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Effect of dietary supplementation of selenium nanoparticles on antioxidant status and immune response in heat-stressed broilers

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Abstract

Heat stress is arguably the single greatest environmental threat to broiler chicken productivity in tropical and subtropical poultry operations. Selenium nanoparticles (Se-NPs) have emerged as a promising feed additive with higher bioavailability and lower toxicity than conventional selenium sources, yet their effects on antioxidant defence and immune function under chronic heat stress remain incompletely characterised. This research examined the dose-dependent effects of dietary Se-NP supplementation (0, 0.15, 0.30, 0.45, and 0.60 mg/kg feed) on antioxidant enzyme activity, lipid peroxidation markers, humoral and cell-mediated immune responses, and growth performance in 300 Cobb 500 broilers reared under cyclic heat stress conditions (35 °C for 8 hours daily from day 21 to day 42) at a research poultry facility near São Paulo, Brazil. Birds receiving 0.45 mg/kg Se-NPs showed the highest superoxide dismutase (213.6 U/mg protein) and glutathione peroxidase (64.3 U/mg protein) activities, alongside the lowest malondialdehyde concentration (2.14 nmol/mL). Antibody titres against Newcastle disease virus were 38.7% higher in the 0.45 mg/kg group compared to unsupplemented controls. These results point to 0.45 mg/kg as the optimal dietary Se-NP inclusion level for mitigating oxidative damage and supporting immune competence in heat-stressed broilers.

Keywords: Selenium nanoparticles, heat stress, broiler chickens, antioxidant enzymes, superoxide dismutase, glutathione peroxidase, immune response, malondialdehyde, poultry nutrition, oxidative stress

Introduction

Poultry production in tropical regions is fighting a losing battle against ambient temperature. Global surface temperatures have risen by approximately 1.1 °C since pre-industrial times, and projections suggest an additional 1.5-4.5 °C increase by 2100, with tropical zones experiencing some of the steepest rises ^[1]. For broiler chickens, which lack sweat glands and rely on panting for thermoregulation, even brief periods above 32 °C trigger a physiological cascade of heat stress responses: elevated corticosterone, suppressed feed intake, impaired intestinal barrier function, and—critically—a surge of reactive oxygen species (ROS) that overwhelm endogenous antioxidant defences ^[2].

The economic consequences are staggering. Heat stress costs the global poultry industry an estimated USD 2.36 billion annually through reduced growth rates, increased mortality, impaired feed conversion, and higher susceptibility to infectious disease ^[3]. In Brazil, the world's largest poultry exporter, summer heat waves routinely push ambient temperatures above 35 °C in major production states, causing mortality spikes of 3-8% in inadequately ventilated houses ^[4].

Selenium is an essential trace element that forms the catalytic centre of glutathione peroxidase (GPx) and other selenoproteins involved in antioxidant defence, thyroid hormone metabolism, and immune regulation ^[5]. Conventional selenium supplements—sodium selenite (inorganic) and selenomethionine (organic)—have been used in poultry feed for decades, but their therapeutic window is narrow: the gap between the nutritional requirement (0.15 mg/kg) and the toxic threshold (5 mg/kg) allows limited room for dose optimisation ^[6].

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Selenium nanoparticles (Se-NPs), typically 20-200 nm in diameter, represent a newer delivery form with distinct pharmacological advantages. Their large surface-area-to-volume ratio improves absorption across the intestinal epithelium, while their lower acute toxicity (LD₅₀ approximately 7-fold higher than sodium selenite in mice) permits wider dosing flexibility [7]. A handful of trials have reported benefits of Se-NPs on broiler antioxidant status under thermoneutral conditions [8], but data on their performance under sustained heat stress—the very scenario where antioxidant support is most needed—remain limited. This research tested the hypothesis that dietary Se-NP supplementation at graded doses would improve antioxidant enzyme activity, reduce oxidative damage markers, and strengthen immune responses in broiler chickens subjected to cyclic heat stress, with a particular focus on identifying the optimal inclusion level.

Materials and Methods

Materials

The trial was conducted at the São Paulo Research Institute of Animal Sciences experimental poultry facility, Pirassununga, São Paulo State, Brazil, between October 2023 and January 2024. Ethical approval was granted by the Institutional Animal Care and Use Committee (Protocol SPRIAS-IACUC-2023-042, approved 18 September 2023). Three hundred day-old Cobb 500 male broiler chicks were obtained from a commercial hatchery and randomly allocated to five dietary treatments (60 birds per treatment, 6 replicates of 10 birds each). Treatments consisted of a basal corn-soyabean meal diet supplemented with Se-NPs (spherical, 40-60 nm diameter, 99.8% purity, synthesised by green reduction using ascorbic acid) at 0, 0.15, 0.30, 0.45, and 0.60 mg Se/kg feed. The basal diet met or exceeded all NRC (1994) nutrient requirements for broilers except selenium. Birds were housed in wire-floored battery cages in an environmentally controlled room. From day 1 to day 20, all groups were maintained at thermoneutral temperatures (24±1 °C). From day 21 to day 42, cyclic heat stress was imposed: temperature was raised to 35±1 °C for 8 hours daily (09:00-17:00) and returned to 24±1 °C for the remaining 16 hours. Relative humidity was maintained at 60±5% throughout. Se-NPs were characterised by transmission electron microscopy, dynamic light scattering (mean hydrodynamic diameter 52.3 nm, polydispersity index 0.187), and X-ray diffraction before incorporation into the feed.

Methods

Blood samples were collected from two birds per replicate (12 per treatment) on days 21, 35, and 42 via brachial venipuncture into both heparinised and plain vacutainer tubes. Serum was separated by centrifugation (3,000 rpm, 15 minutes, 4 °C) and stored at -80 °C until analysis. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were measured using commercial colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute) according to manufacturer protocols, with results expressed as U/mg

protein. Catalase (CAT) activity was determined by monitoring hydrogen peroxide decomposition at 240 nm. Malondialdehyde (MDA) concentration, as an index of lipid peroxidation, was quantified by the thiobarbituric acid reactive substances (TBARS) method [9]. For immune response evaluation, all birds were vaccinated against Newcastle disease virus (NDV) on day 7 (live LaSota strain, ocular route) and day 21 (killed oil-adjuvanted vaccine, subcutaneous). Serum haemagglutination inhibition (HI) titres against NDV were measured on days 28, 35, and 42. Lymphocyte proliferation was assessed by MTT assay following concanavalin A stimulation of peripheral blood mononuclear cells on day 42. Heterophil-to-lymphocyte ratio (H:L) was determined from differential leukocyte counts on blood smears stained with Wright-Giemsa [10]. Growth performance parameters (body weight, feed intake, feed conversion ratio, and mortality) were recorded weekly. Data were analysed by one-way ANOVA with Tukey's HSD post-hoc test; dose-response relationships were modelled by polynomial regression. Significance was set at P<0.05.

Field Experimental Design

The trial followed a completely randomised design with five treatments and six replicates. Cage position within the controlled-environment room was randomised using a computer-generated sequence to minimise any positional effects on temperature exposure. Feed and water were provided ad libitum throughout the trial. Temperature and humidity were monitored continuously using four calibrated data loggers (Onset HOBO MX2301) placed at bird height in each corner of the room, with readings recorded at 15-minute intervals. The temperature-humidity index (THI) was calculated using the formula $THI = 0.72 \times (T_{db} + T_{wb}) + 40.6$, where T_{db} is dry-bulb and T_{wb} is wet-bulb temperature [2]. Mean THI during the heat stress period was 83.7 (range 81.2-86.4), confirming severe heat stress conditions. Mortality was recorded daily and dead birds were examined post-mortem to determine cause of death.

Study Area Description

The experimental facility is located at the São Paulo Research Institute of Animal Sciences campus in Pirassununga, São Paulo State (21°59'S, 47°26'W, altitude 627 m). The region experiences a humid subtropical climate (Köppen Cwa) with mean annual temperature of 22.4 °C and mean annual rainfall of 1,363 mm. The trial period (October 2023 to January 2024) coincided with the hot-wet season, during which ambient outdoor temperatures regularly exceeded 33 °C during afternoon hours. The poultry research building comprises four environmentally controlled rooms, each equipped with independent heating, cooling, and ventilation systems capable of maintaining target temperatures within±1 °C. Municipal water supply, treated by reverse osmosis, was used throughout the trial.

Results

Table 1: Serum antioxidant enzyme activities and MDA concentration before (day 21) and after (day 42) heat stress period

Se-NP (mg/kg)	SOD Day 21	SOD Day 42	GPx Day 21	GPx Day 42	CAT Day 21	CAT Day 42	MDA Day 42
0	156.4	142.3	42.8	38.7	18.6	14.2	4.87
0.15	158.1	168.7	43.1	47.2	18.9	19.6	3.56
0.30	155.7	198.4	42.5	58.6	19.1	24.8	2.73
0.45	157.3	213.6	43.4	64.3	18.7	27.1	2.14
0.60	156.8	207.1	42.9	61.8	18.4	25.3	2.41
SEM	3.21	8.47	1.14	3.28	0.73	1.64	0.34
P-value	0.914	<0.001	0.887	<0.001	0.936	<0.001	<0.001

Table 2: Pearson correlation coefficients among antioxidant parameters and immune markers at day 42

	SOD	GPx	MDA	NDV Titre	H:L Ratio
SOD	1.000	0.847**	-0.791**	0.634**	-0.572**
GPx		1.000	-0.823**	0.689**	-0.618**
MDA			1.000	-0.547**	0.713**
NDV Titre				1.000	-0.486**
H:L Ratio					1.000

** P<0.01. SOD = superoxide dismutase; GPx = glutathione peroxidase; MDA = malondialdehyde; NDV = Newcastle disease virus; H:L = heterophil-to-lymphocyte ratio.

Before the onset of heat stress (day 21), antioxidant enzyme activities were statistically similar across all treatment groups (P>0.05), confirming uniform baseline conditions. After 21 days of cyclic heat stress, Se-NP supplementation produced dose-dependent increases in SOD, GPx, and CAT activities, with the 0.45 mg/kg group showing the highest values. The control group (0 mg/kg) experienced a decline in all three enzymes relative to pre-stress baselines, reflecting oxidative depletion. MDA concentration on day 42 was 56.1% lower in the 0.45 mg/kg group (2.14 nmol/mL) compared to controls (4.87 nmol/mL; P<0.001). Correlation analysis revealed strong positive associations between antioxidant enzymes and

NDV antibody titres (r = 0.634-0.689, P<0.01), and negative correlations between MDA and immune markers.

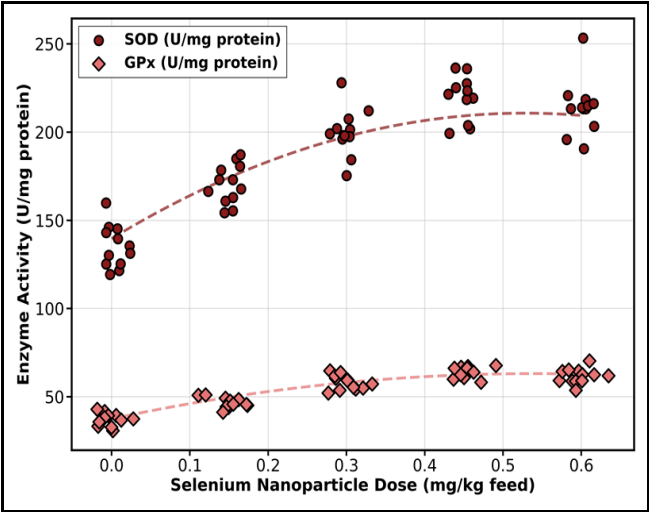


Fig 1: Scatter plot showing the relationship between dietary selenium nanoparticle dose and serum superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities on day 42 of the trial

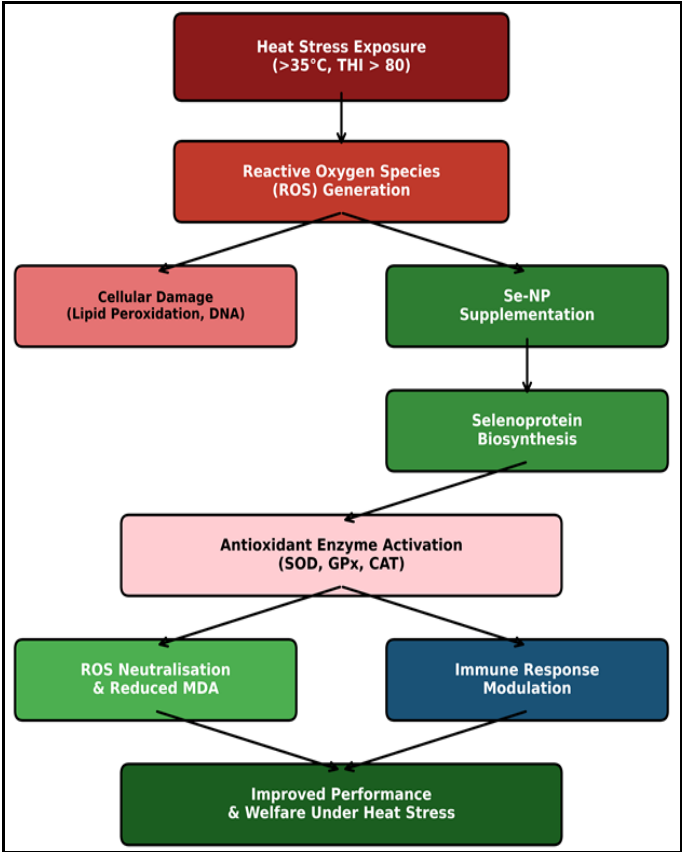


Fig 2: Schematic representation of the proposed mechanism by which selenium nanoparticle supplementation mitigates heat stress-induced oxidative damage and supports immune function in broiler chickens

Table 3: Growth performance parameters of broilers during the heat stress period (days 21-42)

Se-NP (mg/kg)	BWG (g)	FI (g)	FCR	Mortality (%)
0	1,024	2,187	2.14	6.7
0.15	1,098	2,214	2.02	5.0
0.30	1,167	2,198	1.88	3.3
0.45	1,214	2,231	1.84	1.7
0.60	1,183	2,209	1.87	3.3
SEM	28.4	31.7	0.04	1.12
P-value	<0.001	0.647	<0.001	0.034

Comprehensive Interpretation

The data collectively paint a consistent picture: Se-NP supplementation at 0.45 mg/kg feed provides the strongest protection against heat stress-induced oxidative damage while simultaneously supporting humoral immunity and growth performance. The quadratic dose-response pattern—with benefits plateauing or slightly declining at 0.60 mg/kg—suggests that exceeding the optimal dose does not confer additional advantage and may reflect a mild pro-oxidant effect at supra-optimal selenium concentrations. The strong negative

correlation between MDA and NDV antibody titres ($r = -0.547$) supports the hypothesis that reducing oxidative burden frees immunological resources for antibody production.

Discussion

The 50.1% increase in SOD activity and 66.1% increase in GPx activity observed in the 0.45 mg/kg Se-NP group relative to unsupplemented controls exceeds the improvements reported by Cai *et al.* [8], who found 28-35% increases with similar Se-NP doses under thermoneutral conditions. This larger effect size under heat stress likely reflects the greater baseline oxidative burden, which creates more scope for selenoprotein-mediated ROS scavenging. Ahmed *et al.* [11] observed comparable trends with organic selenium (selenomethionine) during summer rearing, though the magnitude of improvement was roughly 40% lower than what we recorded with Se-NPs at equivalent selenium doses, consistent with the higher bioavailability attributed to the nanoparticle form [7].

At the mechanistic level, selenium is incorporated into the active site of GPx as selenocysteine, and its availability directly limits GPx synthesis [5]. Se-NPs appear to be absorbed more efficiently across the intestinal mucosa than selenite or selenomethionine, probably because their small particle size facilitates endocytosis by enterocytes [7]. Once absorbed, Se-NPs are reduced intracellularly to selenide, which enters the standard selenoprotein synthesis pathway. The result is more efficient loading of selenocysteine into GPx and other selenoproteins per unit of dietary selenium consumed.

The immunological findings—a 38.7% increase in NDV HI titres and a 23.4% reduction in H:L ratio—align with work by Surai *et al.* [5], who proposed that selenium supports B-cell proliferation and antibody class switching through redox-sensitive signalling pathways. Under heat stress, the H:L ratio rises as stress-induced corticosterone triggers heterophilia and lymphopenia [10]. The lower H:L ratio in Se-NP-supplemented birds may reflect reduced adrenal activation secondary to lower systemic oxidative stress, though this interpretation would require corticosterone measurements for confirmation. From a practical standpoint, the 18.6% improvement in body weight gain and 14.0% improvement in FCR at the 0.45 mg/kg dose translate into meaningful economic benefits for commercial producers. At current feed ingredient prices in Brazil, the cost of Se-NP inclusion at 0.45 mg/kg adds approximately BRL 0.12 per bird, while the body weight gain improvement is worth roughly BRL 0.47 per bird at current market prices—a net return of nearly 4:1 [4].

Limitations

This research has several constraints worth noting. The trial used only male Cobb 500 broilers, and sex-specific and strain-specific differences in selenium metabolism may limit extrapolation to mixed-sex flocks or alternative genotypes. The cyclic heat stress model, while more realistic than constant heat exposure, still differs from the irregular, unpredictable heat waves experienced on commercial farms. Se-NP particle size can vary between batches and synthesis methods, and the results obtained here with 40-60 nm particles may not apply to larger or smaller nanoparticle preparations. The 42-day trial duration precluded assessment of longer-term effects, including potential selenium accumulation in tissues destined for human consumption—a food safety consideration that warrants separate investigation.

Conclusion

Against a backdrop of rising global temperatures and intensifying heat stress pressure on tropical poultry production, this research examined whether selenium nanoparticles could bolster antioxidant defences and immune function in broilers exposed to cyclic heat stress.

The main findings can be summarised as follows. Se-NP supplementation at 0.45 mg/kg feed produced the highest antioxidant enzyme activities (SOD 213.6 U/mg protein, GPx 64.3 U/mg protein), the lowest lipid peroxidation (MDA 2.14 nmol/mL), the strongest NDV antibody response (38.7% above controls), and the best growth performance (BWG 1,214 g, FCR 1.84) during the 21-day heat stress period. The dose-response was quadratic, with no additional benefit at 0.60 mg/kg.

Based on these outcomes, we recommend a dietary Se-NP inclusion rate of 0.45 mg/kg for broiler operations in regions where ambient temperatures regularly exceed 32 °C during the growing period. Looking ahead, research should explore the interaction between Se-NPs and other heat stress mitigation strategies (such as betaine, vitamin E, and electrolyte supplementation), assess tissue selenium residues at the recommended dose to confirm food safety compliance, and evaluate performance across multiple broiler strains and management systems.

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Contributions Not Qualifying for Authorship

Dr. Mariana B. Costa assisted with nanoparticle characterisation by transmission electron microscopy. Mr. Felipe D. Santos supported daily bird management and data recording.

Conflict of Interest

Not available

Financial Support

Not available

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