



RESEARCH ARTICLE

Comparative Effects of Selenium Sources and Concentrations on Growth, Nutrient Absorption and Biochemistry in Japanese Quails

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ABSTRACT

Selenium nanoparticles (Se-NPs) have gained importance in poultry due to their ability to improve growth performance, bioavailability and digestive efficiency compared to inorganic sodium selenite (SS). This study compared the effects of selenium nanoparticles and inorganic sodium selenite on growth performance, nutrient digestibility, and serum biochemical parameters in Japanese quails. A total of 420 quails (14 days old) were distributed into five groups having 84 birds in triplicate, 1st group as control, fed by basal diet; 2nd and 3rd group were supplemented with Se-NPs (0.2 mg/ kg and 0.4 mg/ kg), 4th and 5th groups were fed with SS (0.2 mg/kg and 0.4 mg/kg), respectively. By using these two levels of supplementation, the study assessed a dose-response relationship, helping to identify any benefits or adverse effects associated with increasing selenium levels. The results showed that quails fed with Se-NPs (0.4 mg/kg) had higher body weight and enhanced digestibility of dry matter, organic matter, crude protein, crude fiber, ether extract, and ash content. Glucose, protein, cholesterol, triglycerides, HDL, and LDL concentrations were significantly lowered ($P < 0.05$) in the quails fed with Se-NPs (0.4 mg/kg). The concentration of hemoglobin, WBCs, platelet count and neutrophil were higher ($P < 0.05$) in the group treated with Se-NPs (0.2mg/kg). This study underscored a dose- response relationship, demonstrating that higher levels of Se-NPs (0.4mg/kg) corresponded to improved growth and metabolic outcomes. In conclusion the result indicated that feeding the quails with Se-NPs (0.4mg/kg) enhanced their growth performance, nutrient digestibility and lowered the cholesterol, triglycerides, HDL, and LDL level in comparison to the SS diet.

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INTRODUCTION

The Japanese quail (*Coturnix coturnix japonica*) is increasingly recognized in poultry production as a valuable source of meat and eggs (Hafeez *et al.*, 2024a; Islam *et al.*, 2024; Sultan *et al.*, 2024) due to its rapid growth, early sexual maturity, and high egg production (Hafeez *et al.*, 2024b; Naz *et al.*, 2024a). These birds reach market in just five to six weeks and require less feed and space compared to other domestic fowl. To ensure their health and sustainability in production, it is crucial to provide a balanced diet rich in essential nutrients, particularly minerals, which are vital for their physiological functions (Imtiaz *et al.*, 2023). Proper mineral supplementation is essential for optimizing their growth and overall well-being (Yatoo *et al.*, 2013).

Nanotechnology is an effective methods now a days to enhance bioavailability and absorption of essential nutrients in the feed (Naz *et al.*, 2024b). It provides a unique opportunity to include nanoparticles as alternative sources of trace minerals in animal diets, with new properties that don't exist in bulk materials or standard mineral salts (Abdel-Wareth *et al.*, 2022). Nanoparticles have an excellent catalytic activity, large surface area, and significant absorption property. Thus, nanotechnology has significant potential for the food consumption and the medical field of poultry. Until now, different nanoparticles such as iron oxide-palladium nanoparticles, palladium nanoparticles, silver nanoparticles, gold nanoparticles, zinc oxide nanoparticles and selenium nanoparticles have been prepared and incorporated in the feed ingredients, highlighting the versatility and promise of nanotechnology

in advancing poultry health and productivity (Shah *et al.*, 2022).

Research into selenium nanoparticles (Se-NPs) is particularly necessary when comparing them to conventional inorganic selenium supplements, such as sodium selenite (SS). While traditional forms of Selenium (Se) can improve growth and health, they often have limitations in bioavailability and absorption efficiency. Inorganic Se sources may lead to variable uptake and can be associated with toxicity risks at higher doses (Zambonino *et al.*, 2023). In contrast, Se-NPs exhibit superior bioavailability, allowing for more efficient utilization by the body and potentially lower effective dosages. This is crucial for poultry, where optimizing nutrient intake while minimizing waste is essential for both health and production efficiency. Moreover, the unique properties of Se-NPs can enhance antioxidant activity, potentially leading to improved health status and reduced oxidative stress in poultry (Prusty *et al.*, 2021).

Se-NPs are recognized as metal-based nanoparticles that are produced every year. They have been effectively synthesized by using a variety of green nanotechnology strategies, including plant extracts, according to Zambonino *et al.* (2021). Green synthesis is an eco-friendly approach for creating nanoparticles that uses both single-celled and multi-celled organisms as natural stabilizing and reducing substances (Naz *et al.*, 2024a). It may provide an affordable substitute for traditional types of selenium fortification since it costumes the offensive palatability and aroma of feed, endures longer resident time, improved solvability, and increased bio-accessibility in the intestinal tract (Giamouri *et al.*, 2023). More effective assimilation and nutrient bioavailability can have advantageous effects on metabolism while preventing mineral discharge into the surroundings (Surai *et al.*, 2017).

Se-NPs indicated greater bioavailability (referring to the extent and rate at which the active ingredient Se is absorbed and utilized by the bird's body) and enhanced antioxidant activity, which is the ability of a substance to neutralize harmful free radicals, thereby reducing oxidative stress and promoting overall health, compared to the bulk state of other chemical forms, with the assistance of decreasing the harmfulness of Se. These properties are crucial for the synthesis of Se-NPs as a feed additive, and they have also been explored for potential homoeopathic applications (Vlaicu *et al.*, 2023).

Excessive dietary Se supplementation is linked to an environmental stress. Many factors influence Se toxicity, including the species of the animal, the dose of Se, the type of supplement, and the period of exposure. Many producers utilize excessive amounts of Se in animal feed with no extra benefits. According to NRC (1994) quails need 0.2 mg/kg Se in its feed. The European legislation limits the use of Se in the feed of animal up to 0.5 mg/kg diet. Despite these guidelines, existing research has not thoroughly examined the specific effects of various Se sources, particularly Se-NPs, at different supplementation levels. This study aims to fill these gaps by investigating the impact of Se-NPs compared to SS on growth performance, nutrient digestibility, and serum biochemical parameters in Japanese quails.

MATERIALS AND METHODS

Synthesis of Selenium Nanoparticles: About 15kg of *Capsicum annuum* was rinsed with double distilled water to eradicate all the adhering impurities. After that, they were cut into smaller pieces and dried at a controlled temperature of 60°C until a constant weight was achieved, ensuring complete moisture removal without degrading the active compound. It was then ground to obtain powder. 500g of powder was mixed into 1000ml ethanol and kept it for one week. Then the solution was filtered by whatman filter paper 41. For 15 minutes, the filtrate was put into rotary evaporator and extracted sample was subjected to vacuum evaporation to attain constant weight (Anaya-Esparza *et al.*, 2021). 50 ml of *C. annuum* extract was mixed with 0.263 g of selenium acid (H_2SeO_3) while wearing personal protective equipment and a lab coat to minimize exposure to hazardous materials. The solution was maintained for 15 h at a pH of 5.4, red color appeared, which was the characteristic color of Se-NPs. The deposits were collected and centrifuged by Benchtop centrifuge for four minutes at 12,000 rpm, and washed many times with acetone, absolute alcohol, and double-distilled water. The solution was desiccated in vacuum at ambient temperature until a stable weight is attained (Fig. 1) (Batra *et al.*, 2017).

Newly synthesized Se-NPs were characterized by UV-Vis Spectrophotometer (Hitachi Spectrophotometer U-2800), measuring in a 200-800 nm range. Fourier Transform Infrared (FTIR) Spectrophotometer (Perkin Elmer) was employed to examine the functional groups present in the extract and to conduct structural analysis of the Se-NPs. This technique covered a complete range of 400 to 4000 cm^{-1} , with a spatial resolution of 4 cm^{-1} , allowing for a detailed understanding of the chemical composition and bonding characteristics of the NPs. The amorphous form and patterns of the Se-NPs were determined using data from measurements obtained at 2 θ by X-ray diffraction (Single Crystal System D8 Advance, Bruker). Scanning was conducted at a speed of 10°/min (Shah *et al.*, 2022).

Animal husbandry: All research was conducted according to the guidelines for the handling and care of laboratory animals set by Government College University, Faisalabad (GCUF). Four hundred and twenty (14 day old) *C. coturnix japonica* chicks were reared under standard conditions in steel cages (75cm×50cm×65cm) for a period of 10 days acclimatization. The quails were distributed into five groups (84 birds in triplicate). As a control group, the 1st group was given normal basal diet. However, 2nd and 3rd groups were supplemented with the feed, containing 0.2 mg/kg and 0.4 mg/kg Se-NPs with respect to their body weight. 4th and 5th groups were fed with the diet, containing 0.2 mg/kg and 0.4 mg/kg of inorganic sodium selenite (SS), correspondingly. The oral gavage needle was used to deliver the treatments once a day, throughout the entire trial period (65 days) of the quails. Temperature (20-25°C) and moisture (70%) were regulated according to the conventional methodology of Japanese quail's management. The quails had unlimited access to water and commercial basal feed that met their developmental needs (NRC, 1994; Table 1).

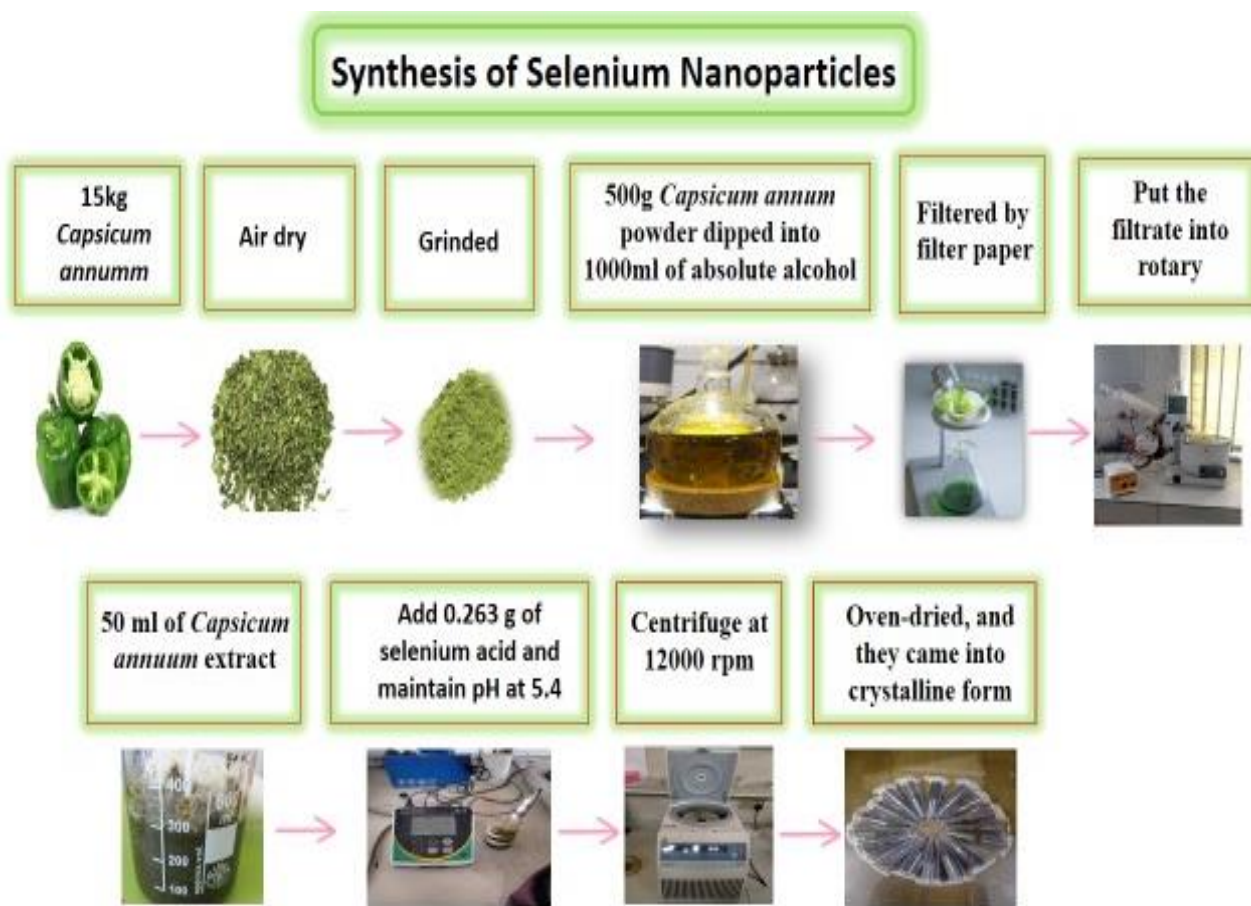


Fig. 1: Green synthesis of Selenium Nanoparticles by *Capsicum annum*.

Table 1: Major components and nutritional contents of Japanese quail diets.

| Components | Growing period | Laying period |
|--|----------------|---------------|
| Soya bean cake (Crude protein 42.9%) | 36 | 28.5 |
| Limestone | 1.65 | 6.1 |
| Corn (7.8% CP) | 52.3 | 53.7 |
| Oil | 1 | 1.1 |
| Lysine | 0.05 | 0.05 |
| Ca(H ₂ PO ₄) ₂ | 0.55 | 0.7 |
| Gluten (59.2% CP) | 7.7 | 5.12 |
| Wheat bran | 0 | 4 |
| Sodium chloride | 0.25 | 0.3 |
| Pre-mix of Vitamin | 0.15 | 0.15 |
| Pre-mix of Mineral | 0.15 | 0.15 |
| Threonine (Thr) | 0.04 | 0 |
| Lysine | 0.05 | 0.05 |
| mycotoxin sequestrants | 0.05 | 0.05 |
| Choline | 0.05 | 0 |
| Methionine | 0.06 | 0.08 |
| Chemical analysis | | |
| Ash percentage | 6.3 | 14.4 |
| Moisture percentage | 13.1 | 11.8 |
| Ether extract percentage | 4.4 | 4.1 |
| Crude protein percentage | 24.1 | 20.2 |
| Ca | 0.8 | 2.5 |
| ME Kcal/kg | 2,972.5 | 2,816.72 |
| Selenium mg per kg | 0.25 | 0.29 |
| P | 0.32 | 0.37 |

Estimation of Growth Performance: Feed intake and body weight were calculated for all Japanese quail in the control and treatment groups, starting from the first day to the next every successive week. Feed intake and Body weight gain data were collected to calculate the feed conversion ratio (Chand *et al.*, 2020).

Feed intake (g): Feed intake (FI) was measured weekly by using given formula.

Feed intake = Feed given (g) - Remaining feed (g)

Body weight gain (g): Body weight gain was recorded every week.

Feed conversion ratio (FCR): FCR was also determined by using given formula.

Feed conversion ratio = Feed intake (g) / Body weight gain (g)

Digestibility of Nutrients %: Upon completion of trial, fecal samples were collected every 24 hours for 5 days from every cage separately, and then analyzed the nutrient digestibility by using standard methods (AOAC, 1995).

Dry matter (DM): Dry matter was measured by using oven at 105 °C for 3 hours.

Organic matter (OM): Organic matter was determined by using formula (dry matter- ash content).

Crude protein (CP): Crude protein was measured by using Kjeldahl apparatus (model No: 808132). K₂SO₄, FeSO₄, and CuSO₄ were combined in a 90:3:7 ratio. In a Kjeldahl flask, 5 g of the digestion mixture, 1 g of the dried sample, and 30 ml of H₂SO₄ were added. The flask was heated until it turned a transparent greenish solution. After cooling, the digested content was diluted with distilled water in a 250 ml flask. The micro Kjeldahl apparatus was washed with 10 ml of this diluted solution and 10 ml of

40% NaOH was added before steam distillation. Ammonia was collected when the pink color turned golden yellow. The solution of boric acid was then titrated against 0.1 ammonium sulfate.

Nitrogen was calculated using the formula: Nitrogen = $\frac{\text{Volume of H}_2\text{SO}_4 \times 0.0014 \times 250}{\text{Volume of H}_2\text{SO}_4}$

Weight of Sample $\times 10$

Crude protein (%) = $N_2 \times 6.25$

Crude fiber (CF): Crude fiber was calculated by using acid digestion method. A Soxhlet apparatus removed fat from one gram of oven-dried sample, which was then digested separately in 1.26% NaOH and H₂SO₄. The samples was transferred to petri dishes and placed in an oven at 105°C for 24 hours. After washing the sample with distilled water, it was ignited at 600°C in a muffle furnace to obtain ash, which was used to calculate crude fiber. CF content was estimated by taking the difference between the weights of the fecal samples.

Ether extract (EE): Ether extract was calculated by Soxtec system (J. P. Selecta). A 3 g sample of ground feces was placed in a thimble, secured with cotton on top, and positioned in the condenser. The extraction cup was filled with 55-75 ml of petroleum ether and clamped into condenser. The extraction mode was set to boiling until the sample boiled, then switched to neutral for 10 minutes. The condenser valves were opened while suspending the thimble above the solvent. After turning off the power and water supply, the extraction cup containing the extract was removed, placed in a desiccator, and weighed. EE(%) was calculated using the formula:

$$\text{EE (\%)} = \frac{\text{Weight of Extract} \times 100}{\text{Weight of Sample}}$$

Ash content: Using an oxidizing flare, 1 g of fecal sample was carbonized until no fumes emerged. The sample was then burned in a muffle furnace (P-176) set at 602°C to ignite it.

Ash content was calculated using the following formula:

$$\text{Ash \%} = \frac{\text{Weight of Ash}}{\text{Weight of Sample}} \times 100$$

Serum biochemical analysis: On the completion of the trial, fifteen quails (five birds/replicate) from each group were selected randomly for the blood collection. About 2ml of blood sample was drain from the wing vein. Centrifuged the blood at 4000 rpm for ten minutes. Separate the serum and kept at -20°C for further biochemical examination. Glucose level was checked by Commercial kit (Singapore Bioscience PTE Ltd, Singapore). The concentration of Protein was measured by using the technique of IFCC (International Federation of Clinical Chemistry and Laboratory Medicine). The cholesterol concentration was determined by enzymatic calorimetric technique by using commercial kits (Tech Diagnostic Technologies, Madrid, Spain). The analyzer was used to measure the levels of HDL and LDL using commercial kits (Bad Cholesterol and Good Cholesterol/ Beta lipoprotein Quantification

Colorimetric or Fluorometric Kits; Bio vision Incorporation., Mountain View, CA, United States. Automated hematology analyzer (Mindray BC-20s) was used to measure the level of Hb, WBC, platelet count, neutrophil, lymphocytes, monocyte, and eosinophil.

Statistical analysis: IBM SPSS Statistics version 21 and Statistics version 8.1 were used for all statistical analyses. The impact of diet on the growth performance, nutrient digestibility, and serum biochemical parameters of Japanese quails was examined using One-way ANOVA (Analysis of Variance). This test was suitable for this data type as it allowed for the comparison of means across multiple treatment groups to determine if there were statistically significant differences among them. When ANOVA indicated significant effects, The Post Hock Turkey test was employed to make pairwise comparisons between the means of different groups. A significance level of $P < 0.05$ was set for overall analysis. Data were represented as the mean \pm standard deviation.

RESULTS

Characterization of synthesized Se-NPs: Fig. 2a displays the Ultraviolet-Visible (UV-VIS) spectroscopy of the newly synthesized Se-NPs and a clear absorption band was formed at 294 nm. This characteristic absorption peak indicated the successful synthesis of Se-NPs, confirming their presence and potential size, as it aligned with the expected optical properties of Se-NPs. To identify the functional group present on the surface of Se-NPs, Fourier transform infrared spectroscopy (FTIR) was performed Fig. 2b. The samples showed a consistently strong and broad signal around 3400 cm⁻¹, which is characteristic of OH/NH groups found in the capsicum molecule. This suggested that the Se-NPs were likely capped with organic molecules, which could enhance their stability and solubility in biological systems. The physical characteristics of Se-NPs were assessed by using X-ray diffraction (XRD) analysis. XRD spectra revealed that there were an extremely wide diffraction peaks in the range of 0-25 at the angle of 2θ. The diffraction peak shows that the synthetic Se-NPs are amorphous in nature and very small in size Fig. 2c. The amorphous structure of Se-NPs was significant as it could enhance their bioavailability in biological systems, allowing for better absorption and utilization by cells. The functional groups could facilitate binding with cellular membranes, potentially enhancing the uptake of Se-NPs by cells and improving their efficacy as a nutrient or therapeutic agent.

Growth performance: Table 2 shows the weekly feed intake (FI) of *C. coturnix japonica* fed with low and high concentrations of Se-NPs and SS. The findings displayed prominent ($P < 0.05$) differences in weekly feed intake between different experimental groups. On average, highest FI (296.32 ± 126.8^a) was shown in the group fed with Se-NPs low dose followed by Se-NPs high dose (288.63 ± 136.4^b) > SS high dose (284.62 ± 138.4^c) \approx SS low dose (284.31 ± 141.0^c) > Control (278.43 ± 136.2^d). Mean values with distinct superscripts (a, b, c, d) indicated substantial variation among groups.

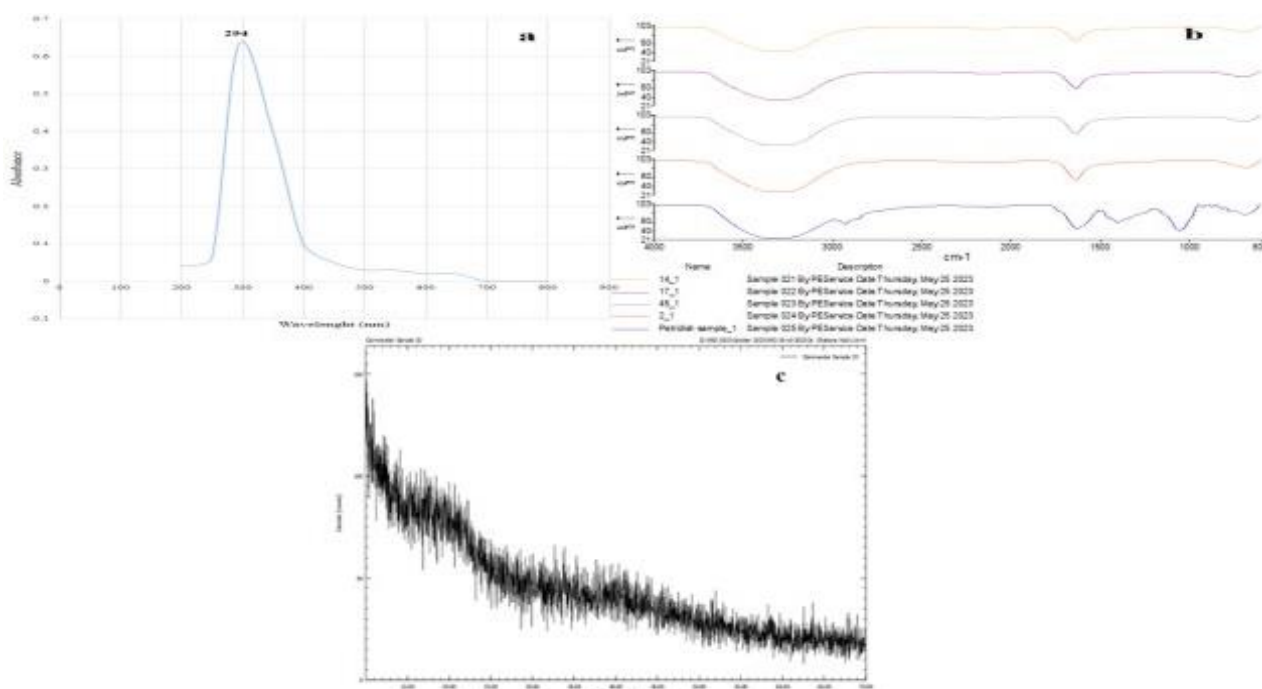


Fig. 2: UV-visible absorption spectra, the synthesized Se-NPs exhibit optical absorption at approximately 294 nm (a); In the FTIR spectra, the range of wavelengths is from 400-4000 cm^{-1} (b); XRD configurations, the presence of wide range diffraction peak at 2θ indicates the formation of the amorphous structure of the Se-NPs (c).

Table 3 displays the weekly body weight of *C. coturnix japonica* fed with low and high concentrations of Se-NPs and SS. The results indicated that considerable ($P < 0.05$) changes happened in the body weight of Japanese quails during first two weeks. However, no significant changes occurred during 3rd, 4th and 5th weeks. On average, highest body weight (104.73 ± 29.36^a) was shown in the group fed with Se-NPs high dose followed by Se-NPs low dose (102.38 ± 29.91^{ab}) > SS low dose (99.83 ± 29.16^{bc}) > SS high dose (97.70 ± 27.71^c) > Control group (92.17 ± 30.46^d). Mean values with different superscripts (a, b, c, d) showed significant variation among groups.

To determine the feed conversion ratio (FCR) across different groups, an analysis of variance (ANOVA) was utilized to assess differences. Table 4 exhibits the weekly FCR of *C. coturnix japonica* fed with low and high concentrations of Se-NPs and SS. Similar to body weight, substantial ($P < 0.05$) changes ensued in the FCR of Japanese quails during first two weeks. However, no significant changes occurred during 3rd, 4th and 5th weeks. Overall, lowest FCR value (2.62 ± 0.65^d) was shown in the group fed with Se-NPs high dose followed by Se-NPs low dose (2.67 ± 0.64^c) \approx SS low dose (2.69 ± 0.71^c) < SS high dose (2.80 ± 0.82^b) < Control (2.87 ± 0.55^a). Mean values with various superscripts (a, b, c, d) showed considerable variations among groups.

Impacts of different doses of Se-NPs and SS on nutrient digestibility of *C. coturnix japonica* are summarized in Table 5. According to the results, there were significant alterations ($P < 0.05$) in DM, OM, CP, CF, EE and ash content. Japanese Quails fed with Se-NPs high dose showed the highest value of DM (82.87 ± 0.78), OM (80.36 ± 0.75), CP (68.12 ± 0.70), CF (58.60 ± 0.54), EE (92.00 ± 0.45) and ash content (63.81 ± 0.28) as compared to other groups.

Table 6 displays the serum biochemical parameters of *C. coturnix japonica* fed with different concentrations of Se-NPs and inorganic sodium selenite (SS). According to the results, there were significant variations ($P < 0.05$) in glucose, protein, cholesterol, triglycerides, HDL, LDL, Hemoglobin (Hb), white blood cells (WBCs), platelets count (PLT) and neutrophils. However, there were non-significant differences in lymphocytes, monocytes and eosinophil numbers. Highest glucose concentration was found in the groups treated with SS low dose (97.33 ± 0.58 mg/dL) followed by SS high dose (96.66 ± 1.15 mg/dL) > SeNPs low dose (95.66 ± 1.15 mg/dL) > Control (95.33 ± 0.58 mg/dL) > SeNPs high dose (93.33 ± 0.58 mg/dL). Japanese Quails fed with Se-NPs high dose showed the lowest value of protein (4.83 ± 0.06 g/dL), cholesterol (287.33 ± 2.51 mg/dl), triglycerides (130.00 ± 17.32 mg/dL), HDL (30.31 ± 0.58 mg/dl) and LDL (130.00 ± 1.00 mg/dl) as compared to others groups. Highest value of hemoglobin (6.33 ± 0.058 g/dL), WBC (4266.67 ± 57.74 / μL), platelet count (16333 ± 577.3 / μL) and neutrophil % (98.33 ± 0.58) were presented in the group treated with Se-NPs low dose as compared to other groups.

DISCUSSION

Selenium performs a critical function in the improvement of antioxidants defense and immunity against oxidative stress and different diseases. Se-NPs also exhibit a variety of advantages such as, minimized cytotoxicity, increased surface area for interaction of bio-molecules, and improved drug stocking capacity (Abdel-Moneim *et al.*, 2022). They also seem to be the preferred food in poultry nutrition. According to reports, they are bioactive compounds that can sustain, physiological processes efficiently against oxidative stress and lipid peroxidation, by boosting host resistance and ultimately

Table 2: Weekly Feed intake (Mean \pm SD) of Japanese quails treated with low and high doses of Se-NPs and SS.

| Weeks | Control | Se-NPs (0.2mg/kg) | Se-NPs (0.4mg/kg) | SS (0.2mg/kg) | SS (0.4mg/kg) | P. value |
|-----------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|----------|
| 1 | 115.36 \pm 1.12 ^e | 130.96 \pm 1.77 ^a | 129.29 \pm 1.78 ^b | 118.65 \pm 1.34 ^d | 120.24 \pm 1.55 ^c | 0.000*** |
| 2 | 174.88 \pm 1.33 ^e | 181.77 \pm 1.15 ^c | 186.37 \pm 1.43 ^a | 178.48 \pm 1.22 ^d | 183.70 \pm 1.14 ^b | 0.000*** |
| 3 | 250.93 \pm 1.34 ^e | 253.05 \pm 1.41 ^c | 256.46 \pm 1.53 ^b | 252.28 \pm 1.51 ^d | 260.99 \pm 1.23 ^a | 0.000*** |
| 4 | 374.40 \pm 1.55 ^d | 380.09 \pm 1.54 ^a | 376.51 \pm 1.38 ^b | 376.47 \pm 1.45 ^c | 358.82 \pm 1.47 ^e | 0.000*** |
| 5 | 476.56 \pm 1.61 ^e | 491.83 \pm 1.32 ^d | 494.13 \pm 1.42 ^c | 495.67 \pm 1.21 ^b | 499.33 \pm 1.35 ^a | 0.000*** |
| Overall mean(g) | 278.43 \pm 136.2 ^d | 296.32 \pm 126.8 ^a | 288.63 \pm 136.4 ^b | 284.31 \pm 141.0 ^c | 284.62 \pm 138.4 ^c | 0.000*** |

SS=Sodium selenite: The means values with distinct superscripts (a,b,c,d,e) in a row exhibit a substantial variation at (P<0.05): ***=Highly significant

Table 3: Weekly Body weight (Mean \pm SD) of Japanese quails fed with low and high doses of Se-NPs and SS.

| Weeks | Control | Se-NPs (0.2mg/kg) | Se-NPs (0.4mg/kg) | SS (0.2mg/kg) | SS (0.4mg/kg) | P. value |
|-----------------|--------------------------------|----------------------------------|---------------------------------|---------------------------------|--------------------------------|---------------------|
| 1 | 48.88 \pm 2.64 ^b | 60.63 \pm 8.25 ^a | 63.38 \pm 8.75 ^a | 57.88 \pm 3.56 ^{ab} | 58.37 \pm 5.95 ^a | 0.001*** |
| 2 | 71.38 \pm 3.54 ^b | 83.38 \pm 10.60 ^{ab} | 87.50 \pm 11.78 ^a | 81.50 \pm 6.57 ^{ab} | 83.50 \pm 7.87 ^{ab} | 0.009** |
| 3 | 93.63 \pm 5.85 | 105.88 \pm 13.08 | 109.13 \pm 12.24 | 106.00 \pm 7.87 | 102.75 \pm 15.54 | 0.095 ^{NS} |
| 4 | 117.50 \pm 6.68 | 127.12 \pm 12.50 | 127.63 \pm 15.32 | 122.63 \pm 10.62 | 116.50 \pm 15.00 | 0.254 ^{NS} |
| 5 | 129.50 \pm 10.38 | 134.38 \pm 9.00 | 136.50 \pm 15.42 | 131.13 \pm 18.86 | 127.38 \pm 15.67 | 0.717 ^{NS} |
| Overall mean(g) | 92.17 \pm 30.46 ^d | 102.38 \pm 29.91 ^{ab} | 104.73 \pm 29.36 ^a | 99.83 \pm 29.16 ^{bc} | 97.70 \pm 27.71 ^c | 0.000*** |

SS=Sodium selenite: The means values with distinct superscripts (a,b,c,d) in a row exhibit a substantial variation at (P<0.05): ***=Highly significant; **=Very significant, NS=Non-Significant

Table 4: Weekly Feed conversion ratio (Mean \pm SD) of Japanese quails supplemented with low and high doses of Se-NPs and SS.

| Weeks | Control | Se-NPs (0.2mg/kg) | Se-NPs (0.4mg/kg) | SS (0.2mg/kg) | SS (0.4mg/kg) | P. value |
|--------------|------------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|---------------------|
| 1 | 2.36 \pm 0.13 ^a | 2.16 \pm 0.26 ^{ab} | 2.04 \pm 0.25 ^b | 2.05 \pm 0.13 ^b | 2.06 \pm 0.26 ^{ab} | 0.026* |
| 2 | 2.45 \pm 0.12 ^a | 2.18 \pm 0.26 ^{ab} | 2.13 \pm 0.23 ^b | 2.19 \pm 0.19 ^{ab} | 2.20 \pm 0.23 ^{ab} | 0.031* |
| 3 | 2.68 \pm 0.18 | 2.39 \pm 0.24 | 2.35 \pm 0.24 | 2.38 \pm 0.89 | 2.54 \pm 0.52 | 0.168 ^{NS} |
| 4 | 3.20 \pm 0.19 | 2.99 \pm 0.28 | 2.95 \pm 0.30 | 3.07 \pm 0.29 | 3.28 \pm 0.50 | 0.227 ^{NS} |
| 5 | 3.68 \pm 0.44 | 3.66 \pm 0.40 | 3.62 \pm 0.23 | 3.78 \pm 0.53 | 3.92 \pm 0.57 | 0.707 ^{NS} |
| Overall mean | 2.87 \pm 0.55 ^a | 2.67 \pm 0.64 ^c | 2.62 \pm 0.65 ^d | 2.69 \pm 0.71 ^c | 2.80 \pm 0.82 ^b | 0.000*** |

SS=Sodium selenite: The means values with distinct superscripts (a,b,c,d) in a row exhibit a substantial changes at (P<0.05): *= significant, ***=Highly significant, NS=Non-Significant

Table 5: Nutrient Digestibility (Mean \pm SD) of Japanese quails supplemented with low and high doses of Se-NPs and SS.

| Nutrient digestibility % | Control | Se-NPs (0.2mg/kg) | Se-NPs (0.4mg/kg) | SS (0.2mg/kg) | SS (0.4mg/kg) | P. value |
|--------------------------|--------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|----------|
| DM | 73.10 \pm 1.71 ^{cd} | 79.77 \pm 0.46 ^{ab} | 82.87 \pm 0.78 ^a | 76.53 \pm 1.54 ^{bc} | 73.03 \pm 1.50 ^d | 0.000*** |
| OM | 75.07 \pm 0.83 ^c | 79.23 \pm 0.10 ^b | 80.36 \pm 0.75 ^a | 72.92 \pm 0.53 ^{cd} | 72.04 \pm 0.85 ^d | 0.000*** |
| CP | 55.23 \pm 3.01 ^c | 62.80 \pm 0.53 ^b | 68.12 \pm 0.70 ^a | 55.25 \pm 1.02 ^c | 53.46 \pm 1.25 ^c | 0.000*** |
| CF | 39.36 \pm 0.80 ^c | 54.19 \pm 0.95 ^b | 58.60 \pm 0.54 ^a | 39.47 \pm 1.00 ^c | 41.13 \pm 1.73 ^c | 0.000*** |
| EE | 81.42 \pm 1.03 ^d | 89.23 \pm 0.62 ^b | 92.00 \pm 0.45 ^a | 84.26 \pm 1.00 ^c | 80.05 \pm 0.25 ^d | 0.000*** |
| Ash | 41.06 \pm 0.43 ^d | 58.17 \pm 0.26 ^b | 63.81 \pm 0.28 ^a | 46.86 \pm 3.90 ^c | 43.11 \pm 1.44 ^{cd} | 0.000*** |

SS=Sodium selenite; DM=Dry matter; OM=Organic matter; CP=Crude protein; CF=Crude fiber; EE=Ether extract: The means values with distinct superscripts (a,b,c,d) in a row exhibit a substantial changes at (P<0.05): ***= highly significant

Table 6: Serum biochemical parameters (Mean \pm SD) of Japanese quails treated with low and high doses of Se-NPs and SS.

| Parameters | Control | Se-NPs (0.2mg/kg) | Se-NPs (0.4mg/kg) | SS (0.2mg/kg) | SS (0.4mg/kg) | P. value |
|----------------------------|--------------------------------|----------------------------------|-----------------------------------|----------------------------------|----------------------------------|---------------------|
| Glucose (mg/ dL) | 95.33 \pm 0.58 ^{ab} | 95.66 \pm 1.15 ^a | 93.33 \pm 0.58 ^b | 97.33 \pm 0.58 ^a | 96.66 \pm 1.15 ^a | 0.002** |
| Protein (g/dL) | 5.46 \pm 0.06 ^a | 6.63 \pm 0.06 ^a | 4.83 \pm 0.06 ^b | 5.43 \pm 0.06 ^a | 5.43 \pm 0.15 ^a | 0.000*** |
| Cholesterol (mg/dL) | 336.67 \pm 4.73 ^b | 382.76 \pm 2.51 ^a | 287.33 \pm 2.51 ^c | 336.67 \pm 7.23 ^b | 327.00 \pm 6.08 ^b | 0.000*** |
| Triglycerides (mg/dL) | 153.33 \pm 5.58 ^a | 164.66 \pm 1.52 ^a | 130.00 \pm 17.32 ^b | 184.66 \pm 6.81 ^a | 143.33 \pm 5.58 ^b | 0.000*** |
| HDL (mg/dL) | 32.33 \pm 0.59 ^b | 46.32 \pm 0.57 ^a | 30.31 \pm 0.58 ^c | 32.31 \pm 0.60 ^b | 32.00 \pm 1.00 ^{bc} | 0.000*** |
| LDL (mg/dL) | 193.00 \pm 1.00 ^a | 136.33 \pm 0.58 ^b | 130.00 \pm 1.00 ^c | 191.33 \pm 0.58 ^a | 193.00 \pm 1.00 ^a | 0.000*** |
| Hemoglobin (g/dL) | 5.87 \pm 0.058 ^c | 6.33 \pm 0.058 ^a | 6.03 \pm 0.058 ^{bc} | 6.20 \pm 0.17 ^{ab} | 6.00 \pm 0.00 ^{bc} | 0.001*** |
| WBC (cells /uL) | 3900 \pm 0.00 ^b | 4266.67 \pm 57.74 ^a | 3766.67 \pm 57.74 ^{bc} | 4133.33 \pm 57.74 ^a | 3666.67 \pm 57.74 ^c | 0.000*** |
| Platelet count (cells /uL) | 11666 \pm 577.3 ^c | 16333 \pm 577.3 ^a | 14333 \pm 577.3 ^b | 15666 \pm 577.3 ^{ab} | 12666 \pm 577.3 ^c | 0.000*** |
| Neutrophil% | 95.66 \pm 0.58 ^b | 98.33 \pm 0.58 ^a | 94.33 \pm 0.58 ^b | 98.33 \pm 0.58 ^a | 94.33 \pm 0.58 ^b | 0.000*** |
| Lymphocytes% | 1.00 \pm 1.00 | 3.00 \pm 1.00 | 2.00 \pm 1.00 | 3.00 \pm 1.00 | 2.00 \pm 1.00 | 0.156 ^{NS} |
| Monocyte% | 1.00 \pm 1.00 | 1.00 \pm 1.00 | 1.00 \pm 1.00 | 1.00 \pm 1.00 | 1.00 \pm 1.00 | 1.000 ^{NS} |
| Eosinophil% | 0.00 \pm 1.00 | 1.00 \pm 1.00 | 1.00 \pm 1.00 | 1.00 \pm 1.00 | 1.00 \pm 1.00 | 0.580 ^{NS} |

SS=Sodium selenite: The means values with distinct superscripts (a,b,c) in a row exhibit a significant variation at (P<0.05). ***=Highly significant; **=Very significant, NS=Non-Significant

increased the growth performance of them (Ibrahim *et al.*, 2020). However, there remains a significant gap in understanding the effects of Se-NPs and SS on growth performance, nutrient digestibility, and serum biochemical parameters in Japanese quails. Variability in experimental conditions such as Se forms, dosage, duration of supplementation, and the specific poultry species studies can significantly influences outcomes. Furthermore, the methods used to assess growth performance and nutrient digestibility may vary, leading to discrepancies in reported results. Our findings contributed to this emerging body of knowledge by providing a comprehensive analysis of the

impacts of Se-NPs compared to SS in Japanese quails. By addressing these gaps in the literature, we advanced our understanding of the potential applications of Se-NPs in poultry nutrition and their implications for improving growth and health status.

Relating to the feed intake of Japanese quails, the findings revealed that feeding them with Se-NPs potently (P<0.05) increased the weekly feed intake, in comparison with other treatment groups. Highest FI was observed in the group supplemented with Se-NPs low dose (0.2mg/kg) as compared to other groups. Se-NPs are able to cross the mucosal barriers of gut wall through paracellular and

transcellular routes. The tight junctions between the epithelial cells and the entry of Se-NPs into intercellular gaps define the paracellular route (Alian *et al.*, 2020). Nevertheless, transcellular transport is achieved by the process of transcytosis, which begins with endocytosis in the apical membrane of the cell and proceeds across the basolateral pole of the cell. According to research by Gangadoo *et al.* (2016), Se-NPs with compacted particle size and shapes are able to transfer nutrients more effectively without developing complicated uptake mechanisms or causing loss of cellular energy.

In this investigation, the results exposed that considerable ($P < 0.05$) changes happened in the weekly body weight of Japanese quails. Highest body weight was observed in the quails treated with high dose Se-NPs (0.4 mg/kg). It's possible that the enhanced growth performance of birds treated with Se-NPs is due to successive impact of diet on the feed utilization and assimilation of nutrition (Alwaleed *et al.*, 2021). It stimulates the production of growth factors, such as insulin-like growth factor (IGF). These factors promote cell growth, proliferation, and tissue repair, contributing to overall tissue health. The present study has shown that taking Se-NPs orally is thought to be the most suitable and economical way to supplement (Mohapatra *et al.*, 2014). According to Surai and Kochish (2019), Se is necessary for the production and expression of almost twenty five distinct selenoproteins that play crucial role in the protection of cell. Selenoprotein N1 (SEPN1) is one of those selenoproteins that is important for the growth of skeletal muscles. Animal growth performance is primarily effected by the impairment in skeletal muscles that is the primary consumable portion of chickens. Selenoproteins mediate the transformation of tetraiodothyronine into triiodothyronine and also necessary for the metabolism of thyroid hormones. Lack of Se causes selenium to be expressed at lower amounts on the mRNA and prevents chicken thyroids from converting T4 to T3. According to the Mahmoud *et al.* (2016), broilers that were fed with 0.3 mg/kg Se-NPs and kept in a warm environment were able to exhibit remarkable growth performance. And it was recognized that this improvement is due to an increase in anti-oxidative and immunological activities, such as the production of glutathione peroxidase mRNA and rise in the impression of the cytokine genetic factor (interleukins II & VI) in their mRNA form. Glut-8, somatomedin 1 and insulin receptors are the three growth factors that have been demonstrated to be enhanced by Se-NPs, according to an experimental that was conducted by Saleh and Ebeid (2019). Furthermore, the addition of Se-NPs to feed at a dose of 0.3 mg/kg increased the rate of growth because of the supplement's stronger absorbing potential, increased catalytic efficacy, FCR, higher bacterial activity, greater bioavailability in the nutrition, and stress reduction in broiler chickens (Surai *et al.*, 2018). According to Hassan *et al.* (2020), Se-NPs' low absorption rate, longer distribution, and increased bioavailability contribute to their good effects on gut health, effective digestion, growth performance, and overall health of poultry products and birds.

The current study revealed that Se-NPs at quantity of 0.2 mg/kg and 0.4 mg/kg have positive effects on Feed conversion ratio. Lowest FCR value was shown in the group fed a diet with Se-NPs high dose (0.4 mg/kg). Lower

FCR indicated that feed is efficiently converted into body weight gain when the quails were supplanted with Se-NPs high dose. The primary effects of Se-NPs on ultimate growth performance are attributed to their physical and chemical characteristics. For the reason that nanoparticles have surface area that is significantly larger than that of micro particles. By increasing the surface area that are available for chemical reactions, it is believed that the smaller particle size of nanoparticles improves mineral absorption, bioavailability, and consumption in the digestive system (Sa'aci *et al.*, 2021). In our study, the quails supplemented with Se-NPs (0.4 mg/kg) had the highest feed intake, body weight, and lowest FCR compared to the other groups. Higher FI provides more energy and nutrients, directly supporting greater body weight gains. In the case of Se-NPs, the increase in FI allowed the birds to maximize their growth potential. A low FCR implies that the birds are converting feed into body weight more efficiently. The combination of high FI and low FCR creates a synergistic effect on overall growth performance. This reciprocal relationship demonstrates how enhancing one parameter (FI) can positively impact another (FCR) and, ultimately, body weight changes (Abd El-Hack *et al.*, 2024).

In this study there were significant alterations ($P < 0.05$) in DM, OM, CP, CF, EE and ash content. Japanese Quails fed with Se-NPs high dose showed the highest value of DM, OM, CP, CF, EE and ash content as compared to other groups. Se-NPs can increase the bioavailability of essential nutrients by enhancing their solubility and stability in biological systems. This means that more nutrients can be absorbed effectively by cells (Javdani *et al.*, 2019). Wang and Yan (2013) revealed that the intestinal epithelial cells have higher protein levels when exposed to a specific dose of Se-NPs. Increased intestinal epithelial cell, intracellular protein may lead to improved feed ingredient metabolism and increased growth enhancement. Selenium has a function in the manufacture of digestive enzymes by acting as a co-enzyme and so increasing their activity, which is another potential explanation. More nutrients are released for absorption by the intestinal epithelial cells when digestive enzymes are activated, increasing the digestibility of nutrients. These findings are in accordance with Elnaggar *et al.* (2020) who identified that feeding the chickens with organic selenium increase the digestibility of DM, CP, CF and EE. Also, significant improvement in digestibility of dry matter, crude protein, crude fiber, ether extract and Ash were obtained from animals feeding with organic Se as compared to other groups (Mariani *et al.*, 2021). However, Amer *et al.* (2019) found no discernible variations in the digestion efficiency ratios of dry matter, organic matter, and crude protein between the groups that received Se treatment and the control group. Furthermore, no obvious alterations in the digestion coefficients of DM, OM, and CP between the organic and inorganic sources of Se were found. In this regard, Sundu *et al.* (2019) found that adding Se from various sources to the diets did not result in an increase in DM digestibility.

Blood constituents can be good bio indicators for the wellbeing of Japanese quails. Regarding the serum biochemical parameters of Japanese quail fed with different amounts of Se-NPs and SS, a significant variations

($P < 0.05$) were seen in the glucose level, protein, cholesterol, triglycerides, HDL, LDL, Hb, white blood cells (WBCs) platelet count (PLT) and neutrophil. Highest glucose level was found in the quails treated with SS low dose as compared to other groups. SS displayed an insulin-like effect in animals. It promote transport and metabolism of glucose by promoting mitogen-activated protein/myelin basic protein kinases (MAPK) pathway and ribosomal S6 protein kinases (Zhao *et al.*, 2022). Similarly, Sarmiento-García *et al.*, 2022 reported that feeding the quails with SS at level of 0.5 mg/kg increase the glucose level in blood.

In our investigation the level of protein, cholesterol, triglycerides, HDL, and LDL were lowest in the group treated with Se-NPs high dose as compared to others groups. Similarly, Emara *et al.* (2019) emphasized that TC, LDL, and triglyceride levels were all decreased by dietary Se-NPs supplementation. The hypolipidemic effect of Se-NPs may enhance overall cardiovascular health in quails, reducing the risk of atherosclerosis and other cardiovascular diseases, which can directly influence longevity (Xiao *et al.*, 2021). The biochemical mechanisms behind the reduced lipid levels involve several pathways. Se promotes the conversion of cholesterol to bile acids, enhancing cholesterol elimination through increased action of cholesterol 7 alpha-hydroxylase. This process not only lowers cholesterol levels but may also help maintain healthier blood vessels, further reducing disease risk (Dalia *et al.*, 2017) El-Deep *et al.* (2017) findings showed that selenium-dependent antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase, may be useful in lowering cholesterol levels and in lowering Hydrogen peroxide and lipid peroxide concentrations. By reducing oxidative stress, these enzymes help to protect cellular integrity, which can prevent the onset of chronic diseases. The enhanced antioxidant status of Se-NPs may also contribute to lower inflammatory responses in quails. Chronic inflammation is linked to numerous health issues, including obesity and metabolic disorders. By mitigating inflammation, Se-NPs can support better health outcomes and reduce the risk of diseases associated with inflammation (Abdelnour *et al.*, 2021). Supplementing with Se-NPs enhanced the concentrations of 14-prostaglandin J2 and 15-deoxy-delta-12, while reducing the amount of sterol regulatory element binding transcription factor 2 (SREBF2) and decreasing the production of cholesterol. These hormonal and enzymatic changes can contribute to better metabolic health and potentially extend the lifespan of quails.

In the present study, the level of hemoglobin and numbers of WBCs, platelet and neutrophil were highest in the groups treated with Se-NP low dose as compared to other groups. The increased Hb levels in quails treated with Se-NPs indicate enhanced oxygen transport throughout the body (Alagawany *et al.*, 2021). This is crucial for energy metabolism and overall physiological functions. Higher Hb values reduces the symptoms of anemia leading to better growth rates and improved performance in quails. Due to its antioxidant characteristics, Se protects the red blood cell membranes against free oxygen radicals and decreases the hemolysis of cells (Çiçek and Özoğul, 2021). Similarly, Selim *et al.* (2015) described that the presence of minute quantity of selenium (0.30 ppm) in broiler diet increased

some hematological indices. According to Talebi and Ghazanfarpoor (2021) hemoglobin (Hb) levels significantly increased in the quail treated with 0.2 mg/kg of Se-NPs. The outcomes of Alagawany *et al.* (2021) showed that Hb level was elevated by 0.4 - 0.6 mg/kg of Se-NPs to the quail diet. By comparing to the control group, Bealish *et al.* (2018) findings showed that Silver Montazah chickens given a feed enriched with Se-NPs (0.25 mg/kg) had the maximum level of Hb, packed cell volume, lymphocytes, and heterophils. According to Eid *et al.* (2023), birds given Se-NPs showed rise in RBCs count, Hb, and hematocrit than the control group. The elevated WBC counts are particularly significant as they indicate a more robust immune response. WBCs are essential for defending against infections and diseases. An increase in WBCs, along with higher platelet and neutrophil counts in the quails treated with Se-NPs, reflects the overall health status of the birds. This suggests that the quails are better equipped to respond to pathogens, potentially reducing the incidence of diseases. In quails, it has been showed that Se-NPs is easily absorbed, and more effective in terms of its bioavailability and impact on animal health, than SS (Saffari *et al.*, 2018).

Conclusions: Green-synthesized Se-NPs proved to be more effective than SS in enhancing growth performance, nutrient digestibility, and various serum biochemical parameters in quails. This improvement reflects the superior bioavailability and lower toxicity of Se-NPs. Notably, the inclusion of 0.4 mg/kg of Se-NPs in the diet led to significantly higher growth rates, positioning these nanoparticles as an excellent dietary source of selenium for producing selenium-enriched meat.

To address the limiting factors of the present research, long-term effects of green-synthesized selenium nanoparticles (Se-NPs) on quail progeny, investigating how maternal dietary supplementation influences offspring growth, health, and selenium bioaccumulation. The duration of studies is crucial for assessing these long-term effects; while 4 to 12 weeks is common for growth studies in quails, extending this to 6 months or more would provide better insights into the impacts of Se-NPs on growth and health, including potential effects on progeny growth, health, and nutrient transfer through eggs.

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