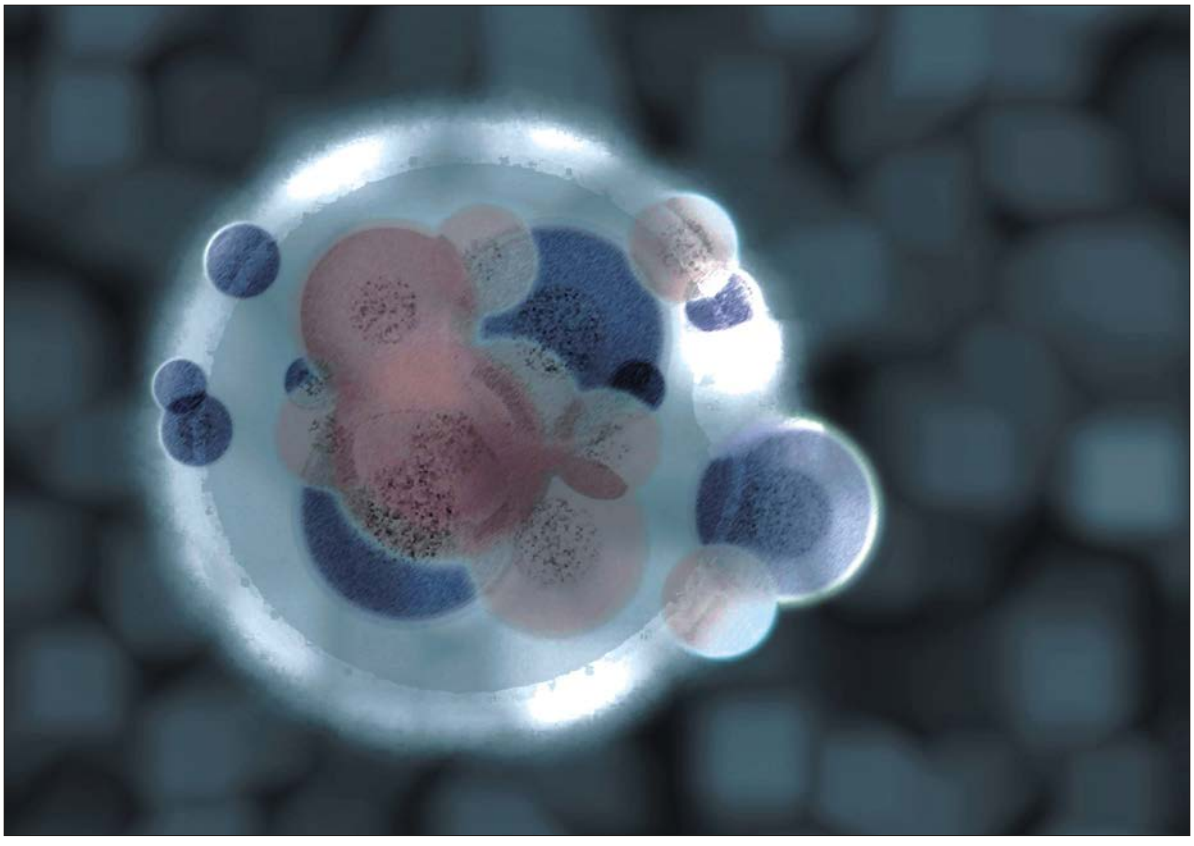

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Effect of Vitamin E and Selenium Supplements on the Antioxidant Markers and Immune Status in Sheep

[1]A.M. Hamam and [2]Hala A.A. Abou-Zeina
[1]Department Of Animal Reproduction and Artificial Insemination
[2]Department of Parasitology and Animal Diseases,
Veterinary Research Division, National Research Center,
P.O. Box 12622, El-Tahrir Street, Dokki, Giza, Egypt

Abstract: Twenty-one Baladi ewes were used to determine the effects of supplemental Vit E and/or Se on natural antioxidants and immunity. The ewes were randomly assigned into three equal groups; group I, was kept as control; group II, was injected I/M with a solution of sodium selenite (1.7 mg/head, once/two weeks for 16 weeks) and group III, treated with 1 mL of Viteselen 15 (contains 1.67 mg sodium selenite plus 150 mg Vit E /mL). The concentrations of Vit E in diet, serum α-tocopherol and β-carotene were determined using HPLC; whole blood- GPX, E-SOD were estimated kinetically and total serum protein and its fractions as well as Se concentration in diet were determined. The results showed that injection of Vit E and Se (group III) significantly increased the serum concentrations of α-tocopherol and β-carotene at all sampling times, while administration of Se alone did not induce significant alterations. Whole blood GPX-activities increased significantly after the administration of Se either alone or in combination with Vit E at week 4 and onwards, while the activity was enhanced by administration of Se alone. No significant changes could be traced concerning E-SOD activity; total serum protein and albumin in both treated groups. It is concluded that administration of Vit E and Se significantly improved the antioxidant status of ewes and the levels of total globulins specifically γ-globulins (Igs).

Key words: Antioxidant, selenium, Vit E immunity, ewes

INTRODUCTION

The term antioxidant has been defined as any substance that delays or inhibits oxidative damage to a target molecule (Gutteridge and Halliwell, 1994), which may be present within the cells, in the cell membranes or in the extra cellular fluid. It may be endogenously produced or derived from the diet (Rumley and Paterson, 1998).

Vitamin E (Vit E) and Selenium (Se) are essential nutrients that have complementary biological functions as antioxidants to minimize cellular damage caused by endogenous peroxides (Kolb *et al*., 1997). These products causing cellular degeneration and necrosis (Gutteridge and Halliwell, 1994). Selenium as an essential component of Glutathione peroxidase (GPX), acts to destroy peroxides before they attack cell membranes (Ebert-Dunig *et al*., 1999), while Vit E was reported to act within the membrane to prevent the formation of fatty acid hydro peroxides (Chow, 2001).

However, Vit E and Se represent only part of the cellular antioxidant defense system. Cytosolic- Super Oxide Dismutase (SOD) enzyme is a vital free radicals scavenger, the activity that protects cells from oxidative stress (Teoh *et al*., 2003). β-carotene is important dietary antioxidant and acts as precursor of Vit A (Young and Lowe, 2001).

In addition to their individual effects, antioxidants interact in synergistic ways and have sparing effects, in which one antioxidant protects another against oxidative destruction. Combinations of antioxidants may be more effective than larger quantities of any single antioxidant (Young and Lowe, 2001).

The antioxidative role becomes very important during the immune response when neutrophils produce large quantities of superoxide and hydrogen peroxide from molecular oxygen to destroy ingested foreign organisms (Ebert-Dumig *et al*., 1999; Chiaradia *et al*., 2002)

Total serum protein concentration and protein electrophoresis could be considered as minimum initial

Corresponding Author: Hala A.A. Abou-Zeina, Department of Parasitology and Animal Diseases, Vet. Division, National Research Center, P.O. Box 12622, El-Tahrir Str., Dokki, Giza, Egypt Fax:+202 3370931

laboratory evaluation of the suspected immune deficiency. The immunoglobulins (mainly IgG) are responsible for most of humoral immunity, with electropholitic mobility in the gamma-band. Animals with immune deficiency disorders are particularly hypogamma globulinamic (Jain, 1993).

Several studies suggest that both humoral and cellular immune functions are improved by Vit E and Se supplementation in different species (Ramos *et al*., 1998 in sheep; Hassan *et al*., 2001 in horses; Kandil and Abou-Zeina, 2005 in dogs). However, the amount of antioxidants needed for maximizing immune competence is higher than the suggested required by NRC (Nockels, 1996).

Therefore, the present study aimed to throw a light on the interaction between Se and Vit E and their effects on the antioxidant and immune status of sheep.

MATERIALS AND METHODS

The design of experiments depends on supplementation with Vit E and/or Se and study their actions on some antioxidants and immune performance of sheep.

Animals: Twenty one clinically healthy non-pregnant, non-lactating Baladi ewes 2 to 3.5 years old were used in the current study. The ewes were raised in hygienic house in Abou-Rawash experimental farm, National Research Center (NRC), each animal was injected s/c with an anthelmintic (Ivomec-Merck) and was routinely examined clinically during the experimental period according to Radostitis *et al*. (1994).

Feeding: Animals were fed on concentrate mixture, which consisted of 65% cotton seed cake meal, 9% wheat bran, 20% rice glue, 2% calcium carbonate, 1% sodium chloride and 3% molasses. Each animal fed at a rate of 600 g/head daily (2%/kg b.w). Wheat straw (Tiben) was offered ad libitum as roughage.

Experimental procedures: The experimental animals were randomly allocated into three equal groups (n = 7). Group I, was kept as control, group II, received Se as sodium selenite (Sigma Co., USA) intramuscularly(I/M) at a dose level of 1.7 mg/head, once/two weeks for 16 weeks (given in 5 mL of sterile normal saline), while group III, received 1 mL of Viteselen 15 (contains 1.67 mg sodium selenite and 150 mg Vit E/mL, (The Egyptian Co. for Chemicals and Pharmaceuticals), by the same way of administration for 16 weeks.

Sampling: Two blood samples were drawn from a jugular vein of each ewe on the day before supplementation commenced (week 0) and every two weeks along the experimental period. One sample was taken into EDTA and kept to cool at about 4°C until the determination of Hb and whole blood-GPX- activity on the following day and a second, without anticoagulant for separation of serum and erythrocytes (washed four times with 0.9% NaCl solution) and were stored at -20°C for later analysis. Sera were assayed for α-tocopherol, β-carotene, total proteins and proteins electrophoresis, while erythrocytes were assayed for SOD- activity.

Analysis: The diet was analyzed for Se using Varian Spectra AA 220 atomic absorption spectrophotometer equipped with a Graphite furance Tube Atomizer (GTA), after sample digestion procedure recommended in the Association of Official Analytical Chemists (AOAC, 1990).

Vit E concentration in diet was estimated using High-Pressure Liquid Chromatography (HPLC) after preparation procedures, consisting of extraction and saponification (McMurray and Blanchflower, 1979).

The GPX- activity in the EDTA–treated whole blood was estimated according to the method of Paglia and Valentine (1967). One unit of enzyme activity was defined as 1 μmol NADPH oxidized per minute and the results are expressed as U GPX/g Hb. The test kits were Ransel supplied by Randox Laboratories (Crumlin, Co. Antrim, UK). The instrument used was a spectrophotometer (UV 2410, Shimatsu, Japan). Hemoglobin concentration was determined using the method of Jain (1993).

The SOD-activity was measured kinetically using a Ransod test kit (Randox Laboratories) as described by Suttle and McMurray (1983).

Determination of serum α-tocopheral and β-carotene concentrations, were performed using HPLC method (Gimeno *et al*., 2001). A total lipid extract from an aliquot of serum sample and containing internal standards of α-tocopherol and β-carotene (Sigma, USA) were injected onto a HPLC with a mobile phase consisted of methanol-water (97:3) and flow-rate, 3 mL min^{-1}. The HPLC instrumentation was from Walters Associates (Hartford, Great Britain) and consists of a pump (model 6000), injector (model UK 6) and column (wakosil C_{18}, 150×0.46 mm, 5 μm particle size). An ultraviolet detector with 280 nm filter was used.

Total serum protein concentrations (n = 3) were estimated by the method of Lowry *et al*. (1951). The serum proteins were fractionated electrophoretically on one dimensional Poly Acrylamide Gel Electrophoresis (PAGE) as shown in Fig. (1A and B). PAGE was carried out according to Laemmli (1970). The albumin and globulins (α, β and γ globulins) were determined by PAGE.

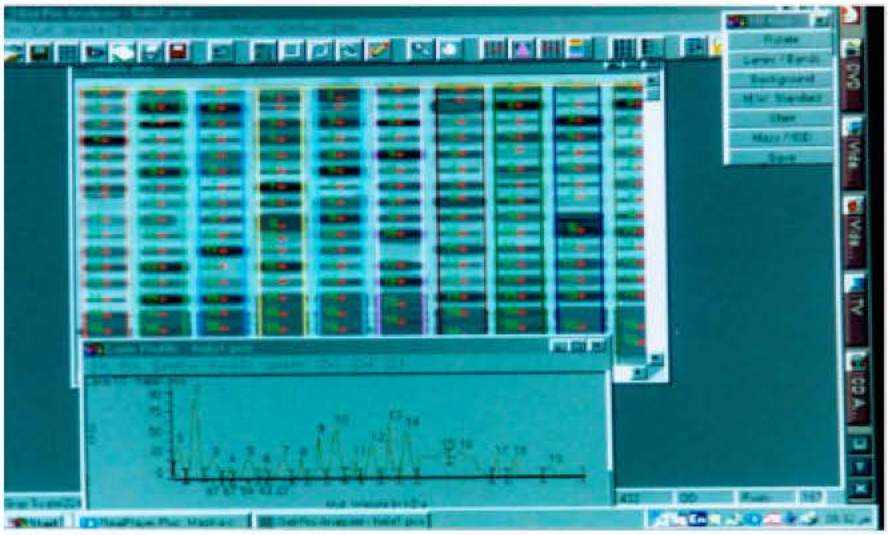

Fig. 1: A: Electrophoretic separation of serum protein fractions on polyacrylamide gel (PAGE) B: Dimentional electrogram of sheep blood serum

Statistical analysis of the obtained data was by the analysis of variance (ANOVA) and means were tested at Least Significant Differences (LSD) (SAS, 1993).

RESULTS AND DISCUSSION

All animals remained clinically normal during the course of the experiment.

The present study monitored the variations in the natural antioxidants levels in the blood of ewes and total serum protein as well as its fractions after the administration of Vit E and/or Se.

The concentration of Vit E and Se in the diet supplement were 15.2 mg kg^{-1} and 0.08 mg kg^{-1} DM, respectively. The diet was considered adequate in Se and Vit E as recommended by NRC (1985), which appears to be based on levels to prevent white muscle disease.

Table 1, shows the antioxidant profile of the experimental animals at different weeks for different trials. A Vit E deficiency in sheep is characterized by serum α-tocopherol concentrations below 1.0 μg mL^{-1} as stated by Puls (1994). The obtained results revealed that serum α-tocopherol levels increased significantly ($p<0.05$) in ewes treated with Vit E/Se (ranged between 3-6 μg mL^{-1} whereas, remained between 2 and 3 μg mL^{-1} in group I and II for most of experimental period ($p>0.05$). These findings were supported by Smith and Allen (1997) and Hatfield *et al.* (2002) in sheep who reported that serum α-tocopherol levels increased linearly with increasing levels of supplemental Vit E. This increase in the level of α-tocopherol of above 3.0 μg mL^{-1}-due to the treatment with Vit E/Se – is found to be better for antioxidant and immune functions (Chawla and Kaur, 2004).

In addition, the present study suggest that the administration of Se alone had no effect on α- tocopherol concentrations, which are in agreement with the findings of Siddons and Mills (1981) and Smith and Allen (1997).

β-Carotene and α-tocopherol may have either antagonistic (Yang *et al.*, 2002) or synergistic (Wang *et al.*, 1995) effects on absorption and metabolism of each other. The present data indicated that treatment with Vit E/Se elevated significantly ($p<0.05$) the levels of β-Carotene in serum of ewes than the other 2 groups (Table 1). Meanwhile, Se alone did not induce a significant alterations in β-Carotene levels. Our findings agree with Wang *et al.* (1995), who recorded that the lymphatic transport of β-carotene was enhanced 4-fold by α-tocopherol at a physiological dose and 12-21 fold at pharmacological dose. They suggest that α-tocopherol has a positive effect on the intestinal absorption of β-carotene. On the other hand Yang *et al.* (2002) found that high concentrations of Vit E may interfere with the absorption of β-carotene as they may compete with each other for binding sites on the lipoprotein molecule.

In the present study the levels of GPX-activities were increased significantly ($p<0.05$) into adequate range (>130) in the blood of ewes treated with Vit E and/or Se compared with control group by week 4 till the end of experiment (Table 1). However, there was significant variance ($p<0.05$) between both supplemented groups by week 8 and onwards. In unsupplemented ewes, GPX-activity remained between 80-110 U g^{-1} Hb which is

Table 1: Antioxidant profile of ewes serum before and after parental administration of vitamin E and/or selenium

Parameters	Groups	Treatments	Concentration/duration (weeks) 0	2	4	6	8
Serum α-tocopherol (μg mL^{-1})							
	I	Contr.	2.73±0.16^{1a}	2.59±0.22^{1a}	2.91±0.10^{1a}	2.47±0.17^{1a}	2.74±0.16^{1a}
	II	Se	2.50±0.17^{1a}	2.53±0.14^{1a}	2.42±0.20^{1a}	2.76±0.13^{1a}	2.28±0.17^{1a}
	III	Se+Vit E	2.65±0.21^{1a}	5.14±0.28^{2b}	5.11±0.29^{2b}	5.27±0.24^{2b}	5.60±0.25^{2b}
β-carotene (μg dL^{-1})							
	I	Contr.	6.56±0.63^{1a}	6.24±0.60^{1a}	7.49±0.61^{1a}	7.25±0.45^{1a}	6.86±0.42^{1a}
	II	Se	8.04±0.76^{1a}	8.15±0.88^{1a}	8.28±0.66^{1a}	8.75±0.70^{1a}	8.56±0.82^{1a}
	III	Se+Vit E	7.93±0.59^{1a}	13.50±1.78^{2b}	11.73±1.42^{2b}	11.38±1.0^{2b}	14.11±1.73^{2b}
GPX activity (U g^{-1} Hb)							
	I	Contr.	93.51±4.48^{1a}	98.38±5.88^{1a}	98.52±8.76^{1a}	90.90±6.17^{1a}	93.63±6.54^{1a}
	II	Se	103.75±7.74^{1a}	110.13±8.86^{1a}	132.01±4.39^{2,c}	160.50±7.11^{2b}	170.74±6.87^{2b}
	III	Se+Vit E	83.82±7.23^{1a}	84.43±6.66^{1a}	115.69±9.32^{1,2,c}	124.19±7.35^{2b}	134.21±5.62^{3bc}
SOD activity (U mL^{-1})							
	I	Contr.	145.0±15.09^{1a}	137.0±12.87^{1a}	142.5±12.9^{1a}	146.0±9.88^{1a}	147.5±12.69^{1a}
	II	Se	148.0±17.68^{1a}	153.5±9.29^{1a}	146.5±10.05^{1a}	150.0±11.11^{1a}	144.5±11.67^{1a}
	III	Se+Vit E	140.5±10.87^{1a}	146.0±13.88^{1a}	150.67±11.92^{1a}	146.5±6.84^{1a}	145.0±11.27^{1a}

Parameters	Groups	Treatments	Concentration/duration (weeks) 10	12	14	16
Serum α-tocopherol (μg mL^{-1})						
	I	Contr.	2.45±0.16^{1a}	2.35±0.20^{1a}	2.58±0.15^{1a}	2.44±0.17^{1a}
	II	Se	2.88±0.21^{1a}	2.78±0.11^{1a}	2.65±0.11^{1a}	2.57±0.13^{1a}
	III	Se+Vit E	5.24±0.19^{2b}	5.57±0.21^{2b}	5.36±0.17^{2b}	4.97±0.31^{2b}
β-carotene (μg dL^{-1})						
	I	Contr.	7.69±0.50^{1a}	6.99±0.68^{1a}	8.26±0.85^{1}	7.45±0.54^{1}
	II	Se	9.16±0.64^{1a}	8.28±0.60^{1a}	9.05±0.65^{1}	9.05±0.68^{1}
	III	Se+Vit E	11.52±0.75^{2b}	12.56±1.42^{2b}	11.39±0.73^{2b}	11.95±0.99^{2b}
GPX activity (U g^{-1} Hb)						
	I	Contr.	89.14±6.79^{1a}	92.47±6.77^{1a}	91.16±6.97^{1a}	90.69±7.47^{1a}
	II	Se	186.14±10.44^{2b}	179.31±8.81^{2b}	183.92±9.61^{2b}	182.97±6.45^{2b}
	III	Se+Vit E	125.23±6.89^{3bc}	150.4±7.25^{3b}	140.66±7.83^{3b}	141.37±7.9^{3b}
SOD activity (U mL^{-1})						
	I	Contr.	144.33±16.02^{1a}	136.5±13.2 9^{1a}	142.33±9.79^{1a}	148.0±12.47^{1a}
	II	Se	152.0±8.13^{1a}	140.5±10.87^{1a}	143.5±9.59^{1a}	144.51±12.09^{1a}
	III	Se+Vit E	148.83±7.36^{1a}	143.0±12.47^{1a}	141.5±7.47^{1a}	145.51±14.62^{1a}

±Standard Error; a,b,c Means within rows with different superscript letters differ ($p<0.05$); 1,2,3 Means within same column with different superscript numbers differ ($p<0.05$)

considered critical and indicative of marginal Se-deficiency, using category suggested by Ransel kit. The obtained results agree with those recorded by Pollock *et al.* (1994), Ramos *et al.* (1998), Abou-Zeina (2002) and Ceballos *et al.* (2003), who noted increased GPX-activity in ruminants following either Se alone or with Vit E supplement. The increase in the GPX-activity was less in ewes supplemented with Vit E/Se, which may be attributed to the fact that Vit E is known to spare the requirement for Se by preventing destruction of membrane lipids thereby, inhibiting the production of hydro peroxides and reducing the amount of GPX formed in the cell (McDowell, 1989).

As shown in Table 1, E-SOD activity didn't show any significant variation among control and/or within treated groups. Similar results were obtained by Walsh *et al.* (1993), who reported that either Vit E or Se had no effect on the activity of SOD in calves and suggested that SOD is a better indication of Copper-status. More information is required to establish the reference values for SOD-activity in sheep.

Figure 2, 3 and Tables 2 and 3, demonstrate total serum protein levels and its fractions electrophoretically in the three trials. The allover values either the tendency to decline or to increase were within the physiological levels recorded by kaneko (1989). No significant changes of clinical importance could be traced concerning total serum protein and albumin concentrations (Fig. 2A and B and Table 2), a result agree with Abou- Zeina (2002).

As shown in Fig. 2 C and Table 2, the Vit E/Se-supplement group had significantly higher total globulins ($p<0.05$) during the weeks 4, 8, 12, 14 than did the control and during the weeks 4, 8, 14 than did the Se- supplement. Moreover, the A/G ratio decreased significantly (Fig. 2 D) during the weeks 4 and 14 compared with that received Se only.

Figure 3 C and Table 3, show that the major changes in total globulins fractions were in the γ-globulins. The γ-globulins concentrations were significantly higher ($p<0.05$) in Vit E/Se- supplement group during the weeks 4, 6 and 8 compared with that of Se group and during the week 4 and onwards compared with control. The authors

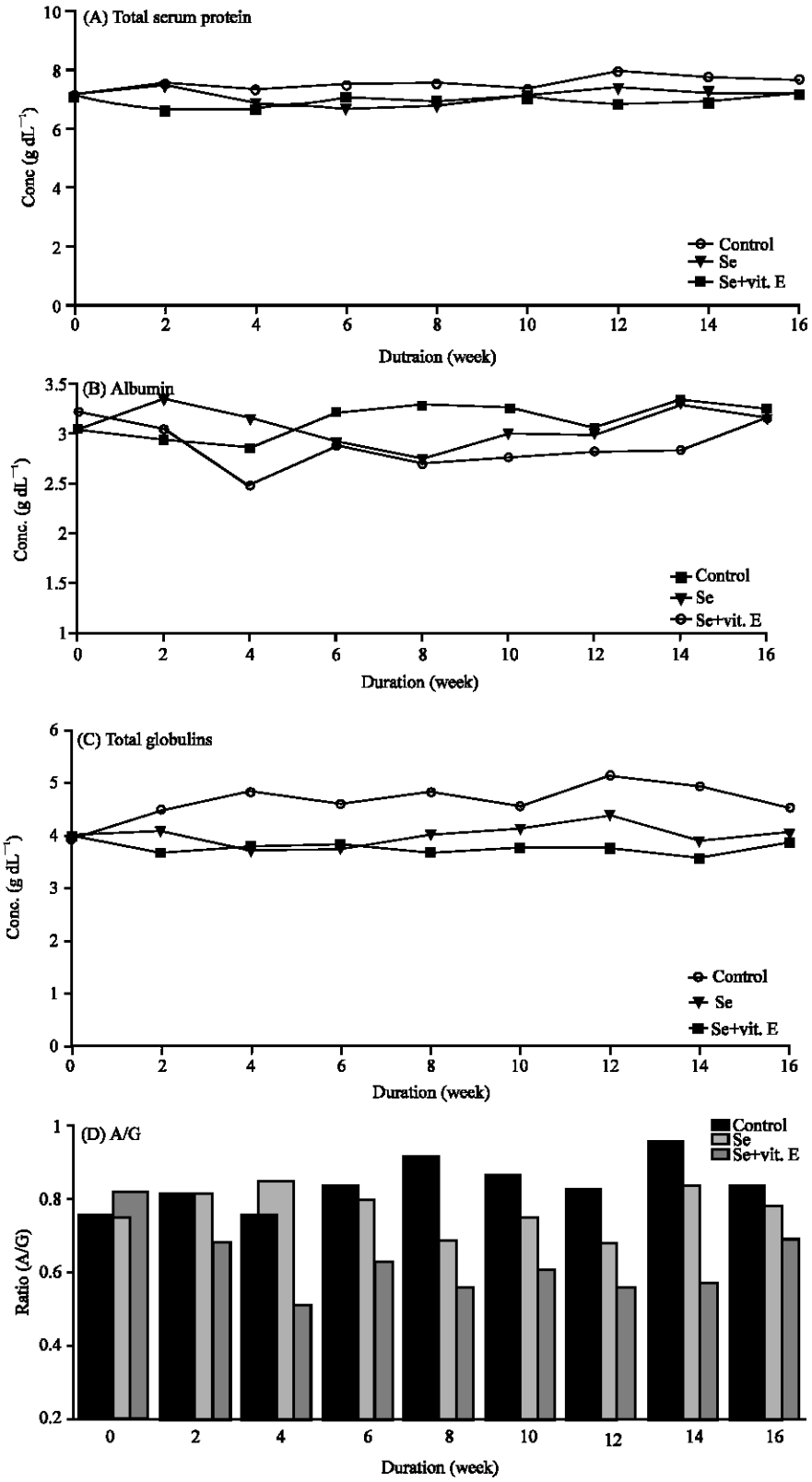

Fig. 2: Total serum protein, albumin and total globulins levels as well as A/G ratio in the blood serum of ewes treated with vitamin E and/or selenium (g dL^{-1})

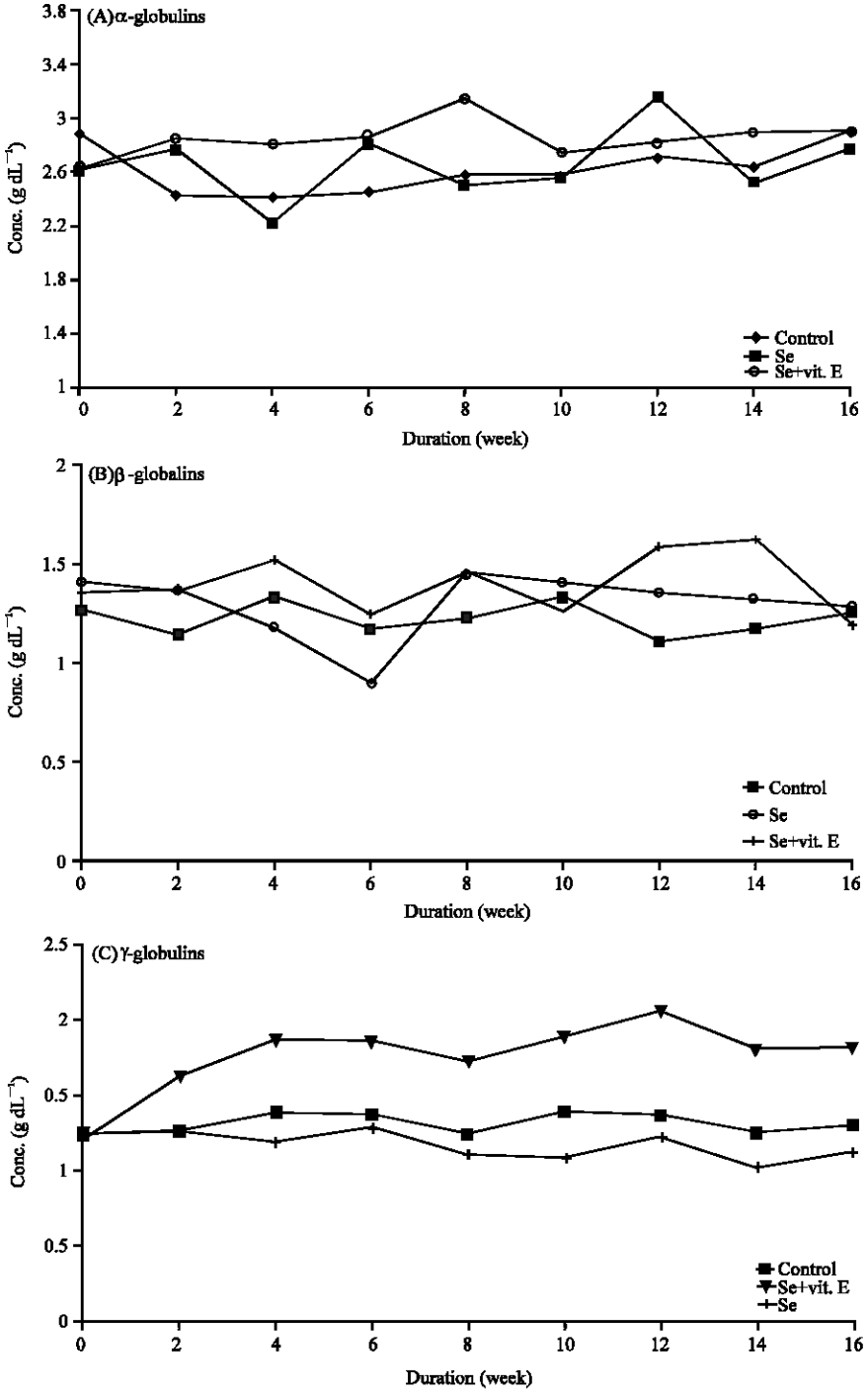

Fig. 3: Electrophoretic patterns of total serum protein of ewes treated with antioxidants (Vit E and/or Se)

attributed this behavior to the fact that animals were exposed to various antigenic agents, resulting in an increase in the Igs production. The Igs, being part of the γ-globulin complex, naturally leads to an increase in this fraction (Jain, 1993). Data from the present study elucidate that sheep-supplemented with Vit E/Se had increased Igs levels. Meanwhile, Se alone did not induce significant alteration in Igs levels. Present results agree with numerous studies which support the view that Vit E and Se together have an important beneficial effect on immunity than the administration of Se alone. For example, Pollock *et al.* (1994), Ramos *et al.* (1998), Hassan *et al.* (2001) and Kandil and Abou-Zeina (2005), who demonstrated a synergistic

Table 2: Total serum protein, albumin and total globulins in the blood serum of ewes treated with vitamin E and/or selenium

Parameters	Groups	Treatments	Concentration/Duration per 2 weeks 0	2	4	6	8
T.S.P (g dL^{-1})							
	I	Control	7.02±0.17^{1a}	6.58±0.37^{1a}	6.63±0.44^{1a}	7.03±0.18^{1a}	6.93±0.53^{1a}
	II	Se	7.05±0.42^{1a}	7.39±0.34^{1a}	6.84±0.32^{1a}	6.62±0.45^{1a}	6.73±0.18^{1a}
	III	Se+Vit E	7.14±0.25^{1a}	7.49±0.54^{1a}	7.30±0.52^{1a}	7.45±0.41^{1a}	7.49±0.52^{1a}
Albumin g dL^{-1} (%)							
	I	Control	3.03±0.30^{1a} (43.15)	2.93±0.05^{1a} (44.7)	2.85±0.49^{1a} (42.64)	3.20±0.31^{1a} (45.41)	3.27±0.49^{1a} (47.0)
	II	Se	3.04±0.31^{1a} (42.95)	3.33±0.25^{1a} (45.0)	3.14±0.25^{1a} (45.82)	2.90±0.25^{1a} (44.03)	2.73±0.29^{1a}(37.43)
	III	Se+Vit E	3.20±0.37^{1a} (44.71)	3.03±0.32^{1a} (40.36)	2.47±0.24^{1a} (33.80)	2.87±0.16^{1a} (38.50)	2.68±0.38^{1a}(35.58)
T. Globulins g dL^{-1} (%)							
	I	Control	3.99±0.25^{1a} (56.85)	3.66±0.41^{1a} (55.30)	3.78±0.11^{1a} (57.36)	3.83±0.18^{1a} (54.59)	3.67±0.41^{1a}(52.99)
	II	Se	4.01+0.11^{1a} (57.05)	4.06±0.13^{1a} (54.97)	3.70±0.19^{1a} (54.18)	3.72±0.44^{1a} (55.97)	4.0±0.19^{1a} (59.49)
	III	Se+Vit E	3.94±0.21^{1a} (55.29)	4.46±0.26^{1ac} (59.64)	4.83±0.34^{2ac} (66.19)	4.58±0.27^{1ac} (61.5)	4.81±0.15^{2bc}(64.42)
A/G	I	Control	0.76±0.12^{1a}	0.82±0.11^{1a}	0.76±0.15^{12a}	0.84±0.11^{1a}	0.92±0.19^{1a}
	II	Se	0.75±0.10^{1a}	0.82±0.05^{1a}	0.85±0.08^{1a}	0.80±0.13^{1a}	0.69±0.10^{1a}
	III	Se+Vit E	0.82±0.13^{1a}	0.68±0.05^{1ab}	0.51±0.04^{2a}	0.63±0.03^{1ab}	0.56±0.06^{1ab}

Parameters	Groups	Treatments	Concentration/duration per 2 weeks 10	12	14	16
T.S.P (g dL^{-1})						
	I	Control	7.0±0.27^{1a}	6.79±0.33^{1a}	6.88±0.39^{1a}	7.12±0.36^{1a}
	II	Se	7.09±0.43^{1a}	7.33±0.54^{1a}	7.15±0.23^{1a}	7.15±0.37^{1a}
	III	Se+Vit E	7.31±0.50^{1a}	7.93±0.36^{1a}	7.73±0.62^{1a}	7.65±0.58^{1a}
Albumin g dL^{-1} (%)						
	I	Control	3.24±0.27^{1a} (46.29)	3.04±0.15^{1a} (45.0)	3.32±0.18^{1a} (48.54)	3.23+0.15^{1a} (45.55)
	II	Se	2.98±0.34^{1a} (42.16)	2.97±0.34^{1a} (40.36)	3.27±0.29^{1a}(45.61)	3.13±0.32^{1a} (43.70)
	III	Se+Vit E	2.75±0.19^{1a} (37.67)	2.81±0.30^{1a} (35.50)	2.82±0.42^{1a} (36.16)	3.14±0.25^{1a} (41.0)
T. Globulins g dL^{-1} (%)						
	I	Control	3.76±0.29^{1a} (53.71)	3.75±0.38^{1a} (55.0)	3.56±0.47^{1a} (51.47)	3.88±0.35^{1a} (54.45)
	II	Se	4.11±0.47^{1a} (57.84)	4.37±0.25^{12a} (59.64)	3.88±0.12^{1a} (54.39)	4.02±0.10^{1a} (56.29)
	III	Se+Vit E	4.56±0.4^{1ac} (62.33)	5.12±0.38^{2bc} (64.51)	4.92±0.20^{2bc} (63.84)	4.51±0.33^{1ac} (58.99)
A/G						
	I	Control	0.87±0.12^{1a}	0.83±0.12^{1a}	0.96±0.16^{12a}	0.84±0.10^{1a}
	II	Se	0.75±0.15^{1a}	0.68±0.07^{1a}	0.84±0.10^{1a}	0.78±0.06^{1a}
	III	Se+Vit E	0.61±0.05^{1ab}	0.56±0.09^{1ab}	0.57±0.06^{2ab}	0.69±0.01^{1b}

±Standard Error; a,b,c Means within rows with different superscript letters differ ($p<0.05$); 1,2,3 Means within same column with different superscript numbers differ ($p<0.05$)

Table 3: Electrophoretic pattern of total serum protein of ewes treated with Vitamin E and /or Selenium)

Parameters	Groups	Treatments	Concentration/Duration per 2 weeks 0	2	4	6	8
α-globulins g dL^{-1} (%)							
	I	Control	1.5±0.08^{1a} (21.29)	1.26±0.18^{1a} (19.09)	1.25±0.12^{1a} (19.08)	1.27±0.10^{1a} (19.38)	1.34±0.11^{1a}(19.33)
	II	Se	1.36±0.21^{1a} (19.27)	1.44±0.29^{1a} (19.34)	1.15±0.24^{1a} (16.86)	1.46±0.18^{1a} (22.0)	1.30±0.20^{1a}(19.51)
	III	Se+Vit E	1.37±0.21^{1a} (19.56)	1.48±0.19^{1a} (19.81)	1.46±0.21^{1a} (19.87)	1.49±0.11^{1a} (20.04)	1.64±0.13^{1a}(22.08)
β-globulins g dL^{-1} (%)							
	I	Control	1.26±0.22^{1a} (17.89)	1.14±0.18^{1a} (17.12)	1.33±0.08^{1a} (20.08)	1.17±0.11^{1a} (16.67)	1.22±0.21^{1a}(17.68)
	II	Se	1.40±0.08^{1a} (19.98)	1.36±0.21^{1a} (18.49)	1.18±0.08^{1a} (17.38)	0.90±0.25^{1a} (13.36)	1.45±0.10^{1a}(21.62)
	III	Se+Vit E	1.35±0.12^{1a} (18.90)	1.37±0.05^{1a} (18.49)	1.52±0.19^{1a} (20.81)	1.24±0.03^{1a} (16.81)	1.46±0.22^{1a}(19.42)
γ-globulins g dL^{-1} (%)							
	I	Control	1.24±0.16^{1a} (17.66)	1.26±0.12^{1a} (19.10)	1.19±0.05^{1a} (18.21)	1.28±0.1^{1a} (18.28)	1.11±0.10^{1a}(15.98)
	II	Se	1.25±0.04^{1a} (17.8)	1.26±0.10^{1a} (17.14)	1.37±0.11^{1a} (19.94)	1.36±0.09^{1a} (20.59)	1.24±0.14^{1a}(18.36)
	III	Se+Vit E	1.19±0.24^{1a} (16.82)	1.61±0.21^{1ab} (21.34)	1.85±0.14^{2,b} (25.51)	1.84±0.12^{2b} (24.64)	1.71±0.09^{2b}(22.92)

Parameters	Groups	Treatments	Concentration/Duration per 2 weeks 10	12	14	16
α-globulins g dL^{-1} (%)						
	I	Control	1.34±0.17^{1a} (19.28)	1.41±0.20^{1a} (20.62)	1.37±0.14^{1a} (19.84)	1.51±0.31^{1a} (21.07)
	II	Se	1.33±0.29^{1a} (18.76)	1.65±0.17^{1a} (22.56)	1.31±0.19^{1a} (18.37)	1.44±0.09^{1a} (20.07)
	III	Se+Vit E	1.43±0.06^{1a} (19.74)	1.47±0.24^{1a} (18.56)	1.51±0.18^{1a} (19.53)	1.51±0.16^{1a} (19.77)
β-globulins g dL^{-1} (%)						
	I	Control	1.33±0.15^{1a} (19.0)	1.11±0.16^{1a} (16.27)	1.17±0.16^{1a} (16.93)	1.25±0.06^{1a} (17.63)
	II	Se	1.40±0.21^{1a} (19.71)	1.35±0.19^{1a} (18.72)	1.32±0.10^{1a} (18.39)	1.28±0.15^{1a} (18.10)
	III	Se+Vit E	1.26±0.13^{1a} (17.19)	1.59±0.22^{1a} (20.12)	1.62±0.10^{1a} (21.20)	1.19±0.09^{1a} (15.84)

Table 3: Continued

Parameters	Groups	Treatments	Concentration/Duration per 2 weeks 10	12	14	16
γ-globulins g dL^{-1} (%)						
	I	Control	1.08 ± 0.17^{1a} (15.43)	1.22 ± 0.19^{1a} (18.11)	1.02 ± 0.21^{1a} (14.68)	1.12 ± 0.10^{1a} (15.75)
	II	Se	$1.37\pm0.05^{1.2a}$(19.37)	$1.36\pm0.30^{1.2a}$(18.41)	1.25 ± 0.15^{12a} (17.63)	1.30 ± 0.15^{12a} (18.13)
	III	Se+Vit E	$1.87\pm0.23^{2.ab}$(25.41)	2.05 ± 0.30^{2b}(25.82)	1.79 ± 0.17^{2ab} (23.15)	1.80 ± 0.24^{2ab} (23.39)

±Standard Error; a,b,c Means within rows with different superscript litters differ ($p<0.05$); 1,2,3 Means within same column with different superscript numbers differ ($p<0.05$)

action between Se and Vit E. On the other hand, our results disagree with that of Larsen (1993) and Ponousis *et al.* (2001), who demonstrated that supplementation with Se alone was more beneficial than supplementation with both Se and Vit E on the production of Igs and antibodies. It has been suggested that Se can protect immune cells for a long period, whereas Vit E has an immediate effect (Pollock *et al.*, 1994). Although the effects of Se and Vit E on the immune system are still unclear, they are most probably attributable to their antioxidant properties. Further investigations are needed to fully clarify that issue.

On the basis of the results of the present study, it is suggested that parenteral supplementation of both Vit E and Se could significantly increase the concentrations of natural antioxidants (α-tocopherol, β-Carotene and GPX) in blood of sheep, hence ensures that they mount adequate immune globulins. It is therefore, concluded that both these nutrients should be administered to sheep in order to improve their immune competence.

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