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Original Research Article

Effects of Selenium Nanoparticles and Chitosan on Meat Quality, Lipid Profile, Tissue Mineral Content and Tibial Bone Morphometry in Heat Stressed Broilers

Key words

selenium;
nano-selenium;
chitosan;
muscle quality;
lipid status;
tibial bone;
broiler;
heat stress

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Abstract: This study aimed to assess the effects of selenium and selenium nanoparticles with chitosan on broiler chickens during heat stress. In this study, total 336 chicks were raised. These birds were split into seven groups, each with six sets of eight birds, depending on the treatments they received. There were two control groups: one with the regular diet (negative control) and another with the regular diet plus heat stress (positive control) known as A and B Groups respectively. The remaining groups were as follows: Group-C (Basal diet+0.3mg/kg selenium), Group-D (Basal diet + 0.3mg/kg nano selenium + heat stress), Group-E (basal diet+300mg/kg chitosan+heat stress), Group-F (basal diet+0.3mg/kg selenium+300mg/kg chitosan+ heat stress) and Group-G (0.3mg nano selenium + 300mg/kg chitosan/+basal diet + heat stress). The various parameters were analyzed, including drip loss, cooking loss, lipid profiles, mineral content, and bone characteristics was significantly improved in Group G, receiving nano selenium and chitosan under heat stress. Moreover, Group G showed higher selenium, calcium, and phosphorus content in breast muscle tissue, along with tibial bone characteristics such as weight, length, wall thickness, density, and medullary canal diameter as compared to group-B. Although weight/length index showed no significant differences, Group G demonstrated the highest Tibiotarsal Index (TTI) and Robusticity Index (RI). These findings suggest the beneficial effects of nano selenium and chitosan supplementation, particularly evident under heat stress conditions.

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Introduction

TDue to the environmental changes, the poultry industry is facing economic and production loss. It is associated that when the temperature crosses the normal range, birds adjust the metabolic heat to maintain their physiological status (1). Heat stress leads to physiological variation accompanied by hormonal changes and reduced feed intake that leads to a loss in production and physiological performance. Heat stress intensively enhances Reactive Oxygen Species (ROS) production in poultry that critically affects the chemical changes that indicates stress in birds (2).

Selenium is an important micro-mineral required in the trace by all animals. It plays a vital role in a wide range of physiological functions, primarily as a well-known antioxidant, used in poultry and small and large ruminants. The main source of this mineral comes from the soil present in animal feed (3).

The emergence of nanotechnology over the past three decades has revolutionized the understanding of drug delivery and its impact on livestock, particularly in poultry. This advancement has significantly contributed to Pathophysiology and treatment options (4). Due to its unique physio-chemical optical electric and thermal properties, selenium is widely used for drug delivery, enhancing bioavailability, as well as production, and performance. These physicochemical properties undergo transformation compared to the original material (5). The application of selenium nanoparticles has already demonstrated several benefits, such as enhanced absorption, enhanced bioavailability, antimicrobial activity, and positive effects on growth performance, Feed Conversion Ratio (FCR), decreased mortality rate, and minimized drip loss (6, 7). Moreover, Selenium supplementation positively effect on plasma lipid status, the lower level of Low Density Lipids (LDL), cholesterol, Triglycerides (TG) and High Density Lipids (HDL) (8).

Skeletal development plays a significant role in body growth. Recently it has been recognized that rapid body growth has adverse effects on bone mineralization (9). The carcass of certain broiler chickens exhibit distinct change in the appearance of breast meat, characterized by pale color and reduced water-holding capacity, as well as dark and dry muscles with altered functional properties (10). These alterations have significant implications for meat quality, leading to substantial economic cost and affecting's carcass characteristics, growth rate, and susceptibility to heat and transport stress during farming practices (11).

The addition of simple and selenium nanoparticles in poultry feed resulted the high absorption and an increase in selenium levels in serum and tissue (12). This improved retention of selenium has been associated with various benefits, including improved the productivity, morpho-histological architecture of intestine, lipid and immune profile, antioxidant profile, mineral absorption, and skeletal muscles and meat quality (13).

Chitosan, a natural polysaccharide derived from marine sources in the form of chitin, has gained considerable attention as a feed additive. It serve as both a drug delivery carrier and source of trace nano minerals (14). Chitosan exhibits multifunctional properties and finds application in animals nutrition (15). Supplementation of chitosan under heat-stressed conditions in poultry contributes to bone parameters (16) immune, antioxidant, performance, disease prevention, gastrointestinal health, and muscles parameters in poultry (17, 18).

Supplementation with selenium and chitosan has shown beneficial effects on poultry health and production. Therefore, this study aims to assess the impact of both selenium and nano-selenium combined with chitosan on lipid profile, meat quality, bone health, and mineral content in broiler chickens under heat stress conditions.

Material and methods

Ethics Statement

All experimental procedures involving the handling of birds in this study were conducted in concordance with the "Guidelines for Experimental Animals". The animal use protocol has been reviewed and approved by the Sindh Agriculture University Animal Ethical and Welfare Committee (DAS/2134/ of 2021).

Experiment Design

This study was performed on 336-day-old Cobb birds, purchased from the local market, and brought to the poultry experimental station, Faculty of Animal Husbandry and Veterinary Science, Sindh Agriculture University Tandojam Sindh. Before the arrival of the chicks, the proper brooding, feeding, temperature, and humidity about 35±1°C and 65% was sustained during brooding time gradually reduced thereafter according to the (19).

After the arrival of the chicks, the birds were weighed and placed together for brooding in deep litter system, all the feeding and management protocols accordance with brooding standards were followed as recommended by Aviagen, (19). On the 21st day, the chicks were divided in seven treatments groups. The treatment groups were as follows: Group-A: Negative control fed with Basal diet under normal temperature, Group-B: Positive control fed Basal diet under heat stress, Group-C: fed with 0.3mg/kg of selenium under heat stress, Group-D: fed with 0.3mg/kg selenium nanoparticles under heat stress, Group-E: fed 300mg/kg of chitosan under heat stress, Group-F: fed with selenium 0.3mg/kg and 300mg/kg of chitosan under heat stress and Group-G: fed with selenium nanoparticles 0.3mg/kg with chitosan 300mg/kg feed under heat stress.

Temperature Management

After arrival of the chicks, the temperature was maintained according the brooding standers recommended by (19). Initially temperature was maintained around 35±1°C on first day and relative humidity was about 65% and gradually decreased to reach a thermoneutral zone (26°C) after weekly reduction of 2.8°C with similar relative humidity at 21st day.

During the experiment, two environmental conditions were utilized from day 21st to 42nd day study period:

Table 1: Ingredients composition of the broiler diet

Ingredients (g/kg)	Starter/grower	Finisher
Corn	344.0	320.0
Rice polish	73.0	80.0
Canola meal	77.0	90.0
Rice tip	145.0	140
Corn gluten 60 %	35.0	40.0
Soybean meal	235.0	240.0
Limestone	11.0	10.0
D-L Methionine	1.2	1.0
L-Lysine	1.9	2.0
Threonine	1.3	1.5
Soy oil	17.0	18.5
DCP	13.0	15.0
Vitamin Premix	2.6	2.0
Molasses	43.0	40.0
Analyzed Nutritive Values		
ME, Kcal/kg	3000	3200
Dry Matter (%)	91.98	91.27
Crude protein (%)	22.93	20.40
Ash (%)	2.11	2.15
Crude fat (%)	4.01	5.05
Crude Fiber (%)	4.11	5.15

*The premix supplied per kg of diet: vit A, 12,000IU; vit-D3, 5000 IU; vit K, 2.55 mg; Vit, B1, 3 mg; Vit B2,7.5 mg; Vit B3, 51 mg, Vit B5, 13.5mg, vit B6, 4.5 mg; Vit B7, 0.2 mg, Vit B12, 0.02 mg; folic acid, 1.5 mg;; choline chloride, 250 mg

thermoneutral conditions (TN) set at 26°C and relative humidity 65±5%, where the cyclic heat stress applied to birds, with temperatures of 35°C for 8 hours and 26°C for the remaining hours of the day.

Vaccination

The vaccinations against infectious Bronchitis virus (IBV) (H120) were administered through drinking water on the first day, while vaccination against Newcastle disease were given on 8th and 21st day. Additionally, the chicks were vaccinated against flu (first day), and infectious bursal disease (IBD071IR; 14 and 23 days) during the period of study.

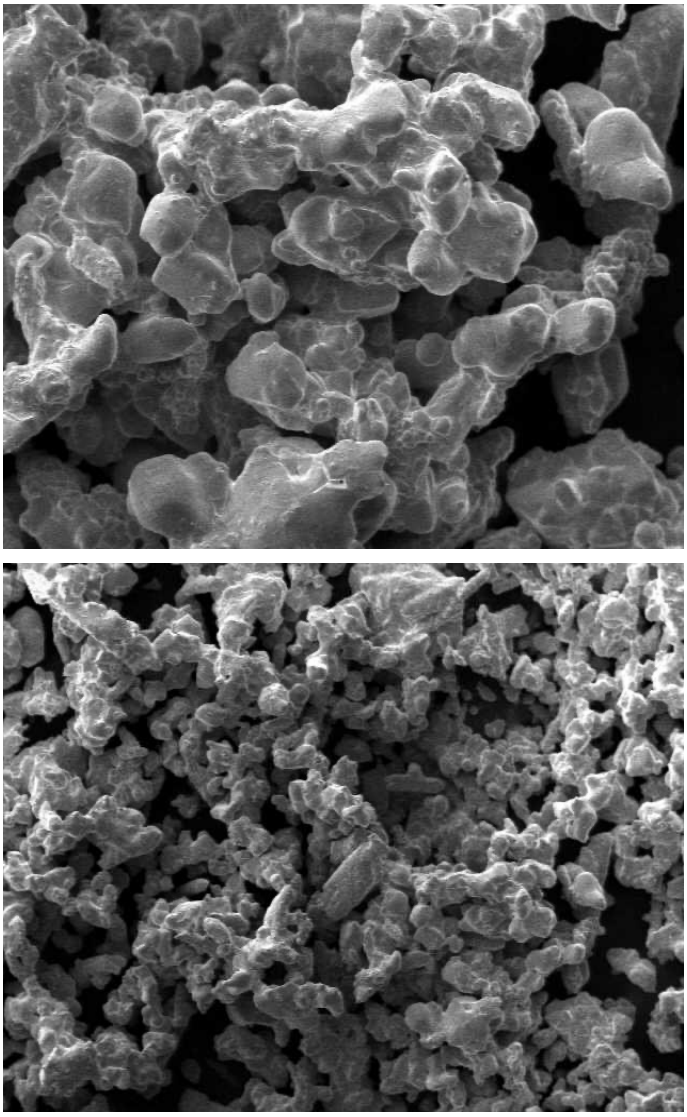

Figure 1: Scanning Electronic Microscopic (SEM) picture of selenium nano particles. Micrographs showing morphological texture of Nano particles on higher (a) and lower (b) resolution

Feeding plan

During the research, the birds were fed with commercial corn based diet according the recommendation by Aviagen, (19) from day 1st to 42nd day of the research. The birds were accessed to water and feed ad-lib. The proposed ratio of ingredients were formulated as recommended by NRC (20) guidelines (Table 1).

Simple Selenium, selenium nanoparticles and chitosan

Selenium (organic Se) was purchased from scientific store and sent to the National Centre of Excellence in Analytical Chemistry (NCEAC), University of Sindh Jamshoro for the

preparation of Nanoparticles, and for the confirmation of the synthesized, accuracy and size (range 100nm) different laboratory test were performed.

Procedure of Synthesis of selenium nanoparticles

Selenium nanoparticles were examined using low- and high-resolution scanning electron microscopy images (SEM), as shown in Figure 1 (a and b). Moreover, the elemental composition of selenium nanoparticles was analyzed using an EDX microanalysis device linked to the scanning electron microscope in chosen portions of SEM slices.

The procedure for the synthesis of simple selenium in nanoparticles separated Bovine serum, Albumin lipase and protease (*Sigma Aldrich Labs*) were purchased. Each protein aliquot (0.1g) was combined with 50 ml of sodium selenite (0.1g) and autoclaved at 121°C, and 15–20 pressure for 20 minutes under standard sterilizing conditions. Nanoparticles were purified by centrifugation at 12,000rpm for 10 minutes, and the supernatant was then discarded. The protein-containing nanoparticles were washed three times with deionized water at room temperature. Fourier-transform infrared spectroscopy and Scanning Electronic Microscopy were performed to verify the size and structure of the nanoparticles, as followed by (21);(22), and (23).

Scanning Electronic Microscopy of selenium nanoparticle

Scanning Electronic Microscopy (SEM) was employed to examine the morphology and form of selenium nanoparticles. A single drop of the nanoparticle suspension was placed on a sample holder for analysis.

Structural Analysis of Nanoparticles through Infrared

This technique was used to determine the surface characterization of selenium nanoparticles by using FTIR (Fourier Transformer Infrared) Spectrophotometer (Shimadzu 8400-Japan 1997). FTIR is a vital analytical technique that is usually used to study the surface functionalities of materials.

Sampling

On the 42nd day of the study, birds were randomly selected from each replications and after blood collection the birds were euthanized by cervical dislocation. The birds from each group (two birds from each replication) were randomly selected for sampling.

Blood samples for lipid status testing

Blood samples were collected form brachial vein before the euthanasia and the sample was shifted in the blood collecting tubes containing EDTA. For obtaining plasma, the blood samples were centrifuge at 1500rpm for 10minutes. Cholesterol concentration was determined by using cholesterol assay kit (Abcam-ab65390) following the methods (24, 25).

Water Holding Capacity (WHC)

The left superficial pectoral muscles about 1cm^3 were wrapped in a labeled in inflated plastic bags. Honikels gravimetric method (26) was used by measuring the WHC in meat through following formula.

Muscles pH

The sample of pectoral muscles were used for pH in two intervals of the time like 0 and 12hrs after slaughter, using the pH meter LOHAND Model-pH2601. The pH was obtained 2.5cm below the depth of surface.

$$\frac{WHC= W_1 - W_2 \times 100}{W_1}$$

Cooking Loss (CL) of meat

The 20gms of breast muscles (*Pectoralis Major*) samples were taken, weighted and cooked in hot water bath on internal temperature about 80°C for Cooking Loss (CL) as followed by (27). The CL was found according to the given formula.

$$\frac{CL\%= \text{Initial Weight -Final Weight} \times 100}{\text{Initial weight}}$$

Drip loss (DL) %

The collected meat sample from pectoral muscle was stored at 4°C for 24H, and expressed at the amount (%) of weight loss, this method was followed by (16, 28).

Bone parameters

The tibia bone of each broiler was separated and labeled carefully. The bone was boiled at 100°C in water for 10 minutes and cooled at room temperature. The bone were unfleshed, and air dried for 24 hours as followed by Harash et al., (29) and Yan et al., (30).

The tibia bone width and length were measured by a digital vernier caliper, and weight through electrical balance. Each bone diameter was measured outside and marked at middle of the bone body (Shaft). After breaking the bone at the mid-shaft, the diameter of the medullary canal was measured with a digital vernier caliper. The bone weight/ length index was determined by dividing the bone weight by its length and from the bone length and cube root of bone weight bone robusticity index was found outby Ndubuisi

et al., (31), Shim et al., (32) and (33)The index of tibiotarsal was determined from the diameter of the diaphysis and medullary canal (34).

Mineral concentration in muscles.

For determination of the concentration of minerals of muscles, the acid digestion of dried ash of pectoral muscles (1gram) was poured in HCl in a ratio of 1:10 and filtered with (Whatman Grade No.1) after the addition of Milli-Q water (Millipore Corporation, Bedford MA, USA) according to the method of (35, 36) and the final solution was adjusted with 50ml distilled water. The Selenium (Se) was analyzed through atomic absorption spectrophotometer (Varian SpectrAA 55B), and Calcium levels were measured through a flame photometer (Systonic-S-935), and phosphorus spectrophotometer.

Statistical Design

Data was presented as Mean ± Standard Error of Mean and was statistically analyzed using SSPSS (Version 20.0) and Turkey test was applied to determine the significant level in groups. The group means was analyzed using one-way ANOVA, with a significant level set at $p < 0.05$.

Results

Morphology and Texture Elucidation of selenium nanoparticles

SEM images show some rectangular and irregular textures of selenium nanoparticles. The EDX spectra disclosed a maximum percentage of selenium which suggests the successful nano selenium synthesis.

Fourier Transformer – Infrared Spectrophotometer (FTIR) studies

The strong absorption peaks at 1649 and 1551cm-1 signify the distinctive vibrations of amide I and C-H groups within the CH2 segments of the protein structure. These features are indicative of the presence of albumin, which serves as the stabilizing and enclosing element around the selenium nanoparticles (Figure 2).

Ultraviolet-Visible spectroscopy studies

The formation of selenium nanoparticles is observed through UV-Vis (Ultraviolet Visible Spectroscopy) with range wavelength from 200-800nm at various intervals. The UV–Visible of C spectrum protein washed with gel (SDS-Page Gel) having 30 kDa molecular weight molecular weight of 30 kDa, in this sense the peak absorption 210nm showed as peak or strong absorption in corresponding to peptide bonds and amino residues at 280nm and these reducing agents help in formation of nanoparticles (Figure 3).

Meat Quality

The effect of selenium, and nano selenium with chitosan on meat quality under heat stress shown in Table- 2. The initial and ultimate pH value of the meat were significantly improved in G followed by group F, D, E, A on the 42nd of study whereas the lowest initial and ultimate pH value (6.20±.03 and 5.43±.04) were observed in group B which was subjected to heat stress. Drip loss and cooking loss were positively affected by group-G exhibited the lowest drip loss (2.35±0.36 %) and cooking loss (35.43±1.56 %) followed by group F, D, E, A as compared with lowest values in group-B fed with basal diet and during heat stress.

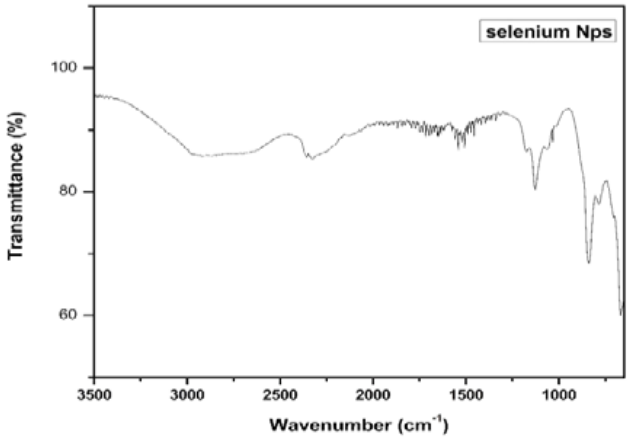

Figure 2: Fourier transformer – infrared spectrophotometer studies showed the strong absorption peaks (1649 and 1551cm-1)

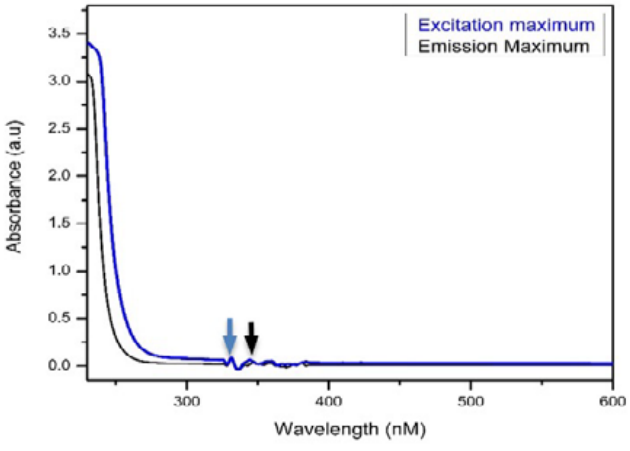

Figure 3: Ultraviolet Visible spectroscopy studies of selenium nanoparticles showing peak absorption 210 and 280nm

Table 2: Effect of selenium and nano selenium with chitosan on muscle quality and serum indices under heat stress

		Groups							P-value
		A	B	C	D	E	F	G	
Meat quality	pHi	6.30±.04^{ab}	6.20±.03^{ab}	6.44±.04^{ab}	6.58±.05^{ab}	6.34±.02^{ab}	6.46±.05^{ab}	6.56±.04^{a}	0.001
	pHf	5.96±.03^{a}	5.43±.04^{b}	5.73±.03^{ab}	5.75±.03^{ab}	5.65±.03^{ab}	5.92±.03^{ab}	6.10±.07^{a}	0.013
	WHC%	4.96 ±.29^{ab}	4.01 ±.33^{c}	4.82±.34^{b}	4.86 ±.17^{b}	4.91 ±.01^{ab}	4.97 ±.13^{a}	4.99 ±.23^{a}	0.012
	DL %	2.71±0.31^{ab}	2.95±0.62^{a}	2.65±0.33^{ab}	2.50±0.39^{bc}	2.56±0.43^{bc}	2.45±.060^{bc}	2.35±0.36^{c}	0.016
	CL %	40.42±2.4^{b}	40.90±1.21^{b}	39.15±0.32^{c}	38.98±2.21^{c}	39.57±0.41^{c}	37.34±2.96^{bc}	35.43±1.56^{d}	0.001
Serum indices	CHOL (mg/dl)	132.3±3.4^{b}	180±6.4^{a}	136.6±4.4^{bc}	130.7±6.3^{bc}	135±3.1^{bc}	115±5.6^{d}	112.6±4.6^{d}	0.023
	HDL (mg/dl)	61.7±2.3^{b}	53.5±1.5^{d}	58.1±1.2^{bc}	60.3±1.3^{bc}	59.3±1.6^{bc}	61.3±1.6^{b}	64.6±2.1^{a}	0.001
	LDL (mg/dl)	81.6±1.2^{b}	93.4±.2.1^{a}	60.2±1.3^{c}	45.4±2.1^{cd}	50.4±2.3^{cd}	41.6±1.3^{cd}	38.4±1.4^{e}	0.000
	TG (mg/dl)	60.2±2.3^{b}	63.3±1.9^{a}	56.6±2.7^{c}	54.8±2.4^{c}	55.8±1.7^{c}	51.9±.6^{cd}	50.2±.8^{d}	0.001

a–dmeans with different superscripts are significantly different(P<0.05) within the same row, HS= Heat Stress; Se= Selenium; Serum Lipids (mg/dL-1), NC* Negative Control and PC* Positive Control: NPs* Nanoparticles; WHC-Water Holding Capacity; pHi= Initial pH at 0 hour; pHu= pH Ultimate pH after 12hours; DL= Droop loss ; CL= Cooking loss, CHOL= Cholesterol, HDL-Hight Density Lipids, LDL- Low Density Lipids, TG- Triglycerides,

Table 3: Effect of selenium and nano selenium with chitosan on minerals in muscles of heat stressed of broiler

	Groups							P-value
	A	B	C	D	E	F	G	
Se (µg/g)	0.11^{f} ±0.03	0.08^{g} ±0.02	0.14^{e} ±0.01	0.21^{c} ±0.01	0.18^{d} ±0.01	0.24^{b} ±0.09	0.29^{a} ±0.01	0.01
Ca (mg/g)	0.22^{f} ±0.10	0.19^{g} ±0.09	0.23^{e} ±0.33	0.27^{c} ±0.06	0.25^{d} ±0.05	0.28^{b} ±0.03	0.29^{a} ±0.04	0.01
P (mg/g)	7.14^{f} ±0.06	6.81^{f} ±0.08	7.24^{de} ±0.06	7.37^{c} ±0.01	7.34^{cd} ±0.01	7.53^{b} ±0.02	7.66^{a} ±0.01	0.01

a–gmeans with different superscripts are significantly different(P<0.05) within the same row, HS= Heat Stress; Se= Selenium;), NC* Negative Control and PC* Positive Control: NPs* Nanoparticles; Se- Selenium, Ca- Calcium, P- Phosphorus

Serum Cholesterol Level

Table 2 presents effect of selenium and nano selenium with chitosan, on lipid status, specifically the cholesterol, under heat stress. The supplementation of nano selenium and chitosan improves the lipid profile in broilers. The cholesterol level was significantly improved in Group followed by group-F, D, C, E and A, whereas the lowest values were found in group-B.

The maximum LDL status was observed in group-G whereas, the minimum LDL level was observed in group-B fed with basal diet and heat stress. The maximum HDL level was observed in group G fed with Nanoselenium and chitosan during heat stress, while the HDL values were decreased in group B subjected to heat stress Triglyceride (TG) levels increased during heat stress, with the highest level observed in group B and the lowest level observed in group G.

Mineral indices

The deposition of minerals in breast muscle tissue content is affected by heat stress because of disturbance in absorption of minerals and physiological status. Table 3 shows breast muscle tissue selenium content was significantly higher (0.29±0.01µg/g) in group G followed by group F, D, E, C and A (0.24±0.09µg/g, 0.21±0.01 µg/g, 0.18±0.01µg/g, 0.14±0.09 and 0.11±0.03 respectively). The lower Selenium was found in group B where birds feed basal diet with heat stress (0.08±0.02µg/g). Whereas the maximum calcium content (0.29±0.04mg/g) was observed in group G followed by F, D, E, C and A resulted (0.28±0.03mg/g, 0.27±0.06, 0.25±0.05mg/g, 0.23±0.33mg/g and 0.22±0.10mg/g) respectively (Fig. 4.2). While the minimum calcium (0.19±0.09mg/g) was observed in group B fed with basal diet and heat stress. Table 3 shows the maximum phosphorous (7.66±0.01mg/g) in group G followed by group F, D, E, C and A calculated as 7.53±0.02mg/g, 7.37±0.01mg/g, 7.34±0.01 mg/g, 7.24 and 7.14±0.06mg/g respectively. While the

Table 4: Effect of selenium and nano selenium with Chitosan on tibia bone morphometry of under heat stress

Parameter	Groups							P-value
	A	B	C	D	E	F	G	
TBW (g)	5.29±.05^{ab}	4.65±.06^{d}	5.20±01^{bc}	5.33±.02^{bc}	5.10±.04^{bc}	5.90±.09^{ab}	6.15±.07^{a}	0.02
TBL (mm)	88.45±.04^{ab}	75.34±.29^{d}	85.33±.04^{c}	90.54±.15^{ab}	83.32±.06^{c}	90.67±.15^{ab}	94.55±.19^{a}	0.01
TBWT (mm)	6.57±.016^{a}	4.98±.049^{c}	5.63±.066^{b}	5..83±.013^{b}	5.59±.151^{b}	6.25.01±.042^{a}	6.65±.024^{a}	0.03
TBD (mm)	6.17±.01^{a}	5.21±.03^{c}	5.82±.05^{b}	6.12±.02^{a}	5.72±.0.8^{b}	6.47±.06^{a}	6.67±.06^{a}	0.01
MCD (mm)	4.10±.03^{ab}	3.41±.20^{c}	3.87±.04^{c}	4.09±.07^{b}	3.78±.30^{c}	4.32±.15^{ab}	4.78±.05^{a}	0.01
W/L index	46.51±.05	44.60±.02	44.91±.03	46.75±.04	45.93±.01	46.87±.13^{a}	46.97±.17	0.41
TTI	40.34±.23^{ab}	37.70±.76^{c}	39.01±.21^{ab}	41.55±.37^{ab}	40.60±.50^{ab}	41.86±.39^{ab}	43.86±.65^{a}	0.02
RI	48.22±.58^{a}	42.48±.20^{c}	46.21±.71^{b}	46.31±.51^{b}	45.53±.44^{b}	46.89±.35^{b}	48.49±.41^{a}	0.00

a–eWith in the same row, means with different superscripts are significantly different(P<0.05). Values represent the Mean ± SEM of five replicates. HS: Heat stress; COS: Chitosan ; Se: Selenium TBW- Tibia Bone Weight (TBL) Tibia Bone Length, TBWT Tibia Bone Wall Thickness; (TBD) Tibial Bone Density (MCD) Medullary Canal Diameter; (W/L I) Weight Length Index (TTI) Tibio Tarsal Index, (RI) Robusticity Index (N/C)*Negative control (PC*) Positive control. : NPs* Nanoparticle.

minimum phosphorous level (6.81±0.08mg/g) was noticed in group B. Results show that mineral indices were much affected by heat stress and selenium nanoparticles and chitosan alleviated the effects of heat stress.

Tibial bone morphometry

The tibial bone morphometry was analyzed to determine the effects of selenium and nano selenium with chitosan under heat stress, as shown in Table 4. Group-G exhibited significantly higher tibia bone weight (6.15±0.7 g) and tibia bone length (94.55±.19 mm) compared to group B, which had the lowest values (4.65±0.06 g and 75.64±0.29 mm respectively). The tibia bone wall thickness was also significantly affected by selenium and nano selenium with chitosan under heat stress. The maximum tibia bone wall thickness (6.65±0.02 mm) was observed in group G, while the minimum tibia bone wall thickness (4.98±0.49 mm) was observed in group B. Tibia bone density showed a statistically significant difference, with the highest density (6.67±0.06 mm) in group G and the lowest density (5.21±0.03 mm) in group B. The maximum medullary canal diameter (4.78±0.05 mm) was observed in group G, while the minimum diameter (3.42±0.20 mm) was observed in group B under heat stress. The weight/length index was not significantly affected by selenium and nano selenium with chitosan. The maximum tibiotarsal index (43.86±0.65) was observed in group G, while the minimum tibial tarsal index (37.70±0.76) was observed in group B. The robusticity index also showed significant differences, with the maximum index (48.49±0.41) in group G and the minimum index (42.28±0.20) in group B under heat stress.

Discussion

Selenium is an important mineral for maintaining animal health (37) as it plays various roles in physiological functions. In this study, we evaluate the effect of selenium and nano-selenium, along with chitosan on heat-stressed broiler. The hypothesis was that supplementation with different minerals and prebiotics would improve the physiological change induced by heat stress.

The results we found align with previous research. Some authors (38-40) had reported significant changes in pH and water holding capacity of muscles due to factors like lactic acid accumulation, glycolysis, ATP hydrolyzation, and hormonal release. Other research (35, 41) indicated that selenium in combination with chitosan affects the pH of the muscles, in the broiler because of the change in the muscles modulation. Wang et al., (39) concluded that heat exposure increased the release of hormones and accelerate the glycolysis resulting decline of pH. Additionally, studies by Oliveira et al., (42); Wang et al., (43), and Bakhshalinejad et al., (44) observed slight variations in meat pH within 24 hours due to the crucial role of muscle in absorbing and retaining water, which greatly impacts meat quality. Mir et al., (45) reported the pH directly influenced on meat quality aspects like tenderness, water holding capacity, color, juiciness, and shelf life and higher pH levels correspond to greater water holding capacity and that color can serve as an indicator of pH levels. However, Boiago et al., (46), and Calvo et al., (47) reported that organic selenium sources raise chicken meat pH, enhancing the muscle's ability to retain water within cells, which is crucial for muscle integrity and reduces drip loss (35, 48).

The drip loss and cooking loss values were significantly improved in group-G followed by Group-F, D, C, E and A fed with different levels of selenium and chitosan. These findings agreed with Zhou et al., (49) who concluded that birds fed 0.3 mg/kg had a lower loss due to improved cell membrane integrity. Other studies by Perić et al.,(48) and Boiago et al., (46), supported selenium's role in maintaining muscle cell integrity, promoting weight retention, and minimizing cooking losses. Cai et al.,(50) also highlighted who selenium nanoparticles improve the muscle's ability to attract and retain water within cells. Mir et al.,(45) emphasized the direct impact of water retention on muscle color and texture, considering it as crucial material for the body.

The Studies related to the muscles was mentioned by Zaboli et al., (51), Mohammadi et al., (52),and Calvo et al., (47) correlated that the breast muscle variability and color in males to pH effects, showcasing implications for shelf life, water holding capacity, and cooking loss. Observations from the study revealed that Group B (Basal Diet and Heat Stress) was highly affected by heat stress, resulting in increased drip and cooking losses. This correlates with findings from (49), (53),(1), (54),(51), and (41) indicating that heat stress decreases water absorption in muscles. Similar conclusions were drawn by Yang et al., (40) and Tahir et al., (55) showing that in broilers, selenium nano and chitosan decrease drip and cooking losses due to their water absorption capabilities.

It was observed from the present study that nano selenium has a noticeable effect on tissue selenium content. The selenium (Se), Calcium (Ca) and Phosphorus (P) content in muscles was significantly higher (P>0,05) in group-F fed with selenium nanoparticles and chitosan. The lowest tissue mineral content was found in group B (BD and HS) it may be due to slow turnover in tissue. Previous research highlighted that a dietary supplement of nano selenium at around 0.3mg/kg influenced both meat and blood selenium content (35, 56). This effect might stem from nanoparticles having better absorption rates compared to regular selenium, leading to higher elimination levels. Additionally (52),(12), and (57) reported that nano selenium enhances selenium concentration in muscle tissues, particularly during heat stress in broilers, echoing findings mentioned by Dukare et al.,(57).

Schrauzer, (58) emphasized that organic selenium deposition in tissues, especially in breast and thigh muscles, surpasses that of inorganic selenium, indicating a higher tendency for organic selenium to accumulate in these specific muscle areas.

The statistical analysis of lipid status within the groups revealed interesting trends. In Group-G, there was a notably favorable outcome for High-Density Lipoprotein (HDL), while Low-Density Lipoprotein (LDL) and Triglyceride levels were significantly higher in Group-B, followed by A, C, D, E, F, and G, in that order.

Selim et al., (2015) noted that LDL serum concentrations remained unaffected prior to heat stress, suggesting minimal changes under those conditions. Furthermore, (59) indicated that cholesterol and triglyceride levels showed significant improvements in trials involving different durations, particularly when a combination of selenium nanoparticles and increased chitosan levels was administered. Studies by Kassim et al., (60) and Bami et al., (41) supported the notion that supplementing selenium (at 0.3mg/kg feed) with chitosan resulted in higher concentrations and improved blood serum indices. Additionally, (61) reported that selenium nanoparticles had the potential to enhance plasma lipoprotein levels, particularly during heat stress, aligning with findings from Ahmad et al., (62).

Bones serve several vital roles in the body, providing support, housing blood cell generation, safeguarding organs, and storing essential minerals. These findings for bone morphometry revealed a direct correlation between bone quality and growth. Group-G showed significant improvements in weight, length, width, diameter, and bone indexes compared to other groups like group F, D, C, E and A. This indicates that the growth rate, muscle quality, and various performance parameters were notably enhanced in the group fed with selenium nano and chitosan compared to those given only selenium. This suggests that mineral intensity impacted the mineral content, length, and width of the tibial bone (63). These results agreed with Savaram et al., (64), Ndubuisi et al., (31), Shah et al., (33), and Rehman et al., (65) indicating that mineral supplementation positively affected tibial strength compared to control groups. Heat stress, as indicated by(32) alters mineral absorption, leading to more porous bones compared to treatment groups. Moreover, Shah et al., (33) and Naz et al., (66) found that mineral supplementation affected tibial length. Savaram et al.,(64) mentioned that a diet low in selenium content reduces bone volume, mineral density, and the weight of bones. Recently, Yang et al.,(67) explored the impact of nano selenium on bone health. They highlighted its importance in selenoprotein synthesis and its crucial role in proper growth and skeletal development. These collective findings underscore the significance of mineral supplementation, especially nano selenium, in enhancing bone quality, growth, and overall skeletal health.

Conclusion

In conclusion, this study demonstrated that supplementation with, selenium nano particles and chitosan had beneficial effects on hematology, muscle quality, pH, water holding capacity, drip loss, cooking loss, tissue mineral content, lipid status, and bone morphometry in heat-stressed broilers. Furthermore, this study highlights the potential

of nano selenium and chitosan as effective interventions to mitigate the negative impacts of heat stress on broiler production and performance.

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Učinki nanodelcev selena in hitozana na kakovost mesa, lipidni profil, vsebnost mineralov v tkivih in morfometrijo golenice pri toplotno obremenjenih brojlerjih

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Izvleček: Namen raziskave je bil oceniti učinke selena in selenovih nanodelcev s hitozanom na piščance brojlerje v času toplotnega stresa. V tej študiji je bilo vzrejenih 336 piščancev. Razdeljeni so bili v sedem skupin, vsaka je vsebovala šest enot, v katerih je bilo nastanjenih po osem piščancev, odvisno od zdravljenja, ki so ga prejeli. Bili sta dve kontrolni skupini: ena z običajno prehrano (negativna kontrola) in druga z običajno prehrano in toplotnim stresom (pozitivna kontrola), imenovani skupini A in B. Druge skupine so bile: skupina C (osnovna prehrana + 0,3 mg/kg selena), skupina D (osnovna prehrana + 0,3 mg/kg nanoselena + toplotni stres), skupina E (osnovna prehrana + 300 mg/kg hitozana + toplotni stres), skupina F (osnovna prehrana + 0,3 mg/kg selena + 300 mg/kg hitozana + toplotni stres) in skupina G (0,3 mg nanoselena + 300 mg/kg hitozana/+ osnovna prehrana + toplotni stres). Analizirani so bili različni parametri, vključno z izgubo vode iz mesa, izgubo vode pri kuhanju, lipidnimi profili, vsebnostjo mineralov in značilnostmi kosti, ki so se v skupini G, ki je prejemala nanoselen in hitozan pod vročinskim stresom, znatno izboljšali. Poleg tega so bile pri skupini G v primerjavi s skupino B ugotovljena višja vsebnost selena, kalcija in fosforja v prsni muskulaturi piščancev ter boljše lastnosti golenice, kot so masa, dolžina, debelina stene, gostota in premer medularnega kanala. Čeprav indeks mase/dolžine ni pokazal bistvenih razlik, sta bila pri skupini G ugotovljena najvišja tibiotarzalni indeks (TTI) in indeks robustnosti (RI). Te ugotovitve kažejo na ugodne učinke dodajanja nanoselena in hitozana, ki so še posebej očitni v razmerah vročinskega stresa.

Ključne besede: selen; nanoselen; hitozan; kakovost mišic; stanje lipidov; golenica; brojlerji; toplotni stres