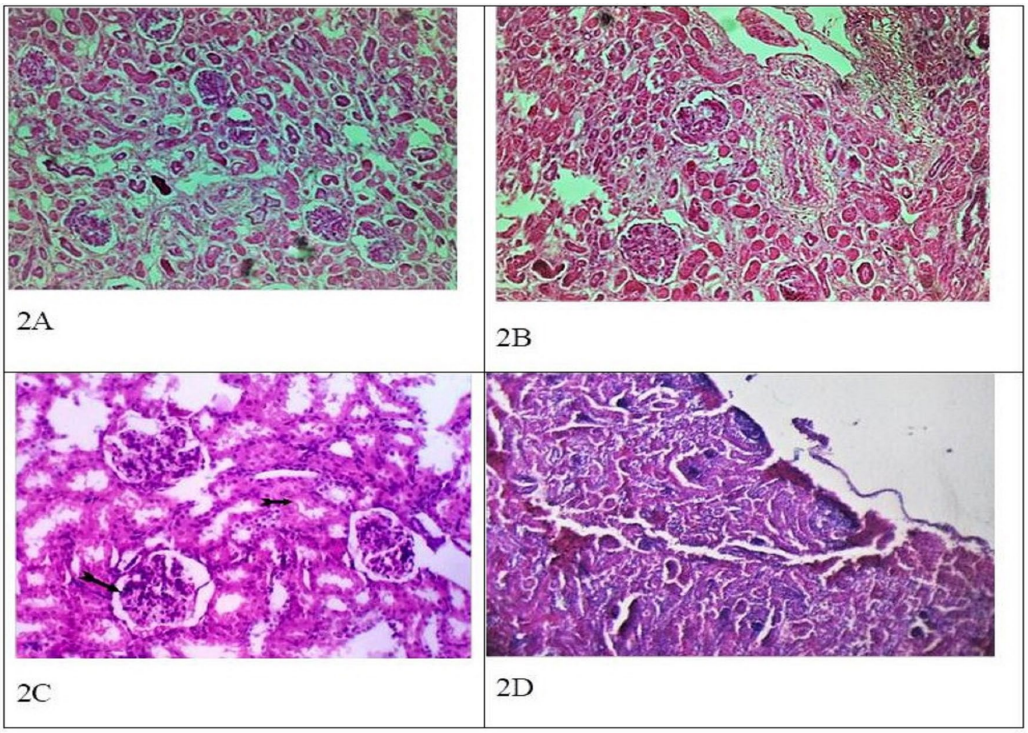

Fig. 2. 2A & 2B) Kidneys of broilers in Gp. 4, which are exposed to heat stress showing interstitial edema, coagulative necrosis of the renal tubules, hyaline casts, and damaged glomeruli. 2C) Kidneys of broilers supplied with nano selenium and exposed to heat stress (Gp. 5) showing focal vacuolar and hydropic degeneration of some renal tubules 2D) kidneys of broilers supplied with selenium and exposed to heat stress (Gp. 6) showing focal interstitial hemorrhage, cloudy swelling of the renal tubules, coagulative necrosis, and glomerular collapse.

nano-elemental selenium (Nano-Se) has garnered more interest than sodium selenite (SS). Examining the effects of dietary supplements with SS and nano-Se on growth performance was the goal of the current investigation. Broiler hens' body weight and gain significantly increased as compared to the control group as a result of the impacts of different dietary treatments (Nano SE) and (Na selenite) on overall growth performance. Our data demonstrated that the application of (Nano-Se) resulted in the maximum feed conversion rate among the groups, These results are in agreement with Hu *et al*. (2012) and Khazaraie and Ghazanfarpoor (2015) who illustrated that weight gain was significantly increased in quail chicks compared with control group and also agree with Salah and Ebeid (2019) who revealed that FCR were enhanced significantly in nano-Se supplemented groups in comparison with the control group. According to Zhang *et al*. (2008), this enhanced performance may be attributable to excellent bioavailability, increased solubility, high cellular uptake, and increased surface activity. This is in contrast to Yoon *et al*. (2007) and Niu *et al*. (2009) who claimed that selenium supplementation has no impact on the growth performance of chickens.

Similar to Ayo and Ogbuagu (2021) and Hassan and Asim (2020), who reported that heat stress impairs erythropoiesis and causes decreased RBCs count, reduced PCV and Hb conc. and negatively affects erythrocytic indices, the results of the present study showed a significant reduction in RBCs, Hb, and PCV levels. This could be because of stressed hens panting, which causes hyperventilation and a rise in blood oxygen tension, which in turn reduces erythropoietin production and, ultimately, reduces RBC production (Ranjan *et al*., 2019). Additionally, the results confirmed the findings of Özge *et al*. (2000) and Ayo and Ogbuagu (2021) by showing a large rise in WBCs count, heterophils and basophil percentages with a significantly lower lymphocyte and monocyte percentage. According to Attia *et al*. (2017), a rise in the total leukocytic count may be caused by an increase in the amount of corticosterone that is circulating, which is thought to be caused by the discharge of leucocytes from the marginated pool into the systemic circulation. Furthermore, heat stress reduces the number of lymphocytes in circulation while increasing the number of heterophils once they are released from the reserve as a result of inflammation, according to Wang *et al*. (2018). In contrast to Medhat *et al*. (2016), who found that adding sodium selenite (15.4 mg/ 10kg ration for 4 weeks) to the broiler diet increased RBC counts due to increased activity of the hematopoietic system, chicks of Gp. 3 showed no significant change in RBC

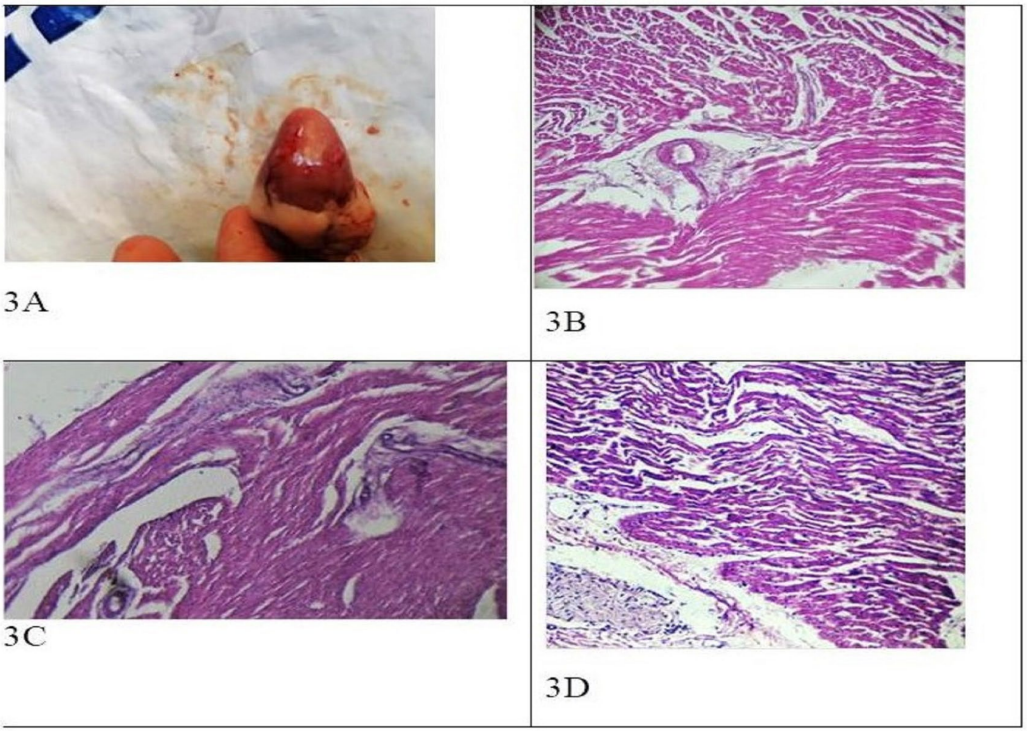

Fig. 3. 3A) Heart of broilers in Gp. 4 and Gp. 6, which was exposed to heat stress showing heart enlargement and severely congested blood vessels. 3B) Heart showing myofibrillar degeneration and diffuse edema. 3C) Heart of broilers in Gp. 5, which was supplied with nano selenium and exposed to heat stress showing focal leukocytic infiltration. 3D) Heart of broilers in Gp. 6 showing mild edema, and myocardial degeneration.

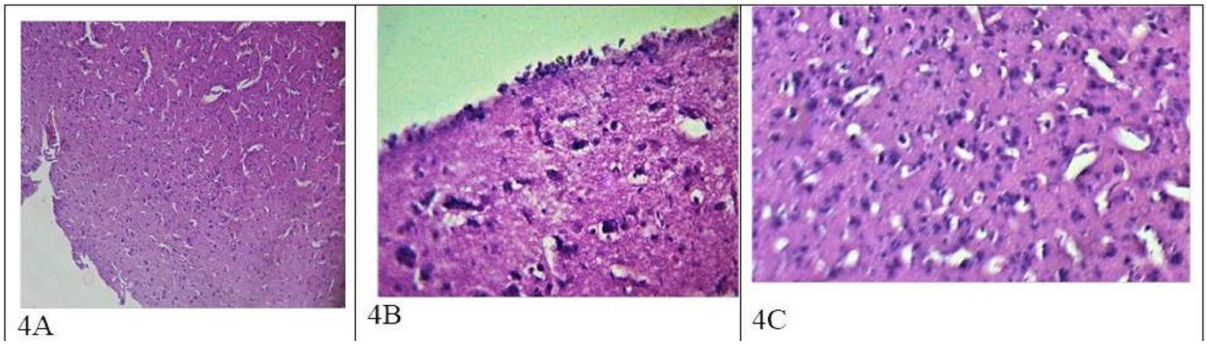

Fig. 4. 4A & 4B) The brain of broilers in Gp. 2 showing neuronal degeneration represented by vacuolation of the cytoplasm and shrunken deeply stained nuclei and submenengial leukocytic infiltrations (mainly lymphocytes) and areas of gliosis, and neuronophagia between the degenerated neurons. 4C) Brain of broilers in Gp. 6, which of broilers supplied with selenium and exposed to heat stress showing focal encephalomalacias represented by shrunken deeply stained nuclei surrounded by gliosis

counts, Hb concentrations, or PCV values, but their leukograms supported the idea that selenium is essential for boosting immune response as evidenced by the significant rise in WBCs, heterophils and monocyte with a decrease in lymphocyte. These results agreed with Shlig (2009); Medhat *et al*. (2016) and Ndubuisi *et al*. (2021). Chicks of Gps. 2 and 5, which supplemented with nano-Se manifested significant increase in RBCs counts, Hb conc. and PCV value. Along with Selim *et al*. (2015); Mohamed *et al*. (2016) and Mohapatra *et al*. (2014) showed that nano-Se appeared to be more successful in boosting several hematological parameters than that of inorganic sodium selenite at a level of 0.3 ppm. According to Ihsan and Qader (2012), nano-Se may be responsible for these outcomes by enhancing the activity of hemopoietic organs. According to Jamima *et al*. (2020), the antioxidant activity of nano-Se also reduces osmotic fragility and erythrocyte disintegration, inhibits oxidative damage to the red blood cell membrane, and prevents erythrocyte breakdown, all of which improve Hb, RBC, and PCV levels. However, in contrast, JunHyung *et al*. (2020), who recorded large increases in WBC count and heterophils and decreases in lymphocyte, found that nano-Se caused considerable increases in these parameters. Mohamed *et al*. (2016) reported significant increases in lymphocyte percentage and significant decreases in heterophil percentage in Sinai chicks supplemented with nano-Se in their diet, in contrast to Fuxiang *et al*. (2008) who mentioned that nano-Se supplementation significantly increased lymphocyte counts and significantly decreased heterophils counts and H/L ratio. The obtained results are supported by the findings of Mohamed *et al*. (2016) who demonstrated that treating chickens with nano-Se under heat stress improved their blood parameters more than supplementing them with (Na selenite). Our findings support those of Mohamed *et al*. (2016); Seliem (2011), and Khan *et al*. (2002) who found a discernible drop in plasma total protein, albumin, and globulin levels in response to heat stress. According to Malheiros *et al*. (2003), these results may be related to an increase in corticosterone that stimulated the gluconeogenesis process. In the current study, Na selenite and Nano-selenium treatment of heat-stressed chickens led to an increase in the levels of plasma total protein, albumin, and globulin compared with Gp. 4; these findings were supported by Ramezani *et al*. (2011). Additionally, Xiao *et al*. (2016) and Gulyas *et al*. (2016) recorded an increase in total protein in nano-selenium treated chickens. Imik *et al*. (2013) noted that heat exposure did not change the serum total protein, albumin, and globulin concentrations in broilers. Our findings were confirmed by Huang *et al*. (2018). In the current study, heat stressed chickens (Gp. 4) elicited a considerable increase in the levels of AST and ALT compared with (Gp. 1). The increase in the levels of the enzymes may be caused by heat damage to the liver, heart, or muscle cells, which changed their permeability and interfered with normal liver functions (Ye *et al*., 2015). However, treatment with Na selenite and Nano-selenium prior to heat stress had no effect on the plasma AST and ALT activity, which was confirmed by Selim *et al*. (2015), while Gps. 5 and 6 elicited a significant decrease in AST, ALT levels compared with Gp. 4. Our findings are consistent with those of Safdari-Rostamabad *et al*. (2017) and Amin *et al*. (2016), who found that Nano-Se supplementation increased liver function and antioxidant enzyme activity while also restoring cellular structure, protecting the rat liver from acetaminophen toxicity. In the current investigation, there were no differences in serum urea and creatinine levels between Gp 2 and 3, and Gp. 1, and our findings were supported by Ibrahim *et al*. (2022). Heat stressed chickens Gp. 4, on the other hand, had higher levels of urea and creatinine than Gp. 1, these findings agreed with Huang *et al*. (2018), who observed that renal parameters were altered during acute heat stress, resulting to acid-base imbalance. The obtained findings agree with Saleh (2014) and Abdel-Wareth *et al*. (2019), who reported that rabbits fed with nano-Se had a significantly lower urea level compared to the control positive group. Treated groups (Gps. 5 and 6) showed a significant decrease in the levels of urea and creatinine compared with Gp. 4. All supplemented groups had significantly lower levels of MDA and higher levels of SOD, which may be related to the actions of NS and S-selenite on antioxidant enzymes that are in charge of removing reactive oxygen species. This is consistent with the findings of (Boostani *et al*., 2015), who claimed that selenium is actively involved in the antioxidant defence mechanisms since it is a necessary component of the enzyme selenium-dependent glutathione peroxidase, which lowers peroxide and safeguards cells. Nano-minerals, according to Yaghmaie *et al*. (2017) and Michalak *et al*. (2022), can also lessen oxidative stress and enhance serum oxidant status by boosting the activity of antioxidant enzymes like SOD. While MDA serves as an indicator of antioxidant status and is a byproduct of oxidative stress, the increase in MDA in Gp. 4 may be caused by the impact of heat stress. This is in agreement with Hassan *et al*. (2020) and Safdari-Rostamabad *et al*. (2017). In NS supplemented groups, lysosome activity, IgG, IgM, and C3 are all much higher. This might be brought on by NS's immunostimulant properties and increased lysosomal activity. Heterophils and lymphocytes—two types of phagocytic cells—may proliferate in conjunction with an increase in C3. The results were consistent with other reports (El-Deep *et al*. 2016; Gangadoo *et al*. 2020; Nabi *et al*. 2020; Michalak *et al*., 2022). As they clarified, adding NS to poultry food boosts fowl's immune capabilities. Keeping NS in the feed of grill chickens at a high ambient temperature reduced its harmful effects. Supplemental NS had a favorable effect on the blood levels of immunoglobulin G and immunoglobulin M in broilers, according to Hassan *et al*. (2020). According to Xiao *et al*. (2016) and Gulyas *et al*. (2016), nano-selenium enhances protein synthesis and folding by raising levels of the eukaryotic translation initiation factor 5A-1, which is one of the mechanisms by which the elevated levels of immunoglobulins are explained. The amounts of IgG and IgM in the serum increased because this substance is a crucial component of the protein production process.

Complex measures to lower body temperature, such as raising heart rate and blood supply to the muscles, brain, and kidneys, are part of the tissue's reaction to heat stress. Vital organs may become significantly swollen as a result (Deaton *et al*., 1996; Yahav *et al*., 1997). The current investigation covered the relationship between microscopic and gross lesions. According to Aengwanichand Suchint (2005), the liver, heart, and kidney vasculatures are markedly engorged and edematous. In accordance with the theory that increased body temperature under heat stress caused endothelial cell damage, and high blood pressure may result in an autonomic response that may cause hemorrhage, our study demonstrated evidence of generalized congestion and hemorrhages of the liver, kidneys, and heart (Aengwanich and Suchint, 2005). Due to a lack of oxygen, liver tissue that has undergone fatty degeneration appears pale, swollen, and necrotic, especially in the centrilobular regions (Cheville, 1999). The localized renal failure, cardiac degeneration, and centrilobular hepatic necrosis of broilers at high environmental temperature leading to hypoxia and ischemia of important organs were discussed by Nakamura *et al*. (1999) and Aengwanich and Suchint (2005). In the present study, neuronal degeneration, vacuolation of the cytoplasm, shrunken profoundly discolored nuclei, and focal liquifactive necrosis of the cerebrum were identified as the effects of ischemia on the brain. The pathological evidence of heatstroke-related cell body shrinkage, nucleus pyknosis, loss of Nissl substance, and ultimately nuclei disappearance was discussed by Lee *et al*. (2013). They claim that this is brought on by an increase in cerebral blood flow. Therefore, hypoxia leads to increased mitochondrial ROS (reaction oxygen species) generation, which triggers apoptosis and activates cellular oxidase and nitric oxide synthesis. Continuously stressful conditions on hypoxic cells caused oxidases to be exported into the extracellular environment, resulting in multifocal cellular damage and leukocytic infiltrations. When compared to broiler chickens treated with selenium, the pathological alterations in the liver, kidneys, and heart of those animals showed little variation in tissue architecture. These findings are consistent with those of Alkhudhayri *et al*. (2018) and Hassan *et al*. (2020), who found that photomicrographs of the heart, liver, spleen, and

bursa of Fabritius in broiler breeds supplemented with nano selenium in drinking water showed a significant improvement to the suffering from environmental challenges and infections compared to those broilers supplemented with selenium supplementation. According to Mousa and Ali (2018), who examined the impact of nano-boron on the liver and kidneys of chicks following an E. coli infection, the ability of nanoparticles to lower heat stress and boost avian immunity is one of their findings.

CONCLUSION

Sodium selenite and nano-selenium are suitable feed additives for controlling adverse effects of heat stress in broilers. However, these beneficial effects were more visible in nano-selenium groups as it enhances the bird's immune status and stimulate ant oxidative status of birds.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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