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Impact of nano-Selenium on biochemical, antioxidant, immunological and histological parameters of broiler chickens during rainy season

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ABSTRACT

Poultry sector supports economies of many nations, especially evolving ones, and provides a consistent and inexpensive protein. Nano-selenium is utilized to increase mineral availability, which aid in broiler growth and maintain their health. The current research was conducted to evaluate the impact of dietary nano-selenium supplement on immunological, haematological, biochemical and histological parameters on Vencobb broiler chicks during rainy season. Two hundred seventy numbers of one-day-old broiler chicks were split in nine groups at random, individually encompassing of three replicates with ten chicks. The control group (T1) was provided a basal diet, T2 received 0.3 mg/kg of inorganic selenium, T3, T4, T5, T6, T7, T8 and T9 received 0.0187, 0.0375, 0.075, 0.15, 0.30, 0.60 and 1.20 mg/kg of nano-selenium, respectively. The results revealed that nano-selenium had no significant (p>0.05) influence on haematological parameters except MCV (%) at third week and MCV (femtolitre) as well as MCHC (%) at the fifth week (p<0.05). No significant differences (p>0.05) were observed among the treatment groups in any of the measured biochemical markers. The glutathione and superoxide dismutase enzyme actions were significantly (p<0.05) higher in T6 than in other groups, but no difference in catalase and lipid peroxidase activities at 5th week. Nano-Se supplementation significantly (p<0.05) increased both cellular and humoral immunity response, however the T6 group showed best result. Our findings revealed that supplementing broiler chickens with nano-Se (0.15 mg/kg) could improve antioxidant activity, boost immunological response and no harmful effect in liver and renal tissues during rainy season.

Keywords: Antioxidant, Broiler, Immune response, Nano-Se, Rainy season

The monsoon season is predominant in several Indian states, leads to numerous stress factors and infectious diseases in poultry birds (Abo-Al-Ela *et al.* 2021; Onagbesan *et al.* 2023). Exposure to low temperature and high humidity increases the risk of cold stress and cold-induced oxidative damage in broilers. Birds exposed to such stress facilitate oxidative damage, immunological dysfunction and impairment of growth performance (Shehata *et al.* 2020; Hafez and Shehata, 2021).

The Indian Council of Medical Research recommends that per capita meat consumption be 11 kg, while India's per capita meat availability is 3.8 kg/year (DAHDF, 2019), which is very low. To overcome this, we need to focus on improving growth performance and immune function of the birds in monsoon season by reducing oxidative stress with some advanced alternatives (nano-Se supplementation) as there are restrictions on the use of antibiotics growth promoter (Abd El-Hack *et al.* 2021).

Selenium (Se) is a vigorous trace mineral that is crucial for poultry health. It is a constituent of selenoproteins, which have a variety of functional activities such as immune response modulation, antioxidant status and growth efficiency (Dalgaard *et al.* 2018). It also increases toxicity in ecosystem if concentration become too high. Recently, nano-selenium (nano-Se) has received a lot of emphasis, since nanoparticles exhibit distinct physical and chemical properties such as specific surface-active centres, high catalytic efficacy and better bioavailability (Hosnedlova *et al.* 2018; Abdelnour *et al.* 2021). In poultry feeds, supplementation of selenium nanoparticles (SeNPs) helps to improve antioxidant capacity, disease resistance, immune status and growth performance which aid to minimize the detrimental effects of different stress factors in rainy season on broiler chickens (Ibrahim *et al.* 2022; Abdel-Moneim *et al.* 2022a). Numerus studies have found that SeNPs enhanced antioxidant status and immune functions of the broiler birds during different stress conditions (Alian *et al.* 2020; Abdel-Moneim *et al.* 2022b). The nano-Se (0.15 ppm) in broiler chickens had improved growth and immune status and protected birds from stress during summer season (Debata *et al.* 2023). The goal of current experiment was to evaluate the effects of various SeNPs doses on the antioxidant activity, immune status,

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haematological and biochemical parameters of broiler chicken during rainy season.

MATERIALS AND METHODS

Nanoselenium synthesis: Selenium nanoparticles (SeNPs) were created using sodium selenite (25mM, 50 ml) and glutathione (4 ml) containing bovine serum albumin (BSA) were combined. As a result, oxidised glutathione and red elemental Se are formed (Zhang et al. 2012). The average size and shape of the Se NPs was 50 nm and spherical shape as observed by Transmission electron microscope (TEM) (Figure 1).

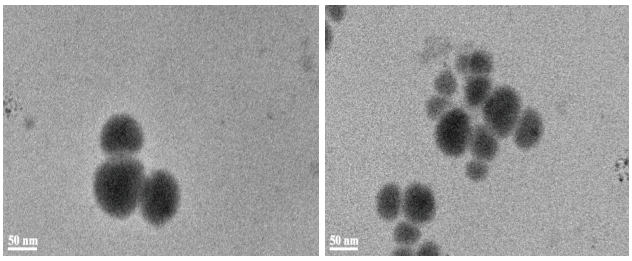

Fig. 1. A) Size of the nano-Se, B) Shape of the nano-Se

Birds experimental procedure: A total of 270 day-old-Vencobb broiler chicks were randomly allotted into nine feeding regimens, each consisting of three replicates (10 birds in each replicate). The dietary treatments of broiler chicks constitute: one basal diet (control) T1, one inorganic selenium (0.3 mg/kg) T2 and seven different levels of nano selenium supplementation. 0.01875 (T3), 0.0375 (T4), 0.075 (T5), 0.15 (T6), 0.30 (T7), 0.60 (T8) and 1.20 (T9) mg SeNPs/kg as feed (BIS, 2007). The feeding schedule was categorized into three phases: pre-starter phase (1-14 days), starter phase (15-24 days) and finisher (25-35 days). The constituent content and approximate composition of the basal diets are revealed in supplementary material (SM 1-2) (Latimer, 2016). Body weight was recorded for individual birds at 0, 7th, 14th, 21st and 35th day.

Blood plasma and serum collection: Five birds from each treatment were picked at random for blood sampling on 3rd and 5th weeks. Blood collection was done early in the morning by pricking wing vein. Five ml of blood were collected in tubes and placed for 1 hour in slanting position for serum collection and immediately transported to lab and centrifuged at 3000×g for 15 minutes. Serum was collected and kept at -20°C.

Serum analysis: Biochemical analysis of serum such as glucose, urea, and cholesterol (mg/dl), triglycerides (mg/dl), total protein, albumin and globulin (g/dl), A/ G ratio, SGPT and SGOT (U/L), calcium (mg/dl) and phosphorus (mg/dl) were analysed using collected sera. The kit was purchased from CPC diagnostic Ltd., Chennai, India.

*Haematological parameters:*Hematological parameters viz., hemoglobin (%), packed and mean cell volume (PCV and MCV), total erythrocyte count and MCHC (mean corpuscular haemoglobin concentration) were analyzed using auto-hematology cell counter.

*Assessment of antioxidant enzymes activity:*After collection and centrifugation of blood samples, to completely remove buffy coat and cell debris, the resultant erythrocyte pellet was washed thrice with 0.9% isotonic saline. The packed erythrocytes were combined with 500 µl of saline solution to make RBC suspension. Hemolysate was equipped by combining RBC suspension with an ice cold EDTA stabilizing solution (Banakar *et al.* 2021). GPx (Paglia, 1967), superoxide dismutase (Madesh and Balasubramanian, 1998) and catalase Aebi (1984) activities were determined.

Cell mediated immune response (CMI): To measure the cellular immunological response, 100 micrograms of phytohaemaglutinin-P (PHA-P) dissolved in normal saline (0.1 ml) were injected intradermally in foot web of broiler birds at the end of 35th day (Edelman *et al.* 1986).

Humoral immune response: This was measured using Sheep red blood cells (SRBC) as test antigens. Birds were inoculated intravenously with packed RBC (0.07 ml) combined with saline (0.93 ml) via wing vein. The SRBC antibody titres were represented as the log2 of reciprocal of the serum concentration at which the greatest number of SRBC agglutinated (Kai *et al.* 1988).

*Histological parameters:*A representative liver and, kidney tissue of five-week-old broiler chickens were preserved in 10% saline solution. Standard histological procedures were used to process tissues that had been formalin fixed. Sections were stained using haematoxylin and eosin technique. Stained slides were examined under light microscope.

Statistical analysis: The data was analysed using SPSS software. The data existing in tables is as means ± standard error.

RESULTS AND DISCUSSION

Impact of nano-Se on body weight and biochemical parameters: The body weight of broiler chicks for groups T1-T9 were: 48.20, 46.68, 47.60, 47.88, 46.48, 47.20, 47.08, 46.20 and 46.96 g, respectively. By the end of fifth week, their weights had increased significantly as 1852, 1873, 1879, 1933, 1962, 1983, 1890, 1877, 1862 g in T1, T2, T3, T4, T5, T6, T7, T8 and T9. Among all groups, T6 showed the highest body weight (1983 g). The outcomes of blood parameters revealed that nano-selenium had no discernible effect on any biochemical indicators. The serum biochemical values of Vencobb broiler birds during the third and fifth week are presented in the SM Table 3-4, respectively. Blood biochemical parameters did not differ between the experimental groups in a statistically way (p>0.05).

Effect of nano-Se supplementation on haematological parameters: According to the findings, haematological parameters were not significantly affected by supplementing with nano-selenium MCV (%) at 3rd week, while MCV (fl) and MCHC (%) were significant ($p<0.05$) at 5th week (Table 1).

Table 1. Haematological parameters of broiler birds (Vencobb) under different dietary treatments after 3rd week and 5th week

Parameter	Treatments (3rd week)									P value
	T1	T2	T3	T4	T5	T6	T7	T8	T9	
HB (%)	9.97 ±0.22	10.10 ±0.15	9.86 ±0.06	10.20 ±0.47	9.96 ±0.46	9.88 ±0.27	9.41 ±0.31	9.24 ±0.29	9.97 ±0.12	0.367
PCV (%)	30.24 ±1.40	29.04 ±0.58	29.82 ±1.72	26.72 ±1.93	32.02 ±1.30	29.35 ±2.98	29.87 ±2.36	27.85 ±1.51	30.50 ±0.58	0.637
TEC (millions/ Cubicmm)	2.64 ±0.07	2.61 ±0.20	2.53 ±0.13	2.27 ±0.15	2.24 ±0.25	2.49 ±0.04	2.47 ±0.10	2.56 ±0.13	2.41 ±0.13	0.499
MCV (femtolitre)	114.91^{a} ±6.50	113.91^{a} ±9.65	119.33^{ab} ±9.61	120.74^{ab} ±13.01	148.97^{b} ±13.96	118.15^{ab} ±12.64	120.93^{ab} ±8.53	109.54^{a} ±7.20	128.09^{ab} ±6.88	0.028
MCHC (%)	33.41 ±2.37	34.85 ±1.08	33.56 ±2.10	39.44 ±4.56	31.36 ±2.02	35.66 ±4.89	32.64 ±3.60	33.71 ±2.55	32.73 ±0.67	0.757
5th week										
HB (%)	10.06abcd ±0.20	10.49 d ±0.27	10.23bcd ±0.45	9.82 abcd ±0.46	10.26cd ±0.38	9.77 abcd ±0.29	9.29 abc ±0.15	9.13 a ±0.32	9.19ab ±0.26	0.035
PCV (%)	30.99 ±1.95	30.64 ±1.01	32.22 ±1.36	30.72 ±0.39	32.72 ±1.28	32.96 ±1.10	34.08 ±1.11	31.12 ±0.54	33.60 ±1.01	0.302
TEC (millions/ cmm)	2.98 ±0.10	2.95 ±0.14	2.72 ±0.11	2.70 ±0.23	2.89 ±0.10	2.75 ±0.12	2.79 ±0.26	2.89 ±0.16	2.59 ±0.13	0.719
MCV (femtolitre)	103.56a ±4.32	104.36a ±3.73	118.87ab ±4.87	116.64ab ±8.81	113.34ab ±4.13	120.14ab ±3.05	127.55b ±14.79	108.90ab ±5.69	130.38b ±4.26	0.046
MCHC (%)	33.02b ±2.23	34.31b ±0.74	31.93 ab ±1.79	32.00 ab ±1.54	31.69 ab ±2.21	29.73ab ±0.99	27.41a ±1.26	29.41ab ±1.39	27.43a ±1.05	0.033

^{abcd} Values bearing different superscripts in a row differ significantly (p<0.05). HB stands for Haemoglobin; PCV: Packed cell volume; TEC: total erythrocyte count; MCV: Mean cell volume; MCHC: Mean corpuscular haemoglobin

At 3rd week, Hb (%), PCV (%), TEC (million/cmm) and MCHC (%) showed non-significant difference (p>0.05) among the treatments. However, MCV (fl) tends to increase (p<0.05) significantly in T5 compared to other treatments at 3rd week. Similarly, at 5th week, PCV (%) and TEC (million/cmm) showed non-significant changes among the treatment groups while, haemoglobin (%) was found higher (p<0.05) in T2 group, MCV (fl) was also greater in T7 and T9, and that MCHC (%) was significantly lower in T7 and T9 over T1 and T2.

Effect of nano-Se supplementation on antioxidant enzymes activity: The concentration of GSH-Px, SOD and CAT wereused as markers for antioxidative capacity and measured at 35th day of the experiment (Table 2). The GSH-Px (moles of $NADPH_2$/ moles of heme/ min) activity was significantly (p<0.05) higher in T6 (4845.92) group than that of all the other dietary treatment groups (Table 2). Similarly, SOD (U/ moles of heme) activity was significantly higher (p<0.05) in T6 (53.69) group than the other treatment groups. Catalase (moles of H_2O_2/ moles of heme/ min) activity ranged from 10.92 (T8) to 16.40 (T4) at 5th weeks of age showing no significant (p>0.05) difference among the treatments.

Impact of nano-Se on immune status: Consequences of

Table 2. Antioxidant enzymes of broiler chicken under different dietary treatments

Parameters	Treatments									P value
	T1	T2	T3	T4	T5	T6	T7	T8	T9	
GPx (moles of $NADPH_2$/moles of heme/min)	3242.43^{a} ±126.74	3598.63^{a} ±177.84	3497.42^{a} ±151.03	3572.12^{a} ±139.09	4295.26^{ab} ±105.96	4845.92^{c} ±287.50	3456.76^{a} ±204.54	3800.28^{ab} ±325.98	3479.30^{a} ±185.45	<0.001
SOD (u/moles of heme)	25.73^{a} ±2.71	41.88^{b} ±3.08	36.45^{b} ±4.91	40.08^{b} ±2.60	41.72^{b} ±3.07	53.69^{c} ±1.63	43.23^{b} ±4.72	43.38^{b} ±3.74	38.45^{b} ±3.38	0.011
Catalase (moles of H_2O_2/moles of heme/min)	15.62 ±2.33	15.28 ±0.90	15.41 ±0.63	16.40 ±1.60	13.84 ±1.35	13.99 ±1.52	14.26 ±1.12	10.92 ±0.86	12.76 ±0.92	0.173

^{abcd} Values bearing different superscripts in a row differ significantly (p<0.05)

Table 3. Immunity status of broiler chicken (5th week) under different dietary treatments

Parameter	Treatments									P value
	T1	T2	T3	T4	T5	T6	T7	T8	T9	
SRBC (log_2)	0.84^{a} ±0.04	1.63^{b} ±0.07	1.64^{b} ±0.07	1.54^{b} ±0.07	1.62^{b} ±0.11	1.89^{c} ±0.04	1.61^{b} ±0.17	1.46^{b} ±0.03	1.47^{b} ±0.07	<0.001
CBH	147.55^{a} ±15.13	175.44^{b} ±3.69	186.35^{b} ±5.52	189.47^{b} ±3.25	181.85^{b} ±5.31	214.09^{c} ±3.24	190.95^{b} ±6.88	186.82^{b} ±11.82	185.80^{b} ±1.75	0.007

[abcd] Values bearing different superscripts in a row differ significantly (p<0.05). SRBC stands for Sheep red blood cells and CBH: Cutaneous Basophilic Hyper Sensitivity

the PHA-P injection induced cell reaction were classified as cutaneous basophil hypersensitivity (CBH). The antibody titres (log_2) against SRBC inoculation and CBH response at 5th week of age of broiler chicks is depicted in the Table 3. The groups supplemented with nano-Se had a considerably higher (p<0.05) CBH response compared to the control and other dietary groups, while T6 group had the highest value (214.09). Similarly, the antibody titre (mean log2 value) in the nano-Se supplemented group was significantly higher (p<0.05) than that in the other dietary treatment, with the highest value in the T6 (1.89) group.

Impact of nano-Se on histological parameters and mortality: The liver and renal tissue sections stained with H & E revealed no abnormalities or alterations in the T1, T2, T3, T4, T5, T6 and T7 groups upon histological testing. However, following the administration of nano-Se in these treatment groups, several aberrant alterations were seen in liver and renal tissues of T8 and T9 (Figure 2).

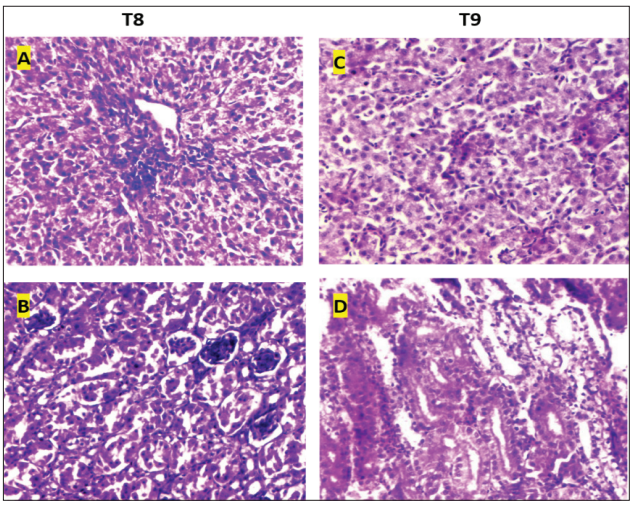

Fig. 2 A and C: Liver perivascular infiltration of inflammatory cell and liver vaculor degeneration of hepatocyte in T8 and T9; B and D: Kidney glomerular atrophy with degeneration and kidney degenerative tubular epithelial lining with mild intestinal congestion in T8 and T9.

The overall mortality during the 5-week experimental period was low and within the acceptable range for broiler chickens, as per BIS (2007) and standard broiler management guidelines. A total of 9 birds (3.3%) across all treatment groups (270 birds) were lost during the trial, with no unusual patterns or treatment-related causes identified.

Enhancing poultry production is crucial to meet the rising global demand for animal protein. Fighting environmental stress in broiler chickens is one of the key factors that determines good poultry production (Akinyemi and Adewole, 2021). Consequently, dietary modifications are a well-accepted method for mitigating the undesirable effects of cold stress in chickens. Nano-selenium has recently attracted consideration due to its superior catalytic effectiveness, better bioavailability, high adsorbing capacity and reduced toxicity as compared to its natural form in chickens (Wang, 2009).

Biochemical profiles of the serum and haematological values give valid information on the health status of the birds and serve as an ideal basis for disease diagnosis (Elarabany, 2018). The biochemical parameters fluctuated with the season as well, but there was no substantial trend in this study to suggest that they were the consequences of changes in response to the environment. This might have been caused by the favourable environment that Vencobb broiler chicks were given during their prestarter stage, along with the addition of nano-Se. Additionally, bird's body and behaviour adapt to their various physiological and biochemical alterations under cold stress. There were no statistical variations in the serum biochemical parameters like total protein and albumin content among all the experimental groups (Alian *et al.* 2020). Selim *et al.* (2015) investigated the effects of various selenium concentration on broiler chickens and reported that the experimental variables had no significant effect on albumin, globulin and plasma proteins. Furthermore, selenium supplementation (0.3mg/kg) had no negative impact on the physiological status of the kidney. Our findings showed that neither the uric acid concentrations nor the serum creatinine levels of the experimental groups differed significantly. Ahmadi *et al.* (2018) also observed that blood biochemical parameters such as glucose and total proteins did not alter after the addition of nano-Se to broiler chicks.

According to the findings, haematological parameters were not significantly affected by supplementing with nano-selenium except Hb, MCV and MCHC. It is well known that selenium deficiency has been related to decreased haematocrit levels due to reduction of glutathione peroxidase and increased in tissues free oxygen radicals such as RBCs that causes oxidative tissue injury (Abdel-Moneim *et al.* 2022b). Selenium has been demonstrated to

contribute to the resistance of red blood cells and a deficit in the mineral has been linked to the development of anaemia (Sadeghian *et al.* 2012). The reason is the anti-oxidant properties of selenium and its capacity to protect body cells from oxidative damage. Packed cell volume serves as a useful indicator of circulating erythrocytes (RBCs) and haemoglobin. Karthiayini and Philomina (2008) observed that overcrowding during the rainy season had no effect on the values of TEC, Hb and PCV of chicks. High dosages of selenium have been proven to boost leukocyte count and enhance immunity against coccidiosis in chickens under humid conditions. Also, it would be regarded appropriate to control infectious diseases by the constructive manipulation of humoral and cell-mediated immune responses with the supplementation of trace minerals to the broiler chicken (Sajadifar *et al.* 2013).

Exposure to cold stress during rainy season induces oxidative stress-induce damage and decreased immunological function in broiler chicks (Mishra and Jha, 2019). Selenium is essential for regulation of oxidative stress due to integration as selenocysteine into GPx and thioredoxin (Yu *et al.* 2005). It is believed that the animal cells first line of defence against free radicals is GPx and SOD (Ighodaro and Akinloye, 2018). It is well known that selenium NPs influence the immune response by modulating cytokine production, maintaining antioxidant defense and reducing oxidative tissue injury (Surai *et al.* 2019). According to our findings, SeNPs, when compared to other forms of selenium, could improve the antioxidant activities of birds during the rainy season via regulating GPx and SOD. These findings could be attributed to more bioavailability of selenium in nano form compared to other forms (Gangadoo *et al.* 2020). Ibrahim *et al.* (2019) also reported when broiler chickens were fed with higher doses of nano-Se, their antioxidant resistance improved respectively. Nano-Se supplementation (0.3 ppm) had significant effect on GPx activity in broiler chicks (Cai *et al.* 2012). Selenium supplementation enhanced laying hen antioxidant capacity due to changes in absorption efficiency and higher bioavailability (Chen *et al.* 2023). However, Alian *et al.* (2020) found that applying various selenium sources containing nano-Se had no influence on the GPx activity in broiler chicks. Liu *et al.* (2023) reported that addition of Selenium dietary yeast improved the antioxidant enzymes and boost Se deposition in broiler tissues, all of which improved broiler meat quality.

PHA-P has been widely utilized to identify cell mediated immune (CMI) responses in birds. The findings of this study revealed that there was a significant difference seen in the nano-Se supplemented groups of broiler poultry for CBH response, which demonstrated a greater cell mediated response (T6-0.15mg Se/kg) over control. Prabakaran *et al.* (2016) reported that indigenous breeds show better CMI response than exotic breeds. Mohammadi *et al.* (2020) revealed that nano-Se supplementation (1.5 mg/kg diet) had no significant effect on toe web swelling after receiving PHA-P for 24-48 hours.

In this study, there was a significant difference in antibody titre against SRBC between the nano-Se supplemented and control group. Because of its increased bioavailability, nanoselenium may have improved the immunological state of broiler birds (Alian *et al.* 2020). Bami *et al.* (2022) found that dietary supplement of nano-Se (0.3 mg/kg) enhanced the antibody response to SRBC. Prabakaran *et al.* (2016) also investigated humoral immune response by injecting SRBC intravenously into CARI red males, an exotic breed of bird that demonstrated a better humoral immune response. The supplementation of nano-Se yielded better results than organic and inorganic forms of selenium, where broilers humoral immune response improved (Mohammadi *et al.* 2020). Nano selenium plays a crucial part in mitigating the harmful effects of heat stress on chicks by enhancing immunological response and antioxidant activities (Eid *et al.* 2023).

The histological analysis of the liver and renal tissue sections revealed no damages in the T1-T7 groups. However, in T8 and T9 groups, there is vacuolar degeneration. The disruption of normal cell metabolism caused by a high nano-Se content may be the cause of degeneration observed in the lobules of liver and kidney. According to Khan *et al.* (2008), broiler treated with selenium (0.3 ppm) showed alternations in the villi of the ilium, spleen and liver lobules. Nano-Se (0.5 mg/kg) exhibits good absorption and minimal toxicity, all without adversely impacting the chicken's growth performance (Bein *et al.* 2023). Notably, the T6 group (0.15 mg/kg nano-selenium), which demonstrated superior antioxidant status and immune responses, exhibited the lowest mortality (only 1 bird out of 30), indicating a potential protective effect of nano-selenium supplementation under the environmental stress conditions of the rainy season).

The present findings demonstrated that adding 0.15 mg/kg of selenium nanoparticles to broilers diets could boost their immune function and increase their antioxidant potential when exposed to cold stress. This may be achieved by modifying their levels of GPx and SOD as well as cellular and humoral immune response. The histological analysis of the liver and renal tissue sections revealed no damage. Therefore, it may be concluded that nano-Se supplementation (0.15 mg/kg) to broiler chickens above and beyond their baseline level will boost their improved antioxidant activity and immune response during rainy season. Further investigation is required to examine the seasonal impact of nano-selenium.

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AUTHOR CONTRIBUTIONS

V.D. performed all experiments and wrote the manuscript, R.K.S. conceptualization and editing the manuscript. K.S, N.P, S.K.M. and S.M. helped in data analysis. All authors read and approved the manuscript.

DECLARATIONS

Ethics Approval and consent to participate

All procedures in bird studies were carried out with the ethical norms of the Institutional Animal Ethics Committee (IAEC), with approval number IAEC, C.V.Sc. & AH (Regd. No.433CPCSEA/CVS/2007) OUAT, Bhubaneswar, Odisha, India. The care and usage of birds, as confirmed by the authors, adhered to the applicable standard operating procedures set forth by the aforementioned ethical committee in order to safeguard animals used in research.

COMPETING INTERESTS

Authors have no competing interests to declare.

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