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Research Article
Effects of Dietary Selenium Sources on Metabolic, Enzymatic and Immunoglobulin Serum Profiles in Growing Rabbits

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Abstract
Objective: This study was conducted to compare different forms of selenium (Se) and its effects on metabolic, enzymatic and immunoglobulin serum profiles in growing rabbits. **Materials and Methods:** Seventy-five mixed sex growing APRI rabbits aged 5 weeks and weighing 662.2±9.07 g was assigned randomly into 5 experimental groups to evaluate the effect of dietary addition of Se in organic and inorganic forms with/or without vitamin E. The five experimental groups were as follows: The first group was fed *ad libitum* a commercial pelleted diet and kept untreated and served as a control, while the other groups (second, third, fourth and fifth) were fed the same diet but addition of 0.3 mg Se-yeast/kg diet as organic Se form (OSe), 0.3 mg OSe/kg diet plus 200 mg vitamin E/kg, 0.3 mg Se/kg diet as inorganic Se form (ISe) and 0.3 mg ISe/kg diet as Na_2SeO_3 plus 200 mg vitamin E/kg, respectively. The study was lasted for 8 weeks during the growing period, from weaning age (at 5 weeks) to marketing age (at 13 weeks). **Results:** The result revealed that addition different forms of Se with/or without vitamin. E caused significant increases in total protein (TP), albumin (Alb) and globulin (Glb) but these increases were still within normal range. No significant differences ($p>0.05$) were observed in the metabolic profile among treated groups. Insignificant differences were found among all experimental groups including the control one for both liver enzymes (ALT and AST). Also, results showed that there was non-significant difference between treated groups and the control group in serum creatinine (CR) and Urea-N. Different dietary Se source caused to increase values ($p\leq0.05$) of serum IgG and IgM concentrations than that of the control group. **Conclusion:** It can be concluded that Se addition in either organic or inorganic form, in the diet of growing rabbit, exerted some benefits on the some metabolic and immunologic profiles which in turn may ameliorate weaned stress.

Key words: Blood parameters, enzymes, immunoglobulin, inorganic selenium, organic selenium, rabbits

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Weaning is a stage in which all young animals suffers from a lot of stress, caused by the separation from the mother, the transfer into different environment and change in the nutrition. This stress can't be easily tolerated by a young rabbits, resulting in increased concentration of cortisol in systemic circulation and hence immune-suppression and increased probability diseases exposure[1,2]. When animals exposed to stress, selenium (Se) is used as antioxidant micro-mineral to decrease the adverse effects of free radicals. In this regard, Se is required for a lot of biochemical functions in both humans and animals[3]; these include antioxidant and immune function[4].

Thus, Se is one of the essential trace mineral[5], serving as an essential co-factor in the antioxidant enzyme glutathione peroxidase (GSH-Px), as well as catalase (CAT) and superoxide dismutase (SOD) in the body, to counteract the damaging effects of reactive oxygen species (ROS) and numerous peroxides in rabbits[6], that are known to increase destructive activity in tissues of rabbits during stress[7].

The source of Se is important to provide enough protection. The dietary micro-mineral Se can be supplied for rabbits in organic (OSe) or inorganic (ISe) form, the latter being traditionally more used. In spite of their requirements are low but if they are not met normal physiological and production status, the antioxidant system may be compromised, causing serious consequences for the animal's metabolism[8]. On the other hand, form of Se either OSe or ISe controls interactions between dietary Se and GSH-Px activity[9].

Vitamin E had been reported to be an excellent biological antioxidant that protects cells and tissues from free radicals which induced lipoperoxidative damage[10]. Both Se and vitamin E are essential and highly efficient antioxidants which help to protect rabbits against oxidation of lipid and protein[11]. The combination of vitamin E and selenium at levels above recommended as nutritional requirements could improve humoral immunity[12].

Serum biochemical profiles provide reliable information on the health status of rabbits[13]. They also reflect the animal responsiveness to its external and internal environments[14].

Very little information is available on using Se in different source for evaluating some physiological indices of rabbits. Also, no mammalian studies showed whether or not it could be used beneficially as immunostimulant than other source of micro-minerals especially during fattening period as ameliorate weaned stress. Therefore, the objectives of this experiment were to compare inorganic with organic sources of Se with/or without Vitamin E and its effects on metabolic, enzymatic and immunoglobulin serum profiles in growing rabbits. Also, the use at studied Se source was planned to discover any toxicity/effects on liver and kidney functions

MATERIALS AND METHODS

Diets and animals: Seventy-five weaned Animal Production Research Institute (APRI) line rabbits (Egyptian line selected for litter weight at weaning according to Abou Khadiga *et al.*[15] were divided randomly into 5 experimental groups of 15 rabbits each of 5 weeks of age with an average live body weight of 662.2±9.07 g.

The five experimental groups were as follows: The first group was fed *ad libitum* a commercial pelleted diet according to NRC[5] recommendations as shown in Table 1 and kept untreated and served as a control, while the other groups (second; third, fourth and fifth) were fed the same diet but addition with 0.3 mg Se-yeast/kg diet as organic Se form (OSe), 0.3 mg Ose/kg diet plus 200 mg vitamin E/kg, 0.3 mg Se/kg diet as inorganic Se form (ISe) (Na_2SeO_3, Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and 0.3 mg ISE/kg diet as Na_2SeO_3 plus 200 mg vitamin E/kg, respectively.

All the experimental animals were healthy and clinically free from internal and external parasites and were kept under the same management and hygienic conditions.

Table 1: Ingredient and chemical composition of experimental diet

Items	Percentage as fed
Ingredients	
Clover hay	40.50
Wheat bran	25.00
Yellow corn	14.00
Soybean meal (44%)	11.00
Molasses	3.00
Vinasse	3.00
Bone meal	1.75
Calcium carbonate	0.70
Sodium chloride	0.55
Vitamins and mineral premix[1]	0.35
DL-methionine	0.15
Calculated chemical composition[2]	
Ash	7.80
Crude protein	18.00
Ether extract	3.00
Crude fiber	14.00
Digestible energy, kcal/kg[3]	2720.00

[1]Vitamins and minerals premix per kilogram diet contains: Vit. A: 6000.0 IU, Vit. D: 900.0 IU, Vit. E: 40.0 mg, Vit. K_3: 2.0 mg, Vit. B_1: 2.0 mg, Vit. B_2: 4.0 mg, Vit. B_6: 2.0 mg. Vit. B_{12}: 10.0 mcg, Nicotinic acid: 50.0 mg, Biotin: 50.0 mcg, Folic acid: 10.0 mg, Choline chloride: 250.0 mg, Zinc: 50.0 mg, Manganese: 85.0 mg, Iron: 50.0 mg, Copper: 5.0 mg, Iodine: 0.2 mg, Selenium: 0.1 mg, Cobalt: 0.1 mg. [2]According to NRC[5] for rabbits. [3]Digestible energy (kcal/kg DM) = 4253-32.6 CF (% DM)-114.4 Ash (% DM). According to Fekete and Gippert[16]

Experimental procedure: Blood samples of growing rabbits were collected during slaughter of 4 rabbits within each experimental group on 13 weeks. The samples were collected into dry clean centrifuge tubes, the serum was separated by centrifugation at 3000 r.p.m. for 20 min and kept in a deep freezer at -20°C until biochemical analysis. Total protein (TP, g/dL), albumin (Alb, g/dL), creatinine (CR, mg/dL) and urea-Nitrogen (Urea-N, mg/dL) levels were estimated according to Doumas *et al.*[17]. Globulin (Glb, g/dL) concentration was obtained by subtracting the values of Alb from the corresponding values of TP.

Serum samples were analyzed for hepatic parameters i.e. Aspartate aminotransferase (AST, U/L) and Alanine transaminase (ALT, U/L) according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFFC)[18]. The levels of immunoglobulin G (IgG, μg mL^{-1}) and immunoglobulin M (IgM, μg mL^{-1}) were also measured in the serum by an ELISA reader (SIRIO S, Italy) using ELISA commercial test kits (Cusabio, rat immunoglobulin M, ELISA kit, catalogue number CSB-E07978r; Cusabio, rat immunoglobulin G, ELISA kit, catalogue number CSB-E079981r, China). All samples were run in duplicate and assayed by the same investigator, who was blind to the experimental situation.

Housing: The study was carried out at the Rabbit Farm of Sakha Experimental Station, Animal Production Research Institute, Agricultural Research Center, Egypt, during breeding season from January to March, 2017. Rabbits were housed separately in individual cages (35×35×60 cm) of conventional universal galvanized wire batteries. All cages were equipped with feeding hoppers, which were made of galvanized steel sheets and nipples for automatic drinking. The batteries were located in a well-ventilated building. Averages of ambient temperature, relative humidity and temperature humidity index inside building were 22.6±0.6°C, 71.0±3.4% and 21.3, respectively, which indicate absence of heat stress.

Statistical analysis: Data of the first and the second experiment were statistically analyzed by one-way ANOVA test according to SPSS[19] computer program using the following fixed model:

$$Y_{ij} = \mu+\alpha_i+e_{ij}$$

Where

Y_{ij} = Observation of the jth rabbit in the treatment I

μ = Overall mean, common element to all observations

α_i = Effect of the treatments (i = 1, 2, 3, 4 and 5)

e_{ij} = Random error component assumed to be normally distributed

Data presented as percentages were transformed to the corresponding arcsine values (Ewens and Grant[20]) before being statistically analyzed. All data were presented as least squares means. For all data analyses, each animal was considered as an experimental unit.

RESULTS AND DISCUSSION

Metabolic profile: Inclusion of different forms of Se with/or without vitamin E caused significant increases in TP, Alb and Glb but these increases were still within normal range as indicated by the non-sign of toxicity (Table, 2). No significant differences ($p>0.05$) were observed in the metabolic profile among treated groups. In conclusion, dietary addition of OSe or ISe led to increases the concentrations of TP in blood serum of rabbits. Similar findings have been observed by El-Mallah *et al.*[21] and Mobaraki *et al.*[22], they showed that addition of vitamin E and/or organic Sel-Plex to diets improves serum TP concentration compared to control group. However, Speranda *et al.*[23], reported that supplementation of a diet with Se had no significant positive effect on TP.

Concerning the effects of Se on TP and its fractions (Alb and Glb) in the present study, can be attributed to the increase in protein synthesis, which resulted in highly live BW and weight gain, as compared to the control group. These improvements may be partially due to the increasing animal resistance to any physiological or physical stress.

Furthermore, serum TP level is a general indication of immune status[24]. Also, increased Glb concentration with Se inclusion, as observed in the present study, may be an indication of increased immunity in the rabbits. Hence the liver will be able to synthesize enough Glb for immunologic action as mentioned by Sunmonu and Oloyede[25]. El-Kholy *et al.*[26] stated that high Glb level signify better disease resistance and immune response. These results are in good agreement with the data in Table 4 which indicates higher percentage of serum immunoglobulin in treated rabbits than the control ones.

Results in Table 2 also show the effect of Se addition on creatinine (CR) and urea-Nitrogen (Urea-N) levels as an indicator of kidney function. The data indicated that there was non-significant difference between treated groups and the control group in serum CR and Urea-N. In both treated and untreated rabbits, levels of CR and Urea-N concentrations are within the normal range of rabbits[27]. In other words, kidney

Table 2: Effect of selenium source and vitamin E addition on metabolic and enzymatic profiles of growing APRI rabbits during fattening period

	Treatments					
Parameters	Control	Organic Se (OSe)	OSe+Vit. E	Inorganic Se (ISe)	ISe+Vit. E	Sig.
Total protein (TP, g/dL)	04.09±0.21^{c}	05.87±0.27^{a}	05.44±0.31^{ab}	05.85±0.29^{a}	05.67±0.20^{ab}	**
Albumin (Alb, g/dL)	02.12±0.10^{b}	03.54±0.11^{a}	03.14±0.09^{a}	03.42±0.10^{a}	03.35±0.10^{a}	*
Globulin (Glb, g/dL)	01.97±0.08^{c}	02.33±0.07^{a}	02.30±0.07^{s}	02.43±0.09^{b}	02.32±0.08^{ab}	*
Aspartate aminotransferase (U/L)	28.21±0.69	27.90±0.70	27.55±0.77	27.17±0.65	28.11±0.71	NS
Alanine aminotransferase (U/L)	14.44±0.77	15.01±0.61	14.61±0.65	14.70±0.57	14.33±0.63	NS
Urea-Nitrogen (mg/dL)	14.73±0.33	14.55±0.99	15.14±1.08	14.00±0.24	14.44±0.34	NS
Creatinine (mg/dL)	00.87±0.07	00.80±0.06	00.82±0.09	00.85±0.07	00.82±0.06	NS

Sig.: Significance, **Significant at 1% level of probability, *Significant at 5% level of probability, NS: Non-significant. Means within the same row bearing different letter superscripts (a-c) are significantly different ($p \leq 0.05$)

Table 3: Effect of selenium source and vitamin E addition on lipid profile of growing APRI rabbits during fattening period.

	Treatments					
Parameters	Control	Organic Se (OSe)	OSe+Vit. E	Inorganic Se (ISe)	ISe+Vit.E	Sig.
Total cholesterol (mg/dL)	67.50±1.21^{a}	43.33±1.25^{b}	44.67±1.28^{b}	40.33±1.21^{b}	41.00±7.21^{b}	*
Triglyceride (mg/dL)	101.20±4.77	101.06±2.95	98.33±2.90	99.00±2.25	100.00±3.57	NS
High density lipoprotein (HDL, mg/dL)	14.02±0.49	13.22±0.58	14.00±0.77	14.06±0.54	13.15±0.92	NS
Low density lipoprotein (LDL, mg/dL)	36.15±0.66^{a}	15.17±0.56^{b}	15.70±0.76^{b}	15.64±0.36^{b}	15.37±0.55^{b}	*

Sig.: Significance, *Significant at 5% level of probability, NS: Non-significant. Means within the same row bearing different letter superscripts (a-c) are significantly different ($p \leq 0.05$)

function seemed to be not affected by any source of Se addition after weaned rabbits till marketing age at 13 weeks.

Enzymatic profile: Although Se is rather of low acute toxicity, in some cases excessive dosages can do harm in various organs and systems[28,29]. For this reason it is necessary to monitor biomarkers of general toxicity. As can be seen in Table 2, insignificant differences were found among all experimental groups including the control one for both liver enzymes (ALT and AST). In contrary to these results, Kanacki and Ruzic[30] showed that serum concentrations of AST and ALT were lower in broilers received OSe than those received ISe. In the present study, values of AST and ALT were within the normal range and indicated that the animals were generally in a good nutritional status and their livers were in a normal health condition.

Serum AST and ALT (Table 2) were determined as an indicator for enzymatic activity related to the rate of protein metabolism and liver function. This result pointed out that growing rabbits could tolerate the addition of Se as either organic or inorganic without any deleterious effects on liver. However, it should be mentioned that toxic Se doses are more than 10 times higher than the physiological requirement[8].

Serum lipid profile: The effect of diets containing different Se source on lipids profile is shown in Table 3. The rabbits from the groups receiving Se either in organic or inorganic form had lower in total cholesterol and LDL than those from the control group. This finding agrees with the results of a study conducted by Amer *et al.*[31]. On the other hand, these results are different from those reported by Ebeid *et al.*[32] who showed that no significant change was noted in terms of the total cholesterol and triglycerides in the Se groups compared to control group.

Also, results indicated that neither Se source nor Vitamin E had any effects on values of triglycerides and HDL. El-Mallah *et al.*[21] reported that addition of vitamin E and/or Se to layer diets did not show significant differences in the TG.

The addition of antioxidants like Se decrease peroxidation of lipid and the severity of atherosclerosis in rabbits[33,34]. In rats lizuka *et al.*[35] cited that Se suppressed free fatty acids, triacylglycerol and total cholesterol concentrations in serum.

Also, several animal studies have also shown that vitamin E supplementation affects lipoprotein metabolism by reducing serum total cholesterol[36]. Qureshi *et al.*[37] demonstrated that vitamin E, particularly the tocotrienol can suppress the synthesis of cholesterol in the liver. This might be an indicator that lipid peroxidation was reduced by addition of either OSe or ISe via enhancing antioxidative action.

Immunoglobulins concentration: Table 4 shows that different dietary Se source increased ($p \leq 0.05$) serum IgG, IgM and IgA concentrations than that of the control group. However, there were insignificant differences in these immunoglobulin concentrations among all treated groups.

In this study, increased in immunoglobulin concentrations with Se addition could be an indication of Se either in organic or inorganic form induces earlier maturation of the humoral

Table 4: Effect of selenium source and vitamin E addition on some humoral immune responses of growing APRI rabbits at different periods during fattening period

	Treatments					
Items	Control	Organic Se (OSe)	OSe+Vit. E	Inorganic Se (ISe)	ISe+Vit.E	Sig
IgG (μg mL^{-1})	4.77±0.22^{c}	6.15±0.23^{ab}	6.34±0.22^{a}	6.15±0.25^{ab}	6.13±0.23^{ab}	**
IgM (μg mL^{-1})	2.02±0.14^{c}	2.89±0.13^{a}	2.66±0.14^{b}	2.80±0.12^{a}	2.85±0.13^{a}	*

Sig.: Significance, **Significant at 1% level of probability, *Significant at 5% level of probability, Means within the same row bearing different letter superscripts (a-c) are significantly different ($p \leq 0.05$)

immune responses. Also, a higher serum IgG level in rabbits treated with Se indicate that Se are effective in improving humoral immunity. Since, Meissonnier *et al*.[38] demonstrated that determinations of the concentrations of serum immunoglobulin such as IgA, IgG and IgM are the most common methods of testing humoral immune responses.

Surai[39] and Rayman[40] have concluded that Se involve in the active site of the enzyme glutathione peroxidase (GSH-Px) in blood, liver and edible tissues which might be connected with enhancing the immune response in mammals. Also, a very important metabolic role of selenium in animals is the presence in the active site of the selenoenzyme (GSH-PX). This enzyme, together with superoxide dismutase and catalase, protects cell against damage caused by free radicals and lipoperoxides[41].

In vitamin E treated groups, Vitamin E has been implicated in stimulation of serum antibody synthesis, particularly IgG antibodies[42]. Together with Se, vitamin E is an important component of the antioxidant defense system which helps to protect the polyunsaturated fatty acids in cell membranes from peroxidative damage[11].

The increasing in the digestion of some nutrients in the treated groups enhances the availability of circulating amino acids for immunoglobulin synthesis by B lymphocytes.

Immunoglobulins are produced in B-cells in bone marrow and the biological characteristics of IgG, IgA and IgM in poultry are similar to those of immunoglobulins in mammals. Since IgG is present at the highest concentration and is responsible for immunologic competence, the immunopotency of serum IgG can be used as an index of humoral immunity. Another possible explanation is that the dietary Se made weaning rabbits more immunostimulant after weaned period.

CONCLUSION

It can be concluded that Se addition in either organic or inorganic form in the diet of growing rabbit exerted some benefits on the some metabolic and immunologic profiles which in turn may be ameliorate weaned stress. Although, non-significant results were observed for the examined parameters, further studies are needed to understand the effects of live yeast and to clarify the effect on the immune response in growing rabbits.

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