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Original Research

Impact of Adding Different Levels of Nanoselenium to Duck Diets on Growth Performance, Immunity Status, Antioxidant Activity and some Blood Parameters

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Abstract

An experiment was designed to investigate the effects of dietary supplementation of various concentrations of nanoselenium (NS) on the growth performance, immune status, antioxidant activity and hematological parameters of ducks. A total of 40 (1 day old) Pekin ducklings were equally divided into groups of 10 ducklings each. The 1st group was considered a control and was fed basal diet ad-libitum without nanoselenium (NS) supplementation; the 2nd group (fed basal diet + 0.2 mg NS/kg diet); 3rd group (fed basal diet + 0.4 mg NS/kg diet) and 4th group (fed basal diet + 0.6 mg NS/kg diet). The selenium content of the unsupplemented control diet was 0.09 mg/kg during the starter period (0-2 weeks) and 0.07 mg/kg during the grower-finisher period (2-10 weeks). All feeds were formulated according to NRC (1994) to meet the nutritional needs of growing ducks. The experiment was extended for 10 weeks. Growth performance parameters such as body weight development, weight gain, feed consumption, feed-to-gain ratio, performance index and mortality were recorded. At the end of experiment, three randomly selected ducks from each group were selected to assess immune status, antioxidant activity, and blood biochemical parameters. The results showed that ducks fed on diets supplemented with different levels of nanoselenium had significantly higher body weight and weight gain ($P<0.05$) and consumed less feed than ducks fed the control diet. The third group had the highest body weight and weight gain and the lowest feed intake, followed by the second and fourth groups respectively. The NS supplemented birds had no mortality throughout the experimental period compared with control group which recorded highest mortality rate (20%). Different levels of NS supplementation improved the feed conversion ratio and performance index, the best values was achieved in 3rd group followed by the 2nd and 4th groups, respectively. Addition of NS to duck diets significantly ($P<0.05$) increased the serum immunoglobulins (IgA, IgG, IgM), glutathione peroxidase (GPx) and superoxide dismutase (SOD) levels, but decreased the malondialdehyde (MDA) levels compared to control. Concerning blood picture, NS supplementation significantly increased white blood cell and lymphocytic counts ($P<0.05$). However, no significant differences in other hematological parameters between tested groups. In conclusion, the present study found that supplementing duck diets with nano-selenium improved growth performance parameters, immune status, antioxidant activity, blood picture and 0.2-0.4 mg/kg is considered the optimum nano-Se supplementation level, and the maximum nano-Se supplementation level for ducks should not exceed 0.6 mg/kg.

KEYWORDS
Ducks, Nanoselenium, Growth performance, Antioxidant, Immunoglobulins.

INTRODUCTION

In Egypt, the poultry sector is one of the major sources of animal protein. Duck production is part of the poultry industry and is very popular in many areas of the world. Ducks are the second largest poultry species in Africa after chickens, similar to pure Egyptian breeds and local developed strains that have been raised for both meat and egg production (Taha *et al*., 2013). Ducks are used for meat production to meet the growing demand for animal protein and duck meat is highly valued for its combination of lean meat characteristics with the dietary qualities of poultry meat. One of the most important considerations for consumers when choosing meat is its health benefits. Duck is an alternative to the widespread use of chicken and turkey. Duck pectoral muscles have a higher intramuscular fat content than chicken and turkey meat (Juodka *et al*., 2016).

Feed composition in poultry diets is one of the important bases considered by research in terms of proportion and type in addition to the main component. Minerals are important ingredients because they are involved in metabolic processes within the body of birds and are considered important cofactors for the completion of metabolic pathways. Selenium is one of the additives that researchers are focusing on because it is involved in regulating various physiological functions such as growth performance, fertility, immunity, meat quality, and protection from oxidative stress (Elnaggar *et al*., 2020). As selenium plays an important role in the synthesis of glutathione peroxidase, selenium played an important positive role in preventing oxidation of cells and cell membranes (Oliveira *et al*., 2014). The major factors that determine the effectiveness of Se are the levels and forms of Se in the diet. Even today, Se remains one of the most debated factors in poultry nutrition. The bioavailability of Se depends on its physical form and occurs in two basic forms in the diet: inorganic and organic. Inorganic selenium sources (such as sodium selenite and sodium selenate) are mainly used in broiler diets (Surai, 2018). However, there is increasing interest in using organic selenium sources (such as selenocysteine and selenomethionine) in poultry nutrition. Another form of Se that has received particular interest recently is nanoselenium (nano-Se).

As for nanotechnology, it is the great scientific progress during the recent decades and it is qualified to enter various fields of life during the next few years, because of the unique properties of nanoparticles, large surface area, surface activity, high stimulating efficiency, great absorption capacity, low toxicity, ease of transport and directing to the target organ, with the possibility of loading and wrapping them with other materials and when the use of antibiotics as a treatment for pathogenic infections in poultry became insufficient due to the resistance of pathogens to them, the demands for the production of nanoparticles and their use as a suitable alternative increased (Hill and Li, 2017). Selenium is among the most important mineral elements to which nanotechnology has been applied and its production in nanoscale form with molecular sizes estimated from (0.2-100 nanometers). Studies have shown that the nanoscale form of selenium is more efficient in preparing body tissues with selenium compared to organic and inorganic sources with low toxicity compared to them, and this is due to several advantages, the most important of which are increased surface area, high catalytic efficiency and high absorption through the intestine, similar to the absorption of amino acids and the arrival of the target organ at the highest speed and low toxicity, and these and other factors affected vital readiness Nanoselenium (Zhang *et al*., 2011; Gangadoo *et al*., 2020). Several studies have illustrated that the dietary Se form and level influences growth performance, meat quality characteristics and antioxidative properties in broilers. However, according to the knowledge of the authors, data on using nano-Se in the diets of ducks are limited. Therefore, the aim of the present study was to determine the effects of dietary supplementation of different levels of nanoselenium on the growth performance, immune status, antioxidant activity and hematological parameters of ducks.

MATERIALS AND METHODS

Ethical Approval

The guidelines used for the care and use of birds have been approved by the Scientific Research Ethics Committee, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, and bear the approval number (06/2023/0040).

Study period and location

The experimental study was conducted for 10 weeks (from February 1, 2023 to April 11, 2023). The study was conducted in the poultry houses experimental units located in the Faculty of Veterinary Medicine, Aswan University, Aswan, Egypt.

Birds, experimental design and diets

A total of 40 (1 day old) asexual Peking ducklings were used in the experiment. The ducklings were one day old of age and nearly had similar initial average weight (42.6–44.0 g). The ducklings were leg-banned, individually weighted and randomly allotted to 4 groups each of 10 ducklings. Birds in the first group fed basal control diet without nano-Se supplementation, while birds in the second, third and fourth groups received 0.2, 0.4 and 0.6 mg/kg of nano-Se in their diets, respectively. Ducklings were fed according to the following two-stage feeding program: starter (0-2 weeks) and grower-finisher (2-7 weeks). A basal control diet was formulated in the form of mash (ground yellow corn, soybean meal 44%, wheat bran, sunflower oil, monosodium phosphate, ground limestone, common salt, methionine, lysine and premix). The three experimental mash diets were formulated from the same feed ingredients as the control diet, but were supplemented with 0.2, 0.4 and 0.6 mg/kg of nano-Se. The selenium content of the unsupplemented control diet was 0.09 mg/kg during the starter period (0-2 weeks) and 0.07 mg/kg during the grower-finisher period (2-10 weeks). Control and three experimental mash diets were formulated according NRC (1994) to meet the nutritional requirements of growing ducks. Diets were offered in mash ad-libitum daily at the morning and evening to all ducks. The physical and calculated chemical compositions of the mash control diet are shown in Table 1.

Table 1. Physical and chemical compositions of the experimental basal diet.

Ingredients	Starter (0-2 weeks)	Grower-finisher (2-10 weeks)
Physical composition (%)		
Yellow corn, ground	40.65	60.16
Soybean meal (44%)	37.66	22.1
Wheat bran	13.94	10.94
Sunflower oil	4.45	3.5
Mono sodium phosphate	0.9	0.9
Limestone, ground	1.5	1.5
Common salt	0.3	0.3
Methionine	0.15	0.15
Lysine	0.15	0.15
Premix*	0.3	0.3
Calculated chemical compostion (%)		
CP (%)	22.14	16.33
ME (kcal/kg)	2900	3000
Calcium	0.67	0.66
Available Phoshorous	0.41	0.37
Methionine	0.48	0.39
Lysine	0.97	0.68
Selenium (mg/kg)	0.09	0.07

*Each 3 kg vitamins and minerals contain: Vit. A, 1200000 IU; Vit. D3, 300000 IU; Vit. E, 700 mg; Vit. k3, 500 mg; Vit. B1, 500 mg; Vit. B2, 200 mg; Vit. B6, 600 mg; Vit. B12, 3 mg; Vit. C, 450 mg; Niacin, 3000 mg; Methionine, 3000 mg; Pantothenic acid, 670 mg ; Folic acid 300 mg; Biotin, 6 mg; Choline chloride, 10000 mg; Magnesium sulphate, 3000 mg; Copper sulphate, 3000 mg; Iron sulphate, 10000 mg; Zinc sulphate, 1800 mg and Cobalt sulphate, 300 mg.

Housing and vaccination

The experimental room was disinfected with 0.5% TH4 and divided into four compartments with a floor area of 2.5 square meters, and sawdust was used as a bedding material. Feeders and water founts were distributed in the experimental compartments. Feed and fresh water were given ad libitum during the whole experimental period. Birds in all groups were housed and kept under the same managerial system and environmental conditions. A cycle of 16 hours light and 8 hours dark were applied throughout the experiment and hygienic disposal of organic washes were followed. The ducklings were vaccinated against avian influenza Virus (H9N2 and H5N1) on day 10 and 15 of age, respectively.

Preparation of nanoselenium (NS)

Selenium nanoparticles were prepared by chemical reduction of sodium selenite with ascorbic acid and subsequently stabilized by coating the prepared nanoparticles with dextrin according to the modified method of Malhotra *et al*. (2014). Characterization of the prepared selenium nanoparticles, such as size, shape, morphology and crystallinity, was performed using various analytical tools such as particle spectroscopy, scanning electron microscopy (SEM) and X-ray diffraction (XRD). The size of the prepared nanoparticles used in our study was 60 nm. Selenium nanoparticles were manufactured by Nano Gate Company in Cairo, Egypt.

Feed analysis

A representative samples of the feed ingredients were chemically analyzed for dry matter (DM(, crude protein) CP(, ether extract (EE), crude fiber) CF), ash and nitrogen-free extract (NFE (according to the methods of Association of Official Analytical Chemists (AOAC, 2022). The contents of ME and Se and the percentage of Methionine and lysine of the feed ingredients were adapted from the feed composition tables of NRC (1994).

Performance measurement

Performance parameters such as body weight development, feed consumption and mortality were recorded weekly. Weight gains, feed-to-gain ratio or feed conversion ratio (FCR%) and performance index (PI%) were calculated according to the recommendations of Soliman and Hassan (2017).

Blood parameters

At the end of the experiment, two blood samples were taken from the wing veins of three randomly selected ducks from each group, one sample with EDTA and the other sample without EDTA. Blood samples with EDTA were collected into well-labeled and sterilized tubes for haematological parameters. EDTA-free blood samples were collected in test tubes, allowed to clot at ambient temperature, centrifuged at 3000 rpm for 15 min, serum separated and stored at −20 °C until further analysis. Serum samples were used to measure immunoglobulins (IgA, IgG, and IgM) with commercial ELISA kits, antioxidant capacity (GPx, SOD, and MDA) with a spectrophotometer, and antibody titers.

Vaccines

Two commercial inactivated oil adjuvant vaccines were used, the first is H9N2 produced by MEVAC company and has batch no. 2204210101 and given at 10-day old by S/C inoculation method, while the second is H5N1 produced by MEVAC company and has batch no. 2204240101 given at 15-day old by S/C inoculation.

Serum samples

Five serum samples were collected from each group at 35 – day old to measure the antibody titer (Ab) using hem agglutination inhibition test (HI test).

Hemagglutination inhibition test (HI)

The HI test was designed to monitor the post-vaccination humoral immune response of each vaccine; using an H9N2 and H5N1 antigens of batch no. 220628 and 210613 respectively, prepared by MEVAC Company. HI test was conducted according to the OIE manual (OIE, 2015).

Statistical Analysis

Raw experimental data were subjected to statistical analysis, from which means and standard errors were calculated. Differences between groups were analyzed by One-Way ANOVA followed by Duncan's multiple comparison Post Hoc tests (Duncan, 1955). Statistical analysis to determine differences between groups were performed using the Statistical Package for the Social Sciences (published by SPSS Inc., 2009) (version 20.0; SPSS Inc., Chicago, IL, USA). A value of $P<0.05$ was considered significant.

RESULTS

Body weight development and weight gain

Ducks weight and weight gain results (Table 2) showed that ducks fed diets supplemented with nanoselenium at various levels of 0.2, 0.4, and 0.6 mg/kg diet exhibited significantly higher body weight and weight gain ($P<0.05$) than those fed the control diet. The highest values were found in the third group fed the diet supplemented with 0.4 mg/kg nanoSe (3338.9 g and 3294.9 g respectively).

Mortality rates

The mortality rates for ducks of the different groups are presented in Table 2. Results showed that the groups fed diets containing different levels of nanoselenium had no mortality throughout the experimental period compared to the control group which recorded the highest mortality rate (20%).

Feed consumption

The data concerning the cumulative feed intake of ducks are shown in Table 2. The results showed that there were significant ($P<0.05$) differences in weekly and total feed intake between different experimental groups. Feeding ducks with different levels of nanoselenium significantly ($P<0.05$) reduced weekly and total feed intake compared to control. The lowest feed intake was achieved in the 3rd group fed a diet supplemented with 0.4 mg nanoselenium (9535.6 g) compared to the control group (9895.3 g).

Table 2. Performance parameters of ducks during the experimental period.

Parameters	Groups			
	1	2	3	4
Initial body weight (g)	43.6±9.3	42.6±7.4	44.0±8.5	43.8±6.9
Final body weight (g)	3099.1±40.8^{b}	3288.0±38.2^{ab}	3338.9±41.8^{a}	3233.8±37.8^{ab}
Body weight gain (g)	3055.5±25.4^{b}	3245.4±26.7^{ab}	3294.9±29.8^{a}	3190.0±27.3^{ab}
Feed intake (g)	9895.3^{a}	9775.2^{ab}	9535.6^{b}	9669.5^{ab}
FCR	3.24±0.27^{a}	3.01±0.22^{ab}	2.89±0.0.17^{b}	3.03±0.19^{ab}
Mortality rate (%)	20%	-	-	-
Performance index (%)	95.65±11.36^{b}	109.24±23.51^{ab}	115.53±18.67^{a}	105.28±21.59^{ab}

*Means with different superscripts within the same raw data are significantly different ($P<0.05$).

Feed conversion ratio

The results in Table 2 indicated that supplementing duck diets with different levels of nanoselenium significantly (P<0.05) improved the FCR. The best feed conversion value (2.89) was recorded in the 3rd group (0.4 NanoSe) followed by the 2nd group (3.01) and 4th group (3.03) which fed on 0.2 and 0.6 NanoSe, respectively compared to the control group (3.24).

Performance index

Data related to the performance index (PI) of the duck during the whole experimental period are shown in Table 2. A significant (P<0.05) difference in performance index values was found between the tested groups and control. Supplementing duck diets with different levels of nanoselenium significantly (p<0.05) improved the performance index (PI). The highest PI was recorded in ducks of the 3rd group (115.53%) followed by rabbits in the 4th and 2nd groups (110.34 and 105.68%). Rabbits in control group achieved the lowest performance index (95.65%).

Immune response indices

Serum immune indices of ducks are shown in Table 3. Supplementation of duck diets with nanoselemium significantly (P<0.05) increased IgA, IgG and IgM. Compared to controls, the highest levels were found in ducks fed 0.6 mg nanoSe/kg diet, followed by ducks fed diets containing 0.4 and 0.2 mg nanoSe, respectively.

Anptioxidant activity

The antioxidant enzyme results in duck serum are shown in Table 3. Ducks fed diet contatining different nanoselenium levels had significantly (P<0.05) higher glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities and lower malondialdehyde (MDA) levels compared to ducks fed the control diet. The highest GPx and SOD values (1.61 and 170.51 U/ml, respectively) were achieved in the fourth group fed the diet containing 0.6 mg nanoSe, followed by the third and second groups fed the diet containing 0.4 and 0.2 mg nanoSe/kg, respectively, compared to the control group, which recorded the lowest values (1.15 and 136.34 U/ml, respectively). Control group had higher MDA values (12.82 nmol/ml) than experimental groups (9.62, 8.67 and 8.21 nmol/ml, respectively).

Hematological parameters

Effects of different levels of nanoselenium on some hematological parameters of ducks are summarized in Table 4. The results revealed that, there were significant (P<0.05) differences in white blood cells (WBCs) and lymphocytic count between ducks fed on different experimental diets and control. However, there weren't significant (P<0.05) differences in red blood cells (RBCs), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT) and other differential leukocytic counts (monocytes, neutrophils, eosinophils and basophils). The 3rd and 4th groups recorded the highest number of WBCs and lymphocytic count followed by the

Table 3. Serum immune index and antioxidant enzymes in ducks fed different experimental diets.

Items	Groups			
	1	2	3	4
IgA (mg/dl)	2.16±0.03^{c}	4.60±0.18^{b}	6.89±0.21^{ab}	7.25±0.43^{a}
IgG (mg/dl)	10.62±0.82^{c}	13.45±1.18^{b}	15.41±1.23^{ab}	16.11±1.37^{a}
IgM (mg/dl)	3.74±0.16^{c}	6.04±0.27^{b}	7.13±0.31^{ab}	7.83±0.56^{a}
GPx (U/mL)	1.15±0.04^{c}	1.47±0.11^{b}	1.53±0.13^{ab}	1.61±0.17^{a}
SOD (U/mL)	136.34±10.35^{c}	153.62±12.46^{b}	160.23±14.23^{ab}	170.51±16.81^{a}
MDA (nmol/mL)	12.82±1.33^{a}	9.62±1.21^{b}	8.67±1.18^{b}	8.21±1.14^{b}

*Means with different superscripts within the same raw data are significantly different (P<0.05).

Table 4. Heamatological picture of ducks fed different experimental diets

Items	Groups			
	1	2	3	4
RBCs (x10^{6}/µl)	2.62±0.15	2.54±0.24	2.45±0.10	2.33±0.09
HGB (g/dl)	21.57±1.02	21.10±0.60	20.79±0.38	20.17±0.23
HCT (%)	39.70±1.67	38.30±2.82	35.40±0.45	34.53±0.67
MCV (fl)	146.00±2.08	151.33±4.06	144.67±4.67	148.67±2.96
MCH (pg)	79.33±1.33	84.00±6.03	79.00±4.51	82.67±2.60
MCHC (g/dl)	54.33±0.67	55.67±2.73	54.33±1.33	55.67±0.88
PLT (10^{3}/µl)	11.33±1.86	14.33±2.33	13.00±9.50	12.00±3.79
WBCs (x10^{3}/µl)	91.23±72.25^{b}	163.83±12.86^{ab}	195.33±2.98^{a}	191.57±5.42^{a}
Lymphocytes (%)	54.00±27.06^{b}	75.33±1.33^{ab}	93.33±1.20^{a}	90.53±1.78^{a}
Monocytes (%)	11.00±5.13	12.33±1.20	14.00±1.00	12.33±1.33
Neutrophils (%)	1.33±0.88	1.00±0.01	1.33±0.33	1.33±0.33
Eosinophils (%)	1.33±0.67	1.67±0.33	1.33±0.33	1.00±0.01
Basophils (%)	1.25±0.15	1.37±0.12	1.46±0.19	1.31±0.11

*Means with different superscripts within the same raw data are significantly different (P<0.05).

2nd group compared with control which achieved the lowest values.

Antibody response for vaccination

The geometrical mean of HI titers log_2 were measured at 35 days of age for both H9N2 and H5N1 vaccines and shown in Table 5. Ducks fed different nanoselenium levels showed significantly higher antibody titers to both H9N2 and H5N1 vaccines. The highest values compared to the control group were found in ducks fed 0.6 mg Nano Se/kg diet, followed by ducks fed diets containing 0.4 mg and 0.2 mg Nano Se, respectively.

Table 5. Antibodies mean titers against H9N2 and H5N1 vaccines at 35 days of age

Items	Groups			
	1	2	3	4
HI titer (log-2) for H9N2 vaccine	2	3	4	3
	2	2	3	3
	3	4	4	5
	3	3	3	5
	2	3	3	4
G.M	2.4	3	3.4	4
HI titer (log-2) for H5N1 vaccine	2	3	3	4
	3	3	4	5
	3	4	3	4
	3	4	4	4
	3	2	4	5
G.M	2.8	0.2	3.6	4.4

DISCUSSION

Nanoselenium supplementation significantly improved the body weight and weight gain in ducks throughout the experimental period. These results may be due to the important role of selenium in human/animal nutrition related to the synthesis of selenoproteins. Selenoproteins have unique catalytic properties, more than half of which are involved in maintaining redox balance and antioxidant defenses. However, it seems unlikely that Nano-Se exerts direct antioxidant effects on biological systems. Additionally, in many studies, the base diet was free from selenium, thus the supplementation of nano-Se was more likely to have positive effects on productivity (Surai, 2002). A possible mechanism is through a beneficial effect on the gut microbiota (Gangadoo *et al.*, 2018) and/or gut anatomy (Ahmadi *et al.*, 2018).

As the final BW was higher in groups 2 and 3 compared to the other groups, this clearly indicates that 0.2-0.4 mg nano-Se per kg of diet is an optimum level and the maximum additive level of nano-Se in duck diet should not exceed 0.6 mg/kg. The results of this study are consistent with those of Baltić *et al.* (2015) who found that ducks fed a Se-rich diet (0.4 mg/kg) had higher live weights at slaughter than ducks fed a Se-poor diet (0 mg/kg) or a Se-rich diet (0.6 mg/kg). Furthermore, Ahmadi *et al.* (2018) observed improved growth performance in broilers supplemented with 0.2, 0.3, 0.4 and 0.5 ppm NS. In addition, higher BWG was reported in broilers at 0.4 mg Nano-Se kg-1 feed concentration (Bakhshalinejad *et al.*, 2018). Furthermore, Eid *et al.* (2022) showed that chicks fed a diet containing 0.3 mg/kg nanoselenium had significantly higher final weights, whereas chicks fed a control diet had significantly lower final weights (2132.2 vs 1996.1 g, p<0.05). On contrary, Giamouri *et al.* (2021) found that throughout the experimental period (6 weeks), broiler body weight and weight gain were unaffected by diets supplemented with 0.4 mg/kg Se nanoparticles compared to unsupplemented control diets. Khajeh Bami *et al.* (2022) showed that different amounts of nanoselenium (0.075, 0.15 and 0.3 mg/kg feed) had no effect on the growth performance of broiler chicks. Furthermore, SEVİM *et al.* (2022) reported that 0.30 mg/kg Nano-Se supplementation to broilers had no effect on growth performance, including BW and BWG.

The experimental groups fed diets containing varying levels of nanoselenium had no mortality throughout the experimental period compared to the control group which showed the highest mortality (20%). These results may be due to selenium supplementation involved in regulating several enzyme systems involved in energy metabolism, synthesis of prostaglandins, and metabolism of the essential fatty acids apric acid and pyrimidine bases. In addition, natural antioxidants may protect the intestinal mucosa from oxidative damage and pathogens. Furthermore, selenium intake appears to provide additional health benefits to the immune system and reduce inflammation and mortality in birds (Ebeid *et al.*, 2013). Also, Barko *et al.* (2017) showed that nanoselenium (NS) stimulates normal intestinal resident populations to directly compete with opportunistic and pathogenic microbes for their receptor sites and prevent their growth and proliferation. Furthermore, NS increased specific immune levels and contributed to a significant reduction in TBC (total bacterial count) and TEC (Total Enterobacteriaceae count). Further, Kheradmand *et al.* (2014) and Yip *et al.* (2014) observed the antibacterial activity of NS against bacterial microorganisms such as Pseudomonas aeruginosa, E. coli and fungal organisms such as Candida albicans and Trichophyton rubrum. Furthermore, Stanley *et al.* (2015); Gangadoo *et al.* (2016) and Shakibaie *et al.* (2017) showed that the use of NS in poultry may have direct antibacterial effects against pathogens such as E. coli and Proteus mirabilis. Consistent with our results, Hu *et al.* (2012) found that supplementation with 0.15–1.20 mg/kg nano-Se in broiler diets improved survival and decreased mortality.

Regarding the feed intake of ducks, the results showed that feeding ducks with different levels of nanoselenium significantly decreased total feed intake compared to controls (P<0.05). The lowest feed intake was recorded in the third group, which was fed a diet enriched with 0.4 mg nanoselenium. Our findings are consistent with those of Rostamabad *et al.* (2016) who reported that nano-Se supplementation at 0.6 and 1.2 mg/kg feed reduced feed consumption and improved FCR in broilers compared to the control group (no Se supplementation). Also, El-Kazaz *et al.* (2020) showed that 0.2 mg/kg Nano-Se significantly reduced total feed intake in Japanese quail. Furthermore, Jamima *et al.* (2020) observed that birds fed 0.15 mg/kg Nano-Se had the lowest feed consumption and superior FCR. Eid *et al.* (2022) showed that broilers fed a diet containing 0.3 mg/kg nanoselenium had significantly lower food intake compared to control (3127.2 vs. 3261.2 g, p < 0.05, respectively). On the other hand, Ibrahim el al. (2019) found that different sources (selenium selenite, selenomethionine, and nano-Se) and Se concentrations (0.3, 0.45, and 0.6 mg Se/kg) had no effect on feed intake in Ross broiler chicks. Saleh and Ebeid (2019) found that broilers fed 0.20 and 0.25 nanoselenium (NS) mg/kg had higher total feed consumption. Furthermore, Sa'aci *et al.* (2021) showed that broilers on 0.20 and 0.25 nano selenium (Nse) mg/kg had higher total feed consumed values. In addition, SEVİM *et al.* (2022) observed that adding 0.30 mg/kg He Nano-Se to broiler diets had no effect on feed intake.

Current study indicates that nano-Se at levels of 0.2, 0.4 and 0.6 mg/kg have beneficial effects on FCR. The best FCR was determined with the diet fed to the group containing 0.4 mg NanoSe. The main effects of nano selenium on final body weight, weight gain, feed intake and FCR may be due to its physical and chemical properties. This is because nanoparticles have a much larger surface area than microparticles. The smaller particle size of nanominerals increases the surface area available for chemical reactions and is thought to improve mineral digestion, bioavailability and utilization in the gastrointestinal tract (Sa'aci *et al.*, 2021). These results were consistent with those of Ahmadi *et al.* (2018) who documented an improvement in FCR in broilers fed a diet enriched with nano-Se (0.3 mg/kg). Also, Saleh and

Ebeid (2019) also found that supplementing broilers with 0.5 mg nano-Se/kg feed improved feed conversion ratio (FCR). Furthermore, Sa'aci *et al.* (2021) showed that broilers fed 0.10, 0.15 and 0.25 mg nanoselenium (NS) had better feed conversion rates. Eid *et al.* (2022) observed that chicks fed a diet containing 0.3 mg/kg nanoselenium had significantly superior FCR, while chicks fed the control diet had the lowest FCR values (1.479 vs. 1.636 g/g, $p < 0.05$, respectively). In contrast, Cai *et al.* (2012) showed that nano-Se does not significantly affect FCR even at high doses (0.3–2 mg kg^{-1}). Also, El-Kazaz *et al.* (2020) observed that supplementing Japanese quail diets with 0.2 mg/kg Nano-Se significantly reduced FCR. Furthermore, SEVİM *et al.* (2022) found that FCR was unaffected by the addition of 0.30 mg/kg Nano-Se to broiler diets.

Supplementing duck diets with different amounts of nanoselenium significantly ($p<0.05$) improved performance index (PI). These results are likely due to the fact that selenium is a trace mineral known to play an important role in promoting biological processes in the body and performance and growth of birds, as reported by Lee *et al.* (2017) and Limaye *et al.* (2018). Kieliszek and Błażejak (2016) also emphasized the importance of selenium as a micronutrient. Selenium helps protect against hydrogen peroxide, detoxifies heavy metals, increases productivity and performance, and improves the immune system. Moreover, the improved performance index in ducks may correlate with the function of selenium on growth rate, as it plays a role in the expression of selenoprotein P and the selenoenzyme type I iodothyronine dei iodinase, which plays an important role in thyroid hormone synthesis and selenium transport (Zhan *et al.*, 2014). Furthermore, our findings of increased growth performance index by nano-Se could possibly be due to increased levels of thyroid hormone, which regulates the body's energy metabolism, and increased protein digestibility (Saleh, 2014). The results obtained are in good agreement with those of Aparna and Karunakaran (2016) who reported that different concentrations (0.1 and 0.2 mg/kg) of nanoselenium increased glutathione peroxidase and malondialdehyde activities, improved oxidative resistance, lipid oxidation, and free radical scavenging, resulting in increased body weight and growth performance index in birds compared to control. Ibrahim el al. (2019) that dietary application of up to 0.6 mg/kg of her nano-Se resulted in the highest growth rate and highest performance index in broiler chicks. On the contrary, El-Kazaz *et al.* (2020) observed that supplementing quail diets with 0.2 mg/kg of nano-Se significantly decreased performance index (PI).

Feeding ducks with varying levels of nanoselenium (0.2, 0.4 and 0.6 mg/kg diet) significantly increased serum immunoglobulin levels (IgA, IgG and IgM). These results may be due to the enhancing effect of nanoselenium on protein synthesis and the elevation of the eukaryotic translation initiation factor 5A-1, which contributed to increased protein synthesis and thus increased IgA, G, and M concentrations (Gulyas *et al.*, 2016 and Xiao *et al.*, 2016). Similar results were reported by Cai *et al.* (2012) who found that serum IgG and IgM levels peaked on day 42 of the experiment in broilers supplemented with 0.30 mg/kg Nano-Se. Also, Boostani *et al.* (2015) observed a significant increase in serum IgG and IgM concentrations in broilers supplemented with 0.3 mg/kg NS. Furthermore, Ahmadi *et al.* (2018) reported that 0.2, 0.3, 0.4 and 0.5 ppm NS dietary supplements improved immunity in broilers. Bakhshalinejad *et al.* (2018) showed that supplementing a day-old chick's diet with 0.4 mg/kg nanoselenium (NS) significantly increased serum IgG concentrations.

Ducks fed different levels of nanoselenium had significantly ($P<0.05$) higher GPx and SOD activities and lower MDA levels compared to ducks fed the control diet. The results were consistent with those of Cai *et al.* (2012) who observed higher serum glutathione levels and lower malondialdehyde levels in broilers fed 0.3 mg/kg Nano-Se compared to control. Also Baltić *et al.* (2015) showed that ducks fed a Se-enriched diet had significantly increased plasma GSH-Px activity compared to ducks fed a diet without added Se. In addition, Aparna and Karunakaran (2016) found that he SOD and GPx cell activity was increased in birds fed selenium nanoparticles (0.1875 mg/kg) compared to control groups. In addition, El-Deep *et al.* (2016) showed that nanoselenium in broiler diets could increase the activity of GSH-Px and SOD, as well as reduce serum MDA levels. Similarly, AZAB *et al.* (2019) found that GPx and SOD activities were significantly increased ($p<0.05$) in groups supplemented with nanoselenium (0.15 mg/kg diet) compared to groups supplemented with the same concentration of sodium selenite. Also, Hassan *et al.* (2020) used 0.3 mg nanoselenium/kg as a dietary supplement in roosters and found significant improvements in antioxidant activity.

The improvement in antioxidant activity in this study may be due to the fact that selenium is an essential trace element that upregulates key components of the antioxidant defense mechanism by regulating the body's glutathione pool and its key selenium-containing antioxidant enzymes. (Jiang *et al.*, 2009). Glutathione peroxidase and superoxide dismutase are the main enzymatic defenses against toxic oxygen-reducing metabolites, and each enzyme plays an important role in free radical regulation (Maestro, 1991). Glutathione is one of the most important intracellular nonenzymatic antioxidants and is believed to be the largest component of the endogenous cellular redox buffer (Hasspieler *et al.*, 1994 and Storey and Braz, 1996). Malondialdehyde is one of the end products of intracellular polyunsaturated fatty acid peroxidation and is a marker of oxidative stress (Gawel *et al.*, 2004). Some literature mentions the role of selenium in activating GSH-Px, which is beneficial for improving antioxidant status (Ebeid *et al.*, 2013). Both glutathione peroxidase and superoxide dismutase are considered essential antioxidants that play an important role in combating toxic oxygen-reducing metabolites (Zhang *et al.*, 2014).

Regarding the hematological characteristics of ducks fed with different amounts of nanoselenium, there was a significant ($P<0.05$) increase in white blood cell count and blood lymphocyte count in nanoselenium-fed ducks compared to control. However, differences in erythrocyte, HGB, HCT, MCV, MCH, MCHC, PLT, and other leukocyte counts (monocytes, neutrophils, eosinophils, basophils) were not significantly different ($P<0.05$). These results are consistent with those of Boostani *et al.* (2015) who reported that supplementing broiler diets with 0.3 mg/kg of her Nano-Se had no significant effect on haematological parameters such as Hb, TEC and PCV. Also, Mohamed *et al.* (2016) showed that the use of chemical nanoselenium (CheSeNP) in Sinai chick diets had no significant effect on eosinophils and monocytes. Furthermore, Alagawany *et al.* (2021) found that supplementation with 0.2, 0.4 and 0.6 g/kg chemical nanoselenium (Che-SeNP) had no effect on granulocytes (neutrophils, eosinophils, basophils), red blood cells (RBCs), hematocrit and mean corpuscular volume in growing quail ($P>0.05$). On the other hand, Jamima *et al.* (2020) found that supplementing broiler diets with 0.15 mg/kg Nano-Se significantly increased levels of hemoglobin (Hb), total red blood cell count (TEC), and concentrated cell volume (PCV), while the effects on white blood cell count and H/L ratio were not significant compared to control. Also, Eid *et al.* (2022) showed that nanoselenium supplementation (0.3 mg/kg) in broiler diets had no significant effect on haematological parameters compared to control.

As selenium is a functional component of selenium-dependent GPx4, improving blood properties protects blood cell properties and components from oxidative membrane damage, and the addition of 1 mg nanoselenium per kg of feed has a positive effect on biomembrane integrity and immune cell performance (Pelyhe and Mézes, 2013).

CONCLUSION

The present study demonstrated that supplementing duck diets with different levels (0.2, 0.4 and 0.4 mg/kg) of nanoselenium improve growth performance parameters, immunity status, antioxidant activity and blood picture. Nanoselenium supplementation shows an optimal response at the level of 0.4 mg/kg, with significant secondary effects. The recommendation of 0.2-

0.4 mg/kg nanoselenium supplementation in duck diet is supported by these results, and the maximum amount of nanoselenium supplementation to ducks should not exceed 0.6 mg/kg.

CONFLICT OF INTEREST

The Authors declare that they have no conflict of interests.

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