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Original Article

Preventive, Behavioral, Productive, and Tissue Modification using Green Synthesized Selenium Nanoparticles in the Drinking Water of Two Broiler Breeds under Microbial Stress

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ABSTRACT

A study was conducted to investigate the influence of selenium nanoparticles (SeNPS) and inorganic selenium supplementation in the drinking water on behavior, performance, and immunity of Arbor Acres® and Ross®308 broilers exposed to *E. coli* O157:H7 1.6 × 10^8 challenge at the 10^{th} day of age.

180 one-day-old female broilers were divided into six groups, each with 30 chicks. G1 and G4 were supplied with 1 mL SeNPS 100 mg.L^{-1}/L, G2 and G5 were supplied with 1 mL inorganic selenium 100 mg.L^{-1}/L, and G3 and G6 were supplied with non-supplemented water. Where, G1, G2, and G3 were Arbor Acres®, while G4, G5, and G6 were Ross®308 broilers. A total of 1280 samples (160 sera, 160 intestinal swabs and 960 organ samples including liver, spleen, bursa, heart, breast muscles, and thymus) were collected in a study period of 38 days.

A highly significant increase (p<0.01) of weight gain, feed conversion, performance index, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, immunoglobulin G and M, total antioxidant capacity, malondialdehyde, and superoxide dismutase was recorded in G4 Ross®308 compared to other supplemented and control groups. G1 Arbor Acres® and G4 Ross®308 broilers, also revealed a highly significant decline (p<0.01) in total bacterial and enterobacteriaceae counts of intestine and breast muscles compared to G2 Arbor Acres® and G5 Ross®308 broilers and to controls. Photomicrographs revealed a higher degree of cellular and tissue protection in G4 and G5 Ross®308 compared to G1 and G2 Arbor Acres® broilers. An improvement from SeNPS supplementation was detected on behavior, performance, bacterial load, immunological, antioxidant profiles, and tissue architecture in broilers breeds with special reference to Ross®308 compared to Arbor Acres® broilers.

INTRODUCTION

The poultry industry worldwide has witnessed large scale intensification proportional to the growing population. Intensification in poultry industry demands proactive preventive measures and enhancing supplemental to maintain high productivity, minimize disease risk, and lessen the influence of environmental stressors. Rearing broilers under adverse conditions produce oxidative stress, including extreme free-radical formation and dispersion, cell destruction, and production losses (Schwean-Lardner *et al.*, 2012). It was established that using complexes containing vitamins, minerals, and selenium improved productivity, increased weight gain by 8.46 %, and improved feed conversion by 3.1% (Trukhachev *et al.*, 2016).

Selenium is a nutritionally essential for chickens, to maintain an optimum health, therefore 0.15 mg/kg diet is suggested for broilers during the growth period. Selenium is an important structure of

Glutathione peroxidase (GSH-Px) which is a strong antioxidant in poultry (Oliveira *et al.*, 2014). Selenium bioavailability depends on its form, including elemental selenium with poor absorption capabilities, inorganic selenium (selenite) which is not biologically active and rapidly excreted from the body, and organic selenium which is more active and plays an important role in biological processes (Suchý *et al.*, 2014). However, there is a common concern that the selenium minimum recommendation is not enough to prevent production losses due to selenium deficiency and that's why, there is continued research into alternative selenium supplementation levels.

Nanotechnology manipulates design, production, devices, and systems via controlling sizes and shapes. Nanomaterials have completely different physical and chemical characteristics compared to the original and raw unmodified chemical forms of selenium (Hartemann *et al.*, 2015), these new characteristics authorize their application in medicinal and nutritional fields (Mohapatra *et al.*, 2014). The success of nanomaterials in many fields contributes to their increased use in poultry industry to enhance productivity, immunity, and combating pathogenic micro-organisms' activity (El Sabry *et al.*, 2018). Nano-selenium has many advantages including high absorption capability, increased surface activity, catalytic efficiency, low toxicity, and reimbursement of the body during oxidative and environmental stressors, as well as alleviate their negative impact (Nasirpour *et al.*, 2017; Purohit *et al.*, 2017).

The present study aimed to investigate the influence of supplementing broiler's drinking water with 1 mL of selenium nanoparticles (Green synthesized selenium nanoparticles – SeNPS – at concentration of 100 mg/ 1L) compared to 1 mL commercial inorganic selenium (Selenite - SEDICO® at concentration of 100 mg/ 1L) on productive performance, behavior, intestinal and muscular bacterial loads (Total bacterial and Enterobacteriaceae counts), biochemical and immunological profile, and histopathological architecture of two broiler breeds (ArborAcres® and Ross®308) exposed to *E. coli* O157:H7 challenge at the 10th day of age.

MATERIALS AND METHODS

Ethical Approval

The procedures in the present study were approved by the Scientific Research Ethics Committee, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt with approval number 2018060.

Experimental birds' microclimate

A total of 180 one-day-old females Arbor Acres® and Ross®308 broilers (ninety of each breed) were purchased from Ismailia-Egypt Poultry Company and housed in a battery system (galvanized iron cages). Birds of each breed were divided into three groups; each group consisted of 30 chicks (five replicates of six birds).

The birds were immunized be mean of mass vaccination in drinking water with Infectious Bronchitis live attenuated virus vaccine (PESTIKAL B1 SPF 1000 dose/ 100 Liter) on the 7th day of age; Infectious Bursal disease live attenuated virus vaccine (SER-VAC D78 Strain 1000 dose/ 100 Liter) on the 14th and 21st days of age, and Newcastle disease live lentogenic virus vaccine (PESTIKAL 1000 dose/100 Liter) on the 16th and 26th days. The experiment lasted for 38 days.

At day-old the rearing temperature was 34 °C and gradually declined by 3.5 °C weekly until achieving 25 °C by the end of the 3rd week. The birds were given ad libitum access to water and a standard corn-soybean basal diet (NRC 1994) based on 23% crude protein over the first fourteen days, and 21.5% crude protein for the remaining 24 days in the fattening cycle. The room was ventilated naturally using V shape windows to stimulate a stack effect. Artificial continuous lightening program for 18 h light and 6 h darkness using Blue LED lights was provided as recommended by Soliman & Hassan (2019). Mortalities, microclimatic temperature and relative humidity were monitored and recorded daily during the experiment.

Synthesis of Selenium nanoparticles

Selenium powder (Merk®) was used as a precursor for the SeNPS and polyvinyl alcohol (PVA) served as a capping agent for the NPS to prevent aggregation and any change in size and shape. Glucose that was commercially produced acts as a reducing agent of selenium; it is assumed that glucose makes reduction and oxidized itself into gluconic acid, this acid may also surround the NPS and act as a stabilizing agent. Firstly aqueous sodium seleno-sulphate solution was prepared by refluxing and heating at 70 °C for an aqueous solution of Na_2SO_3(Merk®) and selenium powder (selenite) for 6h as reported in Gorer & Hodes (1994). The solution was filtered to remove any unreacted materials, then glucose powder (6%) and 1% PVA were added to the solution, refluxing persisted for another 6 h. The color of the solution changed through refluxing from colorless to pale yellow. The colored solution remained stable for months. The synthesized SeNPS were characterized by UV-visible

optical absorption spectroscopy (Shimaduz RF5301PC double beam spectrophotometer), and absorption spectra of the resulting SeNPS were scanned (200-800 cm-1) using 1 cm matched quartz cells. The morphology of the NPS was observed using Transmission Electron Microscopy (HR-TEM) JOEL JEM-2010 operating at an accelerating voltage of 200 kV and equipped with a Gatan digital camera Erlangshen ES500. TEM sample was prepared by dropping a sample suspension as it is after preparation on a coppergrid coated with a carbon film. Preparing SeNPS via this method characterized by obtaining Nanocrystalline SeNPS without post annealing treatment.

Selenium and SeNPS solution supplement

One group (N=30) from each breed was assigned the following treatments: G1 and G4 were supplied with 1 mL SeNPS (green synthesized SeNPS at concentration of 100 mg/L) per each liter of drinking water, G2 and G5 were supplied with 1 mL commercial inorganic selenium (Selenite - SEDICO® at concentration of 100 mg/L) per each liter of drinking water, finally, G3 and G6 were supplied with non-supplemented water. Where, G1, G2, and G3 were Arbor Acres®, G4, G5, and G6 were Ross®308 broilers. The final concentration of both SeNPS and inorganic selenium in the supplemented water was about 0.1 mg per each liter of drinking water on a daily basis of the entire fattening cycle (38 days).

E. coli O157:H7 challenge

E. coli O157: H73.6 × 10^5 CFU culture was purchased from the Animal Health Research Institute – Dokki, the culture was propagated using Mac-Conkey broth at 44 °C / 24 h. Ten microliters from the positive tubes (yellow color development and gas accumulation in inverted Durham's tube) were dropped aseptically onto Eosine Methylene Blue (EMB) agar, and incubated at 37 °C / 24 h (Herigstad *et al.*, 2001). Metallic green colonies were counted and picked up. Four out of the six groups (G1, G2, G4 and G5) were challenged with *E. coli* O157:H7 1.6 × 10^8 on the 10^{th} day of age in drinking water (Kocijancic *et al.*, 2016).

Performance indices

Average live body weight (LBW) was estimated by weighing 28 birds (the number was calculated using Solvin's formula for random sampling) from each group on a weekly basis. Weekly feed intake (FI) was calculated based on total amount consumed in relation to the bird's absolute number in each group. Body Weight Gain (WG), Feed Conversion Ratio (FCR), and Performance Index (PI) were calculated according to Soliman & Hassan (2017).

Behavior recording and observation methods

Twelve broiler chicks from each group were randomly selected for recording the behavioral parameters. Broiler's behavior was recorded for a total of 3 h a day, 1 h starting at 9:00 am, 2:00 pm, and 9:00 pm in a daily pattern for three weeks (1^{st}, 3^{rd}, and the 5^{th}) using a video camera (Panasonic WV Ns202ae) connected to computer through DVR card, and they were suspended at a height of 1.5 m above the floor of each pen (Li *et al.*, 2015).

The recorded videos were replayed in the laboratory, and the performed behaviors, duration, and frequency were recorded every five min/h (12 observations per h) using focal sampling method. They were classified into: feeding, drinking, walking, standing, resting, preening, stretching, pecking, and flapping (Villagra *et al.*, 2014).

Sampling

Sampling was carried out via two slaughters (at the 19^{th} and the 38^{th} day of age). A total of 1280 samples were collected during the study period, including 160 sera, 160 intestinal swabs and 960 organ samples of liver, spleen, bursa, heart, breast muscles, and thymus. Blood samples were collected during slaughters, centrifuged at 4000 rpm for 15 minutes. Non-hemolyzed clear sera were obtained, stored at -20 °C for the biochemical and antioxidant assay (Soliman *et al.*, 2017). Liver, spleen, bursa, heart, and thymus were kept on formalin 10% for histopathological examination. Intestinal swabs, and 3 g of breast muscles were collected in 9 mL buffered peptone water for bacteriological assessment.

Biochemical profile and antioxidant markers

Sera samples were examined for total protein (TP), albumin (ALB), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), urea (UREA), and creatinine (CREAT), as well as for antioxidant markers including: Total Antioxidant Capacity (TAC), Malondialdehyde (MDA), and Superoxide Dismutase (SOD) calorimetrically using UV-1100 spectrophotometer.

Serum immunoglobulin IgG and IgM concentrations were measured by using immunoturbidimetric assay using Sysmex CS-5100 System.

Bacteriological examination

Intestinal swabs, as well as breast muscles samples, were prepared according to APHA (2012). Tenfold serial dilution sup to 10^{-6} were prepared. Total Bacterial Count (TBC) and Total Enterobacteriaceae Count (TEC) were applied on standard plate count (SPC) and Eosine Methylene Blue Agar (EMB), respectively at 37 °C for 24 - 48 h by using the drop plate technique as recommended by Soliman *et al.* (2016) and Kim & Lee (2016). Plates were counted using Dark-field colony counter (Murray *et al.*, 2015).

Histopathological examination

Representative tissue samples from liver, heart, lymphoid organs (spleen, bursa of Fabricious, and thymus) were fixed in 10% buffered formalin saline solution until further processing. Specimens were cut into 5-mm thick sections, put into tissue cassettes, dehydrated by transferring through a series of alcohols increasing concentrations, cleared in two changes of xylene, infiltrated with different grades of melted paraffin in the oven, and finally the sections were cut at 5 µm thickness using rotator microtome. After cutting, sections were floated on warm water bath at 38°C for stretching, mounted on clean slides using egg albumin, and dried on a slide warmer at 38°C (Luna, 1968 and Darboux, 1994). The sections were stained using Hematoxylin and Eosin (H & E). The histological structures of the examined organs were observed using light microscope under (x10) and (x20) magnification.

Statistical analysis

Statistical analysis was carried out using the statistical package for social sciences (SPSS version 20.0) software package (Argyrous, 2005). *E. coli* O157:H7 and total bacterial counts were expressed as logarithms using Microsoft Excel. The obtained data were analyzed statistically using two-tailed Analysis of Variance (ANOVA). In the two-tailed ANOVA, factors including breeds, groups, age of broiler, and slaughter times were used as between-subjects' effects along with their interactions. Mixed model ANOVA was used as the principal statistical mean for the current data. The principle and interaction effects were tested for their statistical significance at 0.05 and 0.01 significance levels. The statistical model was emphasized as following:

$$Y_{ijk} = \mu+\alpha_1+\beta_j+(\alpha\beta)_{ij}+e_{ijk}$$

Where, Y_{ijk} was the measurement of any dependent variable; µ was the overall mean; α_1 was the fixed effect of slaughter time; β_j was the fixed effect of the age of the birds; $(\alpha\beta)_{ij}$ was the interaction effect of slaughter time by age of the birds; e_{ijk} was the random error. The error term, e_{ijk} was approximately NID (0, σ^2_e), normally independently distributed with mean of 0 and variance of, σ^2_e.

RESULTS

UV-Vis spectrophotometry of SeNPS

The absorption spectrum of yellow colored SeNPS (Figure 1) showed appearance of the transition point at around 367 nm, without a clear maximum reflecting the formation of SeNPS. The stabilization of the yellow color during the entire reaction time supported the stabilizing influence of PVA. Morphology and structure of the synthesized SeNPS was observed using TEM imaging, and the image (Figure 2) revealed a spherical shape for individual nanoparticles, with size at 25 nm.

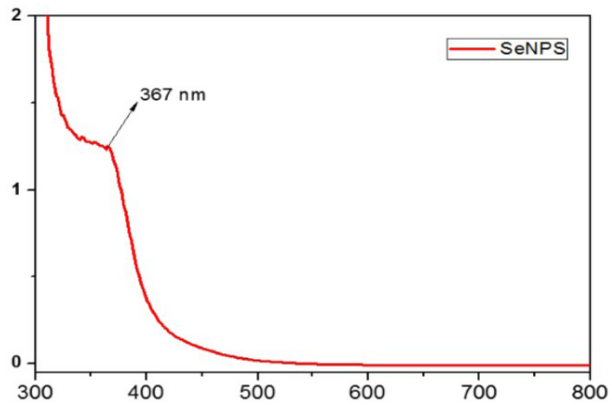

Figure 1 – The absorption spectrum of prepared SeNPS.

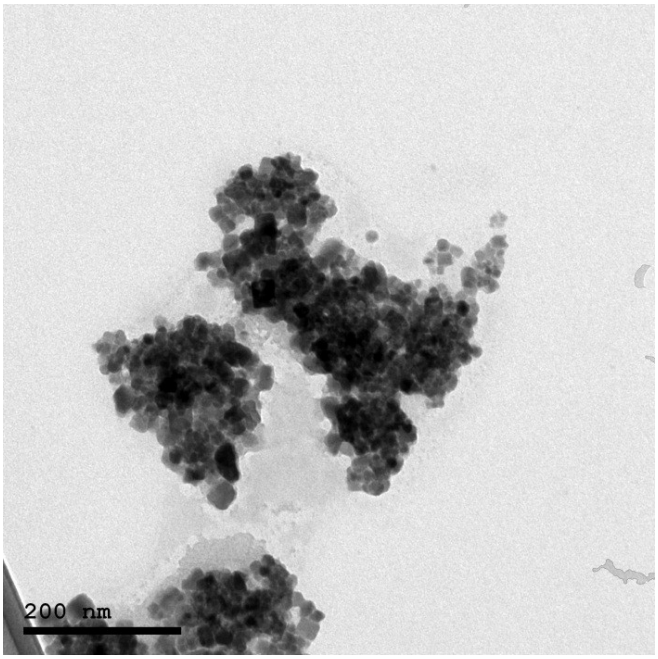

Figure 2 – The TEM image for the prepared SeNPS.

Behavioral observations

Broiler's behavior (Table 1) exhibited a highly significant increase (p<0.01) in the frequencies of feeding, drinking, walking, resting, and in the duration of drinking and walking in G1 and G4 supplemented with SeNPS compared to G2 and G5 supplemented with commercial inorganic selenium and to the controls. A highly significant decrease (p<0.01) was recorded in the duration of feeding and standing among all treated groups in both broilers' breeds compared to the control. A highly significant increase (p<0.01) was declared in walking and drinking duration of G1 and G4 compared to all other supplemented and control groups.

SeNPS (Table 1) were able to produce a highly significant decrease (p<0.01) in standing duration of G1 and G4 when compared to each other, a non-significant decrease in standing duration of G2 and G5, and a non-significant decrease in standing frequency among all groups of both breeds.

Resting frequency showed (Table 1) a highly significant increase (p<0.01) in G1 broilers compared to G2, G5, and G4 with no significant difference between theses latter three groups. The selenium effect was inconsistent having no effect on resting frequency in either breed.

SeNPS was able to significantly decrease (p<0.01) the frequencies of preening, stretching, and pecking in G1, G4, G2, and G5 compared to their controls (Table 1). On the other hand, it was able to increase the frequency of flapping in the same groups compared to their controls.

Biochemical profile

The biochemical profile (Table 2) revealed a highly significant decrease (p<0.01) in TP, ALB, ALT, and AST among treated groups of both experimented broilers' breeds compared to their control. TP revealed (Table 2) a highly significant elevation (p<0.01) in G4 Ross®308 compared to G1Arbor Acres® and to the other treated groups. ALB showed a highly significant increase (p<0.01) in G5 Ross®308 broilers treated with inorganic selenium compared to G2 Arbor Acres® broilers treated with the same dose and concentration of inorganic selenium, and to G1 and G4 treated with SeNPS.

On a time scale, TP showed a highly significant decrease (p<0.01) on the 38 day old broilers compared to the19 day old broilers in G1, G2, G3, and G4, and a highly significant increase on the 38 day old broilers compared to the 19 day old broilers in G5 and G6 (Table 2). ALB revealed a highly significant decrease (p<0.01) on the 38 day old broilers compared to the 19 day old in G1, G2, G4, and G5, no significant differences between the two slaughter times in G3 Arbor Acres® control, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G6 Ross®308 control (Table 2).

Table 1 – Behavioral patterns of different broiler breeds supplemented with selenium and green synthetized selenium nanoparticles.

Groups	Behaviors								
	Feeding	Drinking	Walking	Standing	Resting	Preening	Stretching	Pecking	Flapping
Duration									
G1	137.5^{e}±25.3	67.5^{b}±16.2	80.8^{a}±20.13	9.1^{d}±3.58	614.1^{a}±29.9	----------	----------	----------	----------
G2	148.3^{d}±37.9	66.6^{b}±5.12	81.6^{a}±23.86	27.5^{ab}±7.60	575.8^{a}±48.6	----------	----------	----------	----------
G3	163.3^{c}±46.9	58.7^{c}±17.6	40.8^{c}±9.25	33.3^{a}±13.10	593.3^{a}±56.2	----------	----------	----------	----------
G4	205.0^{b}±30.8	95.0^{a}±19.6	40.0^{c}±6.51	7.5^{d}±2.18	535.0^{a}±35.0	----------	----------	----------	----------
G5	214.1^{b}±46.8	93.3^{a}±30.8	50.0^{b}±11.28	27.5^{ab}±8.36	551.6^{a}±62.1	----------	----------	----------	----------
G6	230.0^{a}±51.5	61.6^{bc}±14.7	20.0^{d}±5.37	30.0^{ab}±10.3	552.5^{a}±55.9	----------	----------	----------	----------
p value	0.002	0.000	0.005	0.001	0.330	----------	----------	----------	----------
Frequency									
G1	1.83^{b}±0.34	2.17^{a}±0.73	3.83^{a}±0.41	0.83±0.32	4.17^{a}±0.37	0.58^{e}±0.15	0.75^{b}±0.22	0.67^{c}±0.45	0.50^{b}±0.23
G2	1.75^{b}±0.33	1.92^{b}±0.19	3.92^{a}±0.60	1.50±0.31	3.17^{b}±0.24	1.00^{c}±0.43	1.08^{a}±0.31	0.42^{d}±0.23	0.97^{a}±0.58
G3	1.42^{c}±0.34	1.42^{d}±0.29	2.50^{b}±0.42	1.00±0.37	3.25^{b}±0.25	1.67^{a}±0.45	1.08^{a}±0 .29	1.50^{a}±0.93	0.17^{d}±0.11
G4	2.25^{a}±0.22	1.83^{b}±0.24	2.00^{c}±0.33	0.75±0.18	3.42^{b}±0.23	0.42^{e}±0.29	0.17^{d}±0.11	0.75^{c}±0.51	0.25^{c}±0.13
G5	2.08^{a}±0.40	1.50^{c}±0.38	1.83^{c}±0.30	0.58±0.15	2.67^{c}±0.19	0.75^{d}±0.00	0.33^{c}±0.19	0.00^{e}±0.00	0.18^{d}±0.02
G6	1.75^{b}±0.33	1.08^{e}±0.19	1.17^{d}±0.30	1.00±0.25	2.92^{c}±0.29	1.28^{b}±0.00	0.33^{c}±0.14	0.92^{b}±0.64	0.17^{d}±0.11
p value	0.001	0.000	0.000	0.086	0.005	0.023	0.000	0.006	0.023

Means carrying different superscripts in the same column are significantly different at (p≤0.05) or highly significantly different at (p<0.01). Means carrying the same superscripts in the same column are non-significantly different at (p<0.05).

G1=Arbor Acres® with 1 mL nano-selenium/1L drinking water, G2=Arbor Acres® with 1 mL selenium/1L drinking water), G3=Arbor Acres® control, G4=Ross®308 with 1 mL nano--selenium/1 L drinking water, G5= Ross®308 with 1 mL selenium/1L drinking water, and G6= Ross®308 control.

A highly significant decline (p<0.01) was detected (Table 2) in ALT of G1 and G2 Arbor Acres® broilers compared to their control with no significant differences between these two groups, and a highly significant decline (p<0.01) of ALT in G4 and G5 Ross®308 broilers compared to their control, with a highly significant increase (p<0.01) in G4 than in G5 broilers. A highly significant decrease (p<0.01) was revealed in AST of G1 treated with SeNPS compared to G2 treated with inorganic selenium and G3 control Arbor Acres® broilers, and in AST of G4 treated with SeNPS compared to G5 treated with inorganic selenium and G6 control Ross®308 broilers.

ALT showed a highly significant decrease (p<0.01) on the 38 day old broilers compared to the 19 day old in G1, G3, and G4, no significant differences between the two slaughter times in G5, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G2 and G6 (Table 2). AST showed a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in all treated and control groups (Table 2).

Urea (Table 2) showed a highly significant increase (p<0.01) in G1 Arbor Acres® and G5 Ross®308, non-significant increase in G2 Arbor Acres®, and a highly significant decrease (p<0.01) in G4 Ross®308 compared to the controls. A highly significant increase (p<0.01) of CREAT levels was noticed in G1, G2, G4, and G5 compared to their controls.

Urea revealed a highly significant decrease (p<0.01) on the 38 day old broilers compared to the 19 day old in G3, G5, and G6, no significant differences between the two slaughter times in G1, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G2 and G4 (Table 2). CREAT showed a highly significant decrease (p<0.01) on the 38 day old broilers compared to the 19 day old in G1, no significant differences between the two slaughter times in G6, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G2, G3, G4 and G5 (Table 2).

Table 2 – Biochemical profile (Mean ±SE) of different broiler breeds supplemented with selenium and green synthetized selenium nanoparticles.

Groups	Slaughters times / days	TP g/dl	ALB g/dl	ALT IU/L	AST IU/L	UREA mg/dl	CREAT mg/dl
G1		11.1^{c}±0.26	1.9^{c}±0.13	4.5^{b}±0.20	38.4^{bc}±0.79	46.8^{a}±1.34	4.7^{a}±0.17
G2		10.1^{d}±0.25	1.8^{c}±0.12	4.5^{b}±0.19	46.3^{a}±0.74	41.6^{b}±1.27	3.2^{b}±0.16
G3		11.9^{b}±0.26	3.6^{a}±0.13	5.1^{a}±0.20	39.3^{b}±0.79	40.3^{b}±1.34	0.8^{d}±0.09
G4		11.7^{b}±0.26	1.5^{c}±0.12	2.8^{d}±0.19	33.3^{d}±0.77	32.4^{d}±1.31	3.0^{b}±0.17
G5		10.4^{d}±0.24	2.7^{b}±0.11	2.3^{e}±0.18	36.3^{bc}±0.72	49.2^{a}±1.22	2.2^{c}±0.16
G6		12.4^{a}±0.26	3.4^{a}±0.12	3.5^{c}±0.19	42.3^{b}±0.77	37.0^{c}±1.31	0.5^{d}±0.02
p value		0.000	0.000	0.002	0.005	0.004	0.005
Groups * Slaughter times							
G1	19^{th}	13.8^{a}±0.46	3.5^{a}±0.07	5.6^{a}±0.73	16.0^{b}±0.92	45.4^{a}±1.03	6.6^{a}±0.76
	38^{th}	8.3^{b}±0.49	0.4^{b}±0.04	3.5^{b}±0.18	60.8^{a}±0.50	48.1^{a}±0.46	2.7^{b}±0.14
G2	19^{th}	13.4^{a}±0.75	2.9^{a}±0.66	4.3^{b}±0.47	23.2^{b}±2.17	14.7^{b}±2.36	1.6^{b}±0.16
	38^{th}	6.9^{b}±0.25	0.7^{b}±0.03	4.7^{a}±0.19	69.4^{a}±0.74	68.5^{a}±2.18	4.8^{a}±0.21
G3	19^{th}	12.8^{a}±0.67	3.6^{a}±0.08	5.7^{a}±0.49	9.8^{b}±1.10	53.6^{a}±4.81	0.5^{b}±0.07
	38^{th}	11.1^{b}±0.06	3.6^{a}±0.02	4.4^{b}±0.05	68.7^{a}±0.64	27.1^{b}±0.33	1.1^{a}±0.05
G4	19^{th}	12.6^{a}±0.42	2.5^{a}±0.47	4.7^{a}±0.57	11.0^{b}±2.08	22.3^{b}±2.59	2.0^{b}±0.31
	38^{th}	10.9^{b}±0.08	0.5^{b}±0.01	0.9^{b}±0.04	55.6^{a}±1.15	42.5^{a}±0.72	4.1^{a}±0.15
G5	19^{th}	9.4^{b}±0.69	3.3^{a}±0.20	2.5^{a}±0.29	12.5^{b}±0.77	51.1^{a}±3.31	0.5^{b}±0.07
	38^{th}	11.4^{a}±0.14	2.2^{b}±0.08	2.1^{a}±0.07	60.1^{a}±0.78	47.2^{b}±0.87	3.9^{a}±0.19
G6	19^{th}	10.8^{b}±0.40	1.9^{b}±0.11	2.1^{b}±0.29	12.6^{b}±1.54	45.2^{a}±1.03	0.7^{a}±0.08
	38^{th}	14.0^{a}±0.12	5.0^{a}±0.02	4.9^{a}±0.08	72.0^{a}±0.25	28.9^{b}±0.44	0.3^{a}±0.04
p value		0.000	0.000	0.000	0.000	0.000	0.000

Means carrying different superscripts in the same column are significantly different at (p≤0.05) or highly significantly different at (p<0.01). Means carrying the same superscripts in the same column are non-significantly different at (p<0.05).

G1=Arbor Acres® with 1 mL nano-selenium/1L drinking water, G2=Arbor Acres® with 1 mL selenium/1L drinking water), G3=Arbor Acres®control, G4= Ross®308 with 1 mL nano--selenium/1 L drinking water, G5= Ross®308 with 1 mL selenium/1L drinking water, and G6= Ross®308 control.

TP=Total Protein, ALB=Albumin, GLOB=Globulin, ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, UREA=Urea, and CREAT=Creatinine. SE=Standard error.

Immunoglobulin concentration

IgG and IgM revealed (Table 3) a synchronized highly significant increase (p<0.01) in G1, G2, G4, and G5 compared to the controls with more significant increase (p<0.01) in G4 and G1 treated with SeNPS, respectively compared to other groups with no significant differences between G1 and G2. The overall average concentrations of IgG and IgM were significantly higher (p<0.01) in Ross®308 (Mean=1625.8 mg/dL) compared to Arbor Acres® broilers (Mean=1607.1 mg/dL).

IgG showed a highly significant decrease (p<0.01) on the 38 day old broilers compared to the 19 day old in G1 and G2, no significant differences between the two slaughter times in G6, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G3, G4 and G5 (Table 3). Meanwhile, IgM revealed a highly significant decrease (p<0.01) on the 38 day old broilers compared to the 19 day old in G1 and G2, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G3, G4, G5, and G6 (Table 3).

Antioxidant profile

TAC (Table 3) revealed a highly significant increase (p<0.01) in G1, G4, G5, G6, G3, and G2, respectively. MDA showed a highly significant increase (p<0.01) in G4, G1, G5, G2, G3, and G6, respectively with no significant differences between Arbor Acres® G3 and Ross®308 G6 controls. SOD revealed a highly significant increase (p<0.01) in G1 and G4 over the other treated and control groups with no significant differences between G1 and G4 and between G2, G3, G5, and G6. Generally, TAC, MDA, and SOD revealed a highly significant increase (p<0.01) in Ross®308 (Means= 1.64 mM/L, 15.6 nmol/ml, and 259.8 U/ml respectively) than Arbor Acres® broilers (Mean= 1.53 mM/L, 13.3 nmol/ml, and 255.3 U/ml respectively) (Table 3).

TAC showed a highly significant decrease (p<0.01) on the 38 day old broilers compared to

Table 3 – Immunoglobulin concentration (Mean ±SE) and Antioxidant profile (Mean ±SE) of different broiler breeds supplemented with selenium and green synthetized selenium nanoparticles.

groups	Slaughters times / days	Immunoglobulin concentration		Antioxidant enzymes		
		IgG mg/dl	IgM mg/dl	TAC mM/L	MDA nmol/ml	SOD U/ml
G1		1739.9^{b}±7.03	414.8^{b}±3.14	1.79^{a}±0.01	21.3^{b}±0.29	260.5^{a}±1.96
G2		1736.0^{b}±6.65	410.5^{b}±2.97	1.33^{f}±0.01	11.6^{d}±0.27	251.2^{b}±1.85
G3		1345.5^{e}±7.03	269.2^{e}±3.14	1.49^{e}±0.01	7.2^{e}±0.29	254.3^{b}±1.96
G4		1769.4^{a}±6.88	425.0^{a}±3.07	1.85^{b}±0.01	24.1^{a}±0.28	267.8^{a}±1.92
G5		1729.9^{c}±6.43	399.0^{c}±2.87	1.58^{c}±0.01	14.7^{c}±0.26	257.2^{b}±1.79
G6		1378.1^{d}±6.88	301.5^{d}±3.07	1.51^{d}±0.01	7.9^{e}±0.28	254.5^{b}±1.92
p value		0.000	0.001	0.000	0.000	0.000
Groups * Slaughter times						
G1	19th	1841.6^{a}±21.6	487.4^{a}±10.0	1.66^{b}±0.01	18.8^{b}±0.67	236.9^{b}±2.98
	38th	1638.2^{b}±11.2	342.2^{b}±4.43	1.93^{a}±0.01	23.8^{a}±0.46	284.1^{a}±0.78
G2	19th	1829.2^{a}±10.3	473.6^{a}±5.68	1.17^{b}±0.00	10.0^{b}±0.33	247.1^{b}±2.10
	38th	1624.8^{b}±7.06	347.4^{b}±2.05	1.50^{a}±0.01	13.2^{a}±0.31	255.2^{a}±1.69
G3	19th	1317.0^{b}±11.1	263.2^{b}±4.04	1.47^{a}±0.00	6.9^{b}±0.27	259.6^{a}±0.89
	38th	1374.0^{a}±7.09	275.2^{a}±1.85	1.51^{a}±0.01	7.4^{a}±0.14	248.9^{b}±4.51
G4	19th	1598.6^{b}±10.2	349.0^{b}±11.6	1.80^{b}±0.02	22.4^{b}±0.53	247.0^{b}±5.25
	38th	1940.3^{a}±9.73	501.1^{a}±1.03	1.89^{a}±0.01	25.9^{a}±0.62	288.6^{a}±0.34
G5	19th	1579.0^{b}±9.75	337.6^{b}±6.50	1.54^{b}±0.01	12.4^{b}±0.26	248.2^{b}±0.82
	38th	1880.8^{a}±5.51	460.4^{a}±1.77	1.61^{a}±0.01	17.0^{a}±0.21	266.2^{a}±1.91
G6	19th	1378.6^{a}±4.29	294.0^{b}±2.98	1.52^{a}±0.00	7.6^{a}±0.12	260.0^{a}±1.13
	38th	1377.7^{a}±3.32	309.1^{a}±1.81	1.50^{a}±0.03	8.2^{a}±0.11	249.1^{b}±2.91
p value		0.003	0.042	0.000	0.000	0.000

Means carrying different superscripts in the same column are significantly different at (p≤0.05) or highly significantly different at (p<0.01). Means carrying the same superscripts in the same column are non-significantly different at (p<0.05).

G1=Arbor Acres® with 1 mL nano-selenium/1L drinking water, G2=Arbor Acres® with 1 mL selenium/1L drinking water), G3=Arbor Acres® control, G4= Ross®308 with 1 mL nano--selenium/1 L drinking water, G5= Ross®308 with 1 mL selenium/1L drinking water, and G6= Ross®308 control.

IgG=Immunoglobulin G, IgM=Immunoglobulin M, TAC=Total Antioxidant capacity, MDA= Malondialdehyde, and SOD=Superoxide Dismutase, SE=Standard error.

the 19 day old in G2, no significant differences between the two slaughter times in G3 and G6, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G1, G4 and G5 (Table 3). MDA revealed a highly significant decrease (p<0.01) on the 38 day old broilers compared to the 19 day old in G2, no significant differences between the two slaughter times in G6, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G1, G3, G4 and G5 (Table 3). SOD showed a highly significant decrease (p<0.01) on the 38 day old broilers compared to the 19 day old in G3 and G6, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G1, G2, G4 and G5 (Table 3).

Bacterial Counts

TBC revealed a highly significant decrease (p<0.01) in intestinal swabs of G5 compared to G4 Ross®308 broilers, and in G1 compared to G2 Arbor Acres® broilers, with no significant differences between G3 and G6 control of the two breeds, and a highly significant decrease (p<0.01) in TBC of breast muscles of G1 compared to G2 Arbor Acres® broilers, and a significant decrease (p≤0.05) in G4 compared to G5 Ross®308 broilers (Table 4).

TEC (Table 4) showed no significant differences between G1 and G2 Arbor Acres® intestinal swabs and breast muscles, and between G4 and G5 Ross®308 broilers intestinal swabs, a highly significant difference (p<0.01) between G4 and G5 Ross®308 broilers breast muscles. TBC and TEC of intestinal swabs and breast muscles revealed a highly significant decrease (p<0.01) in G4 and G5 Ross®308 (Mean=4.1, 3.1, 2, and 1.0 CFU/mL respectively) compared to G1 and G2 Arbor Acres® broilers (Mean=4.5, 3.4, 2.8, and 1.4 CFU/mL respectively).

TBC of intestinal swabs showed no significant differences in G4, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G1, G2, G3, G5 and G6 (Table 4). TBC of breast muscles showed a highly significant decrease (p<0.01) on the 38 day old broilers compared to the 19 day old in G3, G4, and G6, and a highly significant increase (p<0.01) on the 38 day old broilers compared to the 19 day old in G1, G2, and G5 (Table 4).TEC of intestinal swabs and breast muscles showed a highly significant increase on the 38 day old broilers compared to the 19 day old in all treated and control groups (Table 4).

Productive performance

WG/g revealed a significant difference (p≤0.05) between G1 and G2 and between G4 and G5 with no significant differences between G3 and G6 controls (Table 5).WG also revealed a highly significant increase (p<0.01) on the 5th, 3rd, 4th, 2nd, and 1st week of age respectively in G1, G2, and G3 Arbor Acres® broilers, at on the 4th, 3rd, 5th, 2nd, and 1st week of age respectively in G4 Ross®308 broilers, and at on the 5th, 4th, 3rd, 2nd, and 1st week of age respectively in G5 and G6 Ross®308 broilers (Table 5).

FI/g (Table 5) showed a highly significant increase in G4 compared to G5 and G6 Ross®308 control, and no significant differences between G1 and G2 Arbor Acres® broilers with a highly significant difference between the last two groups and G3 Arbor Acres® control. FI revealed (Table 5) a highly significant increase (p<0.01) on the 5th, 4th, 3rd, 2nd, and 1st week of age respectively in G1, G2, G5, and G6 broilers, and on the 4th, 5th, 3rd, 2nd, and 1st week of age respectively in G3 Arbor Acres® control and G4 Ross®308 broilers.

FCR% showed a significant difference (p<0.01) between G1 and G2 with no significant differences between G2 and G3 Arbor Acres® control, and a highly significant difference (p<0.01) between G4 and G5 with no significant differences between G4 and G6 Ross®308 control (Table 5). Meanwhile, on a weekly basis, FCR revealed a highly significant increase (p<0.01) on the 4th, 3rd, 2nd, 1st, and 5th week of age respectively in G1, a highly significant increase (p<0.01) on the 4th, 3rd, 2nd, 5th, and 1st week of age respectively with no significant differences between 2nd and 3rd weeks in G2, a highly significant increase (p<0.01) on the 4th, 5th, 2nd, 3rd, and 1st week of age respectively with no significant differences between the1st and 3rd weeks in G3, a highly significant increase (p<0.01) on the 5th, 2nd, 3rd, 4th, and 1st week of age respectively in G4, a highly significant increase (p<0.01) on the 4th, 5th, 3rd, 2nd, and 1st week of age respectively with no significant differences between the 1st and 2nd weeks in G5, and a highly significant increase (p<0.01) on the 4th, 3rd, 5th, 1st, and 2nd week of age respectively with no significant difference between the 3rd and 5th weeks in G6.

PI showed a significant difference (p<0.01) between G1, G2 and G3 Arbor Acres® broilers, and a highly significant differences (p<0.01) between G4 and G5 with significant differences (p≤0.05) between G4 and G6 Ross®308 control. PI (Table 5) showed also a highly significant increase (p<0.01) on the 5th, 4th, 3rd, 2nd, and 1st week of age respectively with no significant

Table 4 – Logarithm bacterial load (Mean ±SE) in intestine and breast muscles of different broiler breeds supplemented with selenium and green synthetized selenium nanoparticles.

Groups	Slaughters times / days	Intestinal Swabs		Breast Muscles	
		Log. TBC CFU/mL	Log. TEC CFU/mL	Log. TBC CFU/mL	Log. TEC CFU/mL
G1		5.0^{b}±0.049	3.3^{a}±0.127	3.5^{b}±0.043	1.7^{a}±0.072
G2		5.3^{a}±0.047	3.0^{a}±0.120	3.9^{a}±0.040	1.8^{a}±0.068
G3		3.4^{e}±0.049	2.2^{b}±0.127	3.0^{d}±0.043	0.9^{c}±0.072
G4		4.3^{d}±0.048	1.9^{b}±0.124	3.1^{cd}±0.042	0.9^{c}±0.071
G5		4.7^{c}±0.045	2.1^{b}±0.116	3.2^{c}±0.039	1.4^{b}±0.066
G6		3.4^{e}±0.048	2.0^{b}±0.124	3.1^{cd}±0.042	0.9^{c}±0.071
p value		0.000	0.000	0.000	0.000
Groups * Slaughter times					
G1	19th	4.4^{b}±0.25	2.4^{b}±0.61	3.0^{b}±0.09	1.3^{b}±0.36
	38th	5.7^{a}±0.02	4.2^{a}±0.03	4.1^{a}±0.02	2.1^{a}±0.03
G2	19th	3.9^{b}±0.07	1.4^{b}±0.60	3.2^{b}±0.09	0.5^{b}±0.31
	38th	6.6^{a}±0.01	4.5^{a}±0.02	4.6^{a}±0.02	3.1^{a}±0.02
G3	19th	3.1^{b}±0.05	0.0^{b}±0.00	3.5^{a}±0.21	0.0^{b}±0.00
	38th	3.8^{a}±0.03	4.4^{a}±0.01	2.5^{b}±0.04	1.8^{a}±0.01
G4	19th	4.3^{a}±0.14	0.0^{b}±0.00	3.2^{a}±0.06	0.0^{b}±0.00
	38th	4.3^{a}±0.02	3.9a±0.02	3.0^{b}±0.00	1.8^{a}±0.01
G5	19th	3.9^{b}±0.07	0.0^{b}±0.00	3.1^{b}±0.04	0.0^{b}±0.00
	38th	5.5^{a}±0.01	4.3^{a}±0.03	3.3^{a}±0.01	2.7^{a}±0.01
G6	19th	3.0^{b}±0.04	0.0^{b}±0.00	3.3^{a}±0.02	0.0^{b}±0.00
	38th	3.8^{a}±0.01	4.0^{a}±0.00	2.8^{b}±0.02	1.8^{a}±0.01
p value		0.000	0.000	0.000	0.000

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$).

G1=Arbor Acres® with 1 mL nano-selenium/1L drinking water, G2=Arbor Acres® with 1 mL selenium/1L drinking water), G3=Arbor Acres® control, G4= Ross®308 with 1 mL nano--selenium/1 L drinking water, G5= Ross®308 with 1 mL selenium/1L drinking water, and G6= Ross®308 control.

TBC=Total Bacterial Count, TEC=Total Enterobacteriaceae Count, CFU=Colony Forming Unit, SE=Standard error.

difference between the 3rd and 4th weeks in G1, a highly significant increase ($p<0.01$) on the 5th, 3rd, 4th, 2nd, and 1st week of age respectively in G2, a highly significant increase ($p<0.01$) on the 5th, 4th, 3rd, 2nd, and 1st week of age respectively with no significant difference between the 4th and 5th weeks and between the 2nd and 3rd in G3, a highly significant increase ($p<0.01$) on the 4th, 5th, 3rd, 2nd, and 1st week of age respectively in G4, and a highly significant increase ($p<0.01$) on the 5th, 4th, 3rd, 2nd, and 1st week of age respectively in G5 and G6.

The overall means of WG, FI, and PI indicated a highly significant increase ($p<0.01$) in Arbor Acres® (380.08g, 589.1g, and 5.98) compared to Ross®308 broilers (378.88g, 639.0g, and 5.67) respectively, meanwhile FCR revealed a highly significant increase ($p<0.01$) in Ross®308 (1.67%) compared to Arbor Acres® broilers (1.62%).

Histopathological architecture examination

Liver photomicrographs revealed in G1 (Figure 3a) and G2 (Figure 3b) thickening of liver capsule due to fibrinous exudation and mononuclear cell infiltration, hepatic cells showed degeneration with mild vacuolation of cytoplasm and mononuclear cell infiltration, as well as, mild hemorrhage in G2 broilers (Figure 3b). Meanwhile, the liver of G4 (Figure 3d) showed congestion of central vein, the hepatocytes showed mild degeneration, cytoplasmic vacuolation and mononuclear cell infiltration. The liver of G5 (Figure 3e) showed mild perihepatitis due to fibrinous exudation and mononuclear cell infiltration, hepatic cells showed mild degeneration, mild hemorrhage and mononuclear cell infiltration compared to Arbor Acres® control (Figure 3c) and Ross®308 control (Figure 3f).

Heart examination showed moderate fibrinous pericarditis which extend to myocardium resulting in myocardial degeneration with cytoplasmic vacuolation and mononuclear cell infiltration in G1 as revealed (Figure 4a) and G2 side by side to the presence of congestion and mild hemorrhage of cardiac muscle in G2 only (Figure 4b) compared

Table 5 – Performance indices (Mean ±SE) of different broiler breeds supplemented with selenium and green synthetized selenium nanoparticles.

Groups	Age / wk	WG / g	FI / g	FCR %	PI
G1		384.42^{ab}±9.53	608.41^{c}±0.002	1.72^{ab}±	5.72^{bcd}±0.11
G2		393.70^{a}±9.91	604.82^{c}±0.001	1.58^{bc}±	6.19^{ab}±0.19
G3		362.12^{b}±8.86	554.26^{d}±0.001	1.57^{bc}±	6.03^{abc}±0.23
G4		399.68^{a}±9.12	720.69^{a}±0.001	1.83^{a}±	5.48^{cd}±0.23
G5		373.62^{ab}±7.12	557.47^{d}±0.002	1.45^{c}±	6.34^{a}±0.15
G6		363.34^{b}±8.21	638.89^{b}±0.001	1.75^{a}±	5.19^{d}±0.18
p value		0.021	0.000	0.069	0.000
Groups*Age of birds					
G1	1st	74.3^{e}±6.87	104.8^{e}±0.001	1.58^{c}±0.22	0.90^{d}±0.11
	2nd	175.6^{d}±18.28	308.6^{d}±0.001	1.97^{b}±0.24	1.77^{c}±0.26
	3rd	497.9^{b}±36.17	711.9^{c}±0.000	1.49^{d}±0.10	5.67^{b}±0.60
	4th	425.6^{c}±28.60	894.4^{b}±0.001	2.20^{a}±0.16	5.81^{b}±0.40
	5th	748.7^{a}±15.86	1022.2^{a}±0.000	1.37^{e}±0.02	14.43^{a}±0.36
G2	1st	96.1^{e}±6.46	110.4^{e}±0.001	1.19^{d}±0.06	1.27^{e}±0.15
	2nd	245.1^{d}±19.57	323.6^{d}±0.001	1.40^{b}±0.12	3.01^{d}±0.35
	3rd	501.2^{b}±17.98	706.9^{c}±0.000	1.42^{b}±0.05	6.29^{b}±0.26
	4th	351.8^{c}±19.71	862.5^{b}±0.001	2.53^{a}±0.16	5.07^{c}±0.32
	5th	774.3^{a}±28.81	1020.5^{a}±0.001	1.33^{c}±0.04	15.32^{a}±0.73
G3	1st	90.3^{e}±3.38	119.5^{e}±0.000	1.33^{d}±0.04	1.03^{c}±0.06
	2nd	247.5^{d}±10.22	365.5^{d}±0.001	1.49^{c}±0.05	2.61^{c}±0.17
	3rd	519.2^{b}±25.03	685.0^{c}±0.001	1.35^{d}±0.07	6.90^{b}±0.48
	4th	409.4^{c}±29.32	812.6^{a}±0.001	2.07^{a}±0.14	6.62^{b}±0.51
	5th	544.2^{a}±42.41	788.5^{b}±0.001	1.59^{b}±0.22	12.98^{a}±1.15
G4	1st	86.8^{e}±6.86	115.8^{e}±0.001	1.42^{e}±0.13	1.03^{e}±0.12
	2nd	248.6^{d}±12.87	423.3^{d}±0.000	1.75^{b}±0.11	2.26^{d}±0.17
	3rd	553.9^{b}±20.88	901.2^{c}±0.001	1.64^{c}±0.06	5.76^{c}±0.29
	4th	706.5^{a}±13.86	1086.1^{a}±0.002	1.54^{d}±0.03	10.68^{a}±0.28
	5th	402.6^{c}±27.30	1076.9^{b}±0.000	2.78^{a}±0.18	7.68^{b}±0.60
G5	1st	103.6^{e}±3.72	125.8^{e}±0.000	1.22^{d}±0.04	1.23^{e}±0.07
	2nd	286.9^{d}±18.00	361.8d±0.001	1.30^{d}±0.07	3.53^{d}±0.5
	3rd	468.8^{c}±9.95	697.9^{c}±0.001	1.49^{c}±0.03	6.08^{c}±0.19
	4th	474.2^{b}±19.87	780.3^{b}±0.001	1.67^{a}±0.07	8.40^{b}±0.40
	5th	534.6^{a}±19.11	821.4^{a}±0.001	1.55^{b}±0.06	12.47^{a}±0.51
G6	1st	85.6^{e}±5.86	131.2^{e}±0.001	1.60^{c}±0.11	0.88^{e}±0.10
	2nd	258.3^{d}±11.36	363.7^{d}±0.002	1.43^{d}±0.06	2.81^{d}±0.17
	3rd	474.6^{c}±37.10	785.8^{c}±0.002	1.82^{b}±0.25	5.38^{c}±0.55
	4th	484.0^{b}±25.31	955.3^{b}±0.000	2.01^{a}±0.09	6.84^{b}±0.34
	5th	514.2^{a}±23.23	958.3^{a}±0.001	1.89^{b}±0.08	10.03^{a}±0.50
p value		0.000	0.000	0.002	0.000

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$).

G1=Arbor Acres® with 1 mL nano-selenium/1L drinking water, G2=Arbor Acres® with 1 mL selenium/1L drinking water), G3=Arbor Acres® control, G4= Ross®308 with 1 mL nano--selenium/1 L drinking water, G5= Ross®308 with 1 mL selenium/1L drinking water, and G6= Ross®308 control.

WG=Weight Gain, FI=Feed Intake, FCR=Feed Conversion Ratio, and PI=Performance Index, SE=Standard error.

to G3 control (Figure 4c). G4 Ross®308 broilers revealed (Figure 4d) mild pericarditis, mild myocardial degeneration with mild congestion, cytoplasmic vacuolation and mononuclear cell infiltration. Heart of G5 (Figure 4e) showed mild fibrinous pericarditis, myocardial degeneration with mild hemorrhage cytoplasmic vacuolation and mononuclear cell infiltration compared to G6 (Figure 4f).

The spleen of G1 and G2 (Figures 5a and 5b) revealed congestion of splenic sinus, mild hemorrhage with hemosedrosis, and moderate lymphoid depletion compared to G3 (Figure 5c). Meanwhile, G4 in Figure 5d revealed no pathological changes, and G5 showed moderate lymphoid depletion of spleen and hemorrhage (Figure 5e) compared to the normal picture in Ross®308 broilers of G6 (Figure 5f).

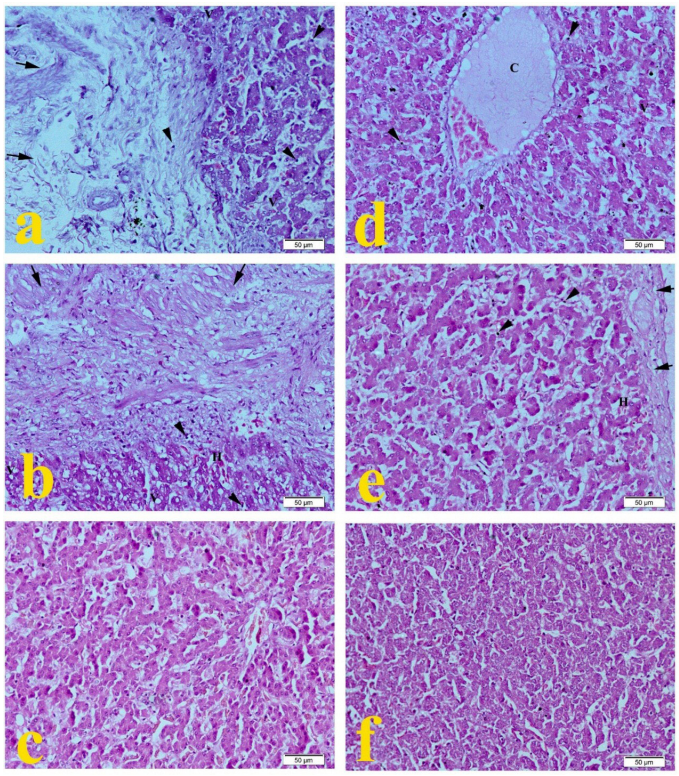

Figure 3 – Representative photomicrographs of liver histopathology (20×): (a) Liver of Arbor Acres® broilers supplemented with 1 mL nano-selenium/1L drinking water showing severe fibrosis (arrow), mononuclear cell infiltration (arrow head), vaculation of hepatocytes cytoplasm (V). (b) Liver of Arbor Acres® broilers supplemented with 1 mL selenium/1L drinking water. (c) Liver of control Arbor Acres® broilers. (d) Liver of Ross®308 broilers supplemented with 1 mL nano-selenium/1L drinking water. (e) Liver of Ross®308 broilers supplemented with 1 mL selenium/1L drinking water. (f) Liver of control Ross®308 broilers. H&E. Bar 50 µm.

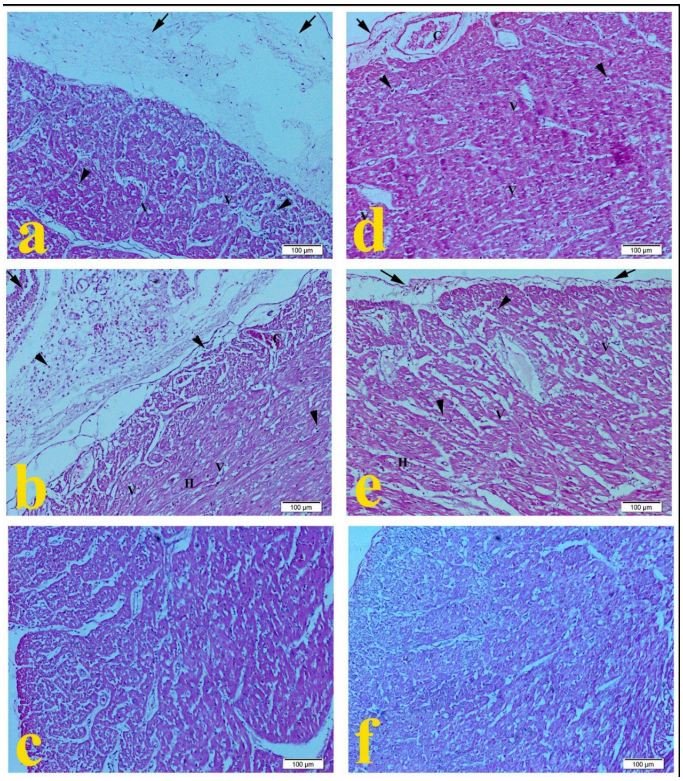

Figure 4 – Representative photomicrographs of Heart histopathology (10×): (a) Heart of Arbor Acres® broilers supplemented with 1 mL nano-selenium/1L drinking water showing fibrinous pericarditis (arrow), mononuclear cell infiltration (arrow head), vaculation of cytoplasm (V). (b) Heart of Arbor Acres® broilers supplemented with 1 mL selenium/1L drinking water. (c) Heart of control Arbor Acres® broilers. (d) Heart of Ross®308 broilers supplemented with 1 mL nano-selenium/1L drinking water. (e) Heart of Ross®308 broilers supplemented with 1 mL selenium/1L drinking water. (f) Heart of control Ross®308 broilers. H&E. Bar 100 µm.

Histopathological examination of the bursa of fabricious revealed severe thickening and hyperplasia of follicular epithelium with severe depletion of lymphoid follicles which are replaced by edematous fluid and fibrous tissue in G1 (Figure 6a). Severe hyperplasia of follicular epithelium with severe lymphoid depletion of lymphoid follicles were revealed in G2 (Figure 6b) in comparison to G3 control (Figure 6c). On the other hand, G4 in Figure 6d showed normal histologic structure of lymphoid follicles with increased interfollicular fibrosis, G5 (Figure 6e) showed moderate lymphoid depletion with mild necrosis of lymphoid follicles with increased interfollicular fibrosis compared to G6 control (Figure 6f).

Photomicrographs of thymus showed moderate depletion of lymphocytes in the cortex and medulla in G1 (Figure 7a), moderate to severe depletion of the lymphocytes in the cortex and medulla in G2 (Figure 7b) in relation to normal architecture in G3 (Figure 7c). G4 (Figure 7d) showed normal histologic structure of both the cortex and medulla as those in G6 Ross®308 broilers (Figure 7f), while G5 showed moderate depletion of lymphocytes in the cortex and medulla (Figure 7e).

DISCUSSION

Selenium has been considered an essential element required for multiple functions in broiler as productivity, growth, fertility, and prophylaxis via increasing immunoglobulin concentrations as reported by Del Puerto *et al.* (2017). Based on the European Union recommendation (2004) selenium has to be supplemented at 0.5 mg/kg dry matter to contribute a notable enhancement of performance, immunity, and antioxidant activity.

Broiler's behavior attested to an improvement in Ross®308 and Arbor Acres® broilers supplemented with 1 mL SeNPS (100 mg/1 L) as represented by higher feeding frequency, drinking duration and frequency, walking duration and frequency, and resting frequency. These finding were in agreement with Omidi *et al.* (2018) who reported that reduced selenium levels may create oxidative stress, contributing to an increase in the risk of behavioral disturbances. In

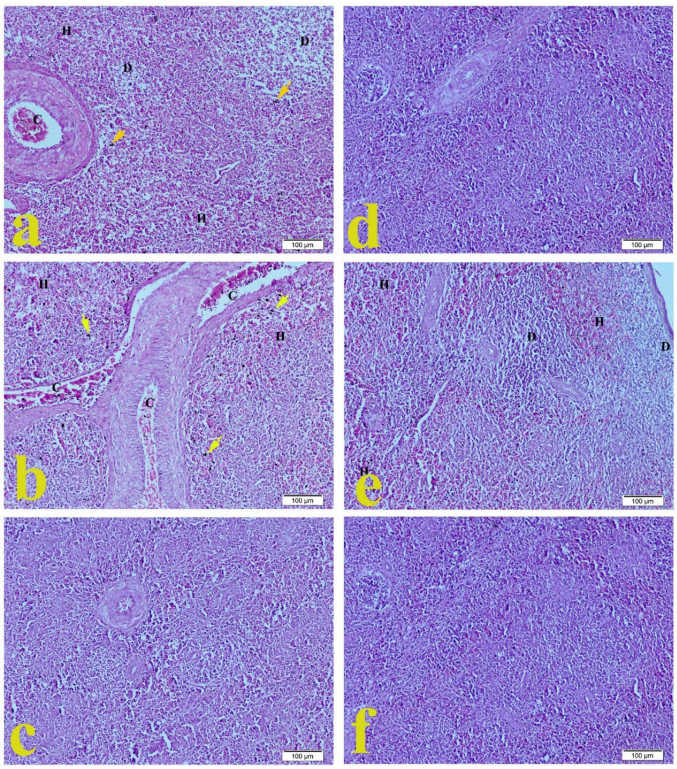

Figure 5 – Representative photomicrographs of Spleen histopathology (10×): (a) Spleen of Arbor Acres® broilers supplemented with 1 mL nano-selenium/1L drinking water showing congestion of splenic sinus (C), depletion of lymphocytes (D), mild hemorrhage (H), and hemosedrosis (yellow arrow). (b) Spleen of Arbor Acres® broilers supplemented with 1 mL selenium/1L drinking water. (c) Spleen of control Arbor Acres® broilers. (d) Spleen of Ross®308 broilers supplemented with 1 mL nano-selenium/1L drinking water. (e) Spleen of Ross®308 broilers supplemented with 1 mL selenium/1L drinking water. (f) Spleen of control Ross®308 broilers. H&E. Bar 100 µm.

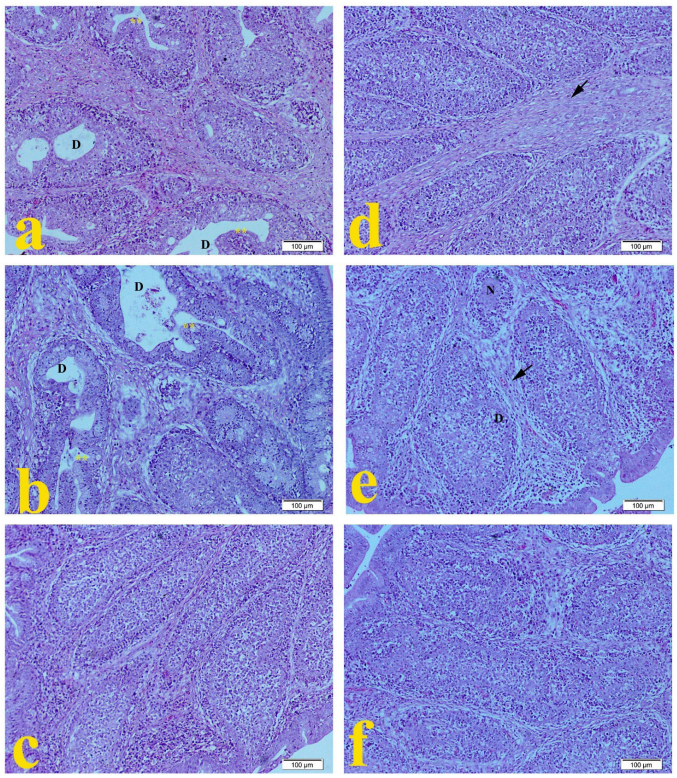

Figure 6 – Representative photomicrographs of Bursa histopathology (10×): (a) Bursa of Arbor Acres® broilers supplemented with 1 mL nano-selenium/1L drinking water showing depletion of lymphocytes (D), hyperplasia of follicular epithelium (**). (b) Bursa of Arbor Acres® broilers supplemented with 1 mL selenium/1L drinking water. (c) Bursa of control Arbor Acres® broilers. (d) Bursa of Ross®308 broilers supplemented with 1 mL nano-selenium/1L drinking water. (e) Bursa of Ross®308 broilers supplemented with 1 mL selenium/1L drinking water. (f) Bursa of control Ross®308 broilers. H&E. Bar 100 µm.

addition, selenium deficiency is linked to temperament deviations of animals (Ferencik & Ebringer, 2003).

The biochemical profile in Ross®308 and Arbor Acres® broilers supplemented with 1 mL SeNPS (100 mg/1 L) revealed significant increase in TP, ALB, ALT, and AST serum concentration with a special reference to Ross®308 broilers groups despite the variation noticed inside each group between the two slaughter times on the 19th and 38th days. The intergroup variation might be attributed to broiler's resistance and breed. The results were in agreement with those of Liu *et al.* (2017) who supplemented broilers with 0.0, 0.2, 0.4, 0.8, 2.0, 4.0, and 8.0 mg SeNPS in two mL 0.9% saline for 14 days, and found a significant increase in ALP, AST, and ALT serum concentrations. Aparna *et al.* (2017) also agreed with our findings, they used 0.075, 0.1125, 0.1875, and 0.225 mg SeNPS/kg feed to 150 broiler chicks and found an improvement in glutathione expression, enhancement in liver enzymes, and serum proteins.

Selenium is known to have immune regulatory functions that reduce the negative impact of stressors. The current study revealed a notable increase in IgG and IgM concentrations in Ross®308 and Arbor Acres® broilers supplemented with 1 mL SeNPS (100 mg/1 L), with more significant increase in Ross®308 broilers. The increase was significantly notable on the 19th day in treated Ross®308 broilers and on the 38th day in treated Arbor Acres® broilers. The results were in agreement to those of Ahmadi *et al.* (2018) who reported a significant increase in immunoglobulin of one-day old male Ross 308 chicks supplemented with 0.1, 0.2, 0.3, 0.4, and 0.5 mg SeNPS/kg feed. Bakhshalinejad *et al.* (2018) were also in agreement with our results, they used 0.4 mg SeNPS/kg for 1200 one-day-old male Ross broilers and found an enhancement in IgG concentrations. Zamani Moghaddam *et al.* (2017) proved a significant increase of immunoglobulin in broilers supplemented with 0.3 mg organic selenium /kg feed.

Glutathione (GSH) and SOD are considered major antioxidant defenses against oxygen reducing metabolites. Meanwhile, TAC and MDA usually increased relevant to oxidative and environmental stressors of broilers (Gangadoo *et al.*, 2018). Nano-selenium at rate of 1 mL (100 mg/1 L) was able to a meliorate TAC, SOD, and MDA activity in the recent study to alleviate the impact of microbial stress induced

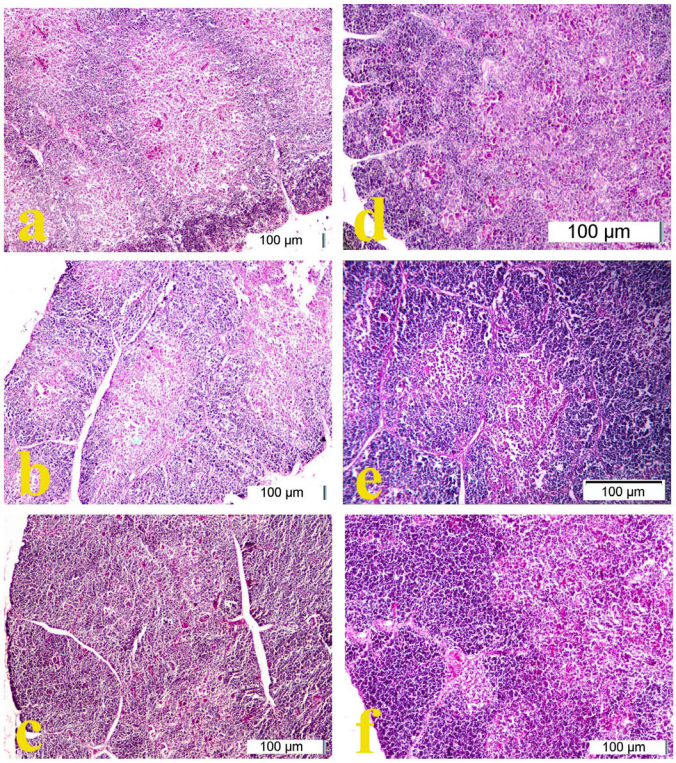

Figure 7 – Representative photomicrographs of Thymus histopathology (10×): (a) Thymus of Arbor Acres® broilers supplemented with 1 mL nano-selenium/1L drinking water showing depletion of lymphocytes. (b) Thymus of Arbor Acres® broilers supplemented with 1 mL selenium/1L drinking water. (c) Thymus of control Arbor Acres® broilers. (d) Thymus of Ross®308 broilers supplemented with 1 mL nano-selenium/1L drinking water. (e) Thymus of Ross®308 broilers supplemented with 1 mL selenium/1L drinking water. (f) Thymus of control Ross®308 broilers. H&E. Bar 100 µm.

by *E. coli* O157:H7 1.6 × 10^8 challenge on the 10^{th} day of age. The results were in agreement with Jiang *et al.* (2017) who reported that 0.3 ppm selenium-Yeast with 1.5% soybean or linseed oil was able to enhance carcass quality and oxidative stability even in the presence of stressors. Bakhshalinejad *et al.* (2018) reported outcomes supporting the current results, they used 0.4 mg SeNPS/kg in male Ross broilers and revealed a significant increase in GSH-Px, TAC, and MDA activity and formation.

TBC and TEC were significantly reduced in the intestine and breast muscles in the current study after supplementing Ross®308 and Arbor Acres® broilers with 1 mL SeNPS (100 mg/1 L). An intergroup variation in TBC and TEC counts with significant increases on the 39^{th} day were attributed to overwhelming microclimatic conditions. The reduction of overall pathogenic bacterial counts might be attributed to the ameliorated immunity conditions. The results agreed with those of Barko *et al.* (2017) who reported that SeNPS was able to modify intestinal microbiota to compete against pathogenic micro-organisms and restrict their survival. Underwood (2014) recommended a dose of 0.9 mg SeNPS/kg to encourage the beneficial growth and proliferation of opportunistic micro-organism in broilers' gut to overcome the growth of the pathogenic ones. The recorded antibacterial action of 1 mL SeNPS (100 mg/1 L) was supported by the in-vitro antibacterial action recorded by Gangadoo *et al.* (2016) using SeNPS against poultry pathogens including *Escherichia coli*. Trang *et al.* (2017) conducted an in-vitro study to evaluate the antibacterial action of SeNPS, in agreement with the current results, they found dose-dependent antibacterial action by using 10 µgSeNPS/mL against *Staphelococcus aureus*, *Listeria monocytogens*, E. coli O157:H7, and *Salmonella*.

The influence of selenium on productive performance in different broiler breeds are somewhat changeable. In our study, supplementing 1 mL SeNPS (100 mg/1 L) had an enhancing effect on the productive performance, despite of the individual variation among groups in relation to age. Similar results had been established to other studies as Senthil *et al.* (2015) and Selim *et al.* (2015), suggesting that addition of SeNPS both in diets or in drinking water was more effective compared to inorganic selenium to get better performance for broilers. However, our results were in contrary with the results recorded by Rao *et al.* (2013) and Chen *et al.* (2013), who recorded in their studies a non-significant difference in growth performance of broilers supplemented with SeNPS.

Our results revealed that 1 mL (100 mg/1 L) of inorganic selenium and selenium nanoparticles (SeNPs) were not able to enforce enough protection against *E. coli* pathognomonic lesions and in Arbor Acres® broilers represented as severe perihepatitis and pericarditis that extended to the myocardium resulting in myocarditis. Changes recorded in the liver and heart in the present study are consistent with those of Manimaran *et al.* (2003). Also, the recorded histopathological lymphoid depletion in bursa, thymus, and spleen was in agreement with the results recorded by Nakamura *et al.* (1990) who reported that *E. coli* infection induce damage in the immune systems of the chickens including lymphocyte depletion in both the bursa and the thymus.

Meanwhile, our results revealed a higher degree of protection against *E. coli* lesions in Ross®308 broilers supplemented with 1 mL (100 mg/1 L) SeNPs, as we recorded mild hepatic lesion, mild pericarditis, and no pathological changes were recorded in the spleen, bursa, and thymus. On the other hand,1 mL (100 mg/1 L) of inorganic selenium in Ross®308 broilers gave moderate protection against *E. coli* lesions as mild perihepatitis, mild pericarditis, and mild to moderate

lymphoid depletion of the spleen, bursa, and thymus. These results came inconsistent with Jia *et al.* (2005) who reported that selenium is an essential trace element that is commonly used as an antioxidant for animals, but there is a very narrow margin between the safe and toxic nutrient levels. Also, Qu *et al.* (2017) reported that, a new source of selenium is selenium nanoparticles (SeNPs) which exhibit the following properties: antioxidant, antibacterial activities, lower toxicity, better absorption and bioactivity. In addition, SeNPs decreased the oxidative stress in the hens, and improved the avian immune system status by alleviating the immunocompetent cells ability for an antigen challenge.

In the same line, selenium is a necessary trace element, which has antioxidant activity, this has been sustained by both clinical and laboratory experiments (Boostani *et al.* 2015).

CONCLUSION

The addition of 1 mL SeNPS (100 mg/L) rather than inorganic selenium (100 mg/L) per liter of drinking water was able to enhance some aspects of behavioral patterns, biochemical profile, immune and antioxidant functions, and decreased TBC and TEC inline with microbial stress from challenge and surrounding microclimate in Arbor Acres® and Ross®308 broilers with enhanced influence in Ross®308 rather than Arbor Acres® broilers.

By the end of this study, results provided too many new question marks to be answered as: can lower doses and concentrations of SeNPS in drinking water produce the same stimulatory influence on the broiler breeds used and other breeds? Can lower doses and concentrations of SeNPS in drinking water minimize the influence of stressors other than biological?

AUTHORS' CONTRIBUTIONS

ESS designed the experiment, participated in the preparation, executing the experiment, and in writing the manuscript. AAA participated in executing the experiment, and in writing the manuscript. RTH conducted the histopathological examination and participated in writing the manuscript, RAH participated in executing the experiment and in writing the manuscript, OMAB prepared SeNPS, ran the characterization, and participated in writing the manuscript. MSH participated in writing the manuscript.

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COMPETING INTERESTS

The author declared no conflict of interest.

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