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Selenium and nano-selenium ameliorations in two breeds of broiler chickens exposed to heat stress

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Abstract

The objective of this study was to compare the effects of synthesized nano-selenium (NS) and commercial inorganic selenium (Se) on immunity, behaviour, and performance of Arbor (AB) and Ross (RB) broilers that were exposed to heat stress of 40 °C for 6 - 8 hours daily over 38 days. Two hundred and ten one-day-old broilers of two breeds were supplemented with 0.5 mL/L of NS or Se in their drinking water. Two hundred sera, 200 intestinal swabs, and 1000 internal organ and tissue samples were collected. Weight gain, performance index, behavioral indices, total antioxidant capacity, malondialdehyde, superoxide dismutase, immunoglobulin G, immunoglobulin M, serum total protein, albumin, alanine aminotransferase, aspartate aminotransferase, and serum creatinine concentrations increased (P <0.01) in RB compared with AB when supplemented with NS. Meanwhile, NS supplementation decreased (P <0.01) water intake and the logarithmic bacterial counts of the intestine and breast in RB and AB, respectively. Histopathology revealed mild leukocytic infiltration and mild vacuolar degeneration in hepatocytes, and focal leukocytic infiltration, mild congestion, and cytoplasmic vacuolation in the myocardium of RB. Photomicrographs showed a mild lymphoid depletion in the spleen, while histopathology of the bursa of Fabricius revealed a normal follicular epithelium and normal lymphoid follicles with mild inter-follicular fibrosis in RB that were supplied with NS as opposed to AB, which expressed more severe pathological affections from heat stress. Thus, NS was more effective than Se in allowing broilers to respond to heat stress.

Keywords: behaviour, immunity, growth traits, tissue architecture
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Introduction

The poultry industry has expanded worldwide owing to increased demands for chicken meat and eggs. Research, as a result of industry growth, has led to improved growth rate, feed efficiency, health status, and reduced carriage of pathogens. However, the research focus has been on enhanced productivity. Vitamin and mineral supplements are used to maintain rapid growth and improve the feed conversion ratio (FCR) and thus lower the amount of feed that is needed to attain market weight (Zhao *et al.*, 2017). Essential elements, which include iron, zinc, chromium, manganese, Se, and molybdenum, are vital to the health of poultry and play important roles in the function of co-enzymes (Peters *et al.*, 2016). Selenium can be supplemented in poultry rations in organic forms (selenomethionine), which have been found to be more suitable than inorganic forms (selenite), which are less efficient (Bolea-Fernandez *et al.*, 2017). Optimum Se concentrations in the ration are essential to good performance and preservation of meat quality during storage (Markovic *et al.*, 2018). Selenium-yeast supplementation of laying Japanese quail also improved the Haugh unit and significantly influenced eggshell and egg quality characteristics (Baylan *et al.*, 2015).

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Nanoparticles are highly absorbable and can augment vaccines and nutrient supplements because of their large surface area to volume ratio and minimal energy loss. Nanoparticles can carry compounds to target organs or systems directly, while avoiding the rapid degradation that has been observed with some antibiotics. Thus, they may provide health benefits (Gangadoo *et al.*, 2016). For example, NS has been considered a potential supplement for broilers as it possesses low toxicity, high catalytic efficiency, and antibacterial activity (Wadhwani *et al.*, 2016; Skalickova *et al.*, 2017).

Heat stress challenges the immune, hormonal, and metabolic systems in broilers (Lara & Rostagno, 2013). During heat stress, thyroid hormones and feed intake (FI) have been shown to be greatly reduced, with blood flowing preferentially to the skin in an attempt to dissipate heat (Gu *et al.*, 2012). Further, intestinal integrity is greatly reduced, allowing enteric pathogens to enter the systemic circulation and thus increase the opportunity for infection (Gu *et al.*, 2012). Under heat stress, broilers express higher levels of seleno-protein with a consequent increase in the requirement for Se. Further, Se can aid in maintaining efficient antioxidant defence mechanisms and minimize or prevent the fatal consequences of heat stress (Surai *et al.*, 2018).

The aim of this study was to investigate the influence of synthesized NS and inorganic Se, which were provided at rate of 0.5 ml of 100 mg/L per litre of drinking water on Ross and Arbor (Aviagen, Inc. Huntsville, Alabama, USA) broilers when they were exposed to heat stress at 40 °C for 6 - 8 hours daily for 38 days. The comprehensive characterization of these effects included monitoring growth, water intake, behaviour patterns, total bacterial and Enterobacteriaceae counts of the intestine and breast muscle, serum biochemical parameters, immunoglobulin concentrations (IgG and IgM), and histopathological characteristics of heart, liver, spleen, and bursa.

Material and Methods

The protocols that were used in this study were approved by the Scientific Research Ethics Committee, Faculty of Veterinary Medicine, Suez Canal University, Egypt (approval number 2018062).

Two hundred and ten one-day-old Arbor and Ross chicks were purchased from the hatchery at Ismailia, Egypt. Birds were housed in a deep litter system (hay). Litter was treated with superphosphate (0.5 g/m^{-2}) to minimize ammonia evaporation and microbial survival according to Soliman *et al.* (2018). Birds were divided into three groups per breed with each group consisting of 35 chicks. The groups were further divided into five replicates of seven birds, and housed in separate isolated rooms with low thermal conduction (K value) and thermal transmission (U value).

Birds were initially brooded at 35 °C with a 0.5 °C daily decrease in temperature until 21 - 25 °C was achieved by the third week. Natural ventilation aids were available in the rooms as V-shaped windows using natural convection. Continuous lightning (23 hours light and 1 hour darkness) was provided with white LED lights as recommended by Soliman and Hassan (2019). The broilers were given ad libitum access to water and provided with a nutritionally balanced standard soybean diet (NRC, 1994). The rations consisted of about 23% protein and 3000 kcal/kg energy in the starter ration, which was provided in the first fourteen days of life, and 21% protein and 3100 kcal/kg in the grower ration, which was provided for the remainder of the experiment (24 days). The experiment was designed to last for 38 days. Mortality of the birds, temperature and relative humidity were monitored and recorded daily. Birds received attenuated live infectious bronchitis virus vaccine (IB-H120 ≥ $10^{3.5}$ EID_{50} / dose) on day 6, attenuated live Infectious bursal disease virus vaccine (VMG91 ≥ $10^{3.0}$ $TCID_{50}$) on days 13 and 21 and lentogenic Newcastle disease virus vaccine (Lasota ≥ $10^{6.0}$ EID_{50}) on days 18 and 26 in de-chlorinated drinking water.

Sodium sulphate and Se powder were refluxed and heated at 70 °C for 6 hours according to Gorer and Hodes (1994) to produce a sodium seleno-sulphate solution. Filtration of the solution followed by adding glucose 6% powder as stabilizing and reducing agent and polyvinyl alcohol to prevent deviations in morphological characters of NS, and protection from aggregation. The solution was refluxed for an additional six hours to develop a pale-yellow colour, which indicated a final stable product. The synthesized NS was examined and identified using transmission electron microscopy and a UV-visible double beam spectrophotometer. Synthesized NS and commercial Se (100 mg/L) were provided to the groups of broilers in their drinking water. The groups of Arbor broilers were designated G1, G2, and G3 and the groups of Ross broilers were designated G4, G5, and G6. Treatments were assigned to the groups as follows: G1 and G4 were supplemented with NS, G2, and G5 were supplemented with Se, and G3 and G6 were controls. Four of the six groups (G1, G2, G4, and G5) were subjected to heat stress at 40 °C for 6 - 8 hours daily using heaters. Behaviour and water intake were monitored during periods of exposure.

A total of 1400 samples, which included 200 sera, 200 intestinal swabs, and 1000 organs and tissue samples, which included liver, spleen, bursa of Fabricius, heart, and breast muscles, were collected at the end of the study. Blood samples were collected and held at 37 °C for 30 min, then centrifuged at 4000 rpm for 20 min. Sera samples were stored at -20 °C for subsequent biochemical, immunological, and antioxidant

assays (Soliman *et al.*, 2017). The liver, spleen, bursa, and heart were kept in formalin for histopathological examination.

Thirty-two birds from each group were weighed at weekly intervals. The number of birds to be weighed (n) was calculated using a simple random sampling design by Solvin's formula:

$$n = N/(1 + Ne^2)$$

given an acceptable error rate (e) of 5% and a total population size of N.

Feed intake and water intake for each bird were calculated by dividing the total amount consumed in each group by the number of birds in this group. Bodyweight gain (BWG), FCR, and the performance index (PI) were calculated according to Soliman and Hassan (2017).

The behaviour of the broilers was evaluated for three hours a day at 9h00, 14h00, and 21h00 during the first, third, and fifth weeks using Panasonic WV Ns202ae video camera (Panasonic India Pvt. Ltd., Haryana, India) suspended 1.5 m above the birds' heads (Li *et al.*, 2015). The duration and frequency of the performed behaviours in each group were recorded every five minutes per hour using focal sampling method when the videotapes were replayed (Villagra *et al.*, 2014).

Tonic immobility (TI) was induced by manual restraint. Each bird was laid on its back in a U-shaped cradle and held motionless by gently pressing its breast for 15 sec. The observer remained still, quiet, and out of sight until birds righted themselves again, and the duration of TI was recorded. If TI was not obtained after three attempts, an observation of 0 seconds was recorded. An observation of 600 seconds was recorded for a bird that remained immobile after 10 min. The protocol was repeated at the first, third, and fifth weeks in ten individually identified broilers from each of the six groups (Sinkalu *et al.*, 2016).

Total protein (TP) (g/dL), albumin (ALB) (g/dL), alanine aminotransferase (ALT) (IU/L), aspartate aminotransferase (AST) (IU/L), urea (mg/dL), and creatinine (Creat) (mg/dL), and the antioxidant markers total antioxidant capacity (TAC) (mM/L), malondialdehyde (MDA) (nmol/mL), and superoxide dismutase (SOD) (U/mL) were measured in serum using Roche Integra 400 Plus chemical analyser (Roche Diagnostics Middle East, Dubai, United Arab Emirates). Serum immunoglobulins (IgG and IgM) (mg/dL) were measured using Roche Elecsys 1010 immunoassay analyser (Roche Diagnostics Middle East, Dubai, United Arab Emirates) as described by Wu *et al.* (2017).

Intestinal swabs and breast muscle samples were prepared as recommended by APHA (2012). Tenfold serial dilutions up to 10^{-8} were prepared. Bacterial counts were performed using a drop plate technique as recommended by Kim and Lee (2016) and Soliman *et al.* (2016). Standard plate count and eosin methylene blue agars were used for the total bacterial count (TBC) and total *Enterobacteriaceae* count (TEC), respectively, at 37 °C for 24 - 48 hours. Plates were counted with a dark-field colony counter (Murray *et al.*, 2015).

All birds were slaughtered at the end of the experiment and tissue samples from the liver, heart, spleen, and bursa of Fabricius were harvested. The samples were fixed in 10% buffered formalin. The specimens were cut into 5-mm thickness, put into tissue cassettes, dehydrated manually by transferring through a series of alcohols with different concentrations, cleared in two changes of xylene, embedded in paraffin wax, cut into 4 µm thick sections, and stained with haematoxylin and eosin (Bancroft, 1990). The histological sections of the examined organs were visualized using a light microscope under (x10) and (x20) magnification and photographed using an Olympus DP-73 microscope digital camera (Olympus MEA FZ-LLC, Dubai, United Arab Emirates).

Statistical analysis was carried out using SAS-STAT version 9.4 for Windows (SAS Institute, Inc., Cary North Carolina, USA). Analysis of variance was used to detect treatment effects with a three-way factorial model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + t_k + (\alpha t)_{ik} + (\beta t)_{jk} + (\alpha\beta t)_{ijk} + e_{ijkl}$$

where: y_{ijkl} was a measurement from the lth a bird of the kth age, and ith breed that was subjected to the jth treatment, µ was overall mean;
α_i was the fixed effect of breed;
β_j was the fixed effect of supplement treatment;
$(\alpha\beta)_{ij}$ was the interaction effect of broiler's breed by supplement treatment;
$(\alpha t)_{ik}$ was the interaction effect of broiler's breed by its age;
$(\beta t)_{jk}$ was the interaction effect of treatment with age;
$(\alpha\beta t)_{ijk}$ was the corresponding three-way interaction; and
e_{ijkl} was random error.

For those dependent variables that were measured only once, the main effect of age and interactions involving age were omitted from the analysis. Total bacterial count and TEC were log-transformed prior to analysis. Pearson's correlation was calculated to reveal the association between immunoglobulin G and M concentrations with TBC and TEC of intestinal swabs and breast muscles, and with antioxidant activity. The differences were considered highly significant at (P <0.01), significant at (P ≤0.05), and non-significant at (P >0.05).

Results and Discussion

On average, the Ross broilers that were supplemented with Se (G4 and G5) gained more weight than any of the other groups, which were similar (Table 1). Feed intake differed among all of the groups. However, those broilers in G5 and G6 consumed substantially more feed than any other groups and thus had higher FCRs. The Ross broilers in G5 and G6 had reduced performance indices relative to their counterpart Arbor broilers. However, when the broilers were supplemented with NS, Ross broilers had a greater performance index than Arbor broilers. Water intake followed a pattern that was similar to the performance index, with Ross broilers in G5 and G6 consuming more water than their counterpart Arbor broilers, and with the NS supplemented Ross broilers consuming less water than the Arbor broilers.

Table 1 Means (± SE) for breed by treatment interaction effects on growth, feed and water intake, and calculate indices for broilers that were supplemented with selenium or nano-selenium under the influence of heat stress

Breed x treatment	*Weight gain, g*	*Feed intake, g*	*Feed conversion ratio*	*Performance index*	*Water intake, ml*
Arbor*NS	$387.4^{ab} \pm 9.7$	$580.9^{c} \pm 0.0$	$1.48^{b} \pm 0.06$	$6.3^{b} \pm 0.19$	$151.2^{c} \pm 6.7$
Arbor*Se	$386.3^{ab} \pm 9.1$	$519.6^{f} \pm 0.0$	$1.43^{b} \pm 0.05$	$7.0^{a} \pm 0.11$	$174.2^{b} \pm 6.1$
Arbor*control	$368.3^{b} \pm 8.8$	$557.0^{d} \pm 0.0$	$1.54^{b} \pm 0.06$	$6.1^{b} \pm 0.18$	$181.1^{b} \pm 6.7$
Ross*NS	$412.8^{a} \pm 9.1$	$543.0^{e} \pm 0.0$	$1.40^{b} \pm 0.06$	$7.5^{a} \pm 0.19$	$138.5^{d} \pm 6.5$
Ross*Se	$407.5^{a} \pm 8.1$	$664.3^{a} \pm 0.0$	$1.74^{a} \pm 0.05$	$6.1^{b} \pm 0.12$	$212.8^{a} \pm 6.7$
Ross*control	$364.2^{b} \pm 8.5$	$643.2^{b} \pm 0.0$	$1.76^{a} \pm 0.05$	$5.1^{c} \pm 0.11$	$198.1^{a} \pm 5.2$
P-value	<0.01	<0.01	<0.01	<0.01	<0.01

^{a,b,c,d} Within each column, means that lack a common superscript are deemed different at P ≤0.05
NS: nano-selenium supplement; Se: inorganic selenium supplement; control: unsupplemented

However, the three-way interaction of breed, supplement treatment, and age was highly significant for WG, FI, FCR, PI, and water intake. Thus, differences in the time trends observed for these measures of performance may be important for a comprehensive interpretation of the data. In general, the broilers all gained weight throughout the experiment (Table 2), with G3 being an aberration in week 4. Because the birds grew over time, feed intake increased with the age of the birds. However, the feed intake of G1, G3, and G6 plateaued or decreased after week 4. For all groups, the performance index increased over time. While weekly fluctuations were noted for the groups at week 5, all of the groups were similar. Water intake also increased as the birds grew over time. However, the magnitude of the increase between weeks 4 and 5 was far less for G4 than for any of the other groups. The overall means for WG and the performance index revealed the greater (P <0.01) of Ross broilers (395.2 g and 6.2, respectively) when compared with Arbor broilers (380.6 g and 6.1, respectively).

Table 2 Weekly means (± SE) for the joint effects of breed and treatment growth, feed and water intake, and calculated indices for broilers that were supplemented with selenium or nano-selenium and under the influence of heat stress

Breed x treatment	*Week*	*Weight gain, g*	*Feed intake, g*	*Feed conversion ratio*	*Performance index*	*Water intake, ml*
Arbor*NS	1	83.1^{d} ± 5.8	109.2^{d} ± 0.0	1.3^{c} ± 0.10	0.9^{e} ± 0.01	50.4^{e} ±11.0
	2	262.5^{c} ± 9.0	331.2^{c} ± 0.0	1.2^{c} ± 0.04	3.0^{d} ± 0.16	95.4^{d} ± 3.7
	3	476.1^{b} ± 23.3	687.0^{b} ± 0.0	1.4^{b} ± 0.08	6.0^{c} ± 0.44	153.3^{c} ±13.0
	4	494.9^{b} ± 31.9	888.6^{a} ± 0.0	1.8^{a} ± 0.12	7.6^{b} ± 0.53	191.8^{b} ± 8.0
	5	620.4^{a} ± 18.4	888.6^{a} ± 0.0	1.4^{b} ± 0.04	13.8^{a} ± 0.46	265.3^{a} ±13.2
Arbor*Se	1	83.8^{c} ± 5.0	108.7^{e} ± 0.0	1.3^{c} ± 0.07	0.9^{e} ± 0.02	47.1^{e} ± 9.7
	2	216.9^{b} ± 23.1	306.5^{d} ± 0.0	1.7^{a} ± 0.36	2.5^{d} ± 0.36	96.1^{d} ± 7.0
	3	549.1^{a} ± 26.1	663.0^{c} ± 0.0	1.2^{c} ± 0.05	7.3^{c} ± 0.43	166.2^{c} ±15.8
	4	542.3^{a} ± 16.4	739.2^{b} ± 0.0	1.3^{c} ± 0.04	10.5^{b} ± 0.37	237.6^{b} ±10.8
	5	539.6^{a} ± 34.9	780.3^{a} ± 0.0	1.5^{b} ± 0.11	13.7^{a} ± 1.10	324·0^{a} ±18.8
Arbor*control	1	91.3^{e} ± 3.4	121.5^{e} ± 0.0	1.3^{b} ± 0.04	1.0^{d} ± 0.06	46.6^{e} ± 9.1
	2	251.2^{d} ± 10.5	367.6^{d} ± 0.0	1.4^{b} ± 0.05	2.6^{c} ± 0.17	97.6^{d} ± 4.1
	3	517.9^{b} ± 22.1	687.0^{c} ± 0.0	1.3^{b} ± 0.05	6.8^{b} ± 0.43	178.7^{c} ±17.8
	4	391.6^{c} ± 26.7	817.6^{a} ± 0.0	2.1^{a} ± 0.14	6.2^{b} ± 0.46	220.7^{b} ±12.5
	5	589.5^{a} ± 23.2	791.5^{b} ± 0.0	1.3^{b} ± 0.05	14.0^{a} ± 0.66	361.7^{a} ±38.9
Ross*NS	1	58.3^{d} ± 4.8	91.7^{e} ± 0.0	1.6^{a} ± 0.15	0.7^{e} ± 0.08	44.0^{e} ± 8.9
	2	225.4^{c} ± 15.3	294.4^{d} ± 0.0	1.3^{b} ± 0.10	2.6^{d} ± 0.26	102.6^{d} ± 4.0
	3	585.4^{b} ± 12.6	669.7^{c} ± 0.0	1.1^{c} ± 0.02	8.0^{c} ± 0.24	146.8^{c} ±18.6
	4	583.5^{b} ± 16.8	796.6^{b} ± 0.0	1.3^{b} ± 0.03	11.0^{b} ± 0.41	186.8^{b} ±11.8
	5	611.5^{a} ± 23.8	862.5^{a} ± 0.0	1.4^{b} ± 0.05	15.0^{a} ± 0.71	212.5^{a} ±15.4
Ross*Se	1	73.9^{d} ± 10.0	127.4^{e} ± 0.0	2.2^{a} ± 0.43	0.8^{e} ± 0.05	52.1^{e} ±11.5
	2	324.0^{c} ± 13.4	400.2^{d} ± 0.0	1.2^{d} ± 0.05	3.6^{d} ± 0.23	113.2^{d} ± 7.9
	3	495.9^{b} ± 21.9	813.6^{c} ± 0.0	1.6^{c} ± 0.08	5.7^{c} ± 0.31	194.9^{c} ±23.6
	4	478.9^{b} ± 12.0	958.3^{b} ± 0.0	2.0^{b} ± 0.05	7.0^{b} ± 0.22	291.2^{b} ±16.4
	5	664.9^{a} ± 33.3	1022.2^{a} ± 0.0	1.5^{c} ± 0.08	13.6^{a} ± 0.83	412.6^{a} ±20.5
Ross*control	1	88.6^{d} ± 5.9	137.2^{e} ± 0.0	1.6^{b} ± 0.10	0.8^{e} ± 0.09	52.6^{e} ± 9.7
	2	261.9^{c} ± 11.4	367.3^{d} ± 0.0	1.4^{c} ± 0.05	2.8^{d} ± 0.17	114.7^{d} ± 8.9
	3	471.8^{b} ± 32.4	789.8^{c} ± 0.0	1.7^{b} ± 0.16	5.2^{c} ± 0.50	202.8^{c} ±18.1
	4	476.0^{b} ± 25.9	985.3^{a} ± 0.0	2.0^{a} ± 0.12	6.6^{b} ± 0.39	257.1^{b} ±10.7
	5	523.0^{a} ± 29.2	963.4^{b} ± 0.0	1.9^{a} ± 0.14	10.1^{a} ± 0.62	363.4^{a} ±19.3
P-value		<0.01	<0.01	<0.01	<0.01	<0.01

^{a,b,c,d,e} Within each column, means lacking a common superscript are deemed different at $P \leq 0.05$
NS: nano-selenium supplement; Se: inorganic selenium supplement; control: unsupplemented

Total protein was reduced (P <0.01) in broilers of both breeds when they received NS (G1 and G4) relative to those birds that were supplemented with Se or in the unsupplemented control groups (Table 3). Within breed, the group that was supplemented with Se was not detectibly different from the unsupplemented control. In the Ross broilers, a similar pattern was observed for AST. However, in the Arbor broilers, the pattern of responses in AST was reversed with the NS-supplemented broilers exhibiting a higher level than G2 and G3, which were again similar. The level of ALB was also reduced (P <0.01) in broilers that were supplemented with NS compared with those that were either supplemented with Se or were not supplemented. However, the level of ALB in birds that were supplemented with Se was lower (P <0.05) than those that were unsupplemented. There was a highly significant reduction (P <0.01) of ALT by broilers in G4

compared with G5 and G6, with no significant difference between G5 and G6. In the Arbor broilers, there was a highly significant reduction (P <0.01) in G1 and G2 compared with G3, with the contrast of G1 and G2 being non-significant. Urea was reduced (P <0.01) in G4 compared with G5 and G6, and in G1 compared with G2, but not compared with G3. The two breeds had similar treatment means for creatinine, with supplemental selenium elevating the levels (P <0.01), but less so in G1 and G4 than in G2 and G5.

Table 3 Biochemical profiles (mean ± SE) of Arbor and Ross broilers supplemented with selenium and nano-selenium under the influence of heat stress

*Breed*treatment*	*TP, g.dL^{-1}*	*ALB, g.dL^{-1}*	*ALT, IU.L^{-1}*	*AST, IU.L^{-1}*	*Urea, mg.dL^{-1}*	*CRT, mg.dL^{-1}*
Arbor*NS	$10.1^{b} \pm 0.40$	$1.0^{f} \pm 0.08$	$1.2^{b} \pm 0.18$	$43.6^{a} \pm 1.7$	$43.7^{b} \pm 0.84$	$2.2^{b} \pm 0.14$
Arbor*Se	$10.3^{b} \pm 0.42$	$1.3^{e} \pm 0.09$	$2.3^{b} \pm 0.19$	$39.7^{b} \pm 1.8$	$48.3^{a} \pm 0.88$	$3.5^{a} \pm 0.15$
Arbor*control	$11.8^{a} \pm 0.45$	$3.3^{a} \pm 0.10$	$4.6^{a} \pm 0.20$	$38.4^{b} \pm 2.0$	$42.2^{b} \pm 0.95$	$0.9^{c} \pm 0.16$
Ross*NS	$10.6^{b} \pm 0.44$	$1.5^{d} \pm 0.09$	$2.6^{b} \pm 0.19$	$31.6^{c} \pm 1.9$	$28.5^{d} \pm 0.89$	$2.3^{b} \pm 0.16$
Ross*Se	$11.8^{a} \pm 0.42$	$2.2^{c} \pm 0.09$	$3.7^{a} \pm 0.20$	$47.1^{a} \pm 2.0$	$48.3^{a} \pm 0.93$	$3.7^{a} \pm 0.15$
Ross*control	$11.5^{a} \pm 0.44$	$2.9^{b} \pm 0.09$	$3.8^{a} \pm 0.20$	$44.3^{a} \pm 2.0$	$37.3^{c} \pm 0.93$	$0.6^{c} \pm 0.16$
P-value	0.010	0.000	0.000	0.000	0.000	0.000

^{a,b,c,d} Within each column, means that lack a common superscript are deemed different at P ≤ 0.05
NS: nano-selenium supplement; Se: inorganic selenium supplement; control: unsupplemented
TP: total protein, ALB: albumin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CRT: creatinine

A synchronized and highly significant increase (P <0.01) of immunoglobulin G, immunoglobulin M, total antioxidant capacity, malondialdehyde, and superoxide dismutase (Table 4) was observed in both breeds of broilers, with NS supplemented G1 and G4 having increased immunoglobulin concentrations and levels of antioxidant enzymes relative to the control groups (G3 and G6). Responses in immunoglobulin and antioxidant levels in G2 and G5 were intermediate between the NS supplemented groups (G1 and G4) and the control groups (G3 and G6).

Table 4 Immunoglobulin concentrations and levels of antioxidant enzymes in broilers supplemented with selenium and nano-selenium under the influence of heat stress

	Immunoglobulin		*Antioxidant enzymes*		
*Breed*treatment*	IgG, mg/dL	IgM, mg/dL	TAC, mM/L	MDA, nmol/mL	SOD, U/mL
Arbor*NS	$1691.9^{c} \pm 7.64$	$375.4^{c} \pm 1.97$	$2.0^{a} \pm 0.012$	$27.4^{b} \pm 0.22$	$273.8^{a} \pm 1.36$
Arbor*Se	$1674.0^{d} \pm 7.45$	$367.7^{d} \pm 1.92$	$1.3^{f} \pm 0.011$	$14.5^{d} \pm 0.21$	$255.3^{c} \pm 1.32$
Arbor*control	$1343.9^{f} \pm 8.07$	$270.4^{f} \pm 2.08$	$1.4^{e} \pm 0.012$	$7.4^{f} \pm 0.23$	$251.4^{c} \pm 1.43$
Ross*NS	$2003.4^{a} \pm 7.54$	$505.9^{a} \pm 1.94$	$1.9^{b} \pm 0.012$	$30.3^{a} \pm 0.21$	$275.1^{a} \pm 1.34$
Ross*Se	$1926.6^{b} \pm 7.90$	$482.8^{b} \pm 2.03$	$1.6^{c} \pm 0.012$	$17.5^{c} \pm 0.22$	$261.4^{b} \pm 1.40$
Ross*control	$1382.5^{e} \pm 7.90$	$305.5^{e} \pm 2.03$	$1.5^{d} \pm 0.012$	$8.2^{e} \pm 0.23$	$256.3^{c} \pm 1.40$
P-value	0.000	0.000	0.000	0.000	0.000

^{a,b,c,d,e,f} Within each column, means that lack a common superscript are deemed different at P ≤0.05
NS: nano-selenium supplement; Se: inorganic selenium supplement; control: unsupplemented
IgG: Immunoglobulin G, IgM: immunoglobulin M, TAC: total antioxidant capacity, MDA: malondialdehyde, SOD: superoxide dismutase

The total bacterial count of intestinal swabs taken from selenium-supplemented broilers increased (P <0.01) relative to G3 and G6 (Table 5). This increase also occurred for TEC from the intestinal swabs of G1 and G2 relative to G3 and for TBC from the breast muscles. However, G4, G5, and G6 were similar in TEC

from the intestinal swabs and TBC from the breast muscles. The TEC levels from breast muscle were similar for G1 and G3, and for G4 and G6, but elevated in G2 and G5 relative to the corresponding control groups.

Table 5 Logarithm bacterial load (mean ± SE) in the intestine and breast muscles of broiler breeds supplemented with selenium and nano-selenium under the influence of heat stress

	Intestinal swabs		*Breast muscles*	
*Breed*treatment*	TBC log CFU/mL	TEC log CFU/mL	TBC log CFU/mL	TEC log CFU/mL
Arbor*NS	$4.6^{b} \pm 0.06$	$3.2^{a} \pm 0.11$	$3.8^{a} \pm 0.02$	$1.5^{b} \pm 0.07$
Arbor*Se	$5.5^{a} \pm 0.05$	$3.5^{a} \pm 0.10$	$4.1^{a} \pm 0.03$	$2.4^{a} \pm 0.06$
Arbor*control	$3.4^{e} \pm 0.06$	$2.2^{b} \pm 0.11$	$3.0^{c} \pm 0.02$	$0.9^{b} \pm 0.07$
Ross*NS	$4.2^{d} \pm 0.05$	$1.9^{b} \pm 0.10$	$3.3^{b} \pm 0.02$	$0.7^{b} \pm 0.07$
Ross*Se	$4.5^{c} \pm 0.06$	$2.3^{b} \pm 0.11$	$3.4^{b} \pm 0.02$	$1.2^{b} \pm 0.07$
Ross*control	$3.4^{e} \pm 0.06$	$2.0^{b} \pm 0.11$	$3.1^{b} \pm 0.03$	$0.8^{b} \pm 0.07$
P value	0.000	0.000	0.000	0.000

^{a,b,c,d,e} Within each column, means lacking a common superscript are deemed different at $P \leq 0.05$
NS: nano-selenium supplement; Se: inorganic selenium supplement; control: unsupplemented TBC: total bacterial count, TEC: total enterobacteriaceae count, CFU: colony forming unit

Pearson correlation coefficients (Table 6) revealed relatively strong (P <0.01) and positive associations between immunoglobulin concentrations and antioxidant levels and among observed levels of the antioxidants. The correlations of the intestinal total bacterial count with the other counts of bacteria were also relatively strong (P <0.01), as was the correction of *Enterobacteriaceae* counts from the intestinal swabs and in breast muscle. The other correlations were substantially weaker. However, the positive correlations of intestinal bacteria count with immunoglobulin levels, and the correlations of superoxide dismutase with the bacterial counts (except in breast muscle) appear to be noteworthy.

Table 6 Correlation coefficients between immunoglobulin levels, traits indicative of bacterial load and antioxidant levels in heat-stressed broilers supplemented with selenium and selenium nanoparticles

Traits	*IgG*	*TBCI*	*TBCB*	*TECI*	*TECB*	*TAC*	*MDA*	*SOD*
IgM		0.257**	0.102	0.074	0.077	0.569**	0.804**	0.518**
TBCI	0.182*	**1**	0.663**	0.502**	0.686**	0.082	0.237*	0.344**
TBCB	0.002	0.663**	**1**	-0.162	0.118	0.018	0.128	-0.01
TECI	0.053	0.502*	-0.102	**1**	0.881**	0.093	0.135	0.374**
TECB	0.032	0.686**	0.118	0.881**	**1**	-0.092	0.030	0.293**
TAC	0.551**	0.082	0.018	0.093	-0.092	**1**	0.887**	0.674**
MDA	0.763**	0.237*	0.128	0.135	0.030	0.887**	**1**	0.672**
SOD	0.512**	0.344**	-0.017	0.374**	0.293**	0.674**	0.672**	**1**

** P <0.01; * P <0.05
IgG above diagonal and IgM below diagonal
IgG: immunoglobulin G, IgM: immunoglobulin M, TBCI: total bacterial count of intestinal swabs, TBCB: total bacterial count in breast muscle, TECI: total Enterobacteriaceae count of intestinal swabs, TECB: total Enterobacteriaceae count in breast muscle, TAC: total antioxidant capacity, MDA: malondialdehyde, SOD: superoxide dismutase

Broilers that were supplemented with NS or Se spent more time feeding, drinking and walking than the corresponding control birds (Table 7). Conversely, the unsupplemented broilers spent more time standing and resting. The duration of tonic immobility was less for the supplemented Arbor broilers and for the NS

supplemented Ross broilers than the corresponding controls. It appears that the Arbor broilers were generally more frequently active than the Ross broilers and that the Ross broilers that were supplemented with NS (G4) were more frequently active than G5 and G6 broilers.

Figures 1, 2, 3, and 4 contain representative photomicrographs of liver heart, spleen, and bursa of Fabricius, respectively. Within each figure, panels a, b, c, d, e, and f illustrate tissues from G1, G2, G3, G4, G5, and G6, respectively.

The Arbor broilers supplied with 0.5 ml NS (G1) exhibited (Figure 1a) moderate perihepatitis and severe fibrosis around central vein with their hepatic cells expressing severe vacuolar degeneration, mild haemorrhage, and leukocytic infiltration compared with G3 broilers (Figure 1c). Heart tissue of the G1 broilers (Figure 2a) showed moderate pericarditis, severe myocardial degeneration, cytoplasmic vaculation, leukocytic infiltration, and mild congestion of the cardiac muscles in comparison with the G3 broilers (Figure 2c)**.** Likewise, spleens of the G1 broilers exhibited moderate lymphoid depletion (Figure 3a) compared with the normal architecture of the G3 broilers (Figure 3c). Finally, the bursa of Fabricius had moderate lymphoid depletion of follicles with hyperplasia of follicular epithelium and interfollicular fibrosis (Figure 4a) compared with its normal appearance in the G3 control broilers (Figure 4c).

Supplementation of 0.5 ml Se led to expression severe congestion of central vein with severe fibrosis, degeneration of hepatic cells, and leukocytic infiltration of the liver in G2 broilers (Figure 1 b) compared with normal architecture of the liver in G3 control broilers (Figure 1c). The heart of broilers in G2 showed severe fibrinous pericarditis, which extended to include the myocardial muscle, with the myocardium showing cytoplasmic vaculation, mononuclear cell infiltration, congestion, and mild haemorrhage of the cardiac muscle (Figure 2b) compared with the image from the corresponding control in Figure 2c. The spleen from a Se supplemented broiler exhibited severe lymphoid depletion in Figure 3b as compared with the normal architecture of an unsupplemented broiler in Figure 3c. In response to Se supplementation, the bursa of Fabricius (Figure 4b) exhibited moderate to severe lymphoid depletion of follicles with hyperplasia of the follicular epithelium and increased interfollicular fibrosis when compared with the appearance of normal tissue from an unsupplemented broiler (Figure 4c).

In Figure 1d, Ross broilers (G4) that were administered 0.5 ml NS expressed focal leukocytic infiltration, and hepatocytes showed mild vacuolar degeneration compared with normal view in Figure 1f. Myocardium showed focal leukocytic infiltration, mild congestion, and cytoplasmic vaculation (Figure 2d) compared with the normal architecture (Figure 2f). The spleen in Figure 3d showed mild lymphoid depletion with mild haemorrhage and no lesions were detected in G6 Ross control broilers (Figure 3f). Bursa of Fabricius samples from the broilers in G4 reflected normal follicular epithelium, and normal lymphoid follicles with mild interfollicular fibrosis (Figure 4d) in reference to the normal picture in Figure 4f.

The Ross broilers responded to Se supplementation by exhibiting mild perihepatitis in the liver, with the hepatic cells showing vaculation of cytoplasm, mild haemorrhage, and leukocytic infiltration (Figure 1e). Their heart tissue showed mild pericarditis, mild vacuolar degeneration of myocardium, mild haemorrhage, and leukocytic infiltration (Figure 2e). The spleen exhibited congestion of the splenic sinus with moderate lymphoid depletion (Figure 3e). Finally, the bursa of Fabricius of a Se-supplemented Ross broilers showed normal follicular epithelium with moderate lymphoid depletion and mild interfollicular fibrosis (Figure 4e). The histological appearance of the liver, heart, spleen, and bursa of Fabricius of broilers in G5 (Figures 1e, 2e, 3e, 4e) compared with the nearly typical histological architecture of the corresponding G6 control broilers (Figures 1f, 2f, 3f, and 4f).

Table 7 Duration and frequency of behaviours and tonic immobility of Arbor and Cobb broilers supplemented with selenium and nano-selenium under the influence of heat stress

	Behaviours									*Tonic immobility*
	Feeding	Drinking	Walking	Standing	Resting	Preening	Stretching	Pecking	Flapping	
Duration										
Arbor*NS	239.1^{a} ± 46.9	67.5^{c} ± 19.5	58.9^{a} ± 9.1	8.3^{e}± 2.7	508.2^{d} ± 57					27.0^{e} ± 2.9
Arbor*Se	223.3^{b} ± 56.5	61.3^{d} ± 9.9	50.0^{b} ± 8.1	15.1^{c}± 5.4	553.3^{b} ± 63					31.0^{d} ± 3.4
Arbor*C.	163.3^{d} ± 46.9	38.3^{e} ± 16.4	40.8^{d} ± 9.2	33.3^{a}± 13.1	593.3^{a} ± 56					51.0^{c} ± 4.8
Ross*NS	224.3^{b} ± 42.7	127.5^{a} ± 24.4	50.0^{b} ± 9.9	11.5^{d}± 3.5	551.6^{b} ± 33					79.0^{b} ± 12.7
Ross*Se	217.5^{c} ± 43.3	105.8^{b} ± 21.8	44.1^{c} ± 4.5	13.5^{c}± 6.6	540.8^{c} ± 55					106.0^{a} ± 19.0
Ross*C.	173.1^{d} ± 46.9	61.6^{d} ± 14.7	23.3^{e} ± 11.3	27.5^{b}± 8.4	552.5^{b} ± 55					107.0^{a} ± 25.0
P-value	0.00	0.002	0.001	0.00	0.04					0.00
Frequency										
Arbor*NS	2.25^{a} ± 0.4	2.00^{a} ± 0.4	6.08^{a} ± 0.7	0.75^{c} ± 0.2	3.83^{a} ± 0.4	1.67^{a} ± 0.5	1.42^{a} ± 0.3	2.92 ± 1.7	0.75^{a} ± 0.3	0.3^{e} ± 0.1
Arbor*Se	2.00^{c} ± 0.3	1.17^{e} ± 0.2	2.83^{b} ± 0.4	1.00^{b} ± 0.3	3.00^{a} ± 0.2	1.33^{c} ± 0.6	0.92^{b} ± 0.3	0.17 ± 0.2	0.50^{b} ± 0.2	0.7^{c} ± 0.2
Arbor*C.	1.42^{e} ± 0.3	1.42^{c} ± 0.2	2.50^{b} ± 0.4	1.75^{a} ± 0.6	3.25^{a} ± 0.3	1.50^{b} ± 0.9	1.08^{b} ± 0.3	1.50 ± 0.9	0.17^{d} ± 0.1	1.0^{b} ± 0.1
Ross*NS	2.08^{b} ± 0.3	1.67^{b} ± 0.2	2.25^{b} ± 0.3	0.58^{d} ± 0.2	3.42^{a} ± 0.2	0.92^{d} ± 0.4	0.58^{c} ± 0.2	0.00 ± 0.0	0.35^{c} ± 0.3	0.5^{d} ± 0.1
Ross*Se	2.00^{c} ± 0.2	1.33^{d} ± 0.2	1.50^{c} ± 0.2	0.50^{d} ± 0.2	2.92^{a} ± 0.3	0.00^{e} ± 0.0	0.08^{e} ± 0.1	0.25 ± 0.2	0.17^{d} ± 0.1	1.0^{b} ± 0.3
Ross*C.	1.83^{d} ± 0.4	1.08^{e} ± 0.2	1.83^{c} ± 0.3	1.00^{b} ± 0.3	2.92^{a} ± 0.3	0.00^{e} ± 0.0	0.33^{d} ± 0.1	0.75 ± 0.5	0.17^{d} ± 0.2	1.3^{a} ± 0.4
P-value	0.00	0.04	0.00	0.03	0.23	0.001	0.00	0.09	0.002	0.00

^{a,b,c,d,e,f} Within each column, means lacking a common superscript are deemed different at $P \leq 0.05$
NS: nano-selenium supplement; Se: inorganic selenium supplement; control: unsupplemented

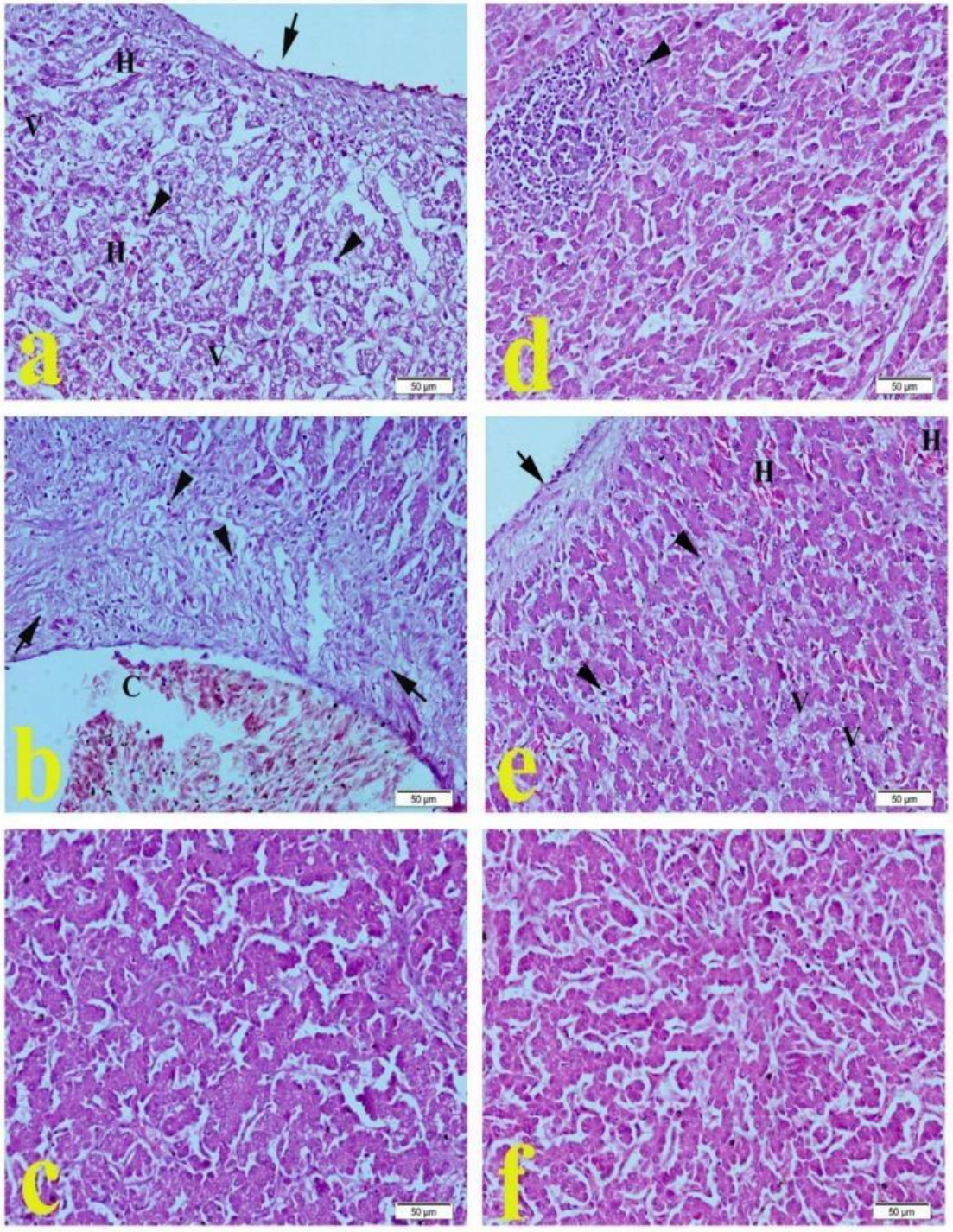

Figure 1 Histopathological sections of the liver stained with haematoxylin-eosin (20×): a) Arbor broilers supplemented with nano-selenium showing severe fibrosis around central vein (arrow), mononuclear cell infiltration (arrowhead), vaculation of hepatocytes cytoplasm (V) and haemorrhage (H); b) Arbor broilers supplemented with selenium showing fibrosis (arrow), mononuclear cell infiltration (arrowhead) and congestion of central vein (C); c) unsupplemented control Arbor broilers; d) liver of Ross broilers supplemented with nano-selenium; e) liver of Ross broilers supplemented with selenium; and (f) unsupplemented control Ross broilers bar 50 µm

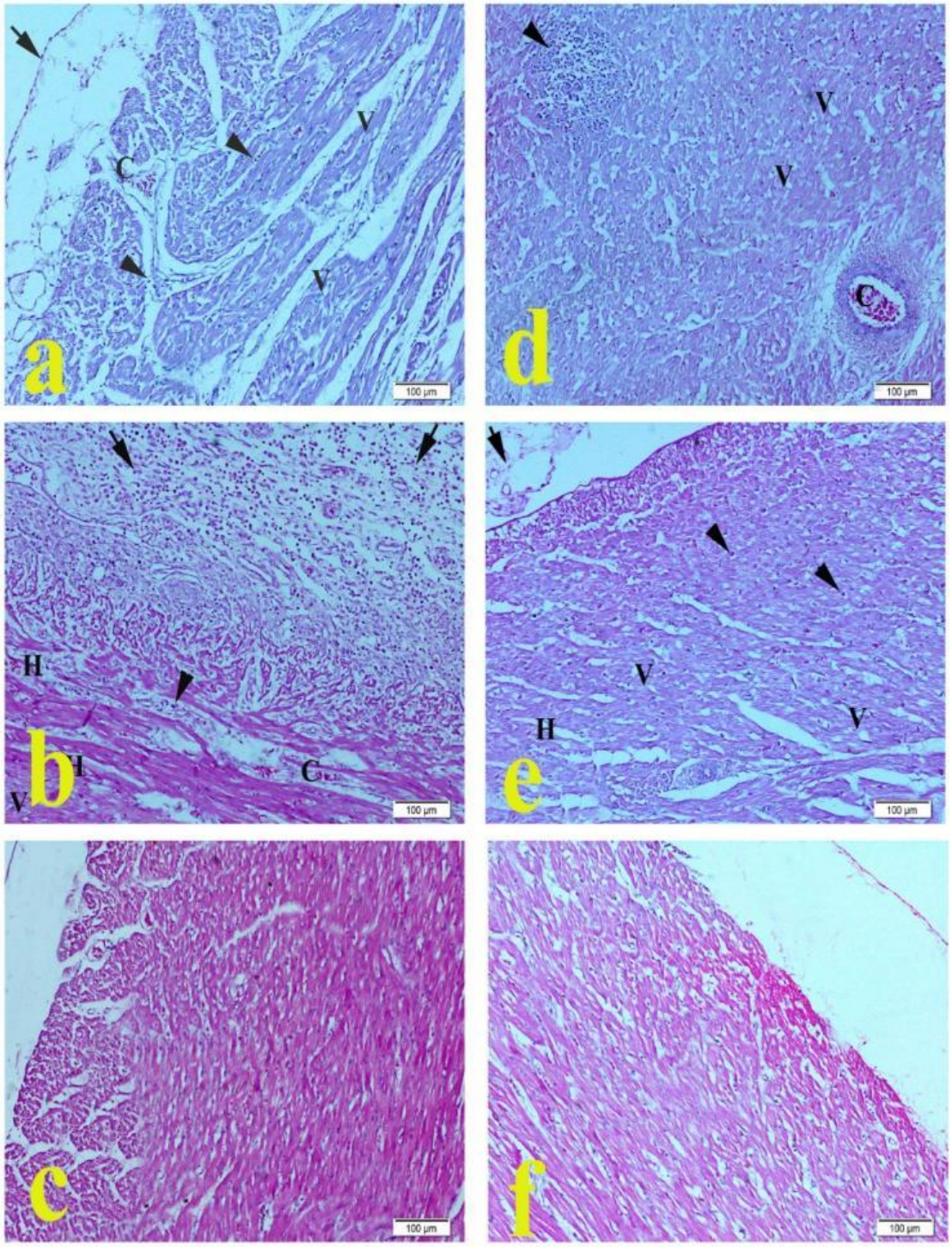

Figure 2 Histopathological section of heart stained with haematoxylin-eosin (10×): a) Arbor broilers supplemented with nano-selenium; b) Arbor broilers supplemented with selenium; c) unsupplemented control Arbor broilers; d) Ross broilers supplemented with nano-selenium; (e) Ross broilers supplemented with selenium; and f) unsupplemented control Ross broilers

Arrow: fibrinous pericarditis; arrowhead: mononuclear cell infiltration; V: vaculation of cytoplasm; C: congestion; bar = 100 µm

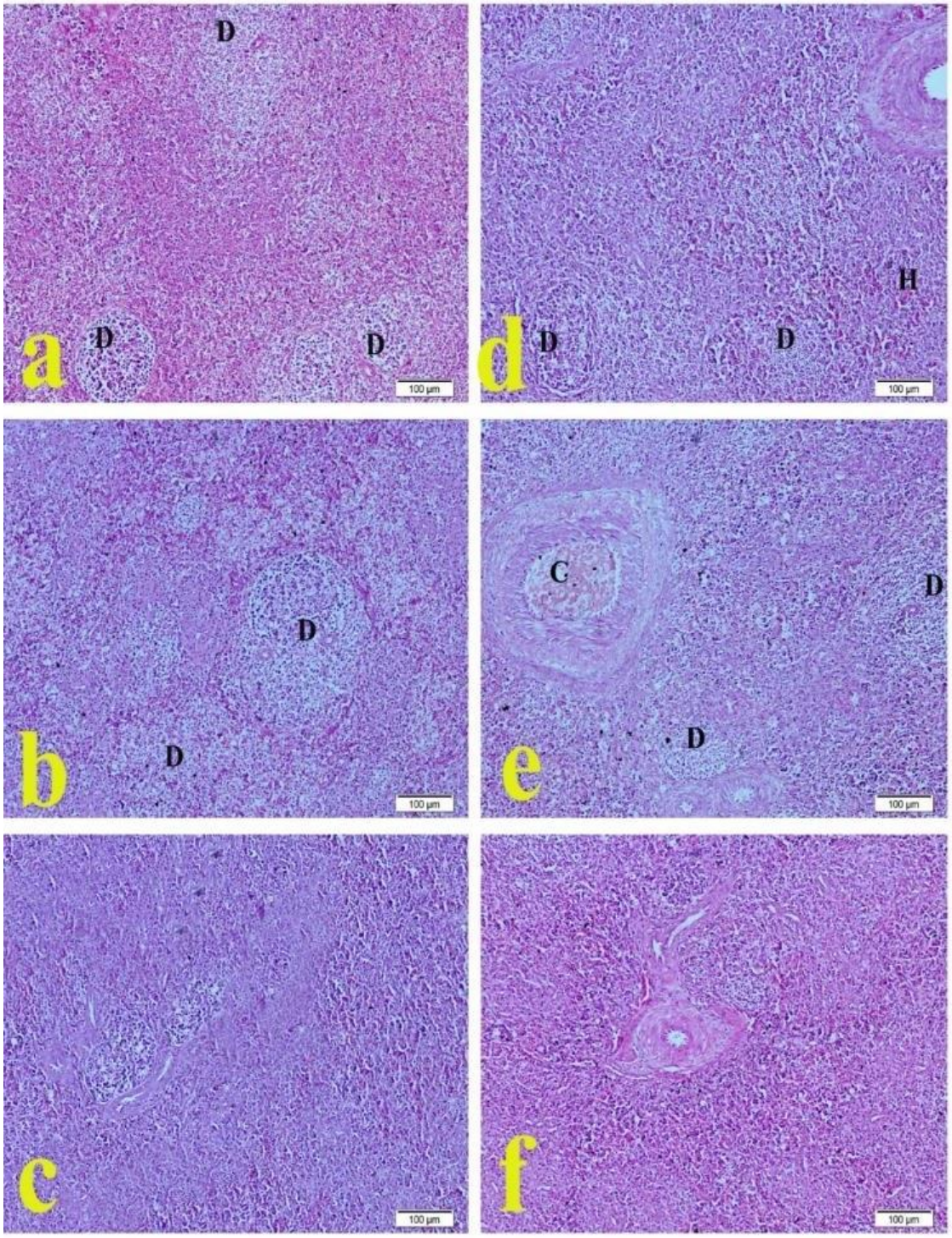

Figure 3 Histopathological section of the spleen stained with haematoxylin-eosin **(10×):** a) Arbor broilers supplemented with nano-selenium; b) Arbor broilers supplemented with selenium; c) unsupplemented control Arbor broilers; d) Ross broilers supplemented with nano-selenium; (e) Ross broilers supplemented with selenium; and f) unsupplemented control Ross broilers
D: depletion of lymphocytes; bar = 100 µm

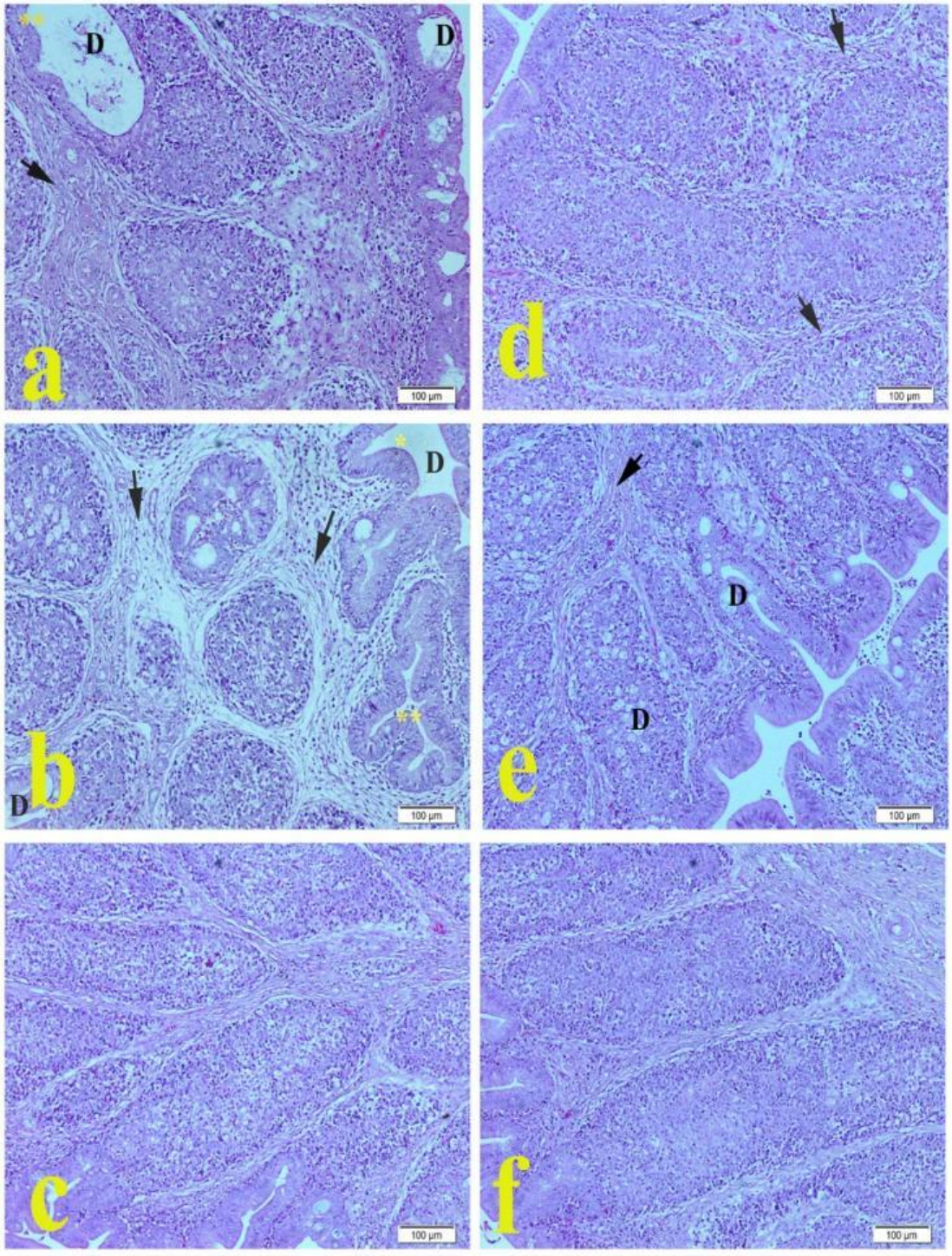

Figure 4 Histopathological section of the bursa stained with haematoxylin-eosin (10×): a) Arbor broilers supplemented with nano-selenium; b) Arbor broilers supplemented with selenium; c) unsupplemented control Arbor broilers; d) Arbor broilers supplemented with nano-selenium; e) Arbor broilers supplemented with selenium; and f) unsupplemented control Ross broilers

Bar = 100 µm; D: depletion of lymphocytes; **: hyperplasia of the follicular epithelium; arrow: increased interfollicular fibrosis

Selenium is an essential element in the nutrition of broilers. It plays an important role in many physiological functions, including calcium regulation, antioxidant redox balance and signalling, thyroid hormone metabolism, protein folding, lipid metabolism, and spermatozoa maturation (Lei, 2017). Selenium deficiency contributes to multiple disorders, especially in the presence of stress that originates from the surrounding micro-environment. These conditions are rarely observed because organic selenium is usually

supplemented in rations composed of soya, oilseed, and grains. The selenite and selenate forms of selenium may also be provided in the vitamin and mineral mixes that are used by the poultry industry, especially post vaccination (Surai & Fisinin, 2014). Lee *et al.* (2017) confirmed that elemental Se was the co-factor that was essential to activate 5'deiodinase enzyme. Later, the 5'deiodinase acts as a key enzyme in the production of triiodothyronine (T3), which is important in controlling energy and protein absorption and thus could regulate animal growth.

In the present study, the productive performance of Arbor and Ross broilers that were exposed to heat stress was improved significantly by supplementation with NS and to a lesser degree with Se in their drinking water. These findings agree with those of Limaye *et al.* (2018), who demonstrated that dietary augmentation of Se with 0.10 to 0.25 mg/kg increased live bodyweight and decreased FCR, compared with diets without supplemental Se and vitamin E. Shabani *et al.* (2018) found that a supplement combination of 200 µg Se and 8×10^9 CFU probiotic per day reduced serum triglyceride concentrations and enhanced weight gain in birds. The current results were inconsistent with those of Zhao *et al.* (2018), who supplemented yellow broilers with 0.15 and 0.30 mg $Se.kg^{-1}$ and did not observe significant improvement in weight gain, but recorded a significant elevation of the glutathione (GSH) activity in the serum. Canoğullari *et al.* (2010) found no significant differences in final bodyweight, feed intake, feed efficiency, egg yield, and egg weight in Japanese quail supplemented with selenium at 0.2 and 0.1 mg/kg of ration.

In this study, supplemental NS alleviated the adverse influence of induced heat stress and minimized water intake. Attia *et al.* (2017), Saleh *et al.* (2018), and Kumbhar *et al.* (2018) also found that supplementation of broilers with antioxidants such as probiotics, trace elements, and vitamins was beneficial in alleviating the adverse effects imposed by a heat stress challenge. However, Mohanty *et al.* (2018) found no effect of the form or level of selenium on weight gain, feed intake or FCR. In addition, Ghalkhanbaz *et al.* (2018) observed that supplemental inorganic selenium at 1 - 8 ppm did not affect the live bodyweight of broilers. They attributed this lack of effect to there being adequate selenium in the feedstuffs provided.

Serum concentrations of total protein, albumin, alanine aminotransferase, aspartate aminotransferase, urea, and creatinine in Ross and Arbor broilers were improved by supplementation with NS in this study. Ahmadi *et al.* (2018) reported a significant decrease in serum albumin with no effects on serum glucose and total protein in broilers supplemented with 0.1, 0.2, 0.3, 0.4, and 0.5 mg NS/kg of ration. Yang *et al.* (2012) and Mohapatra *et al.* (2014) also recorded increased serum levels of glucose, total protein, aspartate aminotransferase, alkaline phosphatase, and globulins in birds supplemented with NS.

The present results revealed a positive influence of supplemental NS on serum concentrations of immunoglobulin G and immunoglobulin M in Ross and Arbor broilers. The enhanced levels of immunoglobulins were explained by Xiao *et al.* (2016) and Gulyas *et al.* (2016) as resulting from nano-selenium increasing protein synthesis and folding because there was a greater level of the eukaryotic translation initiation factor 5A-1. This factor is an important member of the protein synthesis pathway, hence the increased immunoglobulin G and immunoglobulin M serum concentrations. In the current study, immunoglobulins G and M also had weak positive correlations with the total bacterial count of intestinal swabs. The current results were similar to those of Chand *et al.* (2014) and Abudabos *et al.* (2017), who reported significant improvements in immunity with nano-zinc and NS supplementation. Total antioxidant activity and malondialdehyde and superoxide dismutase levels were markedly and equally improved in both Ross and Arbor broilers supplemented with NS compared with Se supplemented and unsupplemented broilers. The significant increase of antioxidant enzymes as reported by Safdari-Rostamabad *et al.* (2017) and Liao *et al.* (2012) was triggered by increased GSH mRNA expression in the liver and alleviated the negative influence of induced heat stress. The results also revealed strong positive correlations between immunoglobulin G and M serum concentrations with antioxidant activity. These results coincide with Gulyas *et al.* (2016), who reported improved GSH activities in liver and serum when feeding NS; they also stated that NS sized 100-500 nm contributed lower toxicity and higher bioavailability, but larger particle sizes of NS can contribute to dietary stress. Cai *et al.* (2012) and El-Deep *et al.* (2016) found increased mRNA GSH peroxidase (GSH-Px) in the liver and reduced malondialdehyde content of the liver and breast muscle when feeding NS to alleviate the impact of high ambient temperature. Markovic *et al.* (2018) recorded improvements in antioxidant activity, performance indices, carcass quality, and the chemical structure of meat from Cobb-500 broilers supplemented with selenium-yeast.

The present study has shown a strong antimicrobial action from augmenting the drinking water with NS when broilers were exposed daily to mild heat stress. Our results agreed with those of Stanley *et al.* (2015), who revealed a significant inhibiting activity of NS on pathogenic microorganisms including *Escherichia coli* and *Staphylococcus*. In a seeming conflict with the aforementioned results, Stanley *et al.* (2015) also reported that NS supplementation increased beneficial bacteria in the gut of broilers, including *Lactobacillus* and *Faecalibacterium*. The present results were compatible with those of Yip *et al.* (2014), who revealed the antifungal properties of NS against *Trichophyton rubrum*. Kheradmand *et al.* (2014) also found

NS to have antimicrobial action against *Candida albicans* and *Pseudomonas aeruginosa*. Shakibaie *et al.* (2015) found antibacterial actions of NS against *Proteus mirabilis,* also in agreement with the current results. The current study provided clear evidence for NS supplementation facilitating tissue and cellular protective influences against environmental stresses.

The photomicrographs of the liver, heart, spleen, and bursa of Fabricius in broiler breeds that were supplemented with NS in their drinking water revealed minimal deviations in the tissue architecture owing to selenium supplementation. However, the Ross broilers exhibited greater adverse effects than Arbor broilers that were supplied with the same dose of traditional commercial Se. These results were inconsistent with those of Alkhudhayri *et al.* (2018), who reported significant and improved histological outcomes from NS supplementation in the face of environmental challenges and infection. Mousa and Ali (2018) studied the influence of nano-boron on the liver of African ostrich chicks after *E. coli* infection and observed the ability of nano-particles to reduce the stress factors with increased resistance.

Nano-selenium supplement relieved the negative influence of heat stress, as shorter duration and smaller frequency of TI was obtained. These findings agreed with those of Sarica and Ozdemir (2018), who reported that TI duration in quail exposed to heat stress at 34 °C was shortened significantly after feeding diets that contained organic Se with tocopherol acetate or oleuropein, and suggested that NS could mitigate the effect of heat stress on poultry in tropical areas. Broilers exhibit an enhanced behavioral profile in the presence of heat stress, which reflects an improvement of ingestive behaviour when they were supplemented with NS. These findings agreed with those of Fischer *et al.* (2008), who reported that Se deficiency caused loss of appetite and reduced efficiency of feed utilization. Behavioral enhancement extended to the activity and alertness of broilers as reflected in their walking more and standing or resting less. Chadio *et al.* (2006) also reported that Se deficiency in animals is characterized by low circulating levels of thyroid hormones that modify activity.

Conclusions

Supplementing broilers' drinking water with NS rather than inorganic commercial selenium resulted in improvements of productive performance, antioxidant activity, immunoglobulin serum concentrations, biochemical profile, tissue architecture, and habituation to some environmental stressors, including heat stress in breeds such as Ross and Arbor broilers.

Authors' Contributions

ESS designed the original experiment, prepared and executed it, analysed the sera samples, and assisted in writing the manuscript. RAH participated in the execution of the experiment, analysed the serum samples, and assisted in writing the manuscript. RTH conducted the histopathological examination, prepared the final photomicrographs, and participated in writing the manuscript. OMAB prepared selenium nanoparticles. AAA participated in the execution of the experiment, the behavioral examination, sample collection, and in writing the manuscript. MSH participated in raising the broilers during the experiment, and in writing the manuscript**.**

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Conflict of Interest Declaration

The authors declare there is no conflict of interest.

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