Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Effect of dietary supplementation by selenium nanoparticles on immunological status of broiler chicken exposed to heat stress and its reflection on carcass quality.

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Received in 13/8/2025
Received in revised from 8/9/2025
Accepted in 23/9/2025

Keywords:

heat stress
selenium nanoparticles
broiler chickens
Salmonella and *E.coli*

ABSTRACT

This study was carried out to evaluate the efficacy of selenium nanoparticles (Se-NPs) at concentration of 0.5 mg/kg ration triggered with heat stress on biochemical parameters, antioxidant biomarkers of broilers and quality of its breast meat kept refrigerated at 2-5°C by determination of both hygiene and chemical quality measurements (total colony count, total coliform count, total mold and yeast count, (pH), Thiobarbituric acid reactive substances (TBARS) and Total Volatile Basic Nitrogen (TVN). Furthermore its effect on certain foodborne pathogens including Salmonella and *E.coli* was investigated. It was found that heat stress greatly reduced serum total protein, albumin, globulin, A/G ratio, glucose, cholesterol, triglyceride and superoxide dismutase (SOD). Meanwhile heat stress significantly increase liver enzymes (serum Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), renal products (uric acid and creatinine), serum malondialdehyde (MDA) of the living broilers and total colony, total coliform and total mold and yeast count of its breast meat. The dietary supplementation of Se-NPs provided a good mitigation action against heat stress which obviously detected in improvement of biochemical, antioxidant parameters and reducing the count of total colony count, total coliform count, mold & yeast count and antibacterial impact on Salmonella and *E.coli*.

INTRODUCTION

On a global scale, chicken has become the most popular meat in terms of both production volume and consumption rate **(Candra et al. 2025)** Poultry production has made significant progress in terms of meat and egg yields **(Lordelo et al. 2020)**. Keeping the optimum circumstances for poultry industry is a challenge for all poultry producers **(Hirakawa et al. 2020)**. One of these obstacles is the ambient temperature that considered a significant determinant in poultry production. Under typical conditions, most poultry species regard the temperature range of 16-25 °C to be their comfort zone. **(Diarra and Tabuaciri 2014).** Heat stress happens once an

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DOI: 10.21608/ejah.2025.461559

animal's body fails to regulate its body temperature because of the absence of sweat glands or feathering **(Akbarian et al. 2016)**. Elevated ambient temperatures cause oxidative injury to broiler liver tissues, further impairing metabolism of lipids **(Emami et al. 2020)**. Some biochemical parameters as glucose, cholesterol, triglyceride, ALT (alanine aminotransferase), AST(aspartate aminotransferase), albumen, serum creatinine level, MDA significantly increased when subjected to heat stress. **(Lu-Ping et al. 2022)**. Moreover, other parameters decreases which include superoxide dismutase SOD **(Salwa et al. 2023).**

With regard to of food safety, heat stress has a correlation with pathogen shedding and carriage in farms **(Dinan and Cryan 2012).** Contamination of poultry by-products is associated with increased feacal shedding **(Burkholder et al. 2008).** Heat stress results in a modification in the gasterointestinal microbiotia and decrease the integrity of the epithelium **(Verbrugghe et al . 2012).** Pervious study revealed that tissue of heat stressed chickens enhanced the attachment of food borne pathogen including Salmonella **(Rostagno 2009).**

Furthermore, heat stress decrease the quality of the meat due to many factors including oxidative reactions of unsaturated muscle lipids, low pH, deteriorated muscle protein and increase thiobarbituric acid, total volatile nitrogen, drift loss, color and shear force **(Baghban et al. 2017 and Ekunseitan et al. 2021).**

Some scientific advances and adaptive solutions are required to face heat stress in broiler industry as providing antioxidants and immune-boosting ingredients to the diet will protect broilers from heat stress **(Apalowo et al. 2024).**

Selenium (Se) is essential microelements in animal diet, it has a role in protecting cell membrane from oxidation **(Oliveira et al. 2014).** Selenium nanoparticles has peculiar advances such as low toxicity, high catalytic activity and absorption capacity. Consequently, the objective of this research was to evaluate the protective impact of Selenium nanoparticles on living and slaughtered broiler chickens against the adverse effect of heat stress.

MATERIALS and METHODS

Preparation of selenium nanoparticles (Se-NPs):

Nanoparticles of selenium have been formed in Nano materials Research and Synthesis Unit, Animal Health Research Institute, Agriculture Research Center, Giza, Egypt **(Vahdati and Tohidi 2020).** The formed Selenium nanoparticles were recognized, and their shape, morphology and particles size were evaluated using HRTEM techniques **(Atul et al. 2010).**

Characterization of selenium nanoparticles (Se-NPs)

The morphology and shape of selenium nanoparticles was performed using TEM characterization **(Atul et al. 2010)**. This resulting product showed a 265 nm transition point without a distinct maximum. (Figure 1).

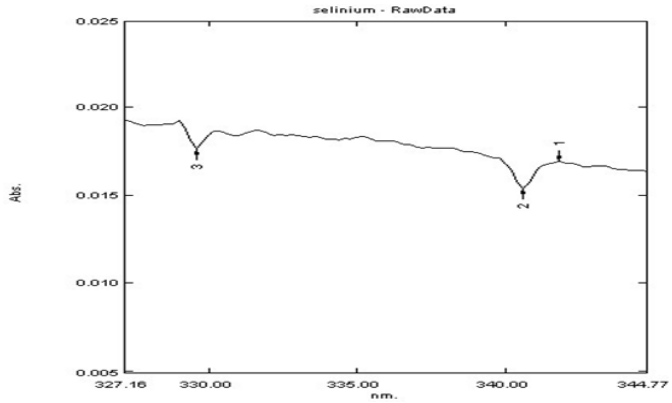

Figure (1) shows the UV spectrum of Se-NPs with absorption peak at 265nm

Experimental broiler chicks

Masa Hen Hatcheries, located in EL Mansoura, Egypt, supplied 100 Arbor Acres commercial broiler chicks that were one day old for this investigation. In fully isolated, sanitized, and disinfected experimental rooms, the chicks were grown on floors with daylight during daytime and some artificial illumination at night. The chickens were fed commercial broiler starter ration and tap water. Common vaccination program was given to the birds.

Experimental design

At one day old, chicks were randomly sorted into four groups, with 25 in each. Group 1 (Control): reared under optimum temperature. Group 2(Se-NPs): fed ration containing (0.5 mg/kg ration) Selenium Nanoparticles from the first day **(Khan et al. 2022)** until the experiment's completion. Group 3 (HS): exposed to 40º C at day 37 for 12 hours. Group 4 (HS+Se-NPs) : fed ration containing (0.5 mg/kg ration) Selenium Nanoparticles from the first day and exposed to 40º C at day 37 for 12 hours.

Samples:

Blood samples were taken from the wing vein and centrifuged at 3000 rpm for fifteen minutes to get serum.
Tissue samples were obtained from breast muscles from each group and divided into 5 portions .

Biochemical examination

At day 38, blood samples were collected and serum total proteins, albumin, serum uric acid, creatinine, triglyceride, cholesterol and glucose levels have been detected using methods based on **Vassault et al. (1999)** and determined using kits obtained from Biomed Diagnostics Co., Cairo, Egypt and spectrophotometer (Spekol 11, Germany).

Serum globulin was determined by subtracting serum albumin from total serum proteins, after which the albumin-to-globulin ratio obtained by splitting albumin by globulin. ALT (alanine aminotransferase) and AST (aspartate aminotransferase) levels in serum activities have been measured based on **Schumann et al. (2010)**. The level of serum malondialdehyde (MDA), serum superoxide dismutase (SOD) were measured according to **Ohkawa et al. (1979)** and **Nishikimi et al. (1972).**

Sensory analysis

Using a five-point rating system that took texture, color, and smell for consideration, the overall approval of the broilers breast muscle was verified. Sensory properties including color change (score 5 indicates no color change; score 1 indicates exceptional color change), smell (score 5 indicates amazing acceptable; score 1 indicates extremely unacceptable/off-odors), and texture (score 5 indicates rigid; score 1 indicates highly soft) were recorded by specialists (7-member trained panel). Overall approval was the middle for these scores, which were as follows: 5 for much acceptable, 4 for good, 3 for average, 2 for doubtful, and 1 for completely unacceptable. According to

shelf life standards, rejection would take place if the sensory characteristics fell under 4.0. **(Ojagh et al. 2010).**

Estimation of Total Colony Count :

According to **APHA (2001),** total colony counts were determined using the pour platting method with plate count agar. The plates were incubated for 48 hours at 35 ± 2°C.

Estimation of Coliform count:

According to **(ICMSF, 1996),** the samples were pour-plated on Violet Red Bile Agar (VRBA); purple-colored colonies typically observed with a surrounding purple zone measuring 0.5mm on un crowded plates were counted after 24 hours of incubation at 37°C.

Estimation of Total Mold and Yeast Count :

According to **APHA (2001),** using Sabouraud's dextrose agar mixed with chloramphenicol and oxytetracycline, the pour platting method was utilized to determine the total mold and yeast counts. The plates required a seven-day incubation period at 25°C.

Isolation and identification of Salmonella:

According to **(ISO 6579-1: 2017/Amend-1:2020)**, prepared sample was incubated in buffered peptone water broth at 37 ± 1°C for 18 ± 2 hours, then transferred to Rappaport Vassilidis broth (RV broth) and incubated at 41.5±1 ° C / 24 ± 3 hrs. One ml of enriched sample was plated on selective XLD agar and Brilliant Green agar and incubated at 37° C / 24 3 hrs. Salmonella colonies then isolated for biochemical confirmation.

Isolation and identification of *E. coli*:

According to **(ISO 16649-2 : 2001)** Eosin Methylene Blue (EMB) agar media was used for isolation. Suspected colonies for *E. coli* were morphologically and biochemically identified.

Estimation of pH value:

The pH value was calculated using the procedure outlined in **Zenebon et al. (2008).**

Estimation of Thiobarbituric acid :

The Thiobarbituric acid values were determined by the method employed by **Ying et al. (2016).**

Estimation of total volatile basic nitrogen (TVB-N):

The total volatile basic nitrogen (TVB-N) was assessed using the procedure outlined in **(Shokri et al . 2015).**

Statistical analysis

Variance analysis was used appropriately to assess the study's results and determine how the control and treated groups differed. At the $P < 0.01$ level, the one-way ANOVA analysis of variance was compared with the Fischer comparison test **(Sudarwati et al. 2019).** The results are expressed as mean ± SD. The trial repeated as triplicate.

RESULTS:

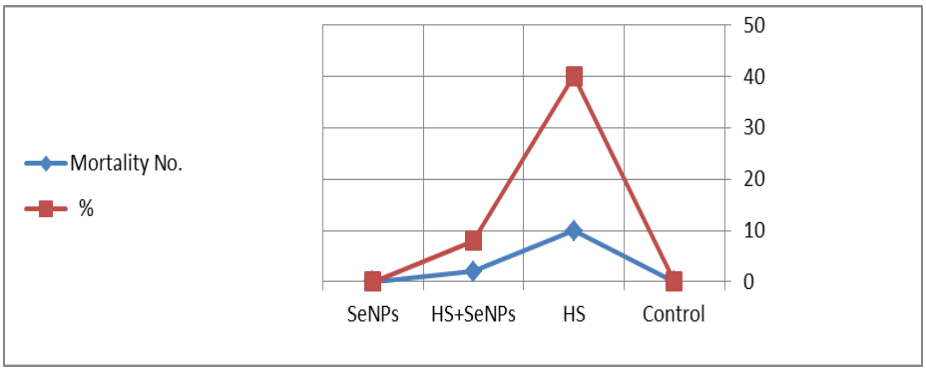

Figure (2) Mortality (no. & %) in different chicken groups.

Table 1. Impact of Selenium Nanoparticles (Se-NPs) and Heat stress (HS) on serum biochemical indicators

Parameter / Group	Total protein	Albumin	Globulin	A/G ratio	glucose	Tri glycer-ide	cholesterol
Control	3.66±0.2^{b}	1.98±0.21^{b}	1.68+0.15^{b}	1.17±0.01^{b}	140±2.9^{b}	140±0.9^{c}	186±0.2^{c}
HS	2.08±0.05^{a}	1.06±0.03^{a}	1.02±0.08^{a}	1.03±0.01^{a}	145±2.5^{c}	150±1.5^{d}	193±1.5^{d}
HS+Se-NPs	4.20±0.09^{c}	2.30±0.02^{c}	1.90+0.03^{bc}	1.21±0.02^{b}	138±0.3^{b}	133±0.7^{b}	180±1.7^{b}
Se-NPs	4.66±0.07^{d}	2.58±0.05^{d}	2.08+0.03^{c}	1.24±0.01^{b}	133±0.3^{a}	128±0.5^{a}	175±1.3^{a}

The result are regarded significant ($p<0.05$) when the same column included differing small letters.

Table 2. Impact of Se-NPs and Heat stress on liver, kidney functions and super oxide dismutase parameters.

Parameter / Group	ALT	AST	Creatinine	Uric acid	MDA	SOD
Control	9.72±0.08^{a}	120±0.9^{a}	0.54±0.02^{a}	5.5±0.01^{a}	3.5±0.05^{b}	92±0.7^{c}
HS	15.25±0.1^{c}	150±1.3^{c}	0.8±0.01^{c}	8.2±0.02^{c}	6.2±0.19^{d}	70±0.34^{a}
HS+Se-NPs	12.76±0.09^{b}	133±1.22^{b}	0.63±0.02^{b}	6.6±0.13^{b}	4.3±0.11^{c}	87±0.52^{b}
Se-NPs	9.23±0.6^{a}	118±1.5^{a}	0.5±0.02^{a}	5.3±0.11^{a}	3.0±0.05^{a}	98±0.25^{d}

The result are regarded significant ($p<0.05$) when the same column included differing small letters.

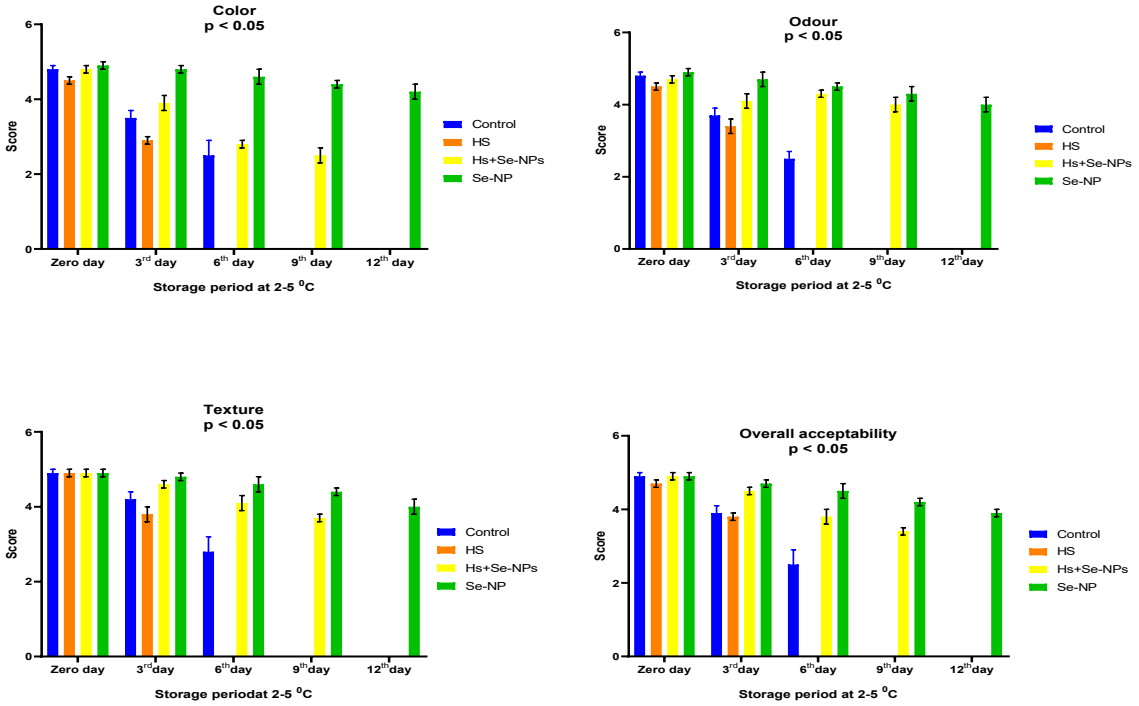

Figure (3) Sensory attributes (color, odor, texture and overall acceptability) of tested groups of chicken breast meat samples stored at chilling temperature (2-5°C)

Table 3. Total colony count of chicken breast meat sample stored at chilling temperature (2-5 °C)

Group Storage period	Control	HS	HS+Se-NPs	Se-NPs
Zero day	4.65±0.04 ^{b}	4.97±0.04^{c}	4.49±0.06^{b}	3.63±0.08^{a}
3rd day	4.83±0.07^{b}	5.87±0.04^{c}	4.67±0.08^{b}	3.88±0.08^{a}
6th day	5.63±0.04^{c}	S	4.91±0.06^{b}	4.36±0.07^{a}
9th day	S	S	5.71±0.04^{b}	4.83±0.09^{a}
12th day	S	S	S	5.3±0.05

The result are regarded significant ($p<0.05$) when the same row included differing small letters.

Table 4. Coliform count of chicken breast meat samples stored at chilling temperature (2-5 °C)

Group Storage period	Control	HS	HS+Se-NPs	Se-NPs
Zero day	1.76±0.02^{bc}	1.92±0.02^{c}	1.61±0.01^{b}	1.36±0.01^{a}
3rd day	1.99±0.01^{b}	2.97±0.01^{c}	1.91±0.03^{b}	1.64±0.01^{a}
6th day	2.73±0.01^{c}	S	2.25±0.02 ^{b}	1.81±0.02^{a}
9th day	S	S	2.83±0.01^{b}	1.91±0.01^{a}
12th day	S	S	S	2.79±0.01

The result are regarded significant ($p<0.05$) when the same row included differing small letters.

Table 5. Total mold and yeast count of chicken breast meat samples stored at chilling temperature (2-5 °C)

Group Storage period	Control	HS	HS+Se-NPs	Se-NPs
Zero day	2.39±0.01^{b}	2.72±0.02^{c}	2.23±0.02^{ab}	2.17±0.01^{a}
3rd day	3.11±0.02^{b}	3.89±0.02^{c}	2.94±0.02^{b}	2.68±0.02^{a}
6th day	4.13±0.02^{c}	S	3.62±0.03^{b}	3.23±0.03^{a}
9th day	S	S	4.21±0.02^{b}	3.87±0.03^{a}
12th day	S	S	S	4.27±0.02

The result are regarded significant ($p<0.05$) when the same row included differing small letters

Table 6. Impact of Se-NPs on Salmonella and *E.coli* isolation from chicken breast meat samples

Group Microorganism	Control	HS	HS+Se-NPs	Se-NPs
Salmonella	+ ve	+ ve	- ve	- ve
E.coli	+ ve	+ ve	- ve	- ve

Table 7. Mean value of pH, TBA (mg/kg) and TVN (mg%) in the examined chicken breast meat samples stored at chilling temperature (2-5 °C)

Group Parameter & storage period		Control	HS	HS+Se-NPs	Se-NPs
pH	Zero day	5.80±0.02[a]	5.62±0.01[b]	5.76±0.01[a]	5.79±0.04[a]
	3rd day	6.28±0.01[b]	6.43±0.03[c]	6.19±0.03[b]	6.08±0.02[a]
	6th day	6.57±0.02[c]	S	6.29±0.01[b]	6.17±0.12[a]
	9th day	S	S	6.51±0.03[b]	6.28±0.04[a]
	12th day	S	S	S	6.43±0.02
TBA (mg/kg)	Zero day	0.53±0.03[b]	0.68±0.0[c]	0.42±0.01[a]	0.39±0.01[a]
	3rd day	0.74±0.01[b]	0.93±0.01[c]	0.67±0.03[b]	0.55±0.01[a]
	6th day	0.98±0.02[c]	S	0.86±0.02[b]	0.71±0.02[a]
	9th day	S	S	0.99±0.0[b]	0.84±0.0[a]
	12th day	S	S	S	0.95±0.02
TVN (mg%)	Zero day	11.7±0.05[c]	16.5±0.0[d]	9.3±0.04[b]	7.6±0.09[a]
	3rd day	16.1±0.02[c]	22.0±0.04[d]	13.5±0.02[b]	10.01±0.05[a]
	6th day	22.0±0.04[c]	S	17.7±0.12[b]	14.31±0.08[a]
	9th day	S	S	23.2±0.04[b]	18.8±0.06[a]
	12th day	S	S	S	21.5±0.04

The result are regarded significant (P<0.05) when the same row included differing small letters S: Spoiled

DISCUSSION:

Numerous alterations in blood biochemistry, oxidant-antioxidant, and meat quality parameters were brought about by raising broiler chickens in a heat-stressed atmosphere **(Cartoni Mancinelli et al. 2023).** Many studies emphasis on using nano-additives to mitigate the adverse effect of heat stress **(Al-Thuwaini et al. 2022).** Selenium nanoparticles has earned attention by its potential advantages in poultry industry **(Mahmoud et al. 2024).**

Mortality rate:

The data showed in figure (2) represent the highest mortality rate was shown in group exposed to heat stress (HS) by (40% (10 chickens dead)) when compared to group **treated** with Se-NPs and exposed to heat stress (HS+Se-NPs) by (8 % (2 chickens dead)). This results was in accordance with **Azoulay et al. (2011)** and **Brossi et al. (2018)** that are declared that the higher death rate among birds subjected to heat stress may be from heat stroke and losing of water in tissues and organs. Groups took Se-NPs and exposed to heat stress showed improvement in the mortality rate and this might be antioxidant effect of Se-NPs which improve tissue structure.

Biochemical profile:

Biochemical parameters of broiler chickens suffered heat stress with or without treatment with Se-NPs was presented in Table (1) the data showed no significant (p<0.05) differences in total protein, albumin, globulin and A/G ratio between the control and Se-NPs groups. However, HS group chickens was significantly (p<0.05) decreased total protein, albumin, globulin and A/G ratio at 12 hours post heat stress compared to those of the control

group. Since the liver is the primary location for protein production, so these results are the implicated feature for impaired liver function due to heat stress **(Huang et al. 2018).** The results obtained are in accord with the research conducted with **Salwa et al. (2023)**. Furthermore, HS+Se-NPs and Se-NPs groups showed a notable amelioration in total protein, albumin, globulin, A/G ratio with heat stressed broiler chickens this findings is in agreement with pervious observations noted by **Abdel-Moneim et al. (2021)** , who documented elevation in total protein, albumin, globulin, A/G ratio with chickens treated with Se-NPs and subjected to heat stress. Se-NPs preserve and boost growth performance by minimizing the bad effect of heat stress on the development of skeletal muscle. **(Tang et al. 2018).**

The biochemical investigation of broiler chickens underwent heat stress with or without treatment of Se-NPs was presented Table 1 that revealed no significant (P<0.05) variations in glucose, triglycerides and cholesterol levels comparing the control and Se-NPs groups. On the other hand, HS group chickens was significantly (P<0.05) increased levels of glucose, triglycerides and cholesterol at 12 hours post heat stress compared to those of the control. This observation of elevation of glucose level is in agreement with **Awad et al. (2019)**, who recorded elevation in glucose level in heat stressed broiler chickens, as catecholamines and glucocorticoides are released in response to stress in birds, this would contribute directly in elevation in concentration of glucose level **(Wasti et al. 2020).** Elevated serum triglyceride in heat stress may be due to its effect in decreasing the capacity for lipolysis together with increasing TG transport from the liver to extrahepatic tissue and fat accumulation in the abdomen **(Lan et al. 2022)**. Hypercholestermia due to heat stress resulted from steatosis of hepatocytes **(Lu et al. 2019)**

Consequently, HS+Se-NPs and Se-NPs groups reported a marked improvement in glucose, triglycerides and cholesterol values, these findings concurred with **(Abdel-Moneim et al. 2021)** who noticed the hypolipimic action of Se-NPs. The reduction in cholesterol and triglyceride may attributed to the capability of Se-NPs to minimize their intake and formation in the host gut **(Hamza et al. 2020)**. On other hand Se-NPs lowers blood cholesterol level by its influence in the function of co enzyme HMG and cholesterol receptor synthesis which have a regulator effect of blood lipids levels **(Safdari-Rostamabad et al. (2017)).**

Liver and kidney functions:

Hepatic and renal functions in heat stressed broiler chickens with or without selenium nanoparticles (Se-NPs) treatment were presented in Table 2, liver and kidney functions did not significantly (P<0.05) affected by dietary Se-NPs supplementation compared to control group. Serum biochemical parameters of liver function test revealed a significant (p<0.05) rising in ALT and AST levels in HS group compared to those of the control group. This results was in line with **Akinyemi and Adewole (2022)**, this alterations may be attributed to hepatobiliary damage due to heat stress **(Will castero et al. 2016).**

Notably, a sig-nificant (P<0.05) decrease in serum levels of ALT, AST was noticed in the HS+Se-NPs and Se-NPs groups when compared with the heat stressed group. And this results was in agreement with **Safdari-Rostamabad et al. (2017)** and **Salwa et al. (2023).**

A significant (P<0.05) elevation in uric acid and creatinine serum level was observed in HS group com-pared to those of the control group. Increased serum creatinine associated with hyperuricemia were in agreement with **Huang et al. (2018)** and **Akinyemi and Adewole (2022)** who demonstrated that alterations resulted from kidney damage due to stress.
Remarkably, a sig-nificant (P<0.05) decrease in serum levels of uric acid and creatinine in the HS+Se-NPs and Se-NPs groups when compared with heat stressed group, indicating that Se-Nps play important role in enhancing the renal function by reducing creatinine and uric acid concentration **(Sheiha et al. 2020)**.

Oxidant-Antioxidant profile.

Data displayed in Table 2 revealed Se-NPs administration didn't result in any significant

(P<0.05) impact on serum oxidant/antioxidant biomarker mean values compared to control group results.

Heat stress result in generating of massive quantities of reactive oxygen species (ROS) which in turn produce cellular oxidative stress, linked to apoptosis damaging to DNA, proteins and cell phospholipid membrane **(Cruvinel et al. 2023**). In this investigation, HS chickens showed a considerable decreases in se-rum superoxide dismutase SOD level, with a considerable raise in the MDA content, in comparison to control group. This results is in line with **Yang et al. (2023)** and **Gouda et al. (2024)**. Trace elements alleviate adverse effect of heat stress by decreasing free radicals and preventing lipid peroxidation **(El-Ratel et al. 2023**). Interestingly, in this study, HS+Se-NPs and Se -NPs groups significantly ameliorated heat stress changes in the oxidant/antioxidant biomarkers, contrast to the matched results of the HS group. This consistent with the end results of **Salwa et al. (2023)**, who claimed that administration of Se-NPs lessen oxidative stress by decrease MDA enzyme activity and enhance serum oxidant status by increasing serum SOD activity to broilers exposed to heat stress.

Sensory evaluation:

As demonstrated in figure (3), The samples obtained from treated group with Se-NPs showed the best acceptance score, followed by HS+Se-NPs samples, according to a significant difference (p<0.01) between groups. Conversely to control group, which was rejected on the 6^{th} day of chilling storage based on sensory evaluation. Heat stress before slaughter can alter muscle metabolism and membrane integrity in broiler chickens, resulting in disappointing meat traits and decrease its quality **(Tang et al . 2013)**. HS group rejected at 3^{rd} day of chilling storage.

Total colony count:

Total colony counts are used to evaluate the microbial load of poultry products for dictating meat quality and providing information on product safety **(Jay 2002)**. The maximum total bacterial count of chilled poultry carcass established by Egyptian Organization for Standardization **(EOS 1651 : 2005)** is 10^5 cfu/g. In this study, results of total colony count shown in Table 3 demonstrated a significant elevation on total colony count in HS group in 3^{rd} , 6^{th}, 9^{th} and 12^{th} days of storage compared to control group. This result correlated to increase lipid peroxidation due to heat stress which can led to spoilage with increase microbial growth **(Ercolini et al. 2006).**

Additionally, the addition of Se-NPs in feed cause notable decrease (p<0.05) on the average values of total colony count in HS+Se-NPs and Se-NPs groups in 3^{rd}, 6^{th}, 9^{th} and 12^{th} days of storage compared to the HS group samples. This results were compatible to **Hassan et al. (2020)**, who identified a notable detrimental effect of Se-NPs on hazardous microbes such as *E coli*. The antibacterial effect of Se-NPs generated from it affects DNA replication, protein synthesis and metabolism and affecting the plasma membrane's function and integrity of bacteria and therefore, decrease its total count **(Pescuma et al. 2023)**. Se-NPs has reduction effect on intestinal total bacterial count and exhibited antibacterial properties against, *E.coli* and Salmonella **(Abdel-Moneim et al. 2022)**.

Total coliform count:

The maximum total coliform count of chilled poultry carcass established by Egyptian Organization for Standardization **(EOS 1651 : 2005)** is 10^2 cfu/g. Data presented in Table 4 revealed noteworthy rising (p<0.05) on the average values of total coliform count in HS group in zero , 3^{rd} , 6^{th}, 9^{th} and 12^{th} days of storage compared to control group. Increase total coliform count is an indicator of inferior quality of chicken meat and presence of enteric pathogen, which constitute public health hazard **(Chaem et al . 2002)**.

Simultaneously, a marked reduction (p<0.05) on the average values of total coliform count in HS+Se-NPs and Se-NPs group samples in zero , 3^{rd} , 6^{th}, 9^{th} and 12^{th} days of storage compared to the HS group samples.

This results correlates to the antibacterial action of Se-NPs **(Hassan et al. 2020)**.

Total mold and yeast count:

Mold and yeast count used to determine meat quality before use **(Salem et al. 2018)**. Mold in food is a term used to express the fungal spore which are prevalent in the environment and enter to the food chain of the bird through dust, air and water. These fungi related to mycotoxin production and their presence in food samples raises significant threats to public health **(Benedict et al. 2016).** The mold and yeast count ranges from 1.87 to 2.52 log cfu/g in the freshly slaughtered chicken meat **(Santosh et al. 2012)**. Results achieved in Table 5 declared that total mold and yeast count showed a significant exaltation in HS group in zero, 3rd, 6th, 9th and 12th days of storage compared to control group.

Coincidentally, decline ($p<0.05$) on the mean values of total mold and yeast count concurrently in HS+Se-NPs and Se-NPs group samples in zero , 3rd , 6th, 9th and 12th days of storage. This results is agreement with **Al-Quwaie (2023)** who declared that using Se-NPs decreased total yeast and mold count and **Kheradmand et al. (2014)** and **Yip et al. (2014)** who revealed that Se-NPs has antifungal properties against *candida albicans* and *Trichophyton rubrum*, respectively. Se-NPs increase superoxide radical level inside fungal cell causing its denaturation and finally its death **(Vera-González** and **Shukla 2020).**

Isolation of Salmonella:

One of the most frequently occurring bacteria responsible for foodborne diseases, widely linked to outbreaks is Salmonella which is responsible for over 1.3 billion reported cases of Salmonellosis and 155,500 deaths worldwide each year **(Sun et al. 2021)**.

Salmonella was recovered from control and HS group samples as tabulated in Table 6. On other side Salmonella wasn't detected in HS+Se-NPs and Se-NPs group samples. Heat stress cause imbalance in the gut microbial community, microbiota dysbiosis and increase prevalence of *E.coli* and Salmonella **(Jayda et al. 2024)**. The results was concurred with **Allam et al. (2024)** who reported that supplementation of Se-NPs reduce colonization and prevalence of Salmonella in the caecum and droppings of broilers.

Isolation of *E.coli*:

E.coli were isolated from HS and control groups' samples (Table 6). On other hands *E. coli* could not be recovered from HS+Se-NPs and Se-NPs groups' samples. This results emphasis that Se-NPs has antibacterial effect against food borne pathogen **(Perumal et al. 2021 and Souza et al. 2022).**

pH estimation:

Fresh chicken breast meat had pH values between 5.69 and 6.13 **(Bae et al. 2014)**. Obtained results this study (Table 7) showed increase in pH value which is a mark of spoilage at 6th day in control group that agreed with **Lee et al. (2022)** who observed rise in pH during the storage process may be as a result of ammonia and amine formation resulting from the proteolytic breakdown of muscle proteins through microbial activity and internal enzymes.

While in case of HS group, we noted that pH chicken breast meat samples was acidic (5.62) in agreement with **(Zhang et al.2019)** who found that higher glycolysis enzyme activity at early postmortem may be the reason for the heat stress group's quick pH fall, heat stress also boosts microbial development, particularly pathogenic bacteria, in chicken intestines and meat, boosting proteolysis and increasing meat pH **(Nawaz et al. 2021)**. Meanwhile meat's pH rises during storage because endogenous enzymes and spoilage bacteria break down proteins, producing basic nitrogenous chemicals that alter the meat's chemical environment and microbiological development **(Sujiwo et al. 2018)** so breast meat samples spoiled at 3rd day of cold storage.

On the other hand, HS+Se-NPs group samples spoiled at 9th day of cold storage in accord

with **(Ibrahim et al. 2019)** who noted that giving heat stressed birds dietary Nano-Se slows down the rise in pH during storage, which suggests that it improves oxidative stability, water-holding capacity, and slows down microbial growth. This results correlated to the impact of Se-NPs in reducing the amount of lactic acid in muscles by enhancing their capacity to eliminate metabolic products and slowing down pH decline **(Bien et al. 2023).**

On other hand, the group treated with Se-NPs spoiled at 12th day of storage this agreed with **(Pardechi et al. 2020)** who found that adding Se-NPs to chicken diets have more positive effects on improving meat quality parameters of broiler breast muscle.

Thiobarbituric acid reactive substances (TBARS) estimation:

Heat stress results in oxidative stress in chickens which has effect on bird's health by increase reactive oxygen species and led to lipid peroxidation **(Emami et al. 2020)**. Thiobarbituric acid used as indicator of lipid peroxidation **(De Leon and Borges 2020)**. In this study results tabulated in (Table 7) the data showed that the mean TBARS values of the examined chicken breast samples was the highest in the HS group at zero, 3rd , 6th, 9th and 12th days of chilling storage in comparison with the control group. This result was matched to other previous studies **Imk et al. (2012)** and **Ruff et al. (2021)**, who observed elevation of TBARS in heat stressed broiler chickens.

The lower TBARS values found in HS+Se-NPs group breast meat samples, while lowest TBARS values in breast meat samples were found in the Se-NPs group samples which were nearly compatible to Egyptian Organization for Standardization **(EOS 1651 : 2005)** for chilled poultry meat with accepted level of TBARS lower than 0.90 mg/kg. This result is an indicative that Se-NPs is efficient antioxidant elements protecting cell membrane from lipid oxidation and contributing in maintaining its integrity by neutralizing free radicals together with decreasing the loss of intracellular fluid **(Aparna and Karunakaran , 2016)**.

Total volatile basic nitrogen estimations (TVBN):

TVBN is utilized as an indicator for quality of meat **(Nemati et al. 2021)**. In this study results documented in Table 7 showed that the average TVBN values of the examined chicken breast samples was the highest in the HS group samples at zero, 3rd , 6th, 9th and 12th days of storage when compared to the control group. This results emphasize that heat stress resulted in affecting the structural integrity and function of cell membranes, producing oxidative changes of protein and nucleic acid **(Emami et al. 2020)**. Thus total volatile basic nitrogen elevated as end product of the breakdown enzymatic protein and non-protein nitrogenous molecules in heat stressed broilers meat **(Nemati et al. 2020).**

The lower TVBN values found in HS+Se-NPs group breast meat samples, while lowest TVBN values in breast meat samples were found in the Se-NPs group samples which were nearly compatible to Egyptian Organization for Standardization **(EOS 1651 : 2005)** for chilled poultry meat with accepted level of TVBN lower than 0.20 mg% in all days storage except in the 12th days when spoilage occurred. The decrease in TVBN confirmed the antioxidant and protective effects of Se-NPs on cells, which inhibit DNA oxidation and protein degradation **(Gao et al. 2012).**

CONCLUSION

The current study declared that Se-NPs supplementation alternate the adverse effect of heat stress , has tissue and cellular protective effect and improve chicken breast meat quality during chilling storage.

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