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ORIGINAL PAPER

AN OPTIMUM LEVEL OF NANO-SELENIUM SUPPLEMENTATION OF A BROILER DIET ACCORDING TO THE PERFORMANCE, ECONOMICAL PARAMETERS, PLASMA CONSTITUENTS AND IMMUNITY*

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Abstract

An optimum nano-Se content in a diet of highly productive broilers has not been defined yet. Meanwhile, there are contradictory reports regarding effects of nano-Se on production traits and the etiology of possible positive effects. The aims of the present study were to test the hypothesis that low levels of nano-Se can improve productivity and metabolic functions during a 42-day-long broiler production cycle, to determine an optimum nano-Selenium (nano-Se) concentration of the diet, and finally to explore the etiology of these effects. One-hundred-eighty 1-day-old, male, Ross 308 broiler chicks were used in a completely randomized experiment, where the birds were placed in experimental pens, in three replicate pens of 10 chicks each, making a total of 18 experimental units. They were assigned diets with different nano-Selenium levels. All birds were fed an almost identical diet from 1 until 42 d, different only in the nano-Se content, namely: 1) control (CON) group 0.0 nano-Se, 2) NS1 group 0.1 mg kg^{-1} dietary nano-Se, 3) NS2 group 0.2 mg kg^{-1} dietary nano-Se, 4) NS3 group 0.3 mg kg^{-1} dietary nano-Se, 5) NS4 group 0.4 mg kg^{-1} dietary nano-Se, 6) NS5 group 0.5 mg kg^{-1} dietary nano-Se, supplied from 1 to 42 day

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of life. The significance level was declared at $P<0.05$. The final bird weight was higher and production cost was lower in NS3 and NS4 groups compared to CON, NS1 and NS5. The weight of an eviscerated carcass was lower in CON group compared to the other groups. The serum blood parameters as well as the weight of organs related with the immune system were not significantly different between groups. Nano-Se supplementation positively affected body weight gain (BWG) and feed conversion ratio (FCR). Higher BWG was achieved owing to better FCR rather than higher feed intake. The supplementation of 0.3-0.4 mg nano-Se kg^{-1} is the optimum level to be added as a feed additive in broiler diets, increasing animal productivity and diminishing environmental impact.

Keywords: broiler, nutrition, nanotechnology, selenium, production traits, immunity.

INTRODUCTION

Selenium (Se) is an essential microelement in animal nutrition, possessing antioxidant capacities. There are many regions of the world that the soil and consequently crops are Se deficient (Gupta, Gupta 2000), having significant implications on animal productivity. Broilers can be used as an animal model to test optimum nutritional needs for productive animals, as large-scale trials with mammalian species demand the sacrifice of many animals. Lately, it has been shown that nanotechnology (Nano) has significant applications in animal nutrition (Rajendran 2013, Skalickova et al. 2017). Nano-Se is of the most effective form (Sanjabi et al. 2018) of Se (i.e., inorganic, organic Se, selenomethionine, Se enriched yeast etc) regarding production parameters. However, inorganic elements in a diet have extremely low bioavailability (Milis et al. 2018), and therefore it is crucial to determine precisely exact amounts of all elements in a ration which can maximize animal productivity whilst diminishing environmental impact. The research dedicated to the evaluation of the performance of commercial broilers fed nano-Se is limited (Wang et al. 2007, Wang 2009, Cai et al. 2012, Hu et al. 2012, Mahmoud et al. 2016, Moghaddam et al. 2017). Higher body weight gain (BWG) has been reported at a level of 0.3 mg Se kg^{-1} feed (Dlouha et al. 2008). Contrarily, other researchers report that nano-Se supplementation does not have a significant effect on the feed conversion ratio (FCR) and BWG (Cai et al. 2012), even at high doses (0.3 till 8 mg kg^{-1}). On the other hand, Hu et al. (2012) report that FCR and BWG are linearly correlated with nano-Se supplementation at levels between 0.15-1.20 mg kg^{-1}. The explanation why these reports are contradictory might be that nano-Se of different particle size is used in different studies. Moreover, many reports do not even mention what size of nano-Se particles was used in the experiments. The aims of the present study were to test the hypothesis that low levels of nano-Se (80 nm particle size) can improve productivity and metabolic functions of 42-day-long broiler production cycle, to determine the ideal nano-Se concentration of the diet and explore the etiology of these effects. Thus, in purpose to evaluate the effects of nano-Se supplementation on BWG and FCR, a trial with 180 one-day old male Ross 308 chicks was conducted, that were fed with gradually elevating levels of nano-Se (0.1; 0.2; 0.3; 0.4 and 0.5 mg kg^{-1}, as feed additive) in excess of basal diet that contained 0.2 mg kg^{-1} inorganic Se.

MATERIALS AND METHODS

This study was carried out at a commercial poultry farm at Rasht, Iran. The study was approved by the Ethic Committee of the Islamic Azad University, and was conducted in compliance with the International Guidelines for research involving animals (Directive 2010/63/EU); care was taken to minimize the number of animals used.

Animals and housing

A total of 180 male Ross 308 broiler chickens were randomly distributed into 6 treatments, with 3 replicates per treatment, having 10 birds *per* replicate. One-day-old chicks were purchased from a local hatchery and randomly assigned into groups with similar mean body weights. Chicks were reared until the age of 42 days. Formula and chemical composition (AOAC 2006) of experimental diets are presented in Table 1. Broiler chickens received feed and water *ad libitum* throughout the trials. Broilers were unable to feed from adjacent cages.

The animals were housed in ground cages size 1.0 x 1.0 m. All broilers were reared in the same environment. Thermo-neutral ambient temperature was maintained in accordance with the standard brooding practice and adopted to the birds' rearing stage (Aviagen 2009). Light was maintained for 23 h on days 1-7 and 40-42, and for 20 h on day, with 4 h of darkness on days 8-39.

Routine vaccination and de-worming were planned by the farm veterinarian, in compliance with the regional veterinary authority. The following vaccines were given: against infectious bronchitis (Infectious Bronchitis Virus – IBV (H120); Razi Co, Iran) at day 1, and Gamboro vaccination (Gamboro – IBD071IR; Razi Co, Iran) at days 14 and 23, Newcastle at days 8 and 21, and influenza at day 1.

Experimental design

A completely randomized design was used, with animal placed in experimental pens assigned different nano-Se levels, in three replicate pens of 10 chicks each, in total making 18 experimental units. Commencing from day one, the groups were as follows:

Group 1 (CON) – control birds; Group 2 (NS1) – 0.1 mg dietary nano-Se

Table 1

Feed ingredients and nutrient analysis of the base diets

Ingredients (%)	Starter (1st -21st day of age)	Grower (22nd-42nd day of age)
Corn	58.6	61.6
Soybean meal (44% CP)	36.2	33.5
Soybean oil	1.4	1.5
Calcium carbonate	0.8	0.9
Gluten meal	0.65	0.16
Dicalcium phosphate	1.30	1.15
NaCl	0.25	0.32
Vitamin premix*	0.25	0.25
Mineral premix**	0.25	0.25
DL-Methionine	0.25	0.30
L-Lysine hydrochloride	0.05	0.07
Total	100	100
Nutrient analysis		
Metabolisable energy (MJ/kg)	12.34	12.55
Crude protein (%)	21.0	20.0
Calcium (%)	0.95	0.90
Available phosphorus (%)	0.47	0.45
Sodium (%)	0.17	0.15
Chloride (%)	0.18	0.17
Lysine (%)	1.12	1.05
Methionine (%)	0.48	0.45
Methionine + cysteine (%)	0.80	0.75
Threonine (%)	0.74	0.70
Selenium (mg kg^{-1})	0.20	0.20

* Supplied per kg of mixture: 1.081 mg *trans*-retinol; 20 mg cholecalciferol; 4 mg α-tocopherol acetate; 800 mg menadione; 720 mg thiamine; 2,640 mg riboflavin; 4,000 mg niacin; 12,000 mg calcium pantothenate; 1,200 mg pyridoxine; 400 mg folic acid; 6 mg cyanocobalamin; 40 mg biotin; 100,000 mg choline; 40,000 mg antioxidant.

** Supplied per kg of mixture: 39,680 mg manganese; 20,000 mg iron; 33,880 mg zinc; 4,000 mg copper; 400 mg iodine; 80 mg selenium.

(particle size 80 nm kg^{-1} feed; Group 3 (NS2) – 0.2 mg kg^{-1}; Group 4 (NS3) – 0.3 mg kg^{-1}; Group 5 (NS4) – 0.4 mg kg^{-1}; Group 6 (NS5) – 0.5 mg kg^{-1}, respectively, from 1 to 42 day of age.

Measurements of broiler performance

Feed intake (FI) and BWG were recorded weekly. FCR (FCR= feed intake (DM)/final BW) and feed cost per kg live weight were calculated based on conventional protocols.

Measurements of broiler carcass traits

At the age of 42 days, after 4 h of fasting for complete evacuation of the gut, one bird from each replicate was euthanized. Care was taken to choose the most representative male birds with respect to body weight compared to the group mean body weight. These animals were used for measuring carcass yield. Birds were fully pecked by dry pecking method. Feet were separated from the carcass in the tibio-tarsal joint. Neck, wingtips, gut and liver were removed, and the empty or edible carcass was weighed.

Measurements of broiler blood metabolites

At the end of the experiment (42 days), one bird from each replicate, in total 3 birds for each experimental group, was randomly selected for blood sampling. Prior to blood collection and slaughter, feed was removed from all the birds for a period of four hours to achieve stabilization of the plasma constituents. Further, all the blood sampling was done in the morning to avoid the variability of the blood parameters to be measured. Care was taken to choose the most representative male birds with respect to body weight compared to the group mean body weight. Blood samples (~5 mL/bird) were collected from the wing vein (*Vena cutanea ulnaris*) into tubes coated with 10 mg of the anticoagulant ethylenediamine tetraacetic acid (EDTA) for plasma separation, and rapidly transferred to the laboratory for analysis (within 2 h of collection). Plasma was harvested after centrifugation (3000 x *g*, for 10 min at room temperature) and stored at -20°C until analysed. Blood parameters analysed in this study included: total cholesterol (Chol), triglycerides (TG), high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, HDL/LDL ratio, and very low density lipoprotein (VLDL) cholesterol. Plasma blood parameters were analysed using a Roche Cobas Integra autoanalyzer (Roche Diagnostics, GmbH, Mannheim, Germany), based on standard protocols using commercial kits from Pars Azmoon (Pars Azmoon Co., Tehran, Iran), according to the manufacturer's instructions, as described elsewhere (Nahavandinejad et al. 2014, Shabani et al. 2015).

Measurements of broiler immune competency

Three birds from each treatment were chosen at random, and blood samples were collected from the brachial vein. Serum was separated by centrifugation (3000 × *g* for 15 min) and the serum was harvested and stored at -20 C until analysis. Antibody titers against infectious bursal disease (IBD) and infectious bronchitis virus (IBV) were measured respectively using commercially available ELISA kits (Bio-check BV, Gouda, Holland) according to Pourhossein et al. (2015). The absorbance of controls and samples were read at 405 nm using an ELISA reader (Bio-Tek Instruments Inc. ELX 800; Winooski, VT, USA). Antibody titers against avian influenza (AI), were measured in birds through hemagglutination inhibition test according to Cunningham (1971).

Statistical analysis

Data were analyzed according to a completely randomized factorial design, with the nano-Se content of the diet being the only independent variable. Comparisons between groups' means were made using the ANOVA procedure, followed by the Tukey's *post hoc* test to separate means, using SAS (1999) software. The Shapiro-Wilks test confirmed normal distribution of data. P values ≤ 0.05 were regarded as statistically significant.

RESULTS

Nano-selenium dietary supplementation significantly improved BWG and FCR both in starter and grower period of the experiment (Ahmadi et al. 2018). CON and NS1 group had inferior FCR compared to NS3 and NS4 groups in 1st, 2nd and 3rd week (Table 2, P<0.05). Feed intake was not different (P>0.05). BWG was higher in NS4 group at 2nd week compared to CON and NS1, and at 5th week compared to CON, NS1 and NS5 groups. NS2, NS3 and NS4 had better FCR compared to CON, NS1 and NS5 at 3rd week (P<0.05). There was no significant effect, in any parameter under study, at 6th week. Final bird weight was higher (Figure 1) and production cost was lower in NS3 and NS4 groups compared to CON, NS1 and NS5 (P<0.05, Table 3). Eviscerated carcass (%) was lower in CON group compared to other groups (Table 3). Serum blood parameters were not significantly different among groups (Table 4, P>0.05). A tendency was found (P<0.1) in Anti-avian influenza hemagglutination, inhibition titers, between groups CON and NS1 compared to the others (Table 5). There were no differences found in the weight of organs (P>0.05) related with the immune system (Table 6).

DISCUSSION

Since final BW was higher in groups NS3 and NS4 in comparison with the others (Table 3), it is a clear indication that 0.3-0.4 mg nano-Se kg^{-1} is the optimum level to be added as a feed supplement in broiler diets. The higher BWG was due to better FCR and not DMI (Table 2).

Table 2

Growth performance parameters of broiler chicken as affected by different levels of dietary nano-selenium supplementation

Specification	CON#	NS1	NS2	NS3	NS4	NS5	SEM	*P*-value
1st week								
Feed intake (g d^{-1})	22.9	22.2	22.5	22.6	21.8	22.8	0.5	0.753
Weight gain (g d^{-1})	20.9	20.8	22.2	22.3	21.6	22.0	0.6	0.382
Feed conversion ratio (%)	1.091^{a}	1.071^{ab}	1.039^{bc}	1.015^{c}	1.010^{c}	1.035^{bc}	0.014	1.010
2nd week								
Feed intake (g d^{-1})	40.2	40.5	39.3	39.9	42.1	40.7	0.953	0.466
Weight gain (g d^{-1})	31.0^{a}	32.2^{ab}	32.8^{ab}	33.7^{ab}	35.7^{b}	34.4^{b}	0.953	0.049
Feed conversion ratio (%)	1.295^{a}	1.259^{a}	1.196^{b}	1.186^{b}	1.181^{b}	1.183^{b}	0.015	0.001
3rd week								
Feed intake (g d^{-1})	79.9	79.1	76.4	80.5	81.4	79.2	1.9	0.565
Weight gain (g d^{-1})	43.5	44.1	44.1	47.0	46.8	44.0	1.1	0.177
Feed conversion ratio (%)	1.837^{a}	1.793^{a}	1.734^{b}	1.714^{b}	1.740^{b}	1.799^{a}	0.016	0.001
4th week								
Feed intake (g d^{-1})	121.5	124.8	127.5	127.0	127.9	128.2	2.3	0.350
Weight gain (g d^{-1})	61.0^{a}	65.8^{b}	69.8^{c}	68.6^{bc}	68.4^{bc}	68.7^{bc}	1.0	0.001
Feed conversion ratio (%)	1.992^{c}	1.896^{b}	1.826^{a}	1.852^{bc}	1.869^{bc}	1.867^{bc}	0.017	0.001
5th week								
Feed intake (g d^{-1})	164.3	162.6	168.0	174.8	167.9	162.8	4.2	0.355
Weight gain (g d^{-1})	79.2^{a}	81.8^{ab}	85.9^{b}	92.5^{c}	87.3^{bc}	82.1^{ab}	2.0	0.006
Feed conversion ratio (%)	2.077^{a}	1.989^{b}	1.955^{bc}	1.888^{c}	1.923^{bc}	1.982^{b}	0.024	0.002
6th week								
Feed intake (g d^{-1})	192.6	194.3	193.9	192.9	196.3	195.8	2.3	0.818
Weight gain (g d^{-1})	79.8	81.2	81.1	80.5	82.7	82.0	1.2	0.594
Feed conversion ratio (%)	2.415	2.393	2.389	2.395	2.375	2.388	0.009	0.161

CON – control, without supplementation, NS1 – supplemented with nano-selenium at 0.1 mg kg^{-1} DM of feed, or at 0.2 mg kg^{-1} of feed (NS2), or at 0.3 mg kg^{-1} of feed (NS3), or at 0.4 mg kg^{-1} of feed (NS4), or at 0.5 mg kg^{-1} of feed (NS5).
Means within each column and week of age with no common superscript differ significantly at $P<0.05$.

It seems that nano-Se improves FCR. The major role of Se in human/ /animal nutrition is related to the synthesis of selenoproteins possessing unique catalytic properties, and more than half of them are involved in redox balance maintenance and antioxidant defenses. Nevertheless, it seems unlikely that nano-Se could have a direct AO effect on the 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 pro-

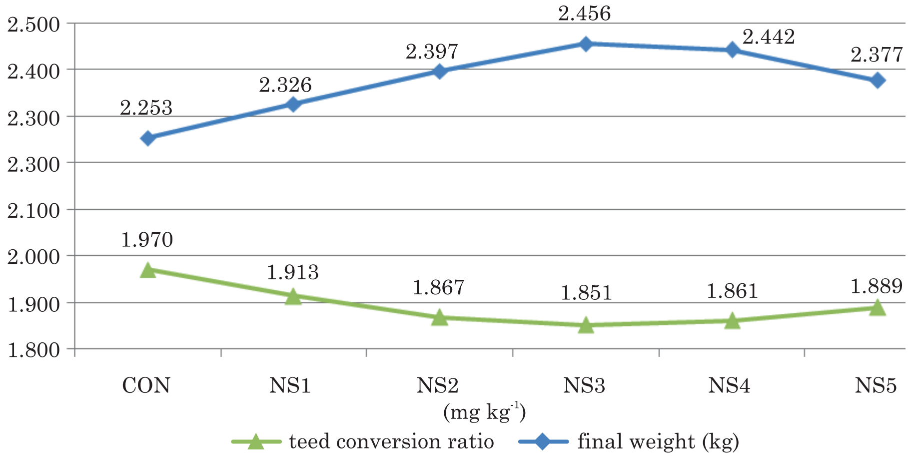

Fig. 1. Feed conversion ratio (%) and final body weight (kg) as affected by nano selenium supplementation, in 42-day-long production cycle: CON – control, without supplementation, NS1 – supplemented with nano-selenium at 0.1 mg kg^{-1} DM of feed, or at 0.2 mg kg^{-1} of feed (NS2), or at 0.3 mg kg^{-1} of feed (NS3), or at 0.4 mg kg^{-1} of feed (NS4), or at 0.5 mg kg^{-1} of feed (NS5)

Table 3

Economical parameters as affected by the different levels of dietary nano-selenium supplementation

Specification	CON	NS1	NS2	NS3	NS4	NS5	SEM	*P*-value
Final weight (g)	2253.3^{a}	2326.3^{b}	2397.7^{cd}	2456.7^{d}	2442.7^{d}	2377.3^{bc}	19.1	0.001
Feed cost per kg live weight (Euro kg^{-1})	0.613^{a}	0.596^{b}	0.582^{cd}	0.577^{d}	0.580^{cd}	0.589^{bc}	0.020	0.001
Eviscerated carcass (%)	65.5^{a}	71.3^{b}	72.8^{b}	72.5^{b}	72.6^{b}	71.6^{b}	1.2	0.006

Explanations under Table 2.
Means within each line with no common superscript differ significantly at $P<0.05$.

Table 4

Mean of blood parameters at 42nd day of age in Ross 308 male broilers fed the different levels of dietary nano selenium from 1st-6th weeks of age

Specification	CON	NS1	NS2	NS3	NS4	NS5	SEM	*P*-value
Total cholesterol (mmol L^{-1})	2.914	2.852	2.524	2.808	3.163	3.488	0.398	0.642
Triglycerides (mmol L^{-1})	0.839	1.077	0.898	1.172	1.218	0.618	0.224	0.438
HDL cholesterol (mmol L^{-1})	0.822	0.812	0.711	0.879	0.665	1.120	0.191	0.639
LDL cholesterol (mmol L^{-1})	1.712	1.596	1.474	1.513	1.947	1.955	0.297	0.770
LDL/HDL	2.22	2.20	2.08	1.70	2.96	2.90	0.83	0.874
VLDL (mmol L^{-1})	0.401	0.510	0.420	0.541	0.559	0.425	0.106	0.821

Explanations under Table 2.
HDL – High Density Lipoproteins, LDL – Low Density Lipoproteins, VLDL – Very Low Density Lipoproteins;

Table 5

Mean of immunity parameters on 42 day of age in Ross 308 male broilers fed the different levels of dietary nano selenium from 1st-6th weeks of age

Specification	CON	NS1	NS2	NS3	NS4	NS5	SEM	*P*-value
IBV (log 10) titer within 38 days after first injection	2.000	2.000	2.000	2.000	2.000	2.000	0.000	0
IBD (log 10) titer within 24 days after first injection	4.667	6.000	4.333	5.000	6.000	6.667	0.850	0.388
AI (log 10) titer within 8 days after injection	3.667	3.000	5.333	5.667	5.333	5.333	0.694	0.087

Explanations under Table 2.
IBV – infectious bronchitis virus, IBD – infectious bursal disease, AI – anti-avian influenza hemagglutination-inhibition

Table 6

Weight and relative weight mean of organs related with immune system on 42 day of age in Ross 308 male broilers fed the different levels of dietary nano selenium from 1st-6th weeks of age

Specification	CON	NS1	NS2	NS3	NS4	NS5	SEM	*P*-value
Spleen weight (g)	1.513	1.443	1.693	1.540	1.543	1.527	0.209	0.975
Relative weight of spleen (%)	0.064	0.062	0.074	0.066	0.067	0.064	0.008	0.922
Bursa of fabricius weight (g)	0.763	1.137	3.660	2.527	1.545	1.180	0.874	0.361
Relative weight of bursa of fabricius (%)	0.032	0.048	0.157	0.106	0.068	0.051	0.044	0.334

Explanations under Table 2.

ductivity (Surai et al. 2017). A possible mechanism is through a beneficial effect on the gut microbiota (Gangadoo et al. 2018) and/or gut anatomy (Ahmadi et al. 2019). Better FCR when the base diet is supplemented with 0.3 mg Se kg^{-1} (organic, inorganic and nano-Se) has also been reported in laying hens (Meng et al. 2018), which can be attributed to higher bioavailability of nano-Se compared to inorganic forms of Se (Li, Wang et al. 2018). Higher BWG has been reported at a level of 0.4 mg nano-Se kg^{-1} feed (Bakheshalinejad et al. 2018). On the other hand, other researchers have demonstrated that nano-Se does not have a significant effect on BWG or on FCR (Cai et al. 2012) even at high doses (0.3 till 8 mg kg^{-1}). In contrast, Hu et al. (2012) reported that FCR and BWG are positively correlated with nano-Se supplementation at levels from 0.15-1.20 mg kg^{-1}.

The current study indicates that nano-Se at the levels of 0.2-0.5 has positive effects on BWG and FCR (Figure 1). The beneficial effect reached a plateau at the level between 0.3-0.4 mg kg^{-1}. Above and below that level, the positive effects were waning, which was reflected by changes in the relative economical index (feed cost per kg live weight; Table 3).

Nano-Se did not affect FI, which is in line with the reports of Li, Zhang et al. (2018), blood constituents, immunity and internal organs related with immunity, indicating that the beneficial effects, at any level of supplementation, cannot be attributed solely to its antioxidant capacities. Bakheshalinejad et al. (2018) have reported that both nano-Se and organic form of Se supplementation positively affect immunity of broilers. In the present study, there was only a tendency (*P*<0.1) of Anti-avian influenza hemagglutination-inhibition titers between CON and NS1 groups in comparison with the other groups (Table 6). The present study indicates that the positive effects of nano-Se on growth parameters could be attributed to gut microbiota, gut anatomy (Ahmadi et al. 2019) or to its role in energy metabolism, which is in line with the reports of Hawkes and Keim (2003) and Steinbrenner (2013) on human subjects.

CONCLUSIONS

Nano-Se at the levels of 0.2-0.5 has positive effects on BWG and FCR. The dose of 0.3-0.4 mg nano-Se kg^{-1} is the optimum amount to be added as a feed supplement in broiler diets. Nano-Se did not affect FI, blood constituents, immunity and internal organs related with immunity. Further research including a larger number of animals.is recommended to analyze parameters of bird health status.

Declaration of interest

The authors report no conflicts of interest.

Acknowledgments

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