DOSE-RESPONSE OF VITAMIN E AND SELENIUM INJECTION ON GROWTH PERFORMANCE, PHYSIOLOGICAL AND IMMUNE RESPONSES OF OSSIMI LAMBS

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ABSTRACT

Eighteen growing lambs averaged 3 months old and 16.92±1.23 kg body weight were used to assess the dose-response of vitamin E and Selenium (Se) injection on growth performance, hemato-biochemical indices, thyroid hormones, immune and antioxidant status. Lambs were randomly allocated into 3 equal groups (6 lambs each). The 1st group served as control and injected with 1.0 ml/head of sterile saline solution (0.9 % NaCl). The 2nd (T1) and the 3rd (T2) groups received vitamin E and Se injection at rate 0.5 ml/head (contained 3.57 IU vitamin E + 0.03 mg Se/head/day) and 1.0 ml/head (contained 7.14 IU vitamin E + 0.06 mg Se/head/day), respectively. The injection solution was administered biweekly and continued for 12 weeks. The results show that lambs received T2 had heavier (P<0.05) final body weight (FBW) than the control and T1. Average daily gain (ADG) was improved (P<0.05) for lambs received T1 and T2 *vs.* control. There was a significant (P<0.05) increase in FBW and ADG of lambs received T2 compared with those of T1. No significant differences in dry matter intake for lambs of T1 and T2 *vs.* control, while averages of feed conversion ratio were improved (P<0.05) for lambs received T2 compared to T1 and control. Lambs of T2 had higher (P<0.05) concentration of blood Hb and PCV % than those of T1 and control. The increase (P<0.05) in RBCs count was dose-dependent for lambs received T1 and T2. Leucocytes count and lymphocytes (%) increased (P<0.01) for lambs received T1 and T2 *vs.* control. Moreover, lambs of T2 had higher (P<0.05) concentrations of serum total protein and globulin than those of T1 and control. Serum cholesterol concentration decreased (P<0.05) for lambs received T1 and T2 *vs.* control. Also, lambs received T1 and T2 had higher (P<0.05) serum immunoglobulin G concentrations than the control. Serum triiodothyronine (T_3) levels and T_3:T_4 ratio increased (P<0.05) for lambs received T1 and T2 *vs.* control. In addition, the results showed that serum total antioxidant capacity increased (P<0.01) for lambs received T1 and T2 vs. control. Lambs received T2 had higher (P<0.05) serum superoxide dismutase activity than those of control and T1. There were dose-dependent increases (P<0.05) in serum glutathione peroxidase activity for lambs received T1 and T2 *vs.* control. No significant differences in neutrophils, eosinophils, basophils, monocytes (%), serum levels of glucose, albumin, AST enzyme and T_4 hormone due to injection of vitamin E and Se. These results show that injection of vitamin E and Se could exert dose-dependent beneficial effects on growing lambs which improve their growth performance concomitant with favourable signs for physiological responses, enhancing their immune function and antioxidant status.

Key words: *Vitamin E plus Selenium, Dose effect, Growing Lambs, Performance, Physiological responses, Antioxidant status*

INTRODUCTION

Vitamin E and selenium (Se) play complementary roles as antioxidants. Glutathione peroxidase (GSH-Px) is an enzyme involved in detoxification of hydrogen peroxide and lipid hydroperoxides while Se, as an essential component of GSH-Px, acts to destroy peroxides before they attach cell membranes, while vitamin E acts within the membrane to prevent the formation of fatty acid hydro peroxides **(Milad *et al*., 2001)**. Se is also a component of enzyme of type I iodothyronine-5´-deiodinase (D1) that is required for the conversion of thyroxin into the biologically active triiodothyronine **(Yatoo *et al*., 2013)**. The mode of action of vitamin E is closely associated with Se metabolism, and it can make up for Se deficiency to a certain

degree **(Makimura *et al.*, 1993)**. As antioxidants, vitamin E and Se have complementary role in the protection of cells against the damage effects of lipid peroxides and free radicals produced during normal metabolism **(Rooke *et al*., 2004)**. Both vitamin E and Se have been recommended to be administered together because they act synergistically and protect the tissues against oxidative damage, which improve immune competence **(Hamam and Abou-Zeina, 2007)**. However, the amount of vitamin E and Se needed for maximizing immune competence is higher than the suggested requirements of NRC **(Nockels, 1996)**. Studies have demonstrated positive growth performance, improved humeral and cellular immune responses and protection against oxidative stress due to supplement of vitamin E and Se **(Ramos *et al*., 1998; Milad *et al*., 2001; Koyuncu and Yerlikaya, 2007)**. Repeated co-administration of Se and vitamin E had a significant positive effect on thyroid activity in ruminants **(Pavlata *et al*., 2004)**. The aim of the present study was to assess the dose-response of vitamin E and Se injection on growth performance of growing Ossimi lambs via monitoring their physiological reactions related to blood haematology, serum biochemical indices, thyroid activity, immune response and antioxidant status.

MATERIAL AND METHODS

Animals and experimental treatments:

Eighteen of growing Ossimi lambs averaged three months old and 16.92±1.23 kg body weight were used to carry out this study at the Farm of Animal Production Department, Faculty of Agriculture, El-Minia University. The experiment was conducted for 12 weeks from December 2013 to March 2014 under winter conditions. The animals were randomly allocated into three equal groups (6 lambs each) of similar initial body weights. The 1st group served as control and injected with 1.0 ml/head of sterile saline solution (0.9 % NaCl). The 2nd (T1) and the 3rd (T2) groups were received vitamin E and Se injection intramuscularly at rates 0.5 ml/head (containing 3.57 IU vitamin E + 0.03 mg Se/head/day) and 1.0 ml/head (containing 7.14 IU vitamin E + 0.06 mg Se/head/day), respectively. Each ml of vitamin E and Se injected solution (Myogaster-E) contained 100 mg vitamin E acetate and 2.0 mg sodium selenite pentahydrate ($Na_2SeO_3.5H_2O$) (eq. to 0.913 mg Se) for veterinary use and manufactured by the VMD-Belgium (Intermedica Vet-Egypt). The injection solution was administered biweekly and continued for 12 weeks as experimental period.

Feeding and management:

The animals were apparently healthy and proved to be free of internal and external parasites. Animals were fed on concentrate feed mixture and rice straw to cover their nutrient requirements according to body weight **(NRC, 1985)**. The concentrate feed mixture contained 15 % yellow corn, 15 % soybean meal, 30% sugar beet pulp, 37 % wheat bran, 2.0 % limestone and 1.0 % common salt. The calculated feeding value of the concentrate mixture was 69.75 % TDN, 17.04 crude protein and 2.53 ME (Mcal/kg). The calculated concentrations of vitamin E and Se in the concentrate feed mixture fed were 11.97 mg/kg DM and 0.17 mg/kg DM, respectively. The requirements of growing lambs for vitamin E and Se are between 24-36 IU/kg DM and 0.20 ppm, respectively **(NRC, 1985)**. Feed was offered twice a day at 8 am and 2 pm and drinking water was available all times. All Parameters were recorded and collected in the morning before animals access to feed or water. Body weights of lambs in all experimental groups were recorded at starting of the experiment then at 2 weeks intervals during the experimental period. Daily feed offered and refused were measured to obtain net feed intake for each animal. Averages of daily weight gain (DWG), dry matter intake (DMI) and feed conversion efficiency (FCE) were calculated for each animal.

Blood sampling and measurements:

At the end of experiment, heparinized blood samples were collected from the jugular vein of each animal at 8:00 am before feeding or drinking. Whole blood samples were analysed after collection for hemoglobin (Hb), packed cell volume (PCV), red blood cell

counts (RBCs) and Leucocytes. The Hb concentration was determined using cyanomethomoglobin method **(Campbell, 1995)**. The PCV was determined using micro-hematocrit tubes with a micro-hematocrit centrifuge at 12000 rpm for three minutes. The RBCs and WBCs were counted using the light microscope. Stained blood smears with Lieshman's stain were prepared for the differential WBCs count **(Dacie and Lewis, 1991)**. Non-heparinized blood samples were collected from the jugular vein of each animal and then were left to clot at room temperature for at least 4 h, then the clots were removed and sera were cleared by centrifugation at 1500×g for 20 min and stored at -20 ºC for later assay. Serum glucose, total protein, albumin, cholesterol and aspartate transaminase (AST) were determined colorimetrically using Bio-diagnostic product kits (Egypt). Serum globulin concentrations were calculated by difference between total protein and albumin concentrations. Serum immunoglobulin G 8as quantified using ELISA kit supplied by WKEA MED Supplies Corporation. The ELISA micro plate was read at 450 nm, using Elisa Reader (ELX 808, Biotek), USA. The assay of serum IgG range was from 0.7 to 30 µg /ml. Serum triiodothyronine (T_3) and thyroxin (T_4) concentrations were determined by ELISA (Enzyme-Linked Immune-Sorbent Assay) technique using EIA kits (Prechek Bio, Inc., Atlaslink technology, California-USA). Triiodothyronine/thyroxin ratio was calculated. Serum total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were analyzed colorimetrically by STAT-LAB SZSL60-SPECTRUM, using Bio-dignostic kits (Bio-dignostic Company, Egypt). The analyses were performed at Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University.

Statistical analysis:

The data were analyzed by least square means analysis of variance using General Linear Models (GLM) procedure of the statistical analysis system **(SAS, 2000)**. The model used to analyze the different traits studied for lambs was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}.$$

Where: Y_{ij}= ij^{th} Observation, µ = Population mean; T_i = Effect of i^{th} treatments and eij= Random error. Duncan's Multiple Range test was used to detect differences between means of the experimental groups **(Duncan, 1955)**.

RESULTS AND DISCUSSION

Growth performance:

The results illustrated in Table (1) show the effect of vitamin E plus Se injection at 0.50 ml/head (T1) and 1.0 ml/head (T2) on growth performance of growing lambs. Lambs received T2 had higher (P<0.05) final body weight (FBW) by 17.0 and 12.0 % than those of control and T1, respectively. Average daily gain (ADG) was improved (P<0.05) by 10.3 and 37.2 % for lambs received T1 and T2 *vs.* control. There was an increase (P<0.05) in FBW and ADG for lambs received T2 *vs.* T1. The improvement represents 11.7 and 24.4 % in FBW and ADG for lambs of T2 respectively above T1. This may indicate that injection of vitamin E plus Se at the doses used improved ADG of the treated lambs. This positive response in FBW and ADG for lambs received T2 was concomitant with a significant (P<0.05) improvement in feed conversion efficiency (FCE) compared to T1 and control. Meanwhile, there were no significant differences in dry matter intake (DMI) for lambs of T1 and T2 *vs.* control.

The results agree with some studies dealt with the effect of vitamin E and Se on animal performance. Supplement with vitamin E was reported to have beneficial effect on improving FBW, ADG, FCE and vitality of lambs **(Shetaewi *et al.*, 1992)**, buffalo calves **(Amer and Hashem, 2008)** and cattle calves **(Hays *et al.*, 1987; Galyean *et al.*, 1999)**. However, Se supplementation alone had no significant effect on ADG of buffalo calves **(El Ayouty *et al.*, 1996)**; growth rate of sheep **(Kumar *et al.*, 2009)** and ADG of goats **(Kamdev *et al.*, 2015)**. Its function is inextricably involved with that of vitamin E. Vitamin E helps protecting membrane integrity of cells, the health status

and subsequently increase the efficiency of growth and production in sheep **(Ali *et al.*, 2004)**. Practically, Se supplementation may indirectly improve animal performance **(Sobiech and Kuleta, 2002)**, possibly by strengthening the immunity of the animals **(Milad *et al.*, 2001)**. Thus, the injection of vitamin E plus Se in buffalo calves improved their growth performance, concomitant with increased thyroid hormone by which the general metabolism is controlled **(El-Barody *et al.*, 2000)**.

Blood hematology:

As shown in Table (2), lambs of T2 had higher (P<0.05) concentration of blood Hb and PCV % than those of T1 and control. The increase (P<0.05) in RBCs count was dose-dependent response for lambs received T1 and T2 (11.5 and 13.9 $x10^6/mm^3$, respectively) *vs*. control (9.5 $x10^6/mm^3$). These results supported the positive effect of vitamin E and Se supplementation on blood hematology as reported in earlier studies in sheep **(Soliman *et al.*, 2001; Makkawi *et al.*, 2012)**, cattle calves **(Mohri *et al.*, 2005)** and buffaloes **(Qureshi *et al.*, 2001)**. The elevation in PCV % may be related to the antioxidant effect of vitamin E and Se that reduce the hemolysis of blood erythrocytes **(Makkawi *et al.*, 2012)**. Supplementation of vitamin E alone increased RBCs, Hb and PCV in lambs **(Shetaewi *et al.*, 1992)**. In case of Se supplement alone to lambs, significant increases in total RBCs count and osmotic resistance of RBCs were observed in sheep **(Faixova *et al.*, 2007)**; while others found no marked effect of Se alone on RBCs count, Hb and PCV in buffalo calves **(El-Ayouty *et al.*, 1996)**. In goats, Se supplementation at 0.3 mg Se/kg DM as sodium selenite had no effect on blood Hb and PCV in male kids **(Kamdev *et al.*, 2015)**. It has been reviewed that vitamin E supplementation led to enhancing erythropoiesis and decreasing the premature erythrocyte hemolysis by reducing the fragility of erythrocytes **(Jiliani and Iqbal, 2011)**. The changes in blood hematological parameters, in the present study, are within the normal physiological value ranges documented for sheep **(Duncan and Prasse, 1986)**. Thus, vitamin E may improve the post-supplemental response of blood Hb, RBCs and PCV levels.

The leukocyte count is an important index for diagnosis of the health status and problems, and may be adopted for assessing physiological changes related to immune response **(Bike, 2003)**. As shown in table 2, the total count of leucocytes increased (P<0.01) by 14.9 and 23.0 % for lambs received T1 and T2 *vs.* control, with no significant differences among lambs of T1 and T2. The leucocytes profile showed a marked increase (P<0.01) in lymphocytes % for lambs received T1 and T2 *vs.* control. While the differences in neutrophils, eosinophils, basophils and monocytes percentages were not significant. The results may be confirmed the positive effect of vitamin E plus Se administration on improving immune function

Table 1: Effects of various doses of vitamin E and Se injection on growth performance of growing lambs (Mean ± SEM).

	Treatments				
Parameters	**Control**	**T1**	**T2**	**SEM**	**Sig.**
IBW (kg)	16.93	16.91	16.93	1.23	NS
FBW (kg)	30.92 b	32.37 b	36.17 a	1.26	*
DWG (kg/day)	0.156 c	0.172 b	0.214 a	0.003	*
DMI (kg/head/day)	1.07	1.12	1.17	0.046	NS
FC (kg feed/kg gain)	6.89 a	6.52 a	5.47 b	0.27	*

a,b,c means within the same row having different superscripts significantly different (* P<0.05). NS = not significant

T1 = Vit E plus Se (0.5 ml/head), T2 = Vit E plus Se (1.0 ml/head) .

IBM = Initial body weight, FBW = Final body weight, DWG = Daily weight gain,

DMI = Dry matter intake, FC = Feed conversion.

Table 2: Effects of various doses of vitamin E and Se injection on blood haematology and differential leukocyte count of growing lambs (Mean ± SEM).

Parameters	Treatments			SEM	Sig.
	Control	T1	T2		
Hb (g/dl)	9.8 b	10.6 b	12.9 a	0.40	*
RBC ($x10^6/mm^3$)	9.5 c	11.5 b	13.8 a	0.08	*
PCV (%)	30.6 b	32.0 ab	34.9 a	1.10	*
Leucocytes ($x10^3/mm^3$)	7.4 b	8.5 a	9.1 a	0.17	*
Neutrophils (%)	29.9	27.74	27.20	4.22	NS
Eosinophils (%)	4.64	4.12	4.10	0.16	NS
Basophils (%)	0.68	0.54	0.55	0.03	NS
Lymphocytes (%)	59.78 b	63.3 a	64.0 a	2.40	**
Monocytes (%)	4.80	4.30	4.20	0.14	NS

a,b,c means within the same row having different superscripts significantly different (* P<0.05 and ** P<0.01). NS = not significant

T1 = Vit E plus Se (0.5 ml/head), T2 = Vit E plus Se (1.0 ml/head).

in sheep **(Makkawi *et al.*, 2012)**, reflecting their adaptability to the adverse effects of environmental conditions **(Soliman *et al.*, 2001)**. Injection of vitamin E and Se were found to be necessary to attain maximum immunologic responses **(Milad *et al.*, 2001).** It has been explained that the higher leucocytes and lymphocyte cell counts due to Se and vitamin E administration could be related to the protection of cell membrane and intracellular organelles by the antioxidant effects of Se and vitamin E and thus increase their lifespan **(Moeini and Jalilian, 2014)**. Similar response of leukocytes count to vitamin E plus Se was also found in adult buffaloes **(Qureshi *et al.*, 2001)**, Friesian heifers **(Suwanpanya *et al.*, 2007)**; and dairy calves **(Mohri et al., 2005)**. In contrast, supplementation of Se alone or vitamin E plus Se had no effect on total count of leucocytes in buffalo calves **(Shinde *et al.*, 2009)**. The neutrophils response was focused in some works. Neither phagocytic index nor percentage of neutrophils differed between vitamin E-injected and non-injected cows **(Hogan *et al.*, 1992)**. Also, Se alone had no effect on neutrophil % in buffalo calves **(El-Ayouty *et al.*, 1996)**, while neutrophil phagocytosis was improved in vitamin E plus Se supplemented-heifers **(Suwanpanya *et al.*, 2007)**. In Awassi rams, neutrophils percentages were found to be decreased with supplemental vitamin E alone *vs.* Se plus vitamin E **(Ammar *et al.*, 2009)**. Neutrophil response could be affected by the different doses of vitamin E and/or Se used, route of administration, physiological status and species of the experimental animals.

The enhanced effect of vitamin E and Se to increase lymphocytes (%) agree with similar response in Ossimi sheep **(Soliman *et al.*, 2001)**, Awassi rams **(Ammar *et al.*, 2009)** and buffaloes **(Qureshi *et al.*, 2001)**. Lymphocyte proliferation was found to be declined following prolonged exposure of lambs to both vitamin E and Se-deficient diet, while their supplementation restored lymphocyte function within a week, suggesting that immunological malfunctions is associated with low Se-vitamin E diets in lambs **(Turner and Finch, 1990)**. The synergistic effect of vitamin E and Se to enhance lymphocyte function and its proliferative responses was detected with cattle **(Pollock *et al.*, 1994)**. In *in vitro* study, exposure of peripheral blood lymphocytes to vitamin E and Se treatment enhanced lymphocyte proliferation, indicating that this response may optimize resistance to diseases **(Ndiweni and Finch, 1995)**. The increase in blood lymphocyte populations may be a good indicator of an immunomodulatory response **(Qureshi *et al.*, 2001)**. Injection of vitamin E alone enhanced lymphocyte populations in calves **(Reddy *et al.*, 1986)**. However, supplementation of Se alone at different levels in buffalo calves did not alter the lymphocyte % **(El-Ayouty *et al.*, 1996)**. So, it appears that the

usefulness of Se to the immune function is increased by giving vitamin E **(Nockles, 1988)**; and the combination of vitamin E and Se were more effective than Se alone to improve immune system **(Fazaeli and Talebian, 2009)**

Serum biochemical's indices:

Injection of various doses of vitamin E and Se had no significant effect on serum glucose concentrations in lambs (Table, 3). Similar response of blood glucose was observed with supplementation of vitamin E plus Se in sheep **(Soliman *et al*., 2001)** and goats supplemented with Se alone **(Kamdev *et al*., 2015)**. This result is supported by **Shinde *et al*. (2008)** who observed that supplementation of vitamin E (300 IU) and/or Se (0.3 ppm) in the diet had no effect on serum glucose levels in buffalo calves.

Serum total protein (TP) and globulin, but not albumin, were increased (P<0.05) for lambs of T2 *vs.* T1 and control (Table, 3). Similar responses of blood TP and globulin to supplement of vitamin E and Se were found in sheep **(El-Shahat and Abd El-Monem, 2011)** and buffalo **(Helal *et al*., 2009)**. In Baladi sheep, supplement of vitamin E plus Se had increased serum globulins, specifically γ-globulins compared to control or Se alone, but the differences in serum TP and albumin were not significant **(Hamam and Abou-Zeina, 2007)**. The insignificant effete of vitamin E plus Se injection on serum albumin could be related to the lack effect of Se supplement alone on serum albumin as previously observed by **Hamam and Abou-Zeina (2007)** and **Kamdev *et al*. (2015)**. Contrary to above, Se and vitamin E supplementation had no effect on serum TP and globulin in buffalo calves **(Shinde *et al*., 2009)**.

The presented results indicated that serum cholesterol concentrations decreased (P<0.05) by 16.0 and 24.0 % for lambs injected with vitamin E plus Se at 0.5 ml (T1) and 1.0 ml (T2), respectively *vs.* control (Table, 3). In agreement with these results, dietary supplementation of Se and vitamin E significantly decreased total plasma cholesterol concentrations and increased high density lipoprotein (HDL) fraction in sheep **(Gabryszuk *et al*., 2007)**. Vitamin E and Se could modify the lipid metabolism via elevating both HDL and triglycerides in cattle **(Falkowska *et al*. 2000)**. Supplementation of vitamin E alone in sheep had no effect on serum cholesterol, triglyceride concentrations or the sum of the two lipid fractions **(Njeru *et al*., 1994)**. However, Se supplementation remarkably lowered serum total cholesterol levels in rats and increase low-density lipoprotein (LDL) receptor activity **(Bunglavan *et al*., 2014)**.

No significant differences observed in serum aspartate transaminase (AST) due to injection of various doses of vitamin E and Se (Table, 3). This result may indicate that the doses of vitamin E plus Se used in this study did not adversely affect normal hepatic metabolism. Similar response of serum AST enzyme activity due to Se supplementation was observed in goats **(Kamdev *et al*., 2015)**.

The results showed that lambs received T1 and T2 had higher (P<0.05) serum immuno-globulin G (IgG) concentrations by 18.0 and 25.2 %, respectively than the control (Table, 3). Although serum IgG concentration tended to increase by 6.2 % for lambs received T2 *vs.* T1; the dose response was not significant. Some studies showed no effect of Se supplement alone on serum IgG levels in goats and their kids **(Kachuee *et al*., 2013)**, while others found significant effect of Se supplementation at various doses on increasing plasma IgG in calves **(Awadeh *et al*., 1998)**. In sheep, vitamin E alone at high doses had increased serum IgG concentrations in lambs **(Gentry *et al*., 1991)**, and in ewes **(Anugu *et al*., 2013)**. The possible positive effect of vitamin E and Se to enhance serum IgG concentrations was also shown in buffalo calves **(Amer and Hashem, 2008)**. Combination of vitamin E and Se improved the status of these micronutrients and their humeral immune response in buffalo calves **(Shinde *et al*., 2007)**. In sheep, supplement of vitamin E and Se, but not Se alone, had increased immunoglobulins levels **(Hamam and Abou-Zeina, 2007)**. There has been a synergistic action between vitamin E and Se to enhance immune response in sheep **(Ramos *et al*., 1998)**. So, the injection of vitamin E plus Se increases the concentrations of natural

antioxidants (*α*-tocopherol and GSH-Px) in blood of sheep, hence ensures that they mount adequate immunoglobulin, improving their immune competence **(Hamam and Abou-Zeina, 2007)**. In contrast to the present results, some reports detected no changes in the mean serum IgG levels among calves from heifers received different doses of vitamin E and Se **(Moeini *et al.*, 2011)**.

Thyroid hormones:

The results showed significant (P<0.05) increases in serum triiodothyronine (T_3) concentrations and T_3:T_4 ratio for lambs received T1 and T2 compared to control (Table, 4). While no significant differences were found in serum thyroxin (T_4) levels due to injection of vitamin E plus Se. Also, there were dose-dependent increases (P<0.05) in serum T_3 levels and T_3/T_4 ratio for lambs received T2 compared with those received T1. These findings may signify that the metabolic activity processes might be enhanced in these injected lambs. The significant increase in T3 levels could probably explained by the fact that type I iodothyronine-5´-deiodinase is a Se-dependent enzyme, since it is responsible for the conversion of T4 into biologically active T3 and its activity is influenced by Se **(Pechova *et al.*, 2012)**. The positive effect of Se on T3 concentration was previously confirmed by **Awadeh *et al*. (1998)**, who noticed the increase of T3 concentrations in cows and calves after high Se intake. Se supplementation resulted in a significant increase in T_3 levels andT_3/T_4 ratio associated with a decrease in T_4 in male Baladi goats **(El-Sisy *et al.*, 2008)**. In cattle, repeated co-administration of Se and vitamin E had a significant positive effect for increased Se intake on higher serum T_3 concentrations and increased T_3/T_4 ratio with vitamin E/Se-injected cows **(Pavlata *et al.*, 2004)**. Moreover, supplementation of buffalo calves with Se alone or vitamin E plus Se significantly increased serum level of T3, but did not affect T4 levels and T4/T3 ratio **(Shinde *et al.*, 2009)**. However, the present results disagree with some studies detected no significant correlation between Se and serum thyroid hormones in sheep **(Nazifi *et al.*, 2008)**.

Serum antioxidant enzymes activity:

The results presented in Table (5) revealed that serum total antioxidant capacity (TAC) increased (P<0.01) by 76.9 and 86.2 % for lambs received T1 and T2, respectively compared to control with no significant difference among lambs of T1 and T2. Lambs received T2 had higher (P<0.05) serum superoxide dismutase (SOD) activity by 32.0 and 24.3 % than those of control and T1, respectively. There have been dose-dependent increases (P<0.05) in serum glutathione peroxidase (GSH-Px) activity for lambs received T1 and T2 *vs.* control. The increment represents 38.2 % in serum GSH-Px activity for T2 above T1. The results clearly indicate that injection of vitamin E plus Se at the doses used could improve antioxidant status in growing Ossimi lambs.

Table 3: Effects of various doses of vitamin E and selenium injection on serum biochemical's indices of growing lambs (Mean ± SEM).

	Treatments				
Parameters	**Control**	**T1**	**T2**	**SEM**	**Sig.**
Glucose (mg/dl)	53.6	56.8	60.4	3.55	NS
Total protein (g/dl)	6.93 b	7.02 b	7.87 a	0.20	*
Albumin (g/dl)	3.20	3.10	3.20	0.18	NS
Globulin (g/dl)	3.73 b	4.02 b	4.67 a	0.10	*
Cholesterol (mg/dl)	70.7 a	61.0 b	57.0 b	5.3	*
AST (U/L)	91.8	83.2	85.8	4.5	NS
IgG (µg/ml)	15.10 b	17.8 a	18.9 a	0.87	*

a,b,c means within the same row having different superscripts significantly different
(* P<0.05 and ** P<0.01). NS = not significant
T1 = Vit E plus Se (0.5 ml/head) , T2 = Vit E plus Se (1.0 ml/head) .

Table 4: Effects of various doses of vitamin E and selenium injection on thyroid hormone concentrations of growing lambs (Mean ± SEM).

Parameters	Treatments			SEM	Sig.
	Control	T1	T2		
T_3 (ng/ml)	1.22 c	1.64 b	1.90 a	0.20	*
T_4 (ng/ml)	41.0	44.0	46.0	2.10	NS
T_3/T_4 ratio	0.030 c	0.037 b	0.043 a	0.001	*

a,b,c means within the same row having different superscripts significantly different (* P<0.05 and ** P<0.01). NS = not significant

T1 = Vit E plus Se (0.5 ml/head) , T2 = Vit E plus Se (1.0 ml/head)

Table 5: Effects of various doses of vitamin E and selenium injection on serum antioxidants enzymes activity of growing lambs (Mean ± SEM).

Parameters	Treatments			SEM	Sig.
	Control	T1	T2		
TAC (mM/L)	0.458 b	0.810 a	0.853 a	0.036	**
SOD (U/ml)	235.5 b	250.7 b	310.8 a	5.77	*
GSH-Px (mU/ml)	3.42 c	5.56 b	7.66 a	0.02	*

a,b,c means within the same row having different superscripts significantly different (* P<0.05 and ** P<0.01). NS = not significant

TAC= Total antioxidant capacity, SOD= Superoxide dismutase,

GPX= Glutathione peroxidise

The present findings are in agreement with those reported by **Hamam and Abou-Zeina (2007)** on Baladi sheep. They found that injection of Se and/or Se plus vitamin E increased (P<0.05) blood GSH-Px activity, but they found no differences in erythrocytes SOD activity. Also, dietary supplementation with 0.8 mg/kg Se and 150 mg/kg vitamin E for 50 d increased (P<0.05) plasma TAC in sheep **(Alhidary *et al.*, 2014)**. In addition, other report in goats **(Abou-Zeina, 2002)** and cattle **(Ceballos *et al.*, 2003)** indicated a significant increase in serum GSH-Px activity following either Se alone or with vitamin E supplementation. In finishing lambs, Se supplementation at 0.10 mg/kg DM increased blood GSH-Px activity **(Qin *et al.*, 2007)**. A trend of higher serum GSH-Px activity was found in Merino lambs with supplement of 0.3 mg Se /kg DM either in inorganic (sodium selenite) or organic (Sel-Plex) form **(Antunovic *et al.*, 2014)**. No differences in the activity of GSH-Px depending on the form of Se supplied were stated in the majority of studies conducted **(Krzyzewski *et al.*, 2014)**. In a study on goats, adding vitamin E at 80 IU/ kid/d can increase serum TAC and activities of serum SOD and GSH-Px **(Hong *et al.*, 2010)**. The antioxidant effect of vitamin E plus selenium supplementation was reflected by a significant increase in plasma GSH-Px and SOD activities, but TAC kept constant, in rats treated with oral administration of vitamin E and Se **(Ghaffari *et al.*, 2011)**. Co-administration of Se and vitamin E showed antioxidant protective effects on oxidative damage of erythrocytes **(Ben Amara *et al.*, 2012)**. It has been suggested that vitamin E may have a controlling effect on oxidative stress through modulation of IL-2 mRNA expression of SOD **(Das *et al.*, 2012)**.

In the present study, the dose-dependent effect of vitamin E plus Se on antioxidant status was clearly seen only with the increase of serum GSH-Px activity. It has been proved, in cattle, that administration of vitamin E and Se resulted in a significant increase in blood GSH-Px activity **(Zhao *et al.*, 2008)**. Among potential antioxidants, Se plays a central role in enzymatic defense pathways against oxidative damage in tissues. The effects of Se are mediated in antioxidant metabolism by GSH-Px in which Se is incorporated in the core of selenocysteine **(Yeh *et al.*, 1997)**. Indeed, vitamin E supplementation increased

antioxidant recycling and improved synergistic antioxidant effect **(Ramos *et al*., 1998)**. It has been stated that antioxidants can be interacted in synergistic ways and have sparing effect in which one antioxidant protect another against oxidative destruction, so combinations of antioxidants may be more effective than larger quantities of any single antioxidant **(Young and Lowe, 2001)**. The synergistic relationship between Se and vitamin E on improving antioxidant status had been evidenced when plasma GSH-Px activity was significantly higher for cows fed Se plus vitamin E than those fed on each micronutrient alone **(Zhao *et al*., 2008)**. Thus, it has been recommended that both vitamin E and Se should be administered together to sheep in order to improve their antioxidant status and immune competence **(Hamam and Abou-Zeina, 2007)**.

CONCLUSION

Based on the present results, injection of vitamin E plus Se to growing Ossimi lambs could exert dose-dependent beneficial effects that improve growth performance concomitant with favourable signs of physiological reactions enhancing their immune function and antioxidant status.

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DOSE-RESPONSE OF VITAMIN E AND SELENIUM INJECTION ON GROWTH PERFORMANCE, PHYSIOLOGICAL AND IMMUNE RESPONSES OF OSSIMI LAMBS

الملخص العربى

تأثيرات الإستجابة- للجرعة للحقن بفيتامين هـ والسيلينيوم على أداء النمو والإستجابات الفسيولوجية والمناعية للحملان الأوسيمى

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أستخدم فى هذه الدراسة عدد ١٨ حمل أوسيمى نامية متوسط وزنها ١٦.٩٢ ± ١.٢٣ كجم وعمرها ثلاثة شهور وذلك بهدف تقييم تأثير الحقن بفيتامين هـ والسيلينيوم بجرعات مختلفة على أداء النمو، المؤشرات البيوكيميائية والهيماتولوجية للدم ، هرمونات الدرقية ، حالة المناعة ومضادات الأكسدة. قسمت الحيوانات عشوائياً إلى ثلاثة مجموعات متساوية (٦ حملان في كل منها). المجموعة الأولى للمقارنة (المجموعة الضابطة) تم حقنها بمحلول فسيولوجى (٠.٩ % كلوريد صوديوم) بمعدل ١.٠ مل/رأس ، بينما المجموعة الثانية (T1) والثالثة (T2) تم حقنهما على التوالى بفيتامين هـ والسيلينيوم بمعدل ٠.٥ مل/رأس (يحتوى على ٣.٥٧ وحدة دولية من فيتامين هـ + ٠.٠٣ ملجم سيلينيوم/رأس/يومياً) و ١.٠ مل/رأس (يحتوى على ٧.١٤ وحدة دولية من فيتامين هـ + ٠.٠٦ ملجم سيلينيوم/رأس/يومياً). وقد تم الحقن كل أسبوعين وأستمر لمدة ١٢ أسبوع ، وقد أظهرت النتائج ما يلى :

- سجلت حملان المعاملة (T2) قيم أعلى معنوياً لمتوسطات وزن الجسم النهائى مقارنة بحملان المعاملة (T1) و المجموعة الضابطة. حملان المعاملة (T1) ، (T2) سجلت قيم أعلى معنوياً لمتوسطات الزيادة اليومية فى الوزن مقارنة بالمجموعة الضابطة. وكان هناك زيادة معنوية فى متوسطات وزن الجسم النهائى ومعدل الزيادة اليومية فى الوزن لحملان المعاملة (T2) مقارنة بالمعاملة (T1). لم تلاحظ أية تغييرات معنوية في إجمالي استهلاك المادة الجافة لحملان المعاملات (T1، T2) مقارنة بالمجموعة الضابطة ، بينما حدث تحسين معنوى ($P < 0.05$) فى معدلات تحويل الغذاء لحملان المعاملة (T2) مقارنة بحملان المعاملة (T1) والمجموعة الضابطة.

- وكذلك أظهرت حملان المعاملة (T2) زيادة معنوية فى تركيز هيموجلوبين الدم والمكونات الخلوية للدم مقارنة بحملان المعاملة (T1) والمجموعة الضابطة. وكان هناك زيادة معنوية مرتبطة بالجرعة فى العدد الكلى لكرات الدم الحمراء لحملان المعاملات (T1، T2) مقارنة بالمجموعة الضابطة. كما لوحظ زيادة معنوية فى العدد الكلى لكريات الدم البيضاء وكذلك النسبة المئوية للكريات الليمفاوية لحملان المعاملات (T1، T2) مقارنة بالمجموعة الضابطة. وقد أظهرت نتائج حملان المعاملة (T2) زيادة معنوية فى تركيزات السيرم من البروتين الكلي والجلوبيولين مقارنة بحملان المعاملة (T1) والمجموعة الضابطة. أظهرت حملان المعاملات (T1، T2) إنخفاض معنوى فى تركيز السيرم من الكوليستيرول بينما كان هناك زيادة معنوية فى تركيز السيرم لكل من الإمينوجلوبيولين (IgG) ، هرمون تراى أيودوثيرونين (T_3) ونسبة هرمونى (T_3 / T_4) وكذلك إجمالى القدرة المضادة للأكسدة (TAC) مقارنة بالمجموعة الضابطة. وكان هناك زيادة معنوية مرتبطة بالجرعة فى نشاط إنزيم الجلوتاثيون بيروكسيديز (GSH-Px) لحملان المعاملات (T1، T2) مقارنة بالمجموعة الضابطة. بالإضافة إلى ذلك فقد أظهرت حملان المعاملة (T2) زيادة معنوية فى تركيزات السيرم من إنزيم SOD مقارنة بالمعاملة (T1) والمجموعة الضابطة. لم تلاحظ أية تغييرات معنوية في نسبة الكريات حمضية الصبغ ، الكريات المتعادلة، الكريات قاعدية الصبغ والكريات الأحادية وكذلك تركيزات سيرم الدم من الجلوكوز ، الألبيومين ، هرمون الثيروكسين (T_4) ومستويات نشاط أنزيم AST لحملان المعاملات (T1، T2) مقارنة بالمجموعة الضابطة.

بناء على النتائج المقدمة، فإن الحقن بفيتامين هـ والسيلينيوم بجرعات مختلفة فى حملان الأوسيمى النامية يمكن أن يؤدى إلى تأثيرات مفيدة مرتبطة بالجرعة تشمل تحسين أداء النمو مصحوباً بعلامات إيجابية في الإستجابات الفسيولوجية وتعزيز الوظيفة المناعية وحالة مضادات الأكسدة.