Journal of Animal and Feed Sciences, 29, 2020, 46–58 *https://doi.org/10.22358/jafs/118209/2020*
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In-ovo and dietary supplementation of selenium nano-particles influence physiological responses, immunological status and performance of broiler chicks

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KEY WORDS: blood analysis, carcass yield, chicks, *in ovo*, nanoparticles, selenium, supplementation

Received: 12 August 2019
Revised: 5 December 2019
Accepted: 9 March 2020

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ABSTRACT. This study aimed to explore impacts of *in ovo* injection and dietary supplementation of selenium nanoparticles (SeNPs) on physiological responses, immunological status and performance of broiler chicks. A total of 210 broiler eggs were divided into 3 *in ovo* groups: not *in ovo* injected and *in ovo* injected with 5 or 10 ppb SeNPs. The hatched chicks from each *in ovo* group were divided into 2 dietary groups, with or without 10 ppb SeNPs/kg ration for 5 weeks. Live body weight and body weight gain were significantly increased in all SeNPs injected or supplemented groups. Feed conversion ratio for the whole feeding period was improved by SeNPs feed supplementation. Serum triglycerides and malondialdehyde contents were significantly decreased, while high-density lipoprotein cholesterol, immunoglobulin status, reduced glutathione content and glutathione reductase activity were increased by both examined routes of SeNPs delivery. It can be concluded that SeNPs *in ovo* injection with a dose up to 10 ppb/egg along with SeNPs diet supplementation at a dose of 10 ppb/kg had a positive effect on performance and physiological, antioxidant and immunological status of broiler chicks.

Introduction

The producers aim to improve poultry production efficiency and achieve high profitability but have noticed that during commercial production, chicks may be exposed to various microbiological challenges, infection by diseases and oxidative stress, and result in economic and production inefficiencies. Therefore, improving the immune function of chicks by enhancing antioxidant status may contribute to the reduction of morbidity and mortality of birds.

Numerous reports have demonstrated that selenium, an important microelement, acts as a natural biological antioxidant that helps in the protection of cellular membranes against oxidative damage and ameliorates bird growth and health (Suraï and Dvorska, 2002; Levkut et al., 2009; Pilarczyk et al., 2012; Khan et al., 2015; 2016). Unfortunately, traditional forms of selenium supplements, in general, have low levels of absorption and increase toxicity (Raza, 2012; Jamil, 2013; Khan et al., 2016). Furthermore, the lack of this microelement leads to numerous metabolic disorders and diseases through its negative effect on the physiological status of the chicks (Cai et al., 2012). Subsequently, delivering a proper amount of this microelement to chicks' body is important for physiological enzymatic, hormonal and immune processes (Keen et al., 2004).

The nanoform of selenium particles (SeNPs), is a good example of applied nanotechnology used in the area of nutritional supplements, exhibits advantages and novel properties better than other forms of this microelement, including greater surface activity, higher solubility, mobility, high cellular uptake and excellent bioavailability (Wang et al., 2007; Zhang et al., 2008). Thus, it can be assumed that both SeNPs *in ovo* injection and SeNPs feed supplementation can be more effective ways of this microelement administration, which would prevent the above-mentioned disadvantages of traditional forms of selenium supplements and elevate the bioavailability and cellular uptake of this element and maintain its health benefits.

Therefore, the objective of the present study was to investigate the effects of SeNPs *in ovo* injection and dietary supplementation, as well as these two routes of delivery interaction on productive performance, stimulation of the antioxidant defence system and immune response of hatched chicks.

Material and methods

Poultry ethics

All of the experiments were carried out according to the guidelines of the Institutional Animal Care and Use Committee for Animal Experiments, which is a member of the Egyptian Network of Research Ethics Committee. The scientific and ethics committee of the Biological Application Department, Egypt) approved all procedures used in this experiment (protocol number 186; date of approval: 14.07.2019).

Experimental procedures

Two trials were performed at the poultry production farm of the Biological Application Department of the Nuclear Research Center (Egypt) during the period from March to May 2017. The first trial was conducted on eggs with or without SeNPs *in ovo* administration and the second one was conducted on post-hatch chicks fed a diet with or without SeNPs addition.

First trial

Eggs collection, incubation and *in ovo* injection with SeNPs. A total of 210 broiler breeder eggs (Hubbard) weighed between 55 and 60 g were obtained from broiler egg production El-Tokhy Company (New Salhia, Egypt) from a maternal flock, 54 weeks of age.

Each egg was dry cleaned using a soft toilet paper, then sprayed with a disinfectant solution, dried with soft tissue paper and numbered. The eggs were set vertically in metal trays. Eggs were incubated at 37.8 °C and 60% relative humidity (RH).The eggs were turned every 2 h through 45° (12 times a day) up to 18 days. At day 14 of incubation the eggs were candled using a candling lamp in a darkened room, and the infertile eggs or eggs containing dead embryos were excluded.

Eggs with live embryos were randomly divided into 3 experimental *in ovo* groups (65 eggs in each): not *in ovo* injected (control group) and *in ovo* injected into the air cell with 5 or 10 ppb SeNPs diluted in 100 µl. SeNPs were obtained from the Department of Physics, Faculty of Science, Mansoura University (Egypt). On day 18, the eggs were transferred to the hatcher set at 37 °C and 90% RH for up to 21 days.

The injection procedure into the air cell was performed as described by Bhanja et al. (2004). The eggs remained outside the incubator for approximately 15 min for injection. Prior injection, both the working bench and the eggs were disinfected with 70% ethanol, the shell was punched at the wide end of the egg to make a hole with a 21-ga needle. Then, the eggs were injected with a 23-ga needle, the site of injection was sealed with adhesive tape and the eggs returned again to the hatcher for the hatching process. The hatched chicks within each group were recorded and weighed individually immediately after hatching using an electronic balance with accuracy $\pm$ 0.01 g.

Second trial

Chicks. Sixty hatched chicks per each group from the first trial were randomly equally divided into two dietary groups (30 chicks each) each assigned to three pens (10 chicks per pen). Chicks in two dietary groups were fed diet with or without SeNPs supplementation at a dose of 10 ppb per kg of ration. SeNPs were added to the ration by diluting 1 ml of solution with the concentration of 10 ppb SeNPs in 20 ml of distilled water. The obtained solution was spread per 100 g of diet and mixed well with another 100 g to reach to homogeneous kilogram ration.

So in the second trial six experimental groups were obtained: R1 (0 × 0) and R2 (0 × 10) – groups without SeNPs *in ovo* injection and without or with SeNPs dietary supplementation, respectively; R3 (5 × 0) and R4 (5 × 10) – groups with SeNPs *in ovo* injection at dose of 5 ppb and without or with SeNPs dietary supplementation, respectively; R5 (10 × 0) and R6 (10 × 10) – groups with SeNPs *in ovo* injection at dose of 10 ppb and without or with SeNPs dietary supplementation, respectively.

Housing and management

All experimental chicks were grown in metal cages (100 × 60 × 50 cm; length × width × height) equipped with manual feeder and automatic nipple drinker. The birds were kept at the same managerial, controlled, clean and hygienic environmental conditions. All groups were allowed *ad libitum* access to water and feed. Throughout the first week, the chicks were exposed to 24 h of light per day, then the exposure was reduced to 22 h per day for the rest of experimental period.

Vaccination against the Newcastle Disease Virus (NDV) was performed on days 21 and 28 of age using an eye dropper (Live Lasota strain; KBNP Inc., Hungnam, South Korea).

Experimental diet

The basal diet was purchased from Salah Attia Co. (Tafahna El-Ashraf, Egypt) and was formulated to meet the recommendations of National Research Council for broiler chicks (NRC, 1994). The basal diet composition and calculated chemical analysis are presented in Table 1.

Table 1. Basal diet ingredients and calculated chemical composition

Indices	Starter	Grower
Ingredients, %		
yellow maize	58.50	62.50
soybean meal (44%)	26.00	23.94
maize gluten meal (62%)	10.00	7.00
vegetable oil	1.50	2.50
limestone	1.12	1.23
di-calcium phosphate	1.75	1.70
premix[1]	0.30	0.30
NaCl	0.30	0.30
L-lysine	0.36	0.36
DL-methionine	0.17	0.17
Calculated composition, g/kg		
ME, kcal/kg	3058	3120
crude protein	22.45	20.20
calcium	0.93	0.95
non-phytate phosphorus	0.46	0.45
methionine	0.62	0.57
lysine	1.28	1.20
TSAA	1.00	0.90

[1] provides each kg of diet: IU: vit. A 12 000, vit. D_3 5000; mg: vit. E 130, vit. K_3 3.605, vit. B_1 3, vit. B_2 8, vit. B_6 4.95,vit. B_{12} 0.17, niacin 60, folic acid 2.083, D-Biotin 200, calcium D-pantothenate 18.333, copper 80, iodine 2, selenium 150, iron 80, manganese 100, zinc 80, cobalt 500 mg per kg; ME – metabolizable energy; TSAA – total sulphur-containing amino acids

Productive performance parameters

Live body weight (LBW) per replicate was recorded individually very week in the early morning before food and water administration using a digital platform balance. Average daily body weight gain (BWG) was calculated weekly and the overall BWG was calculated for the whole experimental period (35 days) at the end of the experimental.

Feed intake and feed conversion ratio

Feed intake per replicate was calculated at the end of a given period by subtraction residual feed (g) from the offered amount. That value was divided by the number of birds per replicate in order to calculate the average amount of feed intake per bird (FI). Feed conversion ratio (FCR) was also calculated (FCR = g feed / g body weight gain).

Carcass characteristics

At the end of the 35-day experimental period, three representative birds were chosen randomly from each pen. Before slaughtering, assigned birds were fasted for 12 h and then individually weighed, slaughtered, defeathered, opened and the hot carcass was weighed and recorded. Edible offals (liver, heart and gizzard) and non-edible offals (proventriculus, thymus, bursa of Fabricius and spleen) were separately weighed and recorded. Carcass yield was calculated as follows:

$$\text{carcass yield} = \frac{\text{empty carcass weight, g} + \text{edible offals weight, g}}{\text{live pre-slaughtering weight, g}} \times 100.$$

Physiological and biochemical parameters

At the end of the experimental period (35 days of age), 3 birds per pen were randomly selected and two blood samples were collected from each bird during slaughtering time. One sample was collected into heparinised tubes to perform the haematological parameters analysis (blood haemoglobin (Hb), erythrocytes (RBCs) and leukocytes (WBCs) number, packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV)). The second sample of blood was collected into non-heparinised tubes and centrifuged at 3400 *g* for 6 min and the sera were separated in Eppendorf tubes and stored at −20 °C until further biochemical measurements.

For humoral immune response assessment, on days 28 and 35 of age (7 and 14 days after vaccination, respectively), blood samples were collected from the wing vein of birds into heparinised tubes, and centrifuged at 2500 *g* for 10 min and the plasma was separated and stored at −20 °C.

Blood and serum biochemical analysis

Serum total proteins, albumin, globulin, glucose, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), uric acid and creatinine concentrations, albumin to globulin ratio (A:G ratio) and aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphates (ALP) activities were measured with a spectrophotometer (UV1601; Shimadzu, Kyoto, Japan) using commercial kits produced by (Stanbio Laboratory, Boerne, TX, USA). Serum triiodothyronine (T_3) hormone concentration was measured by radioimmunoassay (RIA) kit produced by Institute of Isotopes Ltd. (Konkoly, Hungary) and samples were counted on Packard Gamma Counter (GMI, Ramsey, MN, USA).

Antioxidant status

Serum content of reduced glutathione (GSH) and malondialdehyde (MDA) and activity of glutathione reductase were determined using commercial kits supplied by Spinreact Co. (Santa Coloma, Spain).

Humoral immune response: antibody production against Newcastle Disease Virus(NDV)

The anti-Newcastle Disease Virus (NDV) titer in serum collected on days 28 and 35 of age was determined by a haemagglutination inhibition test using ELISA test kit (FLOCK TYPE recNDV, Labor Diagnostik, Leipzig, Germany) as described by Allan and Gough (1974).

Plasma immunoglobulin concentrations

Plasma immunoglobulin (Ig) A, IgM, IgG and total Ig concentrations were determined using chicken-specific IgA, IgM and IgG ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX, USA). Total plasma Ig concentration was calculated by the sum of the respective serum IgA, IgM and IgG concentrations (Mountzouris et al., 2010).

Statistical analysis

Data of the study for all variables were subjected to analysis of variance (ANOVA) as a completely randomized design using the procedure of SPSS software ver. 18 (2010; SPSS Inc., Chicago, IL, USA). Multiple range test method was used to test the statistical differences among treatments according to Duncan (1955). The following model was used:

$$Y_{ijk} = \mu + O_i + I_j + OI_{ij} + e_{ijk}$$

where: Y_{ijk} – the observation, μ – the overall mean, O_i – fixed effect of i^{th} *in ovo* level (i = 0, 5 or 10 ppb/egg); I_j – fixed effect of j^{th} SeNPs diet level (j = 0 or 10 ppb), OI_{ij} – interaction effect of i^{th} *in ovo* injection level with j^{th} diet supplementation level, and e_{ijk} – error of the model.

Results and discussion

Live body weight and body weight gain. It was shown that live body weight (LBW) and body weight gain (BWG) of Hubbard chicks were influenced by SeNPs *in ovo* injection and feed supplementation (Table 2). But the interaction between these two delivery routes was not statistically significant. Both of SeNPs delivery routes significantly improved LBW. The *in ovo* delivery increased LBW in the 1st, 2nd, 3rd and 5th week regardless of the used dose ($P < 0.001$, $P < 0.007$, $P < 0.001$ and $P < 0.03$, respectively for each week). The SeNPs feed supplementation increased LBW in the 3rd, 4th and 5th week ($P < 0.027$, $P < 0.001$ and $P < 0.001$, respectively). Although the interaction between the two examined delivery routes was not stated for LBW, it was found that the highest values of LBW were observed in R6 (10 ×10) and R4 (5 ×10) groups during all experimental periods and the ratio of increment in these groups was 8.97 and 7.24, respectively. The overall BWG was also increased by both routes of SeNPs delivery ($P < 0.035$ and $P < 0.001$, respectively for *in ovo* and dietary delivery). The improvements in LBW and BWG in treated groups may confirm the important role of selenium as a structural component of 5'-deiodinase, which is a key enzyme participating in the thyroxine (T_4) conversion to the active triiodothyronine (T_3), which may influence the body energy and protein uptake, and thus may regulate chick growth (Jianhua et al., 2000). This may also be due to the fact that selenium deficiency leads to nutritional muscular dystrophy and the selenium supplementation prevents such a negative effect. This is in agreement with Cantor et al. (1982) who concluded that selenium supplementation in turkey poults increased body weight and reduced the incidence of gizzard myopathy. A similar trend was also reported by Zhou and Wang (2011) who showed significant improvement in the growth performance of broiler chicks by SeNPs supplementation up to 0.5 mg/kg basal diet. Likewise, Heindl et al. (2010) and Rozbicka-Wieczorek et al. (2012) reported a beneficial effects of selenium-enriched yeast addition into feed on body weight of broiler chickens.

Table 2. Effect of *in ovo* injection and feed supplementation with selenium nanoparticles (SeNPs) on live body weight and body weight gain of broiler chicks at different periods

Indices	Live body weight, g						Body weight gain, g/bird/day
	initial	1st week	2nd week	3rd week	4th week	5th week	1–35 day
Main factors							
in ovo injection (I)							
0	45.3	153[b]	411[b]	796[b]	1367	1977[b]	55.2[b]
5	45.7	168[a]	439[a]	857[a]	1391	2038[a]	56.9[a]
10	45.4	164[a]	443[a]	856[a]	1388	2052[a]	57.3[a]
P-value	0.759	0.001	0.007	0.001	0.376	0.032	0.035
SEM	0.35	2.49	7.74	11.63	13.54	21.48	0.61
feed supplementation (S)							
0	45.6	162	424	821[b]	1355[b]	1977[b]	55.2[b]
10	45.4	162	438	851[a]	1409[a]	2068[a]	57.8[a]
P-value	0.676	0.909	0.133	0.027	0.001	0.001	0.001
SEM	0.29	2.04	6.32	9.50	11.06	17.55	0.50
Interaction (I × S)							
R1 (0 × 0)	45.5	153	414	790	1351	1936	54.1
R2 (0 × 10)	45.2	154	408	803	1383	2018	56.4
R3 (5 × 0)	45.7	166	429	837	1368	2001	55.8
R4 (5 × 10)	45.7	169	449	876	1414	2076	58.0
R5 (10 × 0)	45.5	165	430	837	1346	1994	55.6
R6 (10 × 10)	45.4	163	456	874	1430	2110	59.0
P-value	0.962	0.780	0.282	0.682	0.391	0.780	0.765
SEM	0.49	3.53	10.95	16.45	19.15	30.38	0.86

[a,b] – means with different superscripts within the same column for each main factor or interaction separately are significantly different ($P \leq 0.05$); SEM – standard error of means

Also Khazraie et al. (2015) showed a significant increase in the weight gain of quail chicks fed diet supplemented with SeNPs compared to control one.

Feed intake and feed conversion ratio. As shown (Table 3) the average daily FI of broiler chicks was significantly ($P < 0.05$) for all weeks except 3rd week influenced by *in ovo* injection of SeNPs, and the FI values were higher in the injected groups especially in the first 3 weeks after the hatch. The total feed intake (TFI) was higher only when the higher dose of SeNPs was injected compare to non-injected group. However, in case of using SeNPs as feed additive no significant difference in average daily FI and TFI values between the treated and control group was stated. There was also a significant interaction between the two examined routes of SeNPs delivery for the average daily FI (except for the first week) and TFI. The highest value of FI was observed in R6 (10 × 10) group, which was both *in ovo* injected with10 ppb SeNPs per egg and dietary supplemented with 10 ppb SeNPs per kg of feed. Nevertheless, a different trend was noted for FCR which was influenced by SeNPs feed supplementation indicating significant improvement, except the first and second growing periods (0–7 and 8–14 day, respectively). The *in ovo* injection did not exert effect on FCR except the first period (0–7 day) in which the FCR values were increased in the group with a higher dose injection. The interaction between the two delivery routes was significant only in the period from day 15 to 21. The most favourable FCR values for the whole experimental period were observed for groups R4 (5 × 10), R2 (0 × 10) and R6 (10 × 10) being 1.40, 1.41 and 1.45, respectively.

The improvement in FCR may result from the higher utilization of SeNPs associated with the unique properties of nanoform selenium, such as greater surface activity, higher solubility, mobility, high cellular uptake and excellent bioavailability (Wang et al., 2007; Zhang et al., 2008). It is also known that selenium is involved in the regulation of the energy metabolism and the metabolism of the essential fatty acids, and purinic and pyrimidinic bases (Ebeid, 2013). The selenium influence on T_3 synthesis may be also of important notice as T_3 is a main hormone controlling the body energy and protein anabolism. In agreement with the present results, Saleh (2014) indicated that broiler chickens feed with the dietary mixture of *Aspergillus* probiotic and SeNPs significantly improved FCR and decreased FI. Also, Chen et al. (2013) reported that yeast selenium supplementation (0.15 or

Table 3. Effect of *in ovo* injection and feed supplementation with selenium nanoparticles (SeNPs) on feed intake and feed conversion ratio of broiler chicks at different periods

Indices	Feed intake, g/bird/day						Feed conversion ratio, g feed/g gain					
	0–7	8–14	15–21	22–28	29–35	1–35	0–7	8–14	15–21	22–28	29–35	1–35
	day						day					
Main factors												
in ovo injection (I)												
0	18.3^{c}	49.4^{c}	79.9^{b}	122	133^{b}	80.7^{b}	1.18^{b}	1.34	1.46	1.51	1.53^{a}	1.46
5	20.3^{b}	52.8^{b}	85.8^{a}	118	130^{b}	81.5^{b}	1.17^{b}	1.37	1.44	1.55	1.41^{b}	1.43
10	22.1^{a}	54.3^{a}	84.4^{a}	119	139^{a}	83.7^{a}	1.31^{a}	1.36	1.43	1.56	1.47^{ab}	1.46
P-value	0.002	0.003	0.001	0.052	0.001	0.002	0.004	0.786	0.670	0.379	0.035	0.208
SEM	0.30	0.33	0.89	1.21	1.26	0.48	0.025	0.03	0.019	0.03	0.029	0.012
feed supplementation (S)												
0	20.2	51.9	83.0	120	134	81.8	1.22	1.39	1.47^{a}	1.58	1.51^{a}	1.48^{a}
10	20.3	52.4	83.8	120	134	82.1	1.22	1.33	1.42^{b}	1.51	1.42^{b}	1.42^{b}
P-value	0.687	0.252	0.476	0.938	0.946	0.629	0.856	0.137	0.042	0.065	0.024	0.001
SEM	0.247	0.268	0.726	0.990	1.03	0.390	0.02	0.025	0.015	0.024	0.024	0.01
Interaction (I × S)												
R1 (0 × 0)	18.6	50.1^{c}	79.9^{c}	125^{a}	136^{ab}	81.9^{b}	1.22	1.35	1.50^{a}	1.56	1.62	1.52
R2 (0 × 10)	18.0	48.6^{d}	80.0^{c}	121^{ab}	130^{bc}	79.5^{c}	1.15	1.33	1.42^{bc}	1.45	1.44	1.41
R3 (5 × 0)	20.2	52.7^{b}	87.5^{a}	121^{ab}	128^{c}	81.9^{b}	1.17	1.41	1.50^{a}	1.6	1.42	1.47
R4 (5 × 10)	20.5	53.0^{b}	84.2^{ab}	116^{bc}	132^{bc}	81.1^{bc}	1.16	1.33	1.38^{c}	1.51	1.4	1.40
R5 (10 × 0)	21.7	53.0^{b}	81.7^{bc}	114^{c}	138^{a}	81.7^{bc}	1.27	1.5	1.40^{c}	1.57	1.50	1.47
R6 (10 × 10)	22.5	55.5^{a}	87.2^{a}	124^{a}	139^{a}	85.7^{a}	1.34	1.42	1.46^{ab}	1.56	1.44	1.45
P-value	0.308	0.004	0.014	0.002	0.047	0.001	0.225	0.691	0.013	0.492	0.164	0.061
SEM	0.43	0.46	1.26	1.72	1.78	0.68	0.035	0.043	0.027	0.042	0.041	0.017

$^{a–d}$ – means with different superscripts within the same column for each main factor or interaction separately are significantly different ($P \leq 0.05$); SEM – standard error of means

0.30 mg/kg) could significantly increase broiler daily gains and FCR in comparison to the same doses of sodium selenite. Similarly, Zhou and Wang (2011) showed that SeNPs supplementation up to 0.5 mg per kg of the basal broiler diet effectively improved FCR.

Carcass characteristics. All the examined carcass traits (carcass, breast, thigh, total edible, heart, gizzard, liver, spleen, thymus, bursa of Fabricius and carcass yield as percentage of LBW) were not significantly affected ($P > 0.05$) by *in ovo* injection with different doses of SeNPs or feed SeNPs supplementation, except the carcass weight that was significantly($P < 0.05$) affected by feed supplementation (Table 4). The interaction between the two examined delivery routes was significant ($P < 0.01$) for the carcass yield, and the highest carcass yield was observed in R4 (5 × 10) followed by R2 (0 ×10) and R6 (10 × 10) groups.

In line with the present results, Khazraie et al. (2015) indicated no significant effect of diets with SeNPs addition on carcass composition of chicks. Also Biswas et al. (2006) reported that selenium supplementation did not have any effect on liver and spleen weights. In the same trend, Peng et al. (2009) and Cai et al. (2012) reported no significant differences in the relative weight of immune organs (thymus, bursa of Fabricius and spleen) of broiler chicks receiving SeNPs with the diet.

Haematological parameters. The results revealed that both SeNPs delivery routes and the interaction between did not affected the examined blood constitute parameters (WBCs, RBCs, Hb, PCV, MCH, MCHC and MCV) (Table 5). Lack of significant changes in haematological indices may reflex that no physiologically stressful condition was introduced in treated chicks. Moreover, the obtained haematological results can be a good indicator that chicks were fed on a sufficient doses of SeNPs in the present study.

The obtained findings are not partially in agreement with the results of Boostani et al. (2015) who showed a significant difference in total WBCs number in broiler chicks fed 0.3 mg/kg SeNPs under oxidative stress conditions. The same authors also showed that no difference was found for the number of RBCs, Hb, PCV, MCH and MCHC, which is in line with the results of the present study. Similarly, Chen et al. (2013) showed no significant difference in blood biochemical indexes (WBCs, RBCs, Hb and PCV) in broilers fed different selenium sources. However, Khazraie et al. (2015) reported

Table 4. Effect of *in ovo* injection and feed supplementation with selenium nanoparticles (SeNPs) on carcass characteristics of broiler chicks

Indices	LBW, g	Carcass traits, %										Carcass yield, %
		carcass	heart	gizzard	liver	giblets	thigh	breast	spleen	thymus	bursa	
Main factors												
in ovo injection (I)												
0	2070	72.5	0.48	1.11	2.59	4.18	29.7	42.6	0.12	0.46	0.19	76.7
5	2096	72.2	0.48	1.16	2.38	3.86	29.1	41.8	0.10	0.42	0.18	76.2
10	2055	72.2	0.48	1.21	2.32	3.97	29.1	41.5	0.11	0.46	0.19	76.1
P-value	0.176	0.938	0.983	0.332	0.232	0.422	0.627	0.762	0.305	0.484	0.743	0.844
SEM	14.51	0.73	0.02	0.05	0.11	0.14	0.53	0.78	0.01	0.02	0.01	0.70
feed supplementation (S)												
0	1970^{b}	71.1^{b}	0.47	1.19	2.31	3.97	28.9	42.0	0.11	0.44	0.19	75.1^{b}
10	2178^{a}	73.4^{a}	0.49	1.12	2.55	4.03	29.9	42.0	0.11	0.45	0.18	77.6^{a}
P-value	0.00	0.016	0.278	0.218	0.087	0.203	0.954	0.143	0.451	0.799	0.932	0.008
SEM	11.85	0.59	0.02	0.04	0.09	0.11	0.44	0.64	0.004	0.02	0.01	0.57
Interaction (I × S)												
R1 (0 × 0)	1955	72.1	0.49	1.11	2.65	4.25	28.9	42.8	0.12	0.48	0.19	76.3^{abc}
R2 (0 × 10)	2185	72.9	0.47	1.11	2.53	4.11	30.4	42.4	0.11	0.45	0.20	77.0^{ab}
R3 (5 × 0)	2020	69.4	0.44	1.22	2.20	3.86	28.0	41.3	0.10	0.40	0.18	73.2^{c}
R4 (5 × 10)	2172	74.9	0.51	1.10	2.57	4.18	30.2	42.3	0.11	0.45	0.17	79.0^{a}
R5 (10 × 0)	1935	71.8	0.46	1.25	2.09	3.80	29.9	41.9	0.10	0.46	0.19	75.6^{bc}
R6 (10 × 10)	2176	72.5	0.50	1.16	2.55	4.14	28.9	41.1	0.11	0.45	0.18	76.7^{ab}
P-value	0.103	0.062	0.323	0.650	0.177	0.262	0.717	0.126	0.701	0.436	0.836	0.040
SEM	20.52	1.03	0.03	0.07	0.16	0.16	0.75	1.11	0.01	0.03	0.02	0.98

LBW – live body weight; $^{a–c}$ – means with different superscripts within the same column for each main factor or interaction separately are significantly different ($P \leq 0.05$); SEM – standard error of means

Table 5. Effect of *in ovo* injection and feed supplementation with selenium nanoparticles (SeNPs) on blood haematological parameters of broiler chicks

Indices	RBC, × 10^6/mm^3	WBC, × 10^3/mm^3	Hb, mg/dl	PCV, %	MCH, pg	MCHC, g/100 ml	MCV, µl^3
Main factors							
in ovo injection (I)							
0	4.99	23.5	13.2	33.3	26.5	39.7	66.8
5	5.08	23.3	13.2	34.2	26.3	38.7	67.6
10	4.95	23.5	13.3	33.2	27.0	40.0	67.5
P-value	0.90	0.73	0.98	0.69	0.84	0.64	0.93
SEM	0.21	1.12	0.42	0.87	0.92	1.03	1.56
feed supplementation (S)							
0	5.16	23.2	13.3	34.1	25.9	39.0	66.5
10	4.85	24.3	13.2	33.0	27.2	40.0	68.2
P-value	0.23	0.41	0.78	0.29	0.25	0.35	0.46
SEM	0.18	0.91	0.34	0.71	0.75	0.84	1.27
Interaction (I × S)							
R1 (0 × 0)	4.99	22.7	13.2	34.3	26.5	38.5	68.8
R2 (0 × 10)	4.99	24.3	13.7	32.3	26.6	41.0	64.8
R3 (5 × 0)	5.37	23.0	13.3	35.0	25.1	38.2	65.6
R4 (5 × 10)	4.80	23.7	13.0	33.3	27.3	39.3	69.6
R5 (10 × 0)	5.13	24.0	13.4	33.0	26.2	40.5	64.9
R6 (10 × 10)	4.76	25.0	13.2	33.3	27.8	39.6	70.2
P-value	0.65	0.95	0.95	0.61	0.71	0.12	0.51
SEM	0.30	1.58	0.59	1.23	1.30	1.46	2.21

RBC – red blood cells; WBC – white blood cells; Hb – haemoglobin value; PCV – packed cell volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; MCV – mean corpuscular volume; SEM – standard error of means

Table 6. Effect of *in ovo* injection and feed supplementation with selenium nanoparticles (SeNPs) on serum constituents of broiler chicks

Indices	TP, mg/dl	Albumin, mg/dl	Globulin, mg/dl	A:G ratio	TC, mg/dl	TG, mg/dl	HDL-C, mg/dl	LDL-C, mg/dl	Uric acid, mg/dl	Creatinine, mg/dl	ALT, U/l	AST, U/l	ALP, U/l	Glucose, mg/dl	T_3, ng/ml
Main factors															
in ovo injection (I)															
0	5.77	2.92	2.85	1.05	145^{a}	138^{a}	46.6^{b}	51.4	4.61	0.50	32.3	136	141	226	0.77^{b}
5	5.48	3.01	2.48	1.27	117^{b}	118^{b}	58.2^{a}	40.9	4.48	0.52	29.1	131	152	234	0.87^{a}
10	5.75	2.80	2.95	0.95	132^{ab}	128^{ab}	49.2^{b}	48.6	4.17	0.51	28.5	133	160	231	0.79^{b}
P-value	0.812	0.851	0.262	0.298	0.005	0.011	0.001	0.103	0.641	0.927	0.159	0.677	.070	0.287	0.042
SEM	0.34	0.26	0.21	0.14	4.86	3.91	1.71	3.27	0.34	0.04	1.37	3.90	5.18	3.32	0.03
feed supplementation (S)															
0	5.78	3.08	2.70	1.19	136	141^{a}	48.9^{b}	48.6	4.39	0.51	32.4^{a}	140^{a}	146	222^{b}	0.76^{b}
10	5.55	2.73	2.82	0.99	127	116^{b}	53.8^{a}	45.3	4.45	0.51	27.6^{b}	126^{b}	157	238^{a}	0.86^{a}
P-value	0.572	0.276	0.633	0.236	0.131	0.001	0.030	0.391	0.886	0.962	0.010	0.010	0.086	0.002	0.006
SEM	0.28	0.22	0.17	0.11	3.97	3.19	1.40	2.67	0.27	0.03	1.12	3.19	4.23	2.72	0.02
Interaction (I × S)															
R1 (0 × 0)	5.65	2.95	2.69	1.09	148	167^{a}	48.0^{b}	52.0	4.66	0.51	30.6^{ab}	147	143	225	0.64^{c}
R2 (0 × 10)	5.88	2.88	3.00	1.00	142	110^{c}	45.2^{b}	50.8	4.56	0.49	33.9^{a}	125	140	227	0.91^{a}
R3 (5 × 0)	5.46	3.14	2.32	1.44	122	128^{b}	56.6^{a}	40.5	4.53	0.52	32.7^{a}	139	148	224	0.92^{a}
R4 (5 × 10)	5.51	2.87	2.63	1.09	113	108^{c}	59.8^{a}	41.3	4.42	0.52	25.6^{bc}	122	156	243	0.82^{ab}
R5 (10 × 0)	6.24	3.15	3.09	1.03	138	128^{b}	42.2^{b}	53.7	3.98	0.50	33.8^{a}	134	146	219	0.73^{bc}
R6 (10 × 10)	5.26	2.44	2.81	0.87	126	129^{b}	56.3^{a}	43.8	4.36	0.52	23.2^{c}	132	174	243	0.84^{ab}
P-value	0.430	0.692	0.521	0.789	0.915	0.001	0.013	0.520	0.842	0.930	0.010	0.217	0.151	0.086	0.001
SEM	0.49	0.37	0.29	0.19	6.87	5.53	2.42	4.63	0.47	0.06	1.94	5.52	7.32	4.70	0.04

TP – total protein; A – albumin; G – globulin; TC – total cholesterol; TG – triglycerides; HDL-C – high-density lipoprotein cholesterol; LDL – low-density lipoprotein cholesterol; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphates enzyme; T_3 – triiodothyronine hormone; SEM – standard error of means; $^{a–c}$ – means with different superscripts within the same column for each main factor or interaction separately are significantly different ($P \leq 0.05$)

a significant increase in haemoglobin concentration in quails fed diet supplemented 0.2 mg SeNPs.

Blood biochemical analysis. The results of serum total protein, albumin and globulin concentrations and A:G ratio showed no statistical effect of both examined routes of SeNPs administration as well as no significant interaction between these routes in the Hubbard broiler chicks (Table 6). The obtained results are in accordance with Yang et al. (2012) who observed no effect of selenium in the diet on the serum total protein and globulin levels in chicks. The same results were observed by Selim et al. (2015) who indicated no significant effect on plasma total protein, globulin and albumin contents in broiler chicks fed diet supplemented with SeNPs. In contrary, Mohamed et al. (2016) showed a significant increase in plasma total protein and globulin concentrations in chicken fed SeNPs; however, albumin content was not affected. Also Mohapatra et al. (2014) observed linear and quadratic increase in serum total protein and globulin levels in layer grower birds fed diet supplemented with SeNPs.

Data connected with serum lipid profile showed that both examined SeNPs administration routes and their interaction were significantly affected in broiler chicks (Table 6). While serum levels of total cholesterol (TC) and triglyceride (TG) were decreased in the *in ovo* injected groups compared to the non-injected group, the HDL-C level was increased. There was no effect of SeNPs injection on LDL-C level. In case of SeNPs dietary administration, serum TC and LDL-C levels were not significantly affected. On the other hand, feed supplementation with SeNPs had a significant impact on serum TG and HDL-C levels, with the lower TG content and higher HDL-C level observed in the SeNPs-treated group compared to the non-supplemented one. The interaction between the two delivery routes was significant for both TG and HDL-C levels. The lowest TG levels were stated for R2 (0 × 10) and R4 (5 × 10) groups, while and the highest contents of HDL-C were observed in R3 (5 × 10), R4 (5 × 10) and R6 (10 × 10) groups. Furthermore serum TC and LDL-C levels were not significantly affected by the interaction between the examined delivery routes. However, the lowest TC and LDL-C levels were recorded for R2 (0 × 10), R4 (5 × 10) and R6 (10 × 10) groups. These results are partially consistent with the results of Saleh (2014) who found significant decrease in plasma TG but also TC levels in broiler chickens fed diet with SeNPs addition, while plasma HDL-C content was increased. Simultaneously Elsaid (2015) found significant increase in plasma HDL-C level in chicks fed diet with SeNPs supplementation at a dose of 40 ppb. The same authors stated also a significant decrease in plasma total lipids, TG, TC and LDL-C in the SeNPs-treated group. Radwan et al. (2015) also observed a significant decrease in plasma total lipids, TC and an increase in HDL-C as a result of SeNPs administration. In proportionate with these results, Yang et al. (2012) reported no significant difference in serum TC, TG and HDL-C levels in chicks fed diet supplemented with selenium.

Concerning liver function, serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were analysed (Table 6). The obtained results indicated that the different routes of SeNPs administration had different effects on ALT, AST and ALP activities. The feed supplementation significantly affected ALT and AST activities ($P < 0.01$ for both enzymes) but not ALP activity, while the *in ovo* injection had no significant effect on these enzymes activities. The interaction between the delivery routes was significant ($P < 0.01$) only for ALT activity. The lowest values of ALT activity were recorded in R6 (10 ×10) and R4 (5 × 10) groups, while in the same groups the highest values of ALP were stated. Although some significant elevations in serum ALP, ALT and AST activities were found, the obtained values were in the normal rang according to the previous studies. The obtained finding is in line with the results of Yang et al. (2012), who reported no significant difference in serum AST and ALP activities in chicks fed diet supplemented with selenium. Also, Selim et al. (2015) indicated no significant effect on plasma ALT, AST and ALP activities in broiler chicks fed diet with SeNPs addition. In contrast the results obtained by Elsaid (2015) showed a significant increase in serum ALT and AST activities in chicks fed diet with 40 ppb SeNPs addition per kg. Also Mohapatra et al. (2014) observed linear and quadratic increase in serum AST activity inlayer grower birds fed diet supplemented with SeNPs, whereas ALP activity was decreased. On the other hand, Saleh (2014) noted significant decrease in plasma AST activity in birds fed SeNPs-supplemented diet.

Regarding kidney function, the obtained data revealed that there was no significant of SeNPs *in ovo* injection or feed supplementation on serum levels of uric acid and creatinine. Also the interaction between the two delivery routes was not significant. The insignificant effect on kidney

function expressed by serum levels of uric acid and creatinine may indicate that no stressful condition so called 'toxicity state' was introduced in treated chicks. The above results are in agreement with Yang et al. (2012) who stated no significant different in urea levels in serum of chicks receiving additional selenium in the diet. On the other hand, Elsaid (2015) found a significant decrease in plasma uric acid and creatinine levels in chicks fed diet with SeNPs. In contrary Mohapatra et al. (2014) observed a quadratic increase in serum urea level of layer grower birds fed diet supplemented with SeNPs.

The serum T_3 and glucose levels were also analysed (Table 6). The obtained results showed a significant effect of SeNPs feed supplementation on glucose serum concentration ($P < 0.002$), while no effect of *in ovo* injection was stated. The highest glucose levels were stated in R4 (5 × 10) and R6 (10 × 10) groups, while the lowest level was observed for R5 (10 × 0) group. On the other hand, T_3 level was significantly ($P < 0.04$) increased in the group *in ovo* injected with SeNPs at a dose of 5 ppb. Also the SeNPs addition into the diet caused an increase in serum T_3 levels ($P < 0.006$). The interaction between the two SeNPs delivery routes was also statistically significant ($P < 0.001$). In all treatments, except R5 (10 × 0), T_3 levels were higher in comparison to R1 (0 × 0) group. The significant elevations in serum glucose and T_3concentrations may be associated with the role of selenium as a structural component of 5'-deiodinase, which is a key enzyme participating in the T_4 conversion to the active T_3. In this line Elsaid (2015) found a significant increase in plasma T_3 and T_4 concentrations in birds fed diet supplemented with SeNPs. Similar trends were also illustrated by Choupani et al. (2014) who showed increased plasma T_3 levels and higher T_3:T_4 ratio in SeNPs supplemented group compared with groups supplemented with organic and inorganic selenium. In the study by Jianhua et al. (2000), Se-deficient chickens showed significant reduction in T_3 levels and elevated T_4 levels compared with Se-supplemented chickens. Contrariwise, Boostani et al. (2015) indicated no significant difference in plasma levels of T_3 and T_4 between birds fed diet with organic selenium, inorganic selenium and SeNPs under oxidative stress.

Antioxidant status. The results of serum antioxidant status including reduced glutathione (GSH) and malondialdehyde (MDA) contents and glutathione reductase (GSR) activity showed significant differences depending on SeNPs delivery route (Table 7). GSH level and GSR activity in serum

Table 7. Effect of *in ovo* injection and feed supplementation with selenium nanoparticles (SeNPs) on antioxidant status, immunoglobulins (IgG, IgM, IgA and total Ig) contents and humoral immune response against Newcastle Disease Virus (NDV) of broiler chicks

Indices	Antioxidant status			Immunological status, µg/ml				Humoral immune response, antibody titer against NDV	
	MDA, µmol/ml	GSH, mg/l	GSR, mg/ml	IgA	IgM	IgG	total Ig	1st response	2nd response
Main factors									
in ovo injection (I)									
0	0.238^{a}	0.151^{b}	0.166^{b}	137	75.8^{c}	908^{b}	1122^{b}	3.00^{ab}	1.45
5	0.146^{b}	0.188^{a}	0.195^{ab}	138	132.5^{a}	920^{b}	1191^{b}	3.50^{a}	1.45
10	0.183^{b}	0.197^{a}	0.220^{a}	150	104.8^{b}	1034^{a}	1288^{a}	2.17^{b}	1.48
P-value	0.001	0.001	0.001	0.443	0.001	0.005	0.002	0.044	0.991
SEM	0.01	0.01	0.01	7.72	7.08	23.47	25.88	0.33	0.20
feed supplementation (S)									
0	0.212^{a}	0.152^{b}	0.161^{b}	118^{b}	94.2^{b}	856^{b}	1068^{b}	2.20^{b}	1.46
10	0.166^{b}	0.206^{a}	0.226^{a}	165^{a}	114.6^{a}	1052^{a}	1333^{a}	3.57^{a}	1.47
P-value	0.008	0.001	0.002	0.000	0.029	0.001	0.004	0.005	0.963
SEM	0.01	0.01	0.01	6.30	5.78	19.17	21.13	0.27	0.17
Interaction (I × S)									
R1 (0 × 0)	0.295^{a}	0.122	0.142^{d}	106	75.7	800	981	2.67^{b}	1.43
R2 (0 × 10)	0.180^{bc}	0.180	0.190^{bc}	168	76.0	1016	1263	2.33^{b}	1.47
R3 (5 × 0)	0.134^{c}	0.167	0.170^{cd}	115	125.0	804	1044	2.00^{b}	1.43
R4 (5 × 10)	0.158^{bc}	0.209	0.219^{b}	162	140.0	1036	1338	5.00^{a}	1.47
R5 (10 × 0)	0.205^{b}	0.168	0.171^{cd}	135	82.0	963	1180	2.00^{b}	1.50
R6 (10 × 10)	0.161^{bc}	0.227	0.269^{a}	165	127.7	1104	1397	2.33^{b}	1.47
P-value	0.007	0.644	0.048	0.393	0.110	0.378	0.552	0.030	0.991
SEM	0.02	0.01	0.01	10.92	10.02	33.19	36.60	0.47	0.29

MDA – malondialdehyde content; GSH – reduced glutathione content; GSR – glutathione reductase activity; SEM – standard error of means; $^{a–c}$ – means with different superscripts within the same column for each main factor or interaction separately are significantly different ($P \leq 0.05$)

were significantly ($P < 0.001$ for both parameters) increased while MDA content was significantly ($P < 0.001$) decreased when SeNPs were *in ovo* injected. The same relationships were found for SeNPs feed supplementation ($P < 0.008$, $P < 0.001$ and $P < 0.002$ for MDA and GSH levels and GSR activity, respectively). The significant interaction between routes of SeNPs delivery was found for MDA content and GSR activity. The highest values of GSR activity and GSH content were observed in birds from R6 (10 × 10) and R4 (5 × 10) groups and the lowest ones in R1 (0 × 0) group. On the other hand the highest values of MDA level were observed in birds from groups R1 (0 × 0) and R5 (10 × 0), while the lowest values of MDA were stated in birds from R3 (5 × 0) group.

The obtained results on serum antioxidant status shed light upon the selenium function as a major component of the antioxidant system which participates in controlling the body glutathione pool. The results clarify the vital roles of SeNPs in protecting cells from reactive oxygen species (ROS) abundance by reducing free radicals and lipid peroxidation products (Pilarczyk et al., 2012). The results are in close agreement with Jiang et al. (2009) who mentioned significant increase in plasma antioxidant enzymes activities in broilers fed diet with Se-methionine. Chen et al. (2013) showed also significant elevation in the serum activities of total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px), as well as in the ability to inhibit hydroxyl radical ($OH^{\cdot}$) and total antioxidant capacity (T-AOC) in chickens treated with selenium yeast. Moreover, the same authors showed significant decrease in the contents of MDA in the selenium yeast groups. Similarly, Cai et al. (2012) showed significant quadratic effect of SeNPs on serum MDA level, GSH-Px activity and free radical inhibition in broilers fed diet supplement with 0.30 mg SeNPs/kg diet. Also, Mohamed et al. (2016) stated positive effects on plasma total antioxidant capacity of chickens fed diet supplemented with SeNPs.

Immunoglobulins and humoral immune response against Newcastle Disease Virus (NDV). In relation to immunoglobulins status and immune response against NDV, the main factors effects and their interaction were stated (Table 7). It was observed that SeNPs *in ovo* injection significantly affected immunoglobulins content ($P < 0.001$, $P < 0.005$ and $P < 0.002$ for IgM, IgG and TIg respectively) and 1st week antibody titer against NDV ($P < 0.04$). The IgM content was the highest in birds with *in ovo* injection of SeNPs at a dose of 5 ppb, while in the non-injected group IgM content was the lowest. IgG and TIg levels were elevated only in birds with *in ovo* injection of SeNPs at a dose of 5 ppb. The 1st week antibody titer against NDV was decreased in birds with *in ovo* injection of SeNPs at a dose of 10 ppb in comparison to 5 ppb SeNPs dose. The SeNPs feed supplementation exerted influence on all examined immunoglobulins ($P < 0.0001$, $P < 0.03$, $P < 0.004$ and $P < 0.005$ for IgA, IgM, IgG and TIg, respectively) and 1st week antibody titer against NDV ($P < 0.005$) with higher values in treated group for all parameters. The interaction between two examined SeNPs routes of delivery was significant only for the 1st week antibody titer against NDV.

The improvement in serum immunoglobulins levels and humoral immune response against NDV may be attributed to the important biological role of SeNPs in increasing the concentration of circulating T and B cells, which leads to an increase in leukocyte subpopulation and cellular phagocytic activity. This results are coordinated with Cai et al. (2012) who reported a significant quadratic effect of SeNPs supplementation on serum IgM in of broiler chicks. Also, Swain et al., (2000) reported a significant increase in antibody production against NDV in broiler chicks fed a combination of 150 IU/kg vitamin E and 0.1 ppm Se as Na_2SeO_3. Similar results were suggested by Levkut et al. (2009) who showed a significant elevation in serum IgM, CD44+ and CD45+ concentrations and MHCII+ peripheral blood lymphocytes in broiler chicks fed diet containing increased dose of selenium.

Conclusions

It could be concluded that both the *in ovo* injection of selenium nanoparticles (SeNPs) on day 14 of embryogenesis and the feed supplementation with SeNPs, as well as the combination of these two examined routes of SeNPs delivery, improve post hatch productive performance, lipid profile and antioxidant and immunological status of broiler chicks. Based on the obtained results, the use of SeNPs may be recommended, especially in the form of *in ovo* delivery at a dose of 10 ppb/egg or in a combination of *in ovo* delivery at a dose of 5 ppb/egg and a feed additive at a dose of 10 ppb/kg ration, to improve production efficiency and the physiological and immunological status of hatched chicks.

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