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# Exploring the Nutraceutical Role of Selenium Nanoparticles on Laying Performance, Egg Attributes and Immune Response in Laying Hens

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## ABSTRACT

Current study investigated the dose-dependent effects of dietary selenium nanoparticles (SeNPs) supplementation on productive performance, organ development, hematological parameters and humoral immunity of laying hens. A completely randomized design was employed, allocating 120 twenty-week-old White Fayoumi birds to four dietary groups (n=30 per group) receiving 0.0 (control), 0.5, 1.0, or 1.5 mg/kg SeNPs for 12-weeks of experimental period (21 to 32 weeks of age). Results demonstrated a significant (P<0.05) enhancement in live weight gain and FCR in the group supplemented with 1.0 mg/kg SeNPs compared to the control. Similarly, egg production, egg weight, yolk weight, albumen weight, shell thickness, and egg mass were significantly (P<0.05) elevated in this group. Notably, the 1.0 mg/kg SeNPs group exhibited significantly (P<0.05) increased relative weights of all examined organs. Conversely, the 1.5 mg/kg SeNPs group showed a significant (P<0.05) decline in laying performance and FCR relative to the control. While the 1.0 mg/kg SeNPs group displayed improved trends in certain blood biochemical parameters, significant differences (P<0.05) were only observed for hemoglobin levels. Importantly, supplementation with 1.0 mg/kg SeNPs significantly (P<0.05) increased serum IgM and IgA concentrations, whereas the 1.5 mg/kg SeNPs dosage significantly (P<0.05) reduced these immunoglobulin levels. In conclusion, this study elucidates a critical role for SeNPs supplementation in laying hens, with an optimal inclusion level of 1.0 mg/kg significantly improved productivity, organ development, and humoral immunity. However, exceeding this dosage (1.5 mg/kg) resulted in detrimental effects, highlighting the narrow therapeutic window and the importance of precise SeNPs administration in poultry nutrition.

## INTRODUCTION

The poultry industry plays a critical role in Pakistan's economy, contributing approximately 5.76% to the agriculture sector and 10.4% to the livestock sector (Akhtar et al., 2018). Beyond its economic impact, it serves as a key source of high-quality protein through eggs and meat, while also supporting the livelihoods of rural communities across the country. The industry faces growing challenges stemming from increasing expenses for feed, pharmaceutical treatments, and vaccines, as well as labor costs and various stressors, including the emergence of new diseases (Arain et al., 2018; Nabi et al., 2020). Various stressors, including microbial infections and environmental factors such as temperature changes, combined with pollution, negatively impact poultry health and performance. (Saeed et al., 2017). Studied strategies for managing these issues involve growth promoters (Saeed et al., 2019) combined with nutraceuticals (Saeed et al., 2018) and therapeutic agents (Ashraf et al., 2024) and microminerals (Nabi et al., 2020a) and herbs with their derivatives (Hassan et al., 2023; Saeed et al., 2021) along with antioxidants to optimize performance and immunity (Arain et al., 2024; Changxing et al., 2018).

Selenium (Se) works as an important trace mineral that supports various biological functions in various species.

The two main supplemental forms of selenium in poultry farming include inorganic selenium, which uses sodium selenite as an example, and organic selenium, illustrated by selenomethionine (Nabi et al., 2023). Multiple studies reveal that selenium functions as a key element for immune enhancement and antioxidant protection of biological systems (Huang et al., 2012). Selenium's potency, together with its absorbability into the body, depends greatly on the specific chemical structure it takes. Symptoms related to inorganic selenium supplements derive from their poor absorption characteristics and elevated toxicity levels, making them less effective (Mahan et al., 1999).

SeNPs stand out as new dietary supplements for poultry nutrition because of their better nutrient uptake and lower toxic side effects when compared to classical forms of selenium and other trace elements. Premium biological functions of selenium involve supporting the enzyme glutathione peroxidase (GSH-Px) as a cofactor for protecting cells against reactive oxygen species (ROS), thus reducing oxidative stress (Gangadoo et al., 2016). Scientific research shows dietary addition of nano-selenium supplements or sodium selenite to laying hens supports better egg production metrics and optimized feed efficiency rates while increasing selenium storage in eggs and raising antioxidant enzyme levels and decreasing egg fat and cholesterol levels (Chen et al., 2024). Studies reveal that broiler weight gain increased after SeNPs treatment at various concentration points from 0.15 to 1.20 ppm, showing better safety results versus regular inorganic selenium sources (Hu et al., 2012). Studies show that particular doses of SeNPs lead to better antioxidant protection, together with improved immune responses in animals (Fuxiang et al., 2008).

SeNPs display major potential benefits for poultry growth and health functions because they serve as metabolic co-factors while acting as non-exogenous metallic elements. Previous research evaluated such favorable outcomes across multiple bird species although specific data regarding commercial Fayumi layers remains insufficient. Therefore, this study aims to investigate the biological and immunomodulatory effects of SeNPs in the Faoyumi hens. The findings may contribute to the advancement of SeNPs as a safe and effective alternative feed additive in the poultry industry.

## MATERIALS AND METHODS

### Experimental Design and Management of Birds

A total of 120 Twenty-week-old Fayumi breed of laying pullets (approximately 5 months of age), with an average body weight of 700 ± 80 grams, were selected and randomly assigned to four dietary treatment groups (n = 30 per group), each comprising three replicates of 10 birds. Prior to the experimental period, all birds underwent a one-week acclimatization phase. The study aimed to evaluate the biological effects of selenium nanoparticles (SeNPs) supplementation in laying hens. Four corn–soybean meal-based dietary treatments were formulated with incremental levels of SeNPs: 0.0 (control), 0.5, 1.0, and 1.5 mg/kg. Each diet was provided to three replicate subgroups.

All diets were formulated to meet or exceed the nutrient requirements established by the National Research Council (NRC, 1994). The experimental period extended from 21 to 32 weeks of age and was divided into three production phases: early (21–24 weeks), mid (25–28 weeks), and late (29–32 weeks) laying periods. The composition and chemical analysis of the basal diet are presented in Table 1. The SeNPs used in this study were procured from Nano Shel, Wilmington, Delaware, USA (CAS No. 7782-49-2), and the feed composition analysis was conducted in accordance with NRC (1994) guidelines, following the methodology described by Tiantian Meng et al. (2018).

The experiment maintained a consistent photoperiod of 16 hours of light and 8 hours of darkness per day. The average ambient temperature during the trial was 23.51 °C, with a relative humidity of 58.81%. Birds were housed in a semi-enclosed system at a stocking density of 5 birds per square meter, using floor-laying pens measuring 2 × 1.5 meters.

**Table 1**

*The Ingredient Composition and Nutrient Content of the Baseline Feed for Laying Hens*

| Item | Ingredients (%) |
|---|---|
| Corn | 56.00 |
| Soybean meal (43%) | 27.00 |
| Wheat bran | 4.00 |
| Limestone | 9.00 |
| Dicalcium phosphate | 1.20 |
| NaCl | 0.30 |
| Zeolite powder | 0.50 |
| Premix1 | 1.00 |
| Nano-Se (mg/Kg) | 1.0 |
| Total | 100.00 |
| **Nutrient and energy content (%)[2]** | |
| ME (kcal/kg) | 2670.32 |
| Crude protein | 17.38 |
| Ca | 3.75 |
| Total phosphorus | 0.60 |
| Available phosphorus | 0.36 |
| Methionine | 0.30 |
| Methionine + cystine | 0.57 |
| Lysine | 0.76 |
| Nano-Se (mg/kg) | 1.0 |

The diet provides the following nutrients per kilogram: 12,000 IU of vitamin A, 3,000 IU of vitamin $D_3$, 30 mg of vitamin E, 6 mg of vitamin $K_3$, 3 mg of vitamin $B_1$ (thiamine), 9 mg of vitamin $B_2$ (riboflavin), 6 mg of vitamin $B_6$ (pyridoxine), 0.03 mg of vitamin $B_{12}$ (cyanocobalamin), 0.15 mg of D-biotin, 18 mg of D-pantothenic acid, 1.5 mg of folic acid, 6 mg of nicotinamide, and 18.15 mg of ethoxyquin (antioxidant).

Additionally, the diet includes 50 mg of choline chloride, 10 mg of phytase, and the following minerals: 0.004 mg of calcium, 5.12 mg of copper, 72 mg of iron, 56 mg of zinc, 84.8 mg of manganese, 0.64 mg of iodine, and 0.32 mg of cobalt. Essential amino acids are also supplied, including 0.30 mg of methionine, 0.27 mg of cysteine, and 0.76 mg of lysine.

### Evaluate the Growth and Laying Performance

Throughout the experiment, body weight was recorded weekly for each replicate to calculate weight gain. Individual hens' feed intake and feed conversion ratio (FCR) were measured to determine the amount of feed consumed (g) per gram of egg mass produced (g), expressed as g feed/g egg mass. To assess laying performance, eggs were collected separately from each hen to evaluate the effect of treatments on egg production. At the end of the trial, six birds were randomly selected from each replicate and humanely slaughtered to determine the relative weights of edible and non-edible organs.

### Egg Characteristics

The Digital Egg Tester (DET6000, NABEL Co., Ltd., Japan) were used to measure the egg weight. Daily egg production and individual egg weights were recorded from each treatment group, and the laying rate was calculated as the number of eggs produced per hen for each treatment group. Egg mass was determined by multiplying the total number of eggs by their average weight. Additionally, the number of normal eggs, soft-shelled or cracked eggs, as well as hen mortality and morbidity rates, were systematically monitored and recorded on a daily basis.

### Hematological Analysis of Various Treatment Groups

At the end of the experiment, six birds were randomly selected from each experimental group for blood collection to assess immunological parameters. Blood samples were drawn into EDTA-coated tubes, and hematological parameters including red blood cell count, white blood cell count, and hemoglobin concentration were determined manually using a hemocytometer and Sahli's method. Biochemical parameters such as glucose, cholesterol, and alanine transaminase (ALT) levels were measured following the protocol described by Mondal et al. (2011). Serum samples were stored at −20°C for subsequent biochemical and immunoglobulin analyses. Concentrations of serum immunoglobulin A (IgA) and immunoglobulin M (IgM) were quantified using commercial ELISA kits (Thermo Scientific, Egypt). Humoral immune responses were further evaluated through hemagglutination inhibition (HI) tests to detect antibodies against Newcastle disease virus and avian influenza virus, according to the method described by Eladl et al. (2014).

### Statistical Analysis

All the data were analyzed using one-way analysis of variance (ANOVA), and post hoc Tukey's test. Each group contained thirty (30) white Fumi hens. The collected data were presented as mean and standard error of mean (SEM). The level of significance was set as 0.05 among the different experimental groups.

## RESULTS

### Performance Assessment of Laying Hens

Table 2 summarizes the effects of various doses of selenium nanoparticle (SeNP) supplementation on the performance of laying hens. Group 3, supplemented with 1.0 mg/kg SeNPs, exhibited the highest live weight gain, followed by Group 2, which also outperformed the control group (Group 1). Statistical analysis revealed that Groups 2 and 3 had significantly greater live weight gains compared to the control ($P < 0.05$). In contrast, Group 4, which received a higher SeNP dosage, showed a decline in weight gain over the trial period. Supplementation with 1.0 mg/kg SeNPs significantly improved the feed conversion ratio (FCR) relative to the unsupplemented control ($P < 0.05$). Moreover, hens receiving 1.0 mg/kg SeNPs produced a significantly higher number of eggs and heavier eggs than those in the control group ($P < 0.05$). Although daily feed intake showed no significant differences among groups ($P > 0.05$), a slight increase was observed in the control group. Overall, 1.0 mg/kg SeNP supplementation significantly enhanced the percentage of egg production ($P < 0.05$), primarily attributed to improved egg output.

**Table 2**

*Impact of Nano Selenium Supplementation on the Productive Performance Characteristics of Laying Hens.*

| Parameters | Group 1 | | | Group 2 | | | Group 3 | | | Group 4 | | | SE± LSD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Early | Mid | Last | Early | Mid | Last | Early | Mid | Last | Early | Mid | Last | |
| Live body weight (g) | 789.0$^{k}$ | 1383.8$^{h}$ | 1419.0$^{f}$ | 734.5$^{j}$ | 1252.2$^{g}$ | 1583.7$^{d}$ | 746.7$^{i}$ | 1662.5$^{c}$ | 1803.3$^{a}$ | 732.5j$^{k}$ | 1432.2$^{e}$ | 1697.2$^{b}$ | 2.16<br>4.33 |
| Feed intake (g/day/bird) | 96.6$^{i}$ | 98.2$^{f}$ | 99$^{d}$ | 112.8$^{i}$ | 108.8$^{ef}$ | 104.0$^{c}$ | 108.2$^{h}$ | 102.0$^{e}$ | 111.3$^{b}$ | 101.0$^{g}$ | 105.3$^{e}$ | 103.3$^{a}$ | 6.23<br>12.48 |
| FCR | 2.26$^{b}$ | 2.38$^{b}$ | 2.37$^{a}$ | 2.28$^{c}$ | 2.25$^{d}$ | 2.24$^{e}$ | 2.01$^{e}$ | 2.12$^{a}$ | 2.17$^{a}$ | 2.06$^{a}$ | 2.19$^{a}$ | 2.15$^{a}$ | 1.28<br>2.67 |
| Egg production (%) | 67.0$^{d}$ | 67.2$^{d}$ | 68$^{d}$ | 77.8$^{c}$ | 80.6$^{a}$ | 81.0$^{a}$ | 85.0$^{b}$ | 89.6$^{b}$ | 91.1$^{a}$ | 82.3$^{a}$ | 84.1$^{a}$ | 80.0$^{a}$ | 2.89<br>1.34 |
| Egg mass (g/hen/day) | 47.9$^{b}$ | 46.28$^{b}$ | 45.9$^{b}$ | 49.4$^{a}$ | 48.4$^{a}$ | 46.4$^{a}$ | 47.8$^{c}$ | 45.9$^{c}$ | 46.9$^{d}$ | 48.8$^{b}$ | 47.9$^{b}$ | 47.8$^{b}$ | 1.20<br>2.20 |

### Egg characteristics

The impact of dietary selenium nanoparticle (SeNP) supplementation on egg characteristics is summarized in Table 3. Egg weight, yolk weight, and albumen weight were evaluated across three production phases: early (21–24 weeks), mid (25–28 weeks), and late (29–32 weeks) laying periods. Statistical analysis indicated that supplementation with 1.0 mg/kg SeNPs significantly ($P < 0.05$) enhanced egg weight, yolk weight, and albumen weight compared to the control group. These results suggest a dose-dependent improvement in egg quality traits with higher SeNP inclusion. Moreover, dietary inclusion of SeNPs at 1.0 mg/kg significantly ($P < 0.05$) increased eggshell thickness and weight, whereas lower supplementation levels exhibited minimal or no significant effects relative to the control.

**Table 3**

*Impact of Nano Selenium Supplementation on the Quality Attributes of Eggs Produced by Laying Hens.*

| Parameters | Group 1 | | | Group 2 | | | Group 3 | | | Group 4 | | | S±E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Early | Mid | Last | Early | Mid | Last | Early | Mid | Last | Early | Mid | Last | LSD |
| Egg weight (g) | 45.7± $0.05^{g}$ | 44.9± $0.05^{fg}$ | 45.9± $0.50^{cde}$ | 45.4± $0.16d^{ef}$ | 47.50.4± $0.18^{bcd}$ | 46.4± $0.16^{abc}$ | 49.26± $0.19^{cde}$ | 52.9± $0.04^{ab}$ | 54.6± $0.65^{a}$ | 45.8± $0.15^{h}$ | 49.9± $0.04^{fg}$ | 52.6± $0.65^{efg}$ | 1.96 3.89 |
| Egg yolk weight (g) | 11.7± $0.4^{fg}$ | 12.4± $0.5^{de}$ | 11.4± $0.6^{cde}$ | 12.4± $0.4^{de}$ | 13.74± $0.5^{bcd}$ | 14.1± $0.56^{ab}$ | 12.7± $0.72^{de}$ | 11.39± $0.59^{abc}$ | 13.6± $0.3^{a}$ | 10.7± $0.8^{g}$ | 12.1± $0.4^{efc}$ | 11.1± $0.2^{cde}$ | 0.80 1.58 |
| Egg Albumin weight (g) | 30.5± $0.6^{e}$ | 28.9± $0.3^{cd}$ | 30.8± $1.1^{bc}$ | 29.4± $0.57^{de}$ | 29.4± $1.1^{bc}$ | 27.6± $0.8^{b}$ | 31.0± $1.2b^{c}$ | 36.89± $0.8^{ab}$ | 35.2± $0.7^{a}$ | 30.5± $0.7^{f}$ | 33.2± $0.8^{de}$ | 36.8± $0.7^{de}$ | 1.10 2.19 |
| Egg Shell weight (g) | 3.47± $0.05^{d}$ | 3.58± $0.05^{cd}$ | 3.68± $0.03^{bc}$ | 3.56± $0.27^{cd}$ | 4.38± $0.17^{bc}$ | 4.69± $0.04^{ab}$ | 5.52± $0.08^{cd}$ | 4.62± $0.07^{bc}$ | 5.75± $0.05^{ab}$ | 4.55± $0.13^{e}$ | 4.57± $0.10^{cd}$ | 4.69± $0.04^{cd}$ | 0.21 0.42 |
| Eggshell thickness (mm) | 0.32± $0.1^{d}$ | 0.39± $0.0^{cd}$ | 0.38± $0.0^{bc}$ | 0.35± $0.1^{cd}$ | 0.37± $0.1^{bc}$ | 0.33± $0.1^{ab}$ | 0.42± $0.1^{cd}$ | 0.37± $0.1^{bc}$ | 0.42± $0.1^{a}$ | 0.32± $0.2^{e}$ | 0.33± $0.1^{cd}$ | 0.39± $0.0^{cd}$ | 0.01 0.02 |

### Evaluation of Edible and Non-Edible Organs

The effects of SeNPs supplementation on the weights of edible and non-edible organs across different treatment groups are summarized in Table 4. The data indicate that supplementation with SeNPs at 1.0 mg/kg in group 3 resulted in a significant increase ($P<0.05$) in the weights of the heart (1.82±0.103 g), liver (36.29±0.247 g), gizzard (10.13±0.116 g), spleen (0.519±0.007 g), thymus (2.518±0.008 g), and bursa (1.518±0.008 g). In contrast, no significant differences in organ weights ($P>0.05$) were observed in the other treated groups when compared to the control group (group 1).

**Table 4**

*Represents Impact of Nano Selenium Supplementation on the Edible and Non-Edible Organs of Laying Hen.*

| Edible and non-edible organs (grams) | Treatment Groups | | | |
|---|---|---|---|---|
| | Group 1 | Group 2 | Group 3 | Group 4 |
| Heart | 2.07± $0.574^{a}$ | 1.26± $0.143^{b}$ | 1.82± $0.103^{a}$ | 1.08± $0.079^{b}$ |
| Liver | 40.1± $0.082^{a}$ | 39.09± $0.088^{b}$ | 36.29± $0.247^{c}$ | 31.77± $0.495^{d}$ |
| Gizzard | 12.09± $0.074^{a}$ | 11.82± $0.103^{b}$ | 10.13± $0.116^{c}$ | 10.81± $0.099^{d}$ |
| Spleen | 0.61± $0.008^{a}$ | 0.483± $0.008^{c}$ | 0.519± $0.007^{b}$ | 0.307± $0.008^{d}$ |
| Thymus | 2.286± $0.005^{c}$ | 2.294± $0.014^{c}$ | 2.518± $0.008^{a}$ | 2.306± $0.008^{b}$ |
| Bursa | 1.286± $0.005^{c}$ | 1.294± $0.014^{c}$ | 1.518± $0.008^{a}$ | 1.306± $0.008^{b}$ |

### Evaluation of Hematological Biochemical Metrics

The blood biochemical characteristics of layers fed food supplements containing SeNPs are summarized in Table 5. In Group 3, administration of SeNPs at a dosage of 1.0 mg/kg resulted in significant improvements in several blood biochemical parameters, including WBC count (10.10±0.36), RBC count (46.2±0.20), hemoglobin levels (52.60±0.38), glucose concentration (2.17±0.05), cholesterol levels (2.63±0.12), and SGPT/ALT activity (1.43±0.012). However, no significant changes were observed in the WBC count, glucose, cholesterol, or SGPT/ALT levels across the other groups.

**Table 5**

*Impact of Nano Selenium Supplementation on the Blood Profile of Laying Hens.*

| Blood Parameters | Treatment Groups | | | |
|---|---|---|---|---|
| | Group 1 | Group 2 | Group 3 | Group 4 |
| WBS ($x10^{9}/\mu L$) | 9.37± $0.17^{b}$ | 10.03± $0.14^{ab}$ | 10.10± $0.36^{ab}$ | 10.80± $0.20^{a}$ |
| RBC ($x10^{9}/\mu L$) | 45.6± $0.26^{a}$ | 45.9± $0.65^{a}$ | 46.2± $0.20^{a}$ | 46.7± $0.95^{a}$ |
| Hemoglobin (g/dl) | 50.13± $0.38^{c}$ | 52.17± $0.61^{b}$ | 52.60± $0.38^{b}$ | 54.63± $0.40^{a}$ |
| Glucose (mg/dL) | 2.10± $0.10^{a}$ | 2.13± $0.09^{a}$ | 2.17± $0.05^{a}$ | 2.17± $0.04^{a}$ |
| Cholesterol (mg/dL) | 2.37± $0.16^{a}$ | 2.57± $0.16^{a}$ | 2.63± $0.12^{a}$ | 2.70± $0.05^{a}$ |
| SGPT/ALT (iu/l) | 1.33± $0.07^{b}$ | 1.40± $0.01^{b}$ | 1.43± $0.012^{b}$ | 1.73± $0.03^{a}$ |

### Impact of SeNPs Supplementation on Immune Performance

Supplementation with selenium nanoparticles (SeNPs) enhanced immune responses in laying hens, with groups C, B, and D showing a significant ($p < 0.05$) increase in serum IgM antibody levels as early as 20-24 weeks. In contrast, the control group (A) did not exhibit any significant change ($p > 0.05$) in antibody levels. The most notable increase in antibody levels was observed in group C between 40-44 weeks. Over the course of the study, antibody levels in all treated groups (B and C) significantly increased ($p < 0.05$) from weeks 20 to 44; however, group D displayed a decline in antibody levels from the 28th to the 44th week. Group C showed the most significant rise in IgM levels ($p < 0.05$), followed by group B, with both groups demonstrating similar antibody patterns. In contrast, group D exhibited lower IgM levels compared to group C (Figure 1). Additionally, SeNPs supplementation significantly increased serum IgA levels ($p < 0.05$) in group C, followed by group B, compared to the control group from early to late production. Group D, however, consistently showed reduced IgA levels relative to the control group (Figure 2).

**Figure 1**

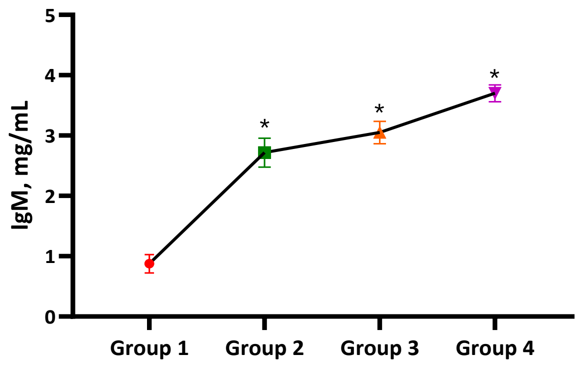


Figure 1 Illustrates serum Immunoglobulin M (IgM) levels concentrations in the treatment groups (B, C, D) and the control group (A) of laying hens. The chicks were monitored during early production weeks (21-24), mid-production weeks (25-28), and late production weeks (29-32), with supplementation of selenium nanoparticles (SeNPs).

**Figure 2**

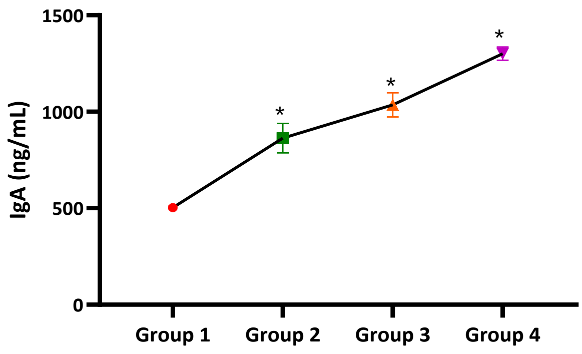


Figure 2 Depicts serum immunoglobulin A (IgA) levels concentrations across the treatment groups of laying hens (B, C, D) and the control group (A). All hens were monitored during early production weeks (21-24), mid-production weeks (25-28), and late production weeks (29-32), with selenium nanoparticles (SeNPs) supplementation.

## DISCUSSION

The experimental findings clearly demonstrate that selenium nanoparticles (SeNPs) supplementation exerts multiple beneficial effects on poultry performance and health. Among the tested doses, supplementation with 1.0 mg/kg SeNPs yielded the most pronounced improvements across key performance indicators, including body weight gain, feed conversion ratio, egg production and quality, organ development, hematological parameters, liver function, and immune response. These findings align with previous studies highlighting both the critical role of selenium in poultry nutrition and the enhanced bioefficacy of its nano-formulated form. Statistically significant increases in live weight were observed in the 1.0 mg/kg SeNP group, with other treated groups also showing improvements relative to the control. These outcomes support the metabolic importance of selenium, as described by the National Research Council (1994), and confirm that pathogen-free cells maintain optimal growth when supplemented with 1.0 mg/kg SeNPs. Consistent with the findings of Zhang et al. (2021), dietary selenium supplementation significantly enhanced broiler weight gain. Additionally, Xu et al. (2022) provided compelling evidence for the growth-promoting effects of nano-selenium in laying hens. Collectively, these results establish SeNPs as a reliable growth enhancer across diverse poultry types.

The data indicate that supplementation with 1.0 mg/kg selenium nanoparticles (SeNPs) led to greater live weight gain compared to the control group; however, this was accompanied by a less favorable feed conversion ratio (FCR). While SeNPs enhanced body weight in poultry, the birds required increased feed intake to achieve this growth, suggesting an altered nutrient utilization profile. These physical performance indicators reflect the influence of selenium on metabolic and nutritional functions in poultry. Gheisar et al. (2020) similarly reported that high selenium supplementation in broiler chicks resulted in reduced feed efficiency, underscoring the importance of optimal dosing for promoting growth without compromising feed utilization. These findings highlight the need for further investigation into the underlying mechanisms and the determination of economically optimal supplementation levels. In terms of reproductive performance, SeNPs supplementation exerted beneficial effects, as evidenced by significant improvements in egg production and egg mass in the group receiving 1.0 mg/kg SeNPs. Scientific evidence confirms selenium's essential role in supporting avian reproduction, particularly through enhancing oogenesis and hatchability (Surai, 2006). These findings align with those of Tufarelli et al. (2021), who demonstrated that selenium-enriched diets improved both the quantity and quality of eggs. Collectively, the results suggest that SeNPs supplementation may serve as a promising strategy to enhance productivity and reproductive efficiency in laying hen operations.

The present study demonstrates significant improvements in various egg quality parameters following dietary supplementation of laying hens with 1.0 mg/kg selenium nanoparticles (SeNPs). The experimental findings reveal that SeNPs enhance overall egg production and nutritional quality, as evidenced by increases in egg weight, yolk weight, and albumen weight. These results are consistent with the findings of Pappas et al. (2021), who reported that dietary selenium supplementation positively influences both egg weight and eggshell quality. SeNPs were also found to markedly improve eggshell characteristics. Supplementation with 1.0 mg/kg SeNPs led to the development of thicker, heavier eggshells with enhanced mechanical strength, reducing the likelihood of shell breakage. This improvement has important economic implications by minimizing egg loss during handling and transport. The outcomes align with the study by Wang et al. (2020), which documented the positive effects of nano-selenium on eggshell integrity. Beyond egg and eggshell quality, this evaluation also considered the physiological effects of SeNPs on internal organ development and general health status. Dietary inclusion of 1.0 mg/kg SeNPs significantly increased the weights of key edible organs, corroborating the findings of Han et al. (2022). Additionally, increased relative weights of non-edible, immune-related organs such as the spleen, thymus, and bursa were observed, supporting the immunomodulatory role of SeNPs as previously reported by Chen et al. (2021).

SeNPs exhibit significant positive effects on hematological parameters. Administration of 1.0 mg/kg SeNPs led to an increase in white blood cell (WBC) and red blood cell (RBC) counts, along with enhanced hemoglobin synthesis and a concomitant reduction in blood glucose and cholesterol levels. These findings align with previous studies by Li et al. (2021) and Zhang et al. (2020), supporting the dual role of SeNPs in enhancing immune function and improving oxygen transport, while modulating metabolic indicators. Furthermore, SeNPs treatment resulted in reduced serum glutamate pyruvate transaminase (SGPT/ALT) levels, indicative of improved liver health, as reported by Kheradmand et al. (2021). This study also highlights the immunomodulatory potential of SeNPs through their influence on humoral immunity. Dietary supplementation with 1.0 mg/kg SeNPs significantly elevated serum IgM and IgA levels during the early laying phase, suggesting enhanced immune responsiveness. These outcomes are consistent with previous findings by Zhang et al. (2021) and Han et al. (2021), who reported similar improvements in antibody titers following selenium supplementation. Collectively, the evidence underscores SeNPs as a promising dietary supplement for laying hens, with 1.0 mg/kg identified as the optimal dose. SeNPs supplementation supports growth performance, egg quality, organ integrity, liver function, and immune health. While further investigation is warranted to evaluate potential impacts on feed conversion ratio (FCR) at higher doses, current data advocate for the inclusion of nano-selenium in poultry nutrition to enhance overall productivity and physiological resilience.

## CONCLUSION

In conclusion, the dietary supplementation of SeNPs at the dose of 1.0 mg/kg to laying hens showed numerous advantages including enhanced productive performance, food efficiency, egg quality, organs weight, and immune defenses. However, higher dose induces negative impact of performance and overall health of laying birds.

### Ethical Statement

This study was carried out under the recommendation of institutional ethical committee (Lasbela University of Agriculture Water and Marine Science, Uthal, Pakistan). All experimental techniques in this investigation followed criteria authorized by the Animal Care Committee. All attempts were made to reduce stress and safeguard the wellbeing of the animal.

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