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EFFECTS OF ADDITION OF SOME FAT SOLUBLE VITAMINS AND SELENIUM ON SEMEN QUALITY AND BLOOD COMPONENTS IN SUDANESE GEZIRA ECOTYPE RAMS

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ABSTRACT

Infertility is a common problem in Sudanese desert sheep, it is due to the severe deficiencies of some nutrients in natural pasture. This study had been carried out at the Faculty of Animal Production, University of Gezira. Twenty four mature and healthy Gezira ecotype rams (Shugor and Dubasi), two ecotype groups Shugor and Dubasi, (12 rams for each)., were brought from local markets with average weight 36.5 ± 2.65 kg, were used to evaluate the effect of fat soluble vitamins and selenium (Se) injection on semen quality and blood components. Each group was subjected to different treatments (A, B, C and D). The first group (A) served as control while the second group (B), third group (C) and fourth group (D) were injected intramuscularly with 3 ml/head Selenium + vitamin E, 3 ml/head vitamin AD3E and 3 ml/head plus 3 ml/head Selenium + vitamin E, respectively. Semen samples were collected monthly using an artificial vagina. Blood samples were analyzed monthly for Hb, RBCs, WBCs and PCV. Sera were analyzed for metabolic indicators plasma testosterone, total protein, albumin, glucose, cholesterol, triglyceride, calcium and phosphorus. The results showed that treated groups recorded higher ($P<0.05$) values in semen characteristics compared to control, and group (D) was superior. Blood parameters had no significant ($P<0.05$) values of red blood cells, white blood cells, hemoglobin and packed cell volume compared to control groups. Treated groups showed significant ($P<0.05$) differences in plasma, total protein, albumin, glucose concentrations, cholesterol, triglyceride and compared with control group. Plasma testosterone were recorded better ($P<0.05$) values in treated groups, moreover Shugor ecotype recorded the best results in all parameters. Generally Dubasi ecotype recorded the best results in most parameters investigated. The study conclude that when rams injected with antioxidant mainly selenium and fat soluble vitamins during summer season were performed better in semen quality and reproductive efficiency. Moreover Dubasi ecotype was more responsive to different treatments than the Shugor ecotype. Also, the current study recommends that treated rams with vitamin AD3E and Selenium injection improve the semen quality and the reproductive efficiency of rams in the Gezira State.

INTRODUCTION

Sudan, the African's country, has one of the largest livestock populations in the continent, which estimated at about (140.5), of which 41.84, 52.14, 43 and 4.4 million head of cattle, sheep, goats and camels respectively (MARF, 2012). Livestock in the Sudan satisfies the internal demand and leaves substantial excess for export, which represents about 22% of the country's total exports. Livestock industry is of great importance to Sudanese economy as it is one of the main sources of food, employment and foreign currency. In recent years, Sudanese sheep namely Sudan desert ecotype, has received great interest as an export commodity to the Arab countries. Ali (2003) mentioned that in spite of this economic importance most sheep are still raised under nomadic conditions with traditional methods of management and natural grazing (FAO, 1985). Over the same period, the number of sheep has grown at 2.8% per year, and so the proportion of sheep in Sudan's livestock population has re-mained constant at about 36%. Sheep therefore play an important social and economic role in the country, and are a valuable strategic resource for both local and export purposes.

Sheep occupies first place in terms of the census of animals in Africa and the Arab world and the increased need at the moment to pay attention to her upbringing to being a small size and a few food needs animals compared to cows as they can live and produce under

harsh environmental conditions. World sheep population is 1138.4 millions, about 206 millions are in Africa (FAO, 1995). Sudan sheep population is about 39.137.000 (Ministry of Animal Recourse 2011).The distribution of Sudanese sheep in the different states is as follows: Northern Kordofan (13.87%), South Kordofan(5.95%), Western Darfur(7.50%), Northern Darfour(7.22%), Gezira (4.75%), and Khartoum (0.85%) while the rest of states produce 59.86% (Ministry of Animal Recourse, 2008). In addition, they estimated sheep meat exportation during the periods between (1997 – 2002) as 705000 tons. (Ministry of Animal Recourse 2011).

Semen evaluation is a useful tool in the selection of males and ejaculates for assisted reproduction. Traditional evaluation techniques, based on the subjective assessment of parameters such as sperm motility and morphology, semen volume or concentration, have long been employed in the diagnosis of male sub fertility and sterility (Verstegen et al., 2002). An in vitro system that could accurately predict field fertility would facilitate stricter selection of AI rams with regard to the semen quality and would provide a valuable tool for increasing conception rate (Donovan et al., 2004).

Vitamins and minerals play an important role in the growth of animals and their reproductive performance. The antioxidants have defined as any substances that delay or inhibit oxidative damage to cellular molecule (Gutteridge et al., 1994). Ovarian inactivity hinders the productive and reproductive performances of farm animals and causes great economic losses, especially in small holder farms. whereas animals are exposed to a lot of stressful conditions. Reproductive performance of farm animals depends on adequate balanced levels of vitamins and essential minerals due to their important roles in cellular metabolism, maintenance and growth (Gutteridge et al., 1994).

Selenium is an anti-oxidant that works in conjunction with Vitamin E to prevent and repair cellular damage in the body. Selenium and/or Vitamin E deficiency has been shown to impair immune response. Selenium is also associated with thyroxin, a thyroid hormone that regulates metabolism, reproduction, circulation and muscle function. Selenium also protects the body from heavy metals by forming complexes to render them harmless. (Lemly, 2002). Most of the soils in the U.S. are marginal to deficient in selenium, but there are areas in the Western U.S. that are abundant in plants that accumulate selenium (for example locoweed). Severe clinical selenium deficiency can result in white muscle disease, characterized by stiffness and heart failure. Marginal selenium deficiencies can result in impaired fertility, silent heats, cystic ovaries and the birth of unthrifty kids with poor immunity. Selenium is highly toxic if excess amounts are consumed. Unfortunately, the amount of selenium required is very close to the toxicity level, thus great care must be taken when supplementing selenium. The maximum level of selenium that can be legally be fed to cattle in the United States is 3mg per head per day or 0.3 ppm in the total ration (dry matter basis). (Lemly, 2002).

MATERIAL AND METHODS

Experimental site: This study had been carried out at the Extension and Rural Development Centre (E. R. D. C), Faculty of Animal Production, University of Gezira (Elmanagil, Gezira State, Sudan). This site lies between latitudes 13.30 and 14.45 longitudinally and 32.45 and 33.15 horizontally at the centre of Sudan. The experimental period lasted for 16 weeks in the hot weather season (from 1st of April to end of July) during this period the highest and lowest temperature were obtained from Elmanagil from the experimental site and are predicted in table (1).

Table (1): The prevailing climatic conditions during the experimental period.

Temperature (°C)			
Time (months)	**Max.**	**Min.**	**Mean**
1	39.6	22.3	30.95
2	42.5	25.6	34.05
3	43.5	31.4	37.45
Mean ±SD	41.87±1.6	26.43±0.65	34.15±0.86

Exprimental Animals: Data were obtained from twenty four rams of two gezira ecotypes sheep (Dubasi and Shugor) at the range of age between 11 to 14 months at puberty with average initial body weight of 36.5 and 36.06 Kg for Dubasi and Shugor respectively.

Experimental ration: Ration was composed of a concentrate feed mixture (CFM), and groundnut hay (GH) according to Kearl (1982) requirements. Dietary allowances were offered twice daily at 7 am. and 5 pm. The CFM composed of 45% Sorghum, 20% Groundnut cake, 22% Wheat bran, 10% Groundnut hull, 1.5 Limestone and 1.5% common salt. Chemical analysis and nutritive value of the ration are presented in table (2). Water was freely available in water troughs whereas, multi mineral licking blocks were available for animals in the stalls.

Table (2): Chemical analysis of feedstuffs as fed and on DM basis.

Chemical analysis (%)	CFM		GH	
	As fed	DM basis	As fed	DM basis
Dry matter	92.7	100	94.2	100
Organic matter	80.5	89.4	76.9	81.7
Crude protein	15.2	16.1	6.2	7.1
Crude fibre	16.8	18.3	33.3	35.2
Ether extract	4.3	5.2	1.4	1.6
NFE	45.3	48.5	39.2	41.2
Ash	11.1	11.9	14.2	14.9

CFM: Concentrate feed mixture. GH: Groundnut hay.

Experimental Procedure: The experimental animals were divided randomly into four comparable groups (3 animals in each group/ecotype). The rams were housed in shaded pens (2x2m) each treatment (3 rams/ treatment). Thereafter, the rams within each group were subjected to one of the following treatments: Group (A) each ecotype: Served as a control group without any vitamins and selenium supplementation. Group (B) each ecotype: The rams were subjected to vitamin E + Selenium intramuscular injection at the rate of 3 ml/head weekly. Group (C) each ecotype: The rams were subjected to vitamin AD3E intramuscular injection at the rate of 3 ml/head weekly. Group (D) each ecotype: The rams were subjected to combination of vitamin E + Selenium and vitaminAD3E intramuscular injection at the rate of 3 ml vitamin E + Selenium and 3 ml vitamin AD3E intramuscular injection/ head weekly.

Each form of vitamin AD3E is a product of Supers Diana SL LTD, Barcelona Spain ®. Each ml. of injection vitamin AD3E contained 100,000 IU vitamin A. 50.000 IU vitamin D3 and 50 mg vitamin E, while each ml. of and vitamin E + Selenium which is a product of Adwia Co. Egypt ®. Fig. (1): contained 150 mg vitamin E acetate and 1.67 mg Sodium selenite (Selenium).

Semen Collection and Evaluation: Semen was collected monthly from each ram in each group. The rams were trained for semen collection into an artificial vagina**,** Semen samples were evaluated using standard methods Boundy(1993). The ejaculate volume (EV) was measured in a graduated tube. The sperm mass motility (SMM) was evaluated by transferring a drop of undiluted semen to a warm slide, placing a cover slip and observing under a microscope (x 40). The assessment of SMM was performed on a scale from 0 (immotile) and 5 (vigorous motility). The sperm individual motility (SIM) was estimated using a scale from 1-10 representing increments of 10%. Sperm cell concentration (SCC) was determined after diluting semen with a 0.05% formaldehyde saline solution (1: 400) and use haemocytometer examined under the microscope (x45) adopting the method for RBC counting. The proportion of live and dead spermatozoa and abnormal sperms percent were determined using Nigrosin-Eosin staining technique by counting 100 spermatozoa under oil immersion objective (x 1000) random fields. Indicator papers (E. Merck Company, Darmstadt, Germany) with the range of 5.2 to 8.0 were used to determine the pH of semen samples.

Blood samples: Blood samples were collected monthly in the morning (08:00hr), before feeding. About 5 ml of blood were drained from the jugular vein using plastic syringes. Collected blood was then transferred into vacuumed capillary tubes in order determine the immediate measurements of haematology (Haemoglobin concentration (Hb), White blood cells (WBCs) count, Red Blood Cells (RBCs) count and Packed cell volume (PCV). And Blood metabolites (blood glucose, total protein, albumin, cholesterol, triglyceride, calcium and phosphorus levels). After coagulation, blood samples were centrifuged for 10 minutes (Hettich EBA 20 – Germany) at 2000 rpm at room temperature, and then the serum was collected and stored at −20°C for analysis. (Blood glucose, total protein, albumin, cholesterol, triglyceride, calcium and phosphorus levels, were determined spectrophotometrically using commercial kits. Blood parameter values were expressed as milligrams per 100 ml. (Kostner et al. 1979). Direct radioimmunoassay technique was adopted for determination of plasma testosterone using kits of "Diagnostic Products Corporation, Los Angeles, USA". biuret method for determination of Blood metabolites was used (Gornall et al 1949, Yaung et al 1975, Tietz, 1986) –Cat.

Statistical analysis: The collected data were subjected to analysis of variance using SPSS 21.0 program package (SPSS, 2010) the model used was GLM and the means were separated by Duncan's multiple range tests (Petrie and Watson, 1999).

RESULTS

Interaction effect of vitamin AD3E and selenium treatments, ecotypes and collection intervals (thee periods) on semen characteristics of Gezira ecotypes rams

Table (3) showed the effect of fat soluble vitamins (AD3E) and selenium on semen quality on Gezira ecotype rams during experimental periods. Data indicated significant ($p<0.05$) differences among the four experimental groups of each ecotype for all traits studied.

Concerning the volume were significant ($p<0.05$) differences among treatments in Shugur at two and three month and in Dubasi at all periods. Generally treatment D recorded the highest estimates. For sperm concentration there was no significant ($p<0.05$) diference in the first period for both ecotypes. While significant ($p<0.05$) differences exist among the four treatments in both breeds in all periods. Generally treatment D recorded the highest estimates and the control group obtaind the lowest values.

For sperm out put and sperm motility the differences occurred in all periods with exception of the first period in Shugor for first trait and Dubasi in the secod trait. treatment D scored the significantly ($p<0.05$) higher estimates for the all traits in both breeds and in all the rest periods. For dead sperms the best results were obtained by treatment D for both ecotypes while the control group recored the higher mortility of sperms.

The results of all parameters studied in this experimental study expressed similar increased pattern with the increasing with the periods except the pH which maintained a stable status during those periods.

Table (3): Interaction effect of vitamin AD_3E and selenium treatments, ecotypes and collection intervals on semen characteristics of Gezira ecotypes rams.

Ecotypes	Periods	Treatments				SEM	Sig
		A	B	C	D		
Ejaculate volume (ml)							
Shugor	0	0.90	0.86	0.87	0.87	0.08	NS
	1 month	1.07	1.30	1.13	1.30	0.11	NS
	2 month	1.17^{b}	1.50^{a}	1.33^{ab}	1.43^{a}	0.06	*
	3 month	1.13^{d}	1.57^{b}	1.37^{c}	1.70^{a}	0.04	*
Dubasi	0	0.83^{a}	0.90^{a}	0.63^{b}	0.90^{a}	0.06	*
	1 month	1.13^{c}	$1.07b^{c}$	1.27^{b}	1.60^{a}	0.07	*
	2 month	0.93^{c}	1.20^{b}	1.53^{a}	1.70^{a}	0.08	*
	3 month	1.20^{c}	1.33^{c}	1.70^{b}	1.97^{a}	0.08	*
Sperm concentrate ($x10^{9}$/ml)							
Shugor	0	1.01	1.02	0.99	1.12	0.14	NS
	1 month	1.93^{b}	2.08^{ab}	2.20^{ab}	2.43^{a}	0.14	*
	2 month	2.06^{c}	2.29^{bc}	2.47^{ab}	2.67^{a}	0.10	*
	3 month	2.06^{c}	2.45^{b}	2.56^{b}	2.83^{a}	0.08	*
Dubasi	0	1.88	1.98	1.89	1.85	0.08	NS
	1 month	1.91^{b}	2.47^{a}	2.38^{a}	2.53^{a}	0.07	*
	2 month	1.94^{d}	2.53^{b}	2.35^{c}	2.75^{a}	0.04	*
	3 month	2.04^{c}	2.77^{a}	2.46^{b}	2.93^{a}	0.05	*
Sperm output (10^{9}/ ejaculate)							
Shugor	0	1.62	1.52	1.61	1.71	0.14	NS
	1 month	2.09^{b}	2.72^{ab}	2.49^{ab}	3.15^{a}	0.30	*
	2 month	2.41^{b}	3.43^{a}	3.29^{a}	3.83^{a}	0.19	*
	3 month	2.33^{c}	3.83^{b}	3.49^{b}	3.81^{a}	0.17	*
Dubasi	0	1.57^{ab}	1.78^{a}	1.20^{b}	1.67^{ab}	0.12	*
	1 month	1.97^{c}	2.63^{b}	3.01^{b}	4.05^{a}	0.15	*
	2 month	1.81^{d}	3.03^{c}	3.61^{b}	4.49^{a}	0.16	*
	3 month	2.46^{c}	3.70^{b}	4.17^{b}	5.76^{a}	0.23	*
Sperm Motility %							
Shugor	0	52.00^{c}	58.00^{b}	65.33^{a}	58.67^{b}	1.34	*
	1 month	55.33^{b}	67.66^{a}	68.67^{a}	72.00^{a}	1.32	*
	2 month	59.67^{c}	72.33^{b}	74.67^{b}	79.33^{a}	1.24	*
	3 month	61.33^{b}	77.33^{a}	76.00^{a}	79.33^{a}	1.19	*
Dubasi	0	58.00	57.00	56.67	58.67	1.84	NS
	1 month	56.67^{b}	58.33^{b}	59.00^{b}	64.33^{a}	0.82	*
	2 month	58.00^{c}	70.67^{a}	65.67^{b}	72.00^{a}	1.49	*
	3 month	59.67^{d}	67.33^{c}	72.67^{b}	76.67^{a}	0.78	*
Dead sperm %							
Shugor	0	18.67	17.33	18.00	17.33	0.87	NS
	1 month	16.00^{b}	14.66^{b}	15.00^{b}	11.00^{a}	0.67	*
	2 month	15.00^{b}	14.33^{b}	12.33^{b}	8.66^{a}	0.87	*

	3 month	11.33^{c}	8.33^{b}	6.67^{b}	4.00^{a}	0.82	*
Dubasi	0	19.00	18.67	16.66	17.00	1.40	NS
	1 month	10.33^{c}	9.33^{c}	8.00^{b}	4.33^{a}	0.41	*
	2 month	13.67^{c}	5.66^{b}	5.00^{b}	2.67^{a}	0.65	*
	3 month	10.00^{c}	4.33^{b}	4.66^{b}	1.67^{a}	0.58	*

a-d Means within rows with no common superscripts are significantly different.
The values in the same row with different superscripts are significantly different (P<0.05).
A: Control group. B: group treated with Selenium + vitamin E. C: group treated with vitamin AD3E.
D: group treated with Selenium + vitamin E and vitamin AD3E. *: significantly

Interaction effect of vitamin AD3E and selenium treatments, ecotypes and collection intervals on blood parameters (haematology). of Gezira ecotype rams

Table (4) shows average hematological values of experimental rams (Shugor and Dubasi) as affected by different treatments. Significant (p<0.05) differences between treatments is found only for hemoglobin concentration at three month in Shugor. For white blood cell count at first and second months in Shugor and at second and third month in Dubasi. For both red blood cell count and packed cell volume at second month in Shugor.

Table (4) Interaction effect of vitamin AD3E and selenium treatments, ecotypes and collection intervals on blood parameters (haematology). of Gezira ecotypes rams.

Ecotypes	Periods	Treatments				SEM	Sig
		A	B	C	D		
Hemoglobin (g/dl)							
Shugor	0	10.33	9.13	9.60	9.27	0.70	NS
	1 month	9.67	8.93	9.70	9.17	0.44	NS
	2 month	10.27	9.40	9.60	9.26	0.31	NS
	3 month	9.87^{ab}	10.40^{a}	9.63^{ab}	9.13^{b}	0.36	*
Dubasi	0	9.93	9.63	9.93	9.40	0.54	NS
	1 month	9.53	9.56	9.90	8.57	0.51	NS
	2 month	10.10	8.53	9.70	8.73	0.51	NS
	3 month	10.17	9.37	9.90	9.23	0.47	NS
W.B.Cs x10^{3}/ml							
Shugor	0	15.47	15.50	15.53	13.30	1.51	NS
	1 month	17.30^{a}	13.53^{b}	17.10^{a}	16.53^{a}	0.89	*
	2 month	12.43^{b}	16.90^{a}	15.77^{ab}	17.00^{a}	1.30	*
	3 month	13.66	15.80	15.90	13.90	2.02	NS
Dubasi	0	10.33^{b}	12.23^{ab}	17.33^{a}	15.33^{ab}	1.68	*
	1 month	12.20	16.50	16.03	15.23	1.46	NS
	2 month	10.53^{b}	18.60^{a}	18.13^{a}	13.50^{b}	1.21	*
	3 month	10.17^{b}	15.40^{ab}	17.00^{a}	13.87^{ab}	1.67	*
R.B.Cs x10^{6}/ml							
Shugor	0	6.05	6.27	6.42	5.10	0.57	NS
	1 month	6.00	6.81	6.62	5.98	0.39	NS
	2 month	6.55^{ab}	7.55^{a}	7.09^{ab}	6.05^{b}	0.34	*
	3 month	7.29	8.05	7.31	6.50	0.46	NS
Dubasi	0	6.61	6.03	6.95	6.35	0.45	NS
	1 month	6.19	7.25	7.13	6.31	0.34	NS
	2 month	7.46	6.48	7.58	7.01	0.46	NS
	3 month	8.03	7.04	8.07	7.38	0.62	NS
Packed cell volume %							
Shugor	0	19.73	20.43	21.00	19.07	1.68	NS
	1 month	19.70	22.77	21.93	19.70	1.41	NS
	2 month	21.47^{ab}	24.97^{a}	23.07^{ab}	19.40^{b}	1.21	*
	3 month	24.30	26.67	24.03	21.00	1.80	NS
Dubasi	0	22.03	20.03	22.87	20.97	1.78	NS
	1 month	21.70	24.47	24.13	21.57	1.33	NS
	2 month	24.93	21.17	25.30	23.23	1.78	NS
	3 month	27.07	22.97	27.20	24.97	2.27	NS

a-b Means within rows with no common superscripts are significantly different.

The values in the same row with different superscripts are significantly different (P<0.05).
*: significantly
A: Control group. B: group treated with Selenium + vitamin E. C: group treated with vitamin AD3E.
D: group treated with Selenium + vitamin E and vitamin AD3E.
Sig: Significant. SEM: Standard error means. NS: no Significant

Interaction effect of vitamin AD3E and selenium treatments, ecotypes and collection intervals (three periods) on serum blood components (biochemical) of Gezira ecotype rams

The data in table (5) and (6) shows the effect of fat soluble vitamins and selenium on blood biochemical profile of studied ecotypes.

Estimates of all studied blood parameters increased gradually with advancement of age. The total protein, albumin and glucose were not significantly ($p<0.05$) different among the experimental periods except the last month. For total protein Treatment D was significantly ($p<0.05$) higher than other treatments. For albumin content the control group was significantly ($p<0.05$) lower than the other treatments. Whereas the glucose content was generally higher in treatment D. Cholesterol contents exhibited significant ($p<0.05$) differences in second and third months in both ecotypes where treatment groups were generally higher than control group. For triglycerides and calcium significant ($p<0.05$) differences were found in Dubasi ecotype at first, second and third month and both the control and C groups reported the highest estimates. On the other hand phosphorus was significantly ($p<0.05$) higher in C group in Shugor at at first, second and third month. For Testosterone there are significant ($p<0.05$) differences among treatment in all months except in the second month whereas D group was significantly ($p<0.05$) different from other groups in Dubasi at last month.

Table (5) Interaction effect of vitamin AD3E and selenium treatments, ecotypes and collection intervals on serum blood componants (biochemical) of Gezira ecotypes.

Ecotypes	Periods	Treatments				SEM	Sig
		A	B	C	D		
Total protein (g/dl)							
Shugor	0	6.19	6.23	6.23	6.15	0.07	NS
	1 month	6.23	6.26	6.25	6.23	0.12	NS
	2 month	6.32	6.31	6.28	6.42	0.16	NS
	3 month	6.29^{b}	6.45^{ab}	6.42^{ab}	6.58^{a}	0.07	*
Dubasi	0	6.21	6.28	6.29	6.22	0.05	NS
	1 month	6.34	6.31	6.30	6.30	0.10	NS
	2 month	6.39	6.38	6.34	6.37	0.13	NS
	3 month	6.39^{b}	6.50^{b}	6.50^{b}	6.62^{a}	0.03	*
Albumin (g/dl)							
Shugor	0	4.14	4.21	4.20	4.17	0.03	NS
	1 month	4.18	4.23	4.26	4.26	0.04	NS
	2 month	4.25	4.29	4.32	4.29	0.04	NS
	3 month	4.41^{b}	4.37^{b}	4.36^{b}	5.60^{a}	0.35	*
Dubasi	0	4.17	4.22	4.24	4.21	0.03	NS
	1 month	4.25	4.27	4.28	4.29	0.04	NS
	2 month	4.29	4.31	4.36	4.34	0.03	NS
	3 month	4.33^{b}	4.41^{a}	4.40^{a}	4.45^{a}	0.02	*
Glucose (mg/dl)							
Shugor	0	49.92	50.56	48.67	51.54	1.26	NS
	1 month	51.45	53.41	51.74	52.55	1.69	NS
	2 month	52.15	55.50	55.59	55.59	1.36	NS
	3 month	49.80^{b}	56.44^{ab}	60.09^{a}	58.83^{a}	2.26	*
Dubasi	0	41.15	42.51	41.70	42.47	1.69	NS
	1 month	45.95	47.69	46.48	49.62	1.12	NS
	2 month	47.59^{b}	52.22^{a}	51.34^{ab}	55.69^{a}	1.35	*
	3 month	50.88^{c}	54.98^{b}	54.53^{b}	58.47^{a}	0.89	*
Cholesterol (mg/dl)							
Shugor	0	41.54	42.27	43.04	40.41	2.09	NS
	1 month	42.49	44.77	43.58	43.47	1.09	NS
	2 month	43.63^{b}	47.53^{ab}	47.58^{ab}	49.59^{a}	1.37	*
	3 month	45.60^{c}	51.66^{b}	53.52^{ab}	55.73^{a}	0.83	*

Dubasi	0	46.62	45.73	44.58	45.55	0.70	NS
	1 month	49.57	52.48	49.50	48.53	0.52	NS
	2 month	51.40^{b}	56.63^{a}	53.58a^{b}	52.53^{ab}	1.25	*
	3 month	53.66^{b}	59.60^{a}	56.47^{ab}	55.41^{b}	1.09	*
Triglyceride (mg/dl)							
Shugor	0	33.37^{ab}	43.35^{ab}	30.91^{b}	53.18^{a}	6.29	*
	1 month	44.98	43.77	47.06	52.36	3.30	NS
	2 month	51.76	54.06	58.24	53.25	3.71	NS
	3 month	47.67	44.71	42.06	45.78	3.29	NS
Dubasi	0	41.89	38.82	47.34	49.79	3.56	NS
	1 month	47.59^{ab}	43.75^{b}	54.63^{a}	52.58^{ab}	2.88	*
	2 month	58.49^{a}	53.63^{a}	43.88^{b}	56.22^{a}	2.91	*
	3 month	53.63^{a}	43.88^{b}	56.22^{a}	55.40^{a}	2.70	*

a-d Means within rows with no common superscripts are significantly different.
The values in the same row with different superscripts are significantly different (P<0.05). *: significantly

Table (6) Interaction effect of vitamin AD3E and selenium treatments, ecotypes and collection intervals on serum blood componants (biochemical) of Gezira ecotype rams.

Ecotypes	Periods	Treatments				SEM	Sig
		A	B	C	D		
Calcium (g/dl)							
Shugor	0	15.81	15.76	16.08	16.22	0.84	NS
	1 month	11.31^{a}	8.26^{b}	10.00^{ab}	9.53^{ab}	0.62	*
	2 month	10.65	10.17	9.95	10.55	0.49	NS
	3 month	10.22	8.50	9.48	9.56	0.70	NS
Dubasi	0	14.34	15.50	13.09	14.98	1.46	NS
	1 month	10.16^{ab}	10.59^{ab}	10.93^{a}	9.27^{b}	0.45	*
	2 month	10.05^{ab}	9.80^{b}	11.34^{a}	10.58^{ab}	0.44	*
	3 month	9.76^{b}	9.26^{b}	10.53^{a}	9.53^{b}	0.43	*
Phosphorus (g/dl)							
Shugor	0	4.62	5.61	4.94	5.09	0.47	NS
	1 month	3.51^{b}	5.25a	3.68^{b}	4.75^{ab}	0.45	*
	2 month	4.34^{b}	6.78a	6.06^{ab}	6.42^{ab}	0.62	*
	3 month	5.28^{b}	7.25a	6.96^{a}	5.49^{b}	0.43	*
Dubasi	0	5.43	4.49	5.36	3.68	0.56	NS
	1 month	4.93	4.39	4.75	5.29	0.61	NS
	2 month	5.63	4.98	5.59	5.12	0.40	NS
	3 month	6.53	6.02	6.22	6.57	0.33	NS
Testosterone (ng/ml)							
Shugor	0	1.18^{b}	1.42a	1.33^{a}	1.37^{a}	0.04	*
	1 month	1.31^{b}	1.45a	1.42^{ab}	1.40^{ab}	0.04	*
	2 month	1.42	1.47	1.47	1.51	0.03	NS
	3 month	1.46^{b}	1.51b	1.57^{a}	1.59^{a}	0.02	*
Dubasi	0	1.03	1.00	1.00	1.18	0.15	NS
	1 month	1.14	1.10	1.16	1.16	0.09	NS
	2 month	1.16	1.25	1.36	1.37	0.07	NS
	3 month	1.26^{c}	1.39^{b}	1.43^{b}	1.56^{a}	0.04	*

a-b Means within rows with no common superscripts are significantly different.
The values in the same row with different superscripts are significantly different (P<0.05).
*: significantly
A: Control group. B: group treated with Selenium + vitamin E. C: group treated with vitamin AD3E.
D: group treated with Selenium + vitamin E and vitamin AD3E.
Sig: Significant. SEM: Standard error means. NS: no Significant.

DISSCUSION

Semen characteristics: Semen volume is one of the important factors in semen evaluation and reproduction performance in the males (Arash, et al 2006). The results of semen characteristics in this study were shown in table (3). The observed increase in the ejaculate volume in group B (Selenium + vitamin E), C (vitamin AD3E) and

D (Selenium + vitamin E and vitamin AD3E) compared with the control group A might be due to the role of vitamin A and vitamin E in maintaining the testes weight within the normal limits and in preservation of the epididymis and sex organs which contribute in producing seminal plasma (Rao and Raja, 1977). The present results agreed with those obtained by Khalid and Ismail (2006); El-Shahat, et al. (2007) and Belal (2009) Who reported that supplementation of vitamin A increased the seminal volume in rams. Also, Khalid and Ismail (2006) reported that semen characteristics were markedly improved when carotene or vitamin A was supplemented to animals. In general obtained results were similar to those reported by Suhair, et al (2012), in Sudanese desert rams and higher than of the previous researchers Arach, et al., (2006) and Ahmed (2012) for Awassi rams.

Sperm cell concentrations were significantly increased in B, C and D groups compared to control group. This effect may be due to the positive effect of vitamins and selenium on the seminiferous tubules leading to normal functioning. This implies that antioxidants, Se + vitamin E, which act synergistically, should be administered jointly (Gabryszuk, 1994). Those results were in agreement with those obtained by Suhair, et al (2012), who reported similar results for Sudanese desert rams. On the other hand the obtained results are lower than that values reported by Arach, et al., (2006) and higher than that the results reported by Hassan., (2010) in Sudan desert rams. Positive effects of Se or Se with vitamin E on fertility were observed by other authors (Scales, 1974; Mihajlovic et al., 1991; Koyuncu et al., 2006),

The spermatogenic output in group B, C and D recoreded higher estimates compared to the control group (A). These results agreed with those reported by Abd El-Latif (2001) and Youssef et al., (2005). Moreover a positive effect of selenium on sperm concentration and morphology has been confirmed also by other authors (Liu et al., 1982; Marin-Guzman et al., 1997).

The sperm motility of the rams in both breeds (Shugor and Dubasi), was significantly ($P<0.01$) different among the experimental groups. Estimates of the trait is higher in treatments compared with the control group. These results agreed with those reported by Suhair, et al (2012). The improvement of sperm motility by administration of vitamins and selenium may be due to their effects on the epithelial cell of male reproductive tract which are responsible for acquiring the spermatozoa the progressive motility (Rabie, 1992). In the present study the results are lower than those values reported by Ahmed, (2011) and higher than that results reported by (Hassan. 2010) in Sudan desert rams. Such discrepancy might be due to differances in managment and environmental condations.

The percentage of dead sperms was significantly different ($P>0.05$) among the experimental groups. Estimates tend to decrease from the control group and reached its lowest value in group D. Those results may be due to the positive effect of vitamin A and selenium on ram's fertility. Marin –Guzman (1997) demonstrated that Se. has a role in the morphology of the sperm tail and possibly in its metabolism. The role of vitamin E as suggested by Brzezinska-Slebodzinska et al., (1995) is that it acts as antioxidant in semen and peroxide damage can alter the morphology of the sperm. The present results agreed with those obtained by Khalil, (2010) whow used vitamin AD3E injection in buffalo male calves but lower than values obtained by Ahmed (2011) who investigate the influence of vitamin E and selenium on semen characteristics and viability in Awassi rams.

Sperm abnormality was significantly ($P>0.05$) higher in the control group than in the other groups. Kupfer et al., (1986) and Ibrahim et al., (1996) indicated that vitamin A supplementation led to reduction in the percentage of abnormal spermatozoa. Also, vitamin A deficiency in bulls resulted in pronounced increase in total sperm abnormalities. This may be due to degeneration in the germinal epithelium of the testes (Hafez, 1987).

Blood haematology: Previous studies had shown that, the composition of blood in animals is affected by various factors, such as seasonal change in environment elements, nutritional status, water balance, physiological state and age of animals (Anderson, 1980). In this study estimates for blood RBCs counts, WBCs counts, Hb and PCV % generally were not significantly different in all treatments. This is in agreement with Elbashier and shadia (2006) but the estimates were lower than the results reported by Wahbi, et al., (1976) and Hind (2007) for Sudanese cows. However, the generally lower blood RBCs counts and PCV % in the current study might be due to different treatments used in this study (Edward, et al 1955).

Blood biochemical's: Table (5) shows no significant differences ($P>0.05$) was observed among all treatments for total protein, albumin and Triglyceride in all periods. These results were in agreement with those obtained by Ahmed (2014) who reported that in goat all serum metabolites values were not significantly ($p>0.05$) different from the control. But these findings disagreed with those obtained by Khalil, et al (2010). The differences in values of the traits among the experimental periods were significant ($p>0.05$). This might be attributed to differences treatments supplies to the animals with the requirement of energy and production, (Khaled et al., 2006). The results showed that there are significant increases in glucose and cholesterol levels in treated groups compared to the control group. These results agreed with those reported by Khalil, et al (2010) in buffalo calves. The reported increase in glucose and cholesterol in supplemented groups of current study could be attributed to the increase in propionate production in rumen and enhanced propionate metabolism by the liver (Elliot, 1980). Obtained results were in a disagreement with results obtained by Badawy

(2003). The highest estimate for calcium was recorded in Shugor and whiles the highest estimate for phosphorus was obtained in Dubasi. These results agree with the report in cows obtained by Harrison et al. (1995).

The effects of fat soluble vitamins (AD3E) and selenium injection treatments during experimental periods on serum testosterone hormone of Shugor and Dubasi ecotype were presented in table (6). Testosterone level in blood of rams for all groups was increased gradually toward the end of the experimental period and this increase was in line with age advances. Such increment might be due to the synergtic affect of vitamin E and vitamin A with the selenium and their role in ameliorating the cell condition for the hormonal secration. These results may be supported by the findings of Khalil, et al (2010). The level of increase for testosterone concentrations was almost parallel for all treated groups throughout the growth period. Obtained estimates were higher than those observed by Abd El-Latif (2001) who found that selenium and vitamins supplementation increased testosterone concentration than that of control group. Also, Youssef et al, (2005) found that vitamin AD3E treatment injection increased concentrations of testosterone. Abd El-Latif (2001) found that testosterone concentration in serum was not significantly influenced by vitamin and selenium treatment in Blade rams. On the other hand, Vitamin E and selenium affect reproductive tissue through their antioxidant roles as well as involvement in prostaglandin synthesis, (Mistry, et al 2012). It is known that there is a positive correlation between testosterone concentration and sexual organs in males. Under the conditions of this experiment, semen quality improved after treatment with Vit AD3E or/in combination with Se and vitamins. This improved in semen quality combined with increased testosterone concentration leads to a direct effect on semen volume. Kaur and Bansal (2004) demonstrated that the levels of testosterone, FSH, LH were significantly reduced during Se deficiency, which has been implicated in testicular dysfunction. Yousef et al. (2003) demonstrated that the treatment of rabbits with Vit E significantly increased reaction time in the libido test. While Kaur and Bansal (2004) demonstrated that levels of plasma testosterone and corticosterone in male rats given a diet deficient in Vit E for 130 days were significantly lower than those in rats given the same diet supplemented with Vit E. the findings in the present study are in agreement with those of other authors who showed that Vit E and Se supplementation leads to improved sperm motility and increased percentage of normal sperm (Yousef et al., 2003).

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