



Laying performance, immune response, serum biochemical parameters and egg quality traits of female turkeys fed diet incorporated with organic selenium

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ABSTRACT

An experiment was conducted to investigate the effects of organic selenium (Se) supplementation on laying performance, immune response, serum biochemical parameters and egg quality traits of female turkeys. Female turkeys (96; 16 wks old) were randomly distributed into 4 treatment groups with 4 replicates and 6 birds each ($4 \times 4 \times 6$) for a period of 24 weeks. The basal diet (T_1) contained 0 mg Se/kg diet and the three experimental diets were supplemented with 0.2, 0.4 and 0.6 mg Se/kg diet (T_2 , T_3 and T_4 respectively). Age at sexual maturity, egg number and egg weight differed significantly in 0.4 mg Se/kg treated group (T_3) than the other dietary treatment groups (T_2 or T_4). Humoral (29th weeks) and *in vivo* cell mediated immune response (30th weeks) were significantly improved in 0.4 or 0.6 mg Se treated group. Se supplemented groups, i.e. T_3 and T_4 decreased significantly in serum cholesterol and uric acid concentration whereas significant increase were recorded in total protein, albumin and alkaline phosphatase (ALP). No significant differences were observed in serum aspartate amino transferase (AST) and alanine amino transferase (ALT) concentration among the experimental groups. Egg quality traits, viz. shape, albumin and yolk index, shell thickness did not differ significantly among Se supplemented groups, whereas albumin and yolk weight and Haugh unit score were significantly higher in 0.4 mg Se/kg treated (T_3) group. Thus, this study demonstrates that that dietary supplementation of 0.4 mg/Se kg diet has a beneficial effect on laying performance, immune responses, serum biochemical and egg quality traits in laying turkeys.

Key words: Egg quality trait, Immune response, Production performance, Selenium, Turkey

Selenium (Se) is a structural component of the glutathione peroxidase enzyme system and it acts as an antioxidant that protects cellular components from oxidative stress (Kurtas 2016). Se is also recognized as having anti-carcinogenic and antiviral properties and probably has an important role in the functioning of the immune system (Surai 2002). Supplementing poultry diets with Se at levels higher than basal requirements (0.2 mg/kg diet) has been shown to enhance primary immune responses by increasing numbers of antibody forming cells and the antibody response to inoculated sheep red blood cells (Biswas *et al.* 2006). Se supplementation of the diet of chickens infected with coccidia produced a change in the numbers of peripheral blood leucocytes, suggesting that Se probably improves resistance to infection through improved immune responses (Pappas *et al.* 2005). Higher doses of Se, such as 0.2 and 0.3 mg, improved performance of broiler chickens but a lower dose 0.1 mg Se, did not have any positive effect (Bonomi 2001). Research activities of different researcher exploring that the effect of selenium on growth and immune response in chickens (Khanal and Knight 2010, Khare and Baghel 2011) and quails (Biswas *et al.* 2006, Surai *et al.* 2006) but, research on the use of selenium in laying turkey

is very limited. Due to a lack of information on the role of organic Se in laying turkey nutrition, its importance as a micronutrient has been described on the basis of work carried out with the chicken.

Taking into consideration all the stated facts, the aim of the present study was to examine the effects that organic Se supplementation to laying turkey's diet in various concentrations (0.2, 0.4 and 0.6 mg/kg) on production performance, immune response, serum biochemical parameters and egg quality traits.

MATERIALS AND METHODS

Experimental birds: The present study was carried out at Avian Nutrition and Feed Technology Division, Central Avian Research Institute (CARI), Izatnagar, India. Seventy-two (72) female turkeys at 16 weeks of age were housed and distributed randomly in to 12 groups (4 treatments $\times$ 3 replicates) of 6 birds in each replicates following completely randomized design (Snedecor and Cochran 1985). All the birds were kept in individual cages in a stair-step two-tier system throughout the experimental period. Birds were allowed to eat and drink *ad lib*. The trial was terminated at 40 weeks of age. The experiment followed the guidelines of "Institutional Animal Ethics Committee (IAEC, CARI, Izatnagar)".

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Experimental diets: A maize-soybean meal based basal diet (195.90 g/kg crude protein and 11.97 MJ ME/kg of diet) was supplemented with 0, 0.2, 0.4 and 0.6 mg Se/kg diet to have four experimental diets (T₁, T₂, T₃ and T₄, respectively). Seleno-methionine (Se-M) was used as a selenium source. The ingredient and chemical composition of basal diet used in this study are presented in Table 1. The dietary supply of nutrients was adequate to meet or exceed the requirement of National Research Council (NRC 1994).

Laying performance: Age at sexual maturity or the age at first lay was measured when the hen started laying eggs. Egg production from each treatment was recorded for a period of 4 wks (37th to 40th). Labeling on each egg was done every day. Egg weight was measured on an analytic balance to the nearest 10 mg.

Table 1. Composition of the basal diet where dietary supplementation of Se were added

Ingredient	Composition (g/kg)	Estimated composition	
Maize	580	ME (MJ/kg)	11.97
Soybean	230	Crude protein (g/kg)	195.9
DORB ¹	103	Total phosphorus (g/kg)	3.5
Rice polish	50	Total calcium (g/kg)	22.5
Rapeseed meal	50	Lysine (g/kg)	11.5
Fishmeal	60	Methionine (g/kg)	4.1
Limestone	8		
Oyster shell	20		
Marble chips	2.4		
Dicalcium phosphate	10		
Sodium chloride	1		
	<i>Amount (g/kg)</i>		
DL-Methionine	0.6		
L Lysine Hydrochloride	1		
Choline chloride	0.5		
Lactose	2		
Toxin Binder	0.5		
Fumaric acid	1		
Sodium acetate	1		
Coccidiostat	0.5		
Mineral mixture (Premix 1)*	1		
Vitamin A, B2, D3, K (Premix 2)*	1.5		
Vitamin B complex (Premix 3)*	0.15		

¹DORB, de-oiled rice bran. *Premix (1, 2 and 3) supplied the following nutrient/kg of complete feed. Premix 1: Each g of mineral mixture contained: 200 mg of FeSO₄·7H₂O, 20 mg of CuSO₄·5H₂O, 200 mg of MnSO₄·H₂O, 150 mg of ZnSO₄·7H₂O, 1 mg of KI. Premix 2: Each g of vitamin A, B2, D3, K (Spectromix, Ranboxy) provided vitamin A (retinol) 540 mg, vitamin B2 (riboflavin) 50 mg, vitamin D3 (cholecalciferol) 400 mg, vitamin K (menadione) 10 mg. Premix 3: Each g of B-complex provided: vitamin B1 (thiamin) 2 mg, folic acid 10 mg, pyridoxine HCl 4 mg, cyanocobalamin 10 µg, nicotinamide 12 mg.

Immune response: To investigate the effect on the humoral immune response, three birds were selected from each of the replicated groups (that is, 9 birds/dietary treatment) at 28 wk of age and were inoculated intravenously with 1.0 ml of a 1% suspension of sheep red blood cell (SRBC). Blood samples were obtained from the jugular vein from all SRBC injected birds at 0 and 6 d post-inoculation. Samples were incubated at 37°C for 1 h to aid clotting and retraction then centrifuged at 15000 g for 5 min for collection of sera. Microtitre plates (U-bottomed) were rinsed with phosphate-buffered saline (PBS; pH 7.6) then dried before the haem-agglutination antibody (HA) titre was estimated by a micro-haemagglutination method (Siegel and Gross 1980) using twofold serial dilutions of sera.

The foot web index (FWI) was used as an index of the cell-mediated immune response. During week 29, three separate birds from each replicate of the Se treatments were selected and 1.0 ml PHA-P mitogen (1 mg/ml PBS) was injected intra-dermally into the left foot web. Sterile PBS (1.0 ml) was injected into the right foot web to serve as a control. A micrometer was used to measure changes in the thickness of both foot webs. Measurements were made at 0 and 24 h after the injection, as described by Cheng and Lamont (1988). Foot web swelling was calculated by subtracting skin thickness at 24 h post-injection from that at 0 h pre-injection.

Serum biochemical parameters: Blood samples from 10 birds / treatment were randomly collected in 40 weeks into sterile glass tubes without anticoagulant. Test tubes containing the blood were kept in slanted position at room temperature for half an hour to facilitate separation of serum. Serum was separated by centrifugation at 1512 g for 10 min and decanted into plastic vials, and then stored at –20°C for estimation of serum enzymes, i.e. alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), kidney function test (creatinine and uric acid), total cholesterol, total protein and albumin. All blood biochemical parameters were analysed with commercial available biochemical kits (Span Diagnostics, India).

Egg quality traits: Two hundred forty eggs (20 eggs × 4 treatments × 3 replicates = 240 eggs) were collected for this experiment. The egg quality traits such as shape index, shell thickness, albumin index and yolk index and Haugh unit scores were estimated according to standard procedure. After measuring the external characters, the eggs were broken open on the egg breaking stand and the contents were poured into a petri-dish for measuring their qualities. The height of the thick albumin and yolk were measured using Ames tripod stand micrometer. The length and width of the thick white and yolk were measured using a vernier caliper and the mean diameters were calculated. Thereafter, yolk was gently separated from the albumin, adherent albumin was removed by rolling the yolks over a filter paper and the yolk weight was recorded. The egg shell was washed to remove the adhering albumin and their thickness was

measured with an Ames micrometer (Ames 25 M5). Yolk index was calculated as ratio of yolk height to yolk width while Haugh unit (Haugh 1937) was determined based on albumen height and egg weight.

Statistical analysis: The data obtained from the experiment were subjected to one way analysis of variance for a completely randomized design, using the SPSS software-20 by the methods of Snedecor and Cochran (1985). The significant mean differences were tested as described by Duncan (1955) with significance level defined at $P < 0.05$.

RESULTS AND DISCUSSION

Laying performance: Production performances in terms of age at sexual maturity, egg number and egg weight were significantly different ($P < 0.05$) in 0.4 mg Se/kg diet supplemented group (T_3) comparison to the T_1 , T_2 or T_4 group (Table 2). The results indicate that production performance was significantly improved by diets containing 0.4 mg Se/kg diet. Though, there was no concrete publications on the effects of dietary organic Se on the production performance of laying turkey, but there has been considerable research on other avian species, such as chicken (Gjorgovska *et al.* 2012, Zuberbuehler *et al.* 2002) and quails (Chitra *et al.* 2013). Some of these reports suggest that feeding diets containing 0.25 mg (Pappas *et al.* 2005) or 0.56 mg (Swain and Johri 2000) Se produced improvements in production performance in the chicken, whereas others have reported no improvement (Biswas *et al.* 2006). The results of the present findings are in agreement with the findings of Correia *et al.* (2000), who reported that supplementation of selenium in layer diets significantly influenced egg production in quails. Scheideler *et al.* (2010) also reported that level of dietary Se (0.75 ppm/kg) did significantly affect the egg production. The NRC (1994) reported that high levels of dietary Se (5–20 mg/kg diet) can be toxic; however, in this trial, significant benefits were observed by increasing dietary Se well above the basal diet level of 0.2 ppm. Moreover, the US Food and Drug Administration (FDA) limits commercial feed production practices to a maximum of 0.3 ppm of Se added to complete feeds. Increasing Se level up to 0.4 mg

significantly increased both egg production and egg weight compared with those in hens fed the control diet.

Selenium is involved in many biochemical and physiological processes in human and animal organisms, including those related to reproduction. Particularly relevant to age at sexual maturity is the antioxidant enzyme glutathione peroxidase (GSH-Px), a selenium dependent enzyme that serves to protect cellular membranes and organelles from peroxidative damages. Glutathione peroxidase assists in the maintenance of ovary and oviductal function in female as well as progesterone biosynthesis (Gallo *et al.* 2003). In the present study, dietary addition of selenium increased female sexual activity manifested by significantly improved the age at sexual maturity (ASM), as well as increased egg production in turkey birds.

Immune response: The haem-agglutination antibody titre to SRBC, an index humoral immunity, was increased ($P < 0.05$) by 0.4 mg Se/kg concentration of dietary Se (Table 2). The mitogenic response to PHA-P measured as the FWI, an index of cell-mediated immunity, also was significantly increased ($P < 0.05$) by Se supplementation at 0.4 mg Se/kg diet (Table 2). The intermediate concentration of Se (T_3), elicited a greater response than that in the other two Se supplemented (T_2 and T_4) and control group (T_1). The highest antibody titres to the non-replicating antigen, sheep red blood cell (SRBC), were obtained with a supplement of 0.4 mg Se/kg diet (T_3). Optimal responses in chickens were achieved with only 0.1 mg Se/kg diet (Pappas *et al.* 2005). This suggests that effective dietary Se contents for enhancing immunity in turkey are about 10 to 20 times greater than those required for chickens. Our results confirm those of other workers (Panda and Rao 1994) who reported improved immune function in chicken fed on diets containing increased levels of Se. The FWI was also influenced by supplementing dietary Se. This result suggests that Se could play a crucial role in the immune responses in laying turkey. Similar trends have been reported for the chicken by Aharon *et al.* (1998) and Surai (2002); however, our results are not consistent with the findings of other workers (Raza *et al.* 1997). How the Se enhances immune responses till date it is not clear; one probability is through greater glutathione peroxidase activity which may protect

Table 2. Effect of dietary supplementation of organic selenium (Se) on production performance and immune response in laying turkeys

Parameter	T1	T2	T3	T4	SEM	P value
<i>Production performance</i>						
Body weight at 16 wks (g)	2450	2455	2460	2445	25.25	NS
Body weight at 40 wks (g)	3525	3545	3610	3570	30.14	NS
Body weight gain (40-16 wks)	1075	1090	1150	1125	10.20	NS
Age at sexual maturity (d)	205 ^b	198 ^b	178 ^a	183 ^{ab}	—	<0.05
Egg production (%)	67.20 ^a	69.25 ^a	83.55 ^b	71.30 ^{ab}	2.64	<0.05
Egg weight (g)	66.39 ^a	68.35 ^{ab}	74.61 ^b	68.85 ^{ab}	1.75	<0.05
<i>Immune response</i>						
¹ HA titre (log ₂)	3.17 ^a	4.05 ^{ab}	5.99 ^b	4.89 ^{ab}	0.20	<0.05
Foot web index (mm ²) [#]	0.27 ^a	0.28 ^a	0.39 ^b	0.36 ^b	0.03	<0.05

Table 3. Effect of dietary supplementation of organic selenium on serum biochemical parameters in laying turkeys

Parameter	T1	T2	T3	T4	SEM	P value
Protein (g/dl)	4.02 ^a	4.53 ^{ab}	5.15 ^b	4.87 ^{ab}	0.87	<0.05
Albumin (g/dl)	3.33 ^a	3.68 ^{ab}	4.09 ^b	3.77 ^{ab}	0.02	<0.05
ALT (IU/L)	27.65	26.73	26.82	27.33	2.06	NS
AST (IU/L)	19.90	20.02	20.43	20.17	2.12	NS
ALP (IU/L)	80.55 ^a	83.67 ^{ab}	87.89 ^b	84.52 ^{ab}	7.57	<0.05
Cholesterol (mg/dl)	138.25 ^b	137.47 ^b	131.75 ^a	134.44 ^{ab}	6.33	<0.05
Uric acid (mg/dl)	4.26 ^b	4.18 ^{ab}	3.55 ^a	3.82 ^a	0.11	<0.05
Creatinine (mg/dl)	0.15	0.14	0.13	0.14	0.02	NS

*T1, control; T₂, 0.2 mg Se/kg feed; T₃, 0.4 mg Se/kg feed; T₄, 0.6 mg Se/kg feed. Mean values bearing the same superscripts in a row do not differ significantly (P<0.05). NS, non-significant.

the membranes and organelles of the lymphocytes from the detrimental effects of pro-oxidants. Likewise, Se may also be involved in modifying the metabolism of arachidonic acid to prostaglandin precursors or related compounds, enhancing immune responses by reducing the endogenous production of prostaglandin (Das 2018).

Blood biochemical parameters: Results presented in Table 3 showed that serum total protein, albumin and ALP concentrations significantly (P<0.05) increased for groups fed 0.4 mg Se (T₃) compared with the control (T1). However, significant decreased (P<0.05) were observed in serum cholesterol and uric acid concentration in T₃ supplemented groups compared with control (Table 3). While, Se supplementation had no significant effect on serum ALT, AST and creatinine concentration. The results of present study are supported with the finding of Mohapatra *et al.* (2014) who reported that dietary Se supplementation increased the concentration of protein and albumin in laying chickens, whereas, Bunglavan *et al.* (2014) reported that selenium supplementation as did not affect serum total protein and albumin concentration. In the same way, Yang *et al.* (2012) reported that supplemented with 0.3 ppm Se for 42 days didn't affect serum albumin and protein level compared to control group in broiler chickens. Moreover, in the present study, dietary Se did not affect the ALT, AST and creatinine level in laying birds. This is supported by the finding of Peric *et al.* (2009) who found substantial reduction in both ALT enzymes activity in chicken fed dietary Se. The same results were obtained by Biswas *et al.* (2011), who found a decrease in ALT and AST activities in

chicks supplemented with 0.5 mg and 1 mg/kg of Se in their diet. However, Okunlola *et al.* (2015) and Gruzauskas *et al.* (2013) indicated that serum ALT and AST increased with no differences in total protein, albumin, uric acid and creatinine in poultry supplemented with 0.5 mg of selenium. The blood enzymes ALT, AST, ALP are used as indicators of liver and kidney oxidative damage, the serum reduction of the enzymes and creatinine levels means increasing protection against oxidative damage through an improved redox status. Animal antioxidant system is significantly influenced by animal nutrition, and dietary Se supplementation is necessary to up-regulate the body's glutathione pool and its Se-containing antioxidant enzymes (Jiang *et al.* 2009). Accordingly, dietary Se can improve antioxidant system and increase GSHPx activity in all tissues of chickens (Zhang *et al.* 2014). However, Glutathione peroxidase and superoxide dismutase are the main enzymatic antioxidants against toxic oxygen reduction metabolites (Del Maestro 1991). Studies on laying turkeys, concerning the relationship between serum biochemical parameters and Se supplementation have not been published so far and, to our knowledge, the present report is the first one on this subject.

Egg quality traits: The result of the present study showed that albumin weight, yolk weight and Haugh unit score were significantly higher (P<0.05) in T₃ group (0.4 mg Se) compared with control group (T1). However, no significant differences (P>0.05) were observed in shell thickness, albumin index and yolk index among the control and Se treated groups (Table 4). This result exhibit that,

Table 4. Effect of dietary supplementation of organic selenium (Se) on egg quality traits in laying turkeys

Parameter	T1	T2	T3	T4	SEM	P-value
Albumen weight (g)	39.92 ^a	40.84 ^{ab}	42.32 ^b	40.91 ^{ab}	0.75	<0.05
Yolk weight (g)	17.12 ^a	18.85 ^{ab}	20.25 ^b	19.10 ^{ab}	0.12	<0.05
Shape index (%)	73.20	73.55	74.50	73.80	1.10	NS
Yolk index (%)	38.16	39.25	39.53	39.38	0.48	NS
Albumen index (%)	7.04	7.33	7.62	7.45	0.08	NS
Haugh unit	78.05 ^a	81.28 ^{ab}	83.35 ^b	81.30 ^{ab}	3.56	<0.05
Shell thickness (mm)	0.317	0.323	0.311	0.327	0.003	NS

*T1, control; T₂, 0.2 mg Se/kg feed; T₃, 0.4 mg Se/kg feed; T₄, 0.6 mg Se/kg feed. Mean values bearing the same superscripts in a row do not differ significantly (P<0.05). NS, non-significant.

independently of the dietary supplementation with trace minerals, there were no changes in egg quality. In the present study, egg quality traits, i.e. albumin weight, yolk weight and Haugh unit (HU) score were differed significantly ($P < 0.05$) when 0.4 mg Se/kg diet were supplemented. The results of the present study are in agreement with the study of Fernandez *et al.* (2011) with layers in which they showed that birds fed diets containing Se bound to an organic molecule presented higher HU. On the contrary, Sechinato *et al.* (2006) observed that the dietary inclusion of Se (trace minerals) did not have any influence on HU. Attia *et al.* (2010) reported that the Se had no significant effect on any traits of egg quality and they also concluded that Se content of the basal diet was adequate to support egg production of good quality. However, Payne *et al.* (2005) and Gajcevic *et al.* (2009) indicated that eggs produced by hens fed a diet with organic Se had higher HU values than eggs of hens fed the NRC recommended level of Se. Sahin *et al.* (2003) and Chitra *et al.* (2013) also reported that different levels of dietary selenium supplementation did not show any significant difference in egg quality traits, i.e. shape index, albumen index, yolk index, etc.

The results of the present study allow concluding that supplementing dietary organic Se @ 0.4 mg/kg diet, indices of the production performance, immune system indicate a positive effect on immune responses, serum biochemical parameters and some egg quality traits in laying turkey; however, further study of this problem is required.

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