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EFFECT OF TREATMENT WITH NANO-Se AND VITAMIN E ON SEMEN QUALITY AND SOME BLOOD PARAMETERS OF BUCK RABBITS

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ABSTRACT

This study aimed to investigate the effects of Nano-selenium (Nano-Se), organic selenium (Org-Se), vitamin E and their combination on semen quality and some blood parameters of New Zealand White bucks rabbits (NZW). A total number of 24 NZW with 2671± 185 initial body weight and 24 weeks of age were used in the study. The bucks were randomly allotted to three experimental main groups. The 1st group fed basal diet and served as a control. The 2nd group fed basal diet and orally treated with 0.5 mg Org- Se / kg body weight (BW). The 3rd group fed basal diet and orally treated with 0.5 mg Nano-Se /kg BW. The experimental groups were divided according to Vitamin E administration into two sub groups. The 1st sub group was left as a control (without vitamin E administration) and the 2nd sub group orally treated with 250 mg Vit. E/ kg Bw. Physical semen characteristics and hematological and biochemical blood parameters were studied.

Mean values of physical semen characteristics were significantly higher (P < 0. 01) for buck rabbits fed diets with selenium sources and vitamin E as compared with those fed on basal diet (control). RBCs, WBCs, blood platelets, hematocrit, MCV and MCHC, were within the normal physiological range and the differences were not significantly affected by treatment with selenium sources. However, Hb, MCH and lymphocytes percentages were significantly increased (P < 0.05 and P < 0.01) for buck rabbit's fed basal diet with Nano selenium. The mean values of RBCs, Hb, hematocrit, MCHC and lymphocytes percentages were significantly higher (P < 0.05 and < P 0.01) in buck rabbits fed basal diet with vitamin E than un-treated rabbits. However, the mean values of blood platelets and neutrophils percentages were significantly higher (P <0.05 or P<0.01) in buck rabbits fed basal diet without vitamin E than those fed diet with vitamin E. Total protein and globulin were significantly increased (P<0.05 and P < 0.01) while, albumin and

AL/GL ratio, were not significantly affected by selenium sources. Total protein was significantly improved (P<0.01) in buck rabbits fed basal diet with vitamin E when compared with un-treated with vitamin E. Total cholesterol concentrations and triglycerides were significantly lower (P<0.05 or P < 0.01) for rabbits fed basal diet with selenium sources or vitamin E than control group. ***.*** *ALT was increased (P < 0.01) in rabbits fed basal diet with Nano selenium when compared with the other groups. The mean values of creatinine and blood urea-N were not significantly affected by selenium sources and Vit. E treatment. Testosterone concentrations in seminal plasma and blood serum were significantly increased (P < 0.05 and P < 0.01) in buck rabbits fed basal diet and treated with Nano selenium group compared with the other groups. The mean values of testosterone concentration in seminal plasma and blood serum were insignificantly affected by treatment with Vit E. The highest values of testosterone concentration in seminal plasma and blood serum were recorded with buck rabbits fed basal diet and treated with Nano- Se plus Vit E groups.*

In conclusion*, orally treatment of buck rabbits with selenium sources (Nano-Se or Org-Se) without or with vitamin E had improved semen quality and hematological and biochemical blood parameters. From the results of present experiment, it is recommended to support the commercial basal diet of buck NZW rabbits by orally treatment of rabbits with 0.5 mg Organic- Se / kg Bw to maintain their health status, protect them from heat stress and improve their semen quality in hot climate of Egypt in summer season.*

Key word: *Nano-selenium*, Vit. E, *semen quality*, *blood parameters*, *bucks rabbits***.**

INTRODUCTION

Heat stress is the most obvious limitation to rabbit production in hot climate area. Heat stress evokes a series of drastic changes in their biological functions that lead to impairment of production and reproduction (Marai *et al*., 1991; Fernandez *et al*., 1994 and Marai and El-Kelawy, 1999). Such detrimental effects are obviously seen during summer, and are reflected in limiting the breeding season of rabbits (Marai *et al*.1996). In North African countries rabbit industry has developed rapidly, and applied research on vital aspects of rabbit production such as economics, housing, management, nutrition and reproduction are urgently needed to ensure a high success rate of rabbit development, which could have a favorable economic and nutritional impact. Dietary selenium is an essential trace element for normal animal growth,

fertility, immunity physiology and metabolism as well as external and internal organs (Mohapatra *et al*., 2014). Selenium improves productive performance and antioxidant status of animals, particularly in hot summer environments (Mahima *et al*., 2012).

Selenium can be supplemented in the form of organic, inorganic, and nanoparticles; however, nanoparticles of selenium possess comparable efficiency, high bioavailability, high catalytic efficiency, strong adsorbing ability, and low toxicity compared with other selenium sources (Wang *et al.*, 2009).

Vitamin E is one of the best antioxidants for the removal of oxidative stress in male reproductive system. Its use increases the reproductive functions and efficiency of male reproductive system. The deficiency of this vitamin leads to degeneration of germinal epithelium and Leydig cells in seminiferous tubules (Muhammad Zubair, 2017).

Therefore, the present study was carried out to study the effects of dietary inclusion of Nano-selenium (Nano-Se), organic- Se, with or without vitamin E on semen quality and health status of New Zealand White (NZW) bucks rabbits, under heat environmental of Egypt in summer.

MATERIALS AND METHODS

The experimental work of the present study was carried out at Rabbits Research Unit, Department of Animal and Poultry Production, Faculty of Technology and Development, Zagazig University, Zagazig, Egypt. The experimental work was initiated in July 2019 and terminated in, August 2020. The present experiment was conducted at sexual maturity of bucks (24 weeks of age). The semen quality and chemical analysis of ingested feed were performed at Central Lab for Soil, Foods and Feedstuffs (International accredited Laboratory, has ISO 17025, since 2012) belongs to Faculty of Technology & Development, Zagazig University, Zagazig, Egypt. Analysis of blood samples, were conducted in private medical Lab., Zagazig, Egypt.

A total number of 24 NZW bucks rabbits with (2671± 185 g). initial body weight and 24 weeks of age were used. The bucks were randomly allotted to three experimental main groups. The 1st group fed basal died and served as a Control. The 2nd group fed basal diet and orally treated with 0.5 mg Org- Se / kg body weight (BW). The 3rd group fed basal diet and orally treated with 0.5 mg Nano-Se /kg BW. The experimental groups were divided according to Vitamin E administration into two sub groups. The 1st sub group was left as a control (without vitamin E administration) and the 2nd sub group orally treated with 250 mg Vit. E/ kg BW.

Samples from the basal diet were taken for chemical analysis to determine crude protein, crude fiber, ether extract and nitrogen free extract according to the International Standard Methods (ISO). Moisture content was determined according to ISO 6496: 1999, crude ash according to ISO 5984:2002, crude protein according to ISO 5983-1:2002, crude fat according to the method described in Official Journal of the European Union (EN), 2009, L54/ 37, Volume 52, and crude fiber was according to the method described in Official Journal of the European Union (EN), 2009, L54/ 40, Volume 52. The diets were formulated to meet the nutrient requirements of rabbits for reproduction according to NRC (1977). Ingredients and chemical composition of the basal diet are shown in Table 1.

Semen samples were collected once weekly for eight consecutive weeks using an artificial vagina device as described by Walton (1958). Each ejaculate was taken to measure physical semen characteristics (ejaculate volume, wave motion, progressive liner motility, sperm concentration, live and abnormal spermatozoa and total sperm output) according to El-Gaafary (1987).

In the end of experiment, three rabbits from each group were slaughtered and blood samples (5 ml/each rabbit) were collected. Each sample was taken into two tubes; the first was heparinized and the second tube was non-heparinized. The heparinized blood samples were used to test the hematological parameters (red blood cells (RBC_S), hemoglobin (Hb), haematocrit (HCT), mean cell volum (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), white blood cells (WBCS), neutrophils, lymphocyte and platelets). Non-heparinized blood samples were immediately centrifuged at 3000 r.p.m. for 15 minutes and serum was separated, frozen under -20°C, and kept for biochemical analysis (total protein, albumen, globulin, albumen globulin ratio, triglycerides, cholesterol, aspartate amino-transferase (AST), alanine amino-transferase (ALT), Urea-N and creatinine) and testosterone.

Statistical analysis:

Data of the present study were statistically analyzed using Least Squares Analysis of Variance according to Snedecor and Cochran (1982) using the General Linear Model Program of SPSS (2014) using the following fixed model for bucks:

$$Yij = \mu + Si + V_j + SV_{ij} + eij$$

Where, Yij = The observed value of a given dependent variable, μ = Overall adjusted mean, Si = Forms of selenium effect (i = 1, 2 and 3), V_j =

Table 1: Ingredients and chemical analysis of the basal diet.

Ingredient	**% of total diet**
Yellow corn	16.00
Barley	18.00
Wheat bran	26.00
Soybeans	19.3
Alfalfa hay	18.00
Limestone	2.00
Salt	0.50
Methionine	0.10
Anti Mycotoxins	0.10
Total	**100**
Chemical analysis:	
Moisture	10.65
Crude protein	17.85
Ether extract	2.30
Crude fiber (cf)	13.40
Ash	8.25
Nitrogen free extract (NFE)	47.55
Lysine*	0.99
Total Calcium(ca)	0.88
Total phosphorous (P)	0.52
Meth+cysteine*	0.65

* It was calculated according to **NRC (1977)**.
Natural ingredients contain 0.38mg Se/kg feed and 38.4 mg Vit E / kg feed.

Vit. E effect (J= 1 and 2), SV_{ij} = Interaction effect (ij=1,2 ….and 6), eij = Random error.

The differences between LSM (least square means) were analyzed by Duncan's New Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

Physical semen characteristics of NZW buck rabbit.

Data in Table 2 shows that semen quality (ejaculate volume, wave motion, progressive linear motility, sperm concentrations, abnormal spermatozoa percentages, live spermatozoa percentages and total sperm

Table 2: Effect of selenium sources and vitamin E administration and their interaction on semen quality of NWZ buck rabbits (mean± SE).

Items	Traits						
	Ejaculate volume(ml)	Wave motion(score)	Progressive linear motility (%)	Live spermatozoa percentage (%)	Abnormal spermatozoa percentage (%)	Sperm concentration ($x10^6$)	Total sperm output ($x10^6$)
Selenium sources:							
Control	0.57± o,o3^b	2.94±0.14^b	69.58±1.95^b	72.06±1.46^c	23.94±1.52^a	186.82±16.60^c	101.06±9.15^c
Org – Se	0.78±0.05^a	3.47±0.14^a	77.64±1.81^a	82.58±1.18^b	13.89±0.98^b	232.43±10.24^b	177.59±13.66^b
Nano – Se	0.76±0.05^a	3.72±0.11^a	81.67±1.24^a	86.53±1.30^a	11.75±0.76^b	304.52±14.28^a	231.70±17.23^a
Significant test	**	**	**	**	**	**	**
Vit. E supplementation:							
Without Vit. E	0.80±0.04	3.24±0.11	76.76±1.28	79.04±1.23	16.72±1.05	209.19±11.04	173.27±14.14
With Vit. E	0.61±0.03	3.52±0.11	75.83±1.76	81.74±1.45	16.33±1.29	273.33±13.61	166.96±12.55
Significant test	**	NS	NS	NS	NS	**	NS
Interaction effects:							
Control without Vit. E	0.63±0.04^b	2.83±0.19^c	70.56±1.89^{bc}	73.17±1.90^c	21.72±2.17^b	147.29±12.07^c	92.76±11.11^c
Control With vit. E	0.51±0.04^b	3.06±0.21^{bc}	68.61±3.45^c	70.94±2.24^c	26.16±2.06^a	226.36±28.38^b	109.35±14.59^c
Org. Se without Vit. E	0.91±0.07^a	3.39±0.18^b	78.33±2.14^a	80.22±1.65^b	15.77±1.48^c	212.53±13.84^b	190.48±18.43^{ab}
Org. S with With vit. E	0.65±0.07^b	3.56±0.20^{ab}	76.94±2.97^{ab}	84.94±1.53a^b	12.00±1.17^{cd}	252.32±13.91^b	164.69±20.22^b
Nano Se without Vit. E	0.87±0.07^a	3.50±0.17^{ab}	81.39±1.89^a	83.72±2.11^b	12.67±0.94^{cd}	267.73±19.37^b	236.56±27.81^a
Nano Se with Vit. E	0.65±0.04^b	3.94±0.13^a	81.94±1.67^a	89.33±1.24^a	10.83±1.18^d	341.30±17.47^a	226.83±21.12^a
Significant test	**	**	**	**	**	**	**

a ,b,c,d Means are bearing different letters in classification, differ significantly at P< 0.05.

NS = Not significant and ** = P< 0.0.

Means±SE of 6 samples for each buck group.

output) were significantly (P<0.01) improved for bucks rabbits fed basal diet plus org-Se or Nano-Se as compared with control group. These results are in agreement with those obtained by Rezvanfar *et al*., (2013). Feeding Nano-Se increased ejaculates volume, wave motion and sperm motility in Californian male rabbits. Also, Saber Abd-Allah and Hashem (2015) found that dietary supplementation with Nano-Se increased live sperm, sperm concentrations and total sperm output in rats. The same author added that increased number of sperm in rat's epididymis is due the increase of cell population in the seminiferous tubules and increased sperm vitality and motility after selenium nanoparticles administration.

The mean values of ejaculate volume were significantly increased (P<0.01) in buck rabbits fed basal diet without vitamin E than those supplemented with vitamin E. However, the mean values of sperm concentrations were significantly higher (P<0. 01) in buck rabbits fed basal diet and orally treated with vitamin E when than non-treated rabbits. On the other hand, the mean values of live spermatozoa percentages, wave motion, progressive linear motility, abnormal spermatozoa percentages and total sperm output were not significantly affected by vitamin E treatment (Table 2).

The interaction effects between orally treatment with selenium source and vitamin E revealed that there were significant differences (P <0.01) in all semen quality traits studied each of ejaculate volume, wave motion, progressive linear motility, sperm concentrations, abnormal spermatozoa percentages, live spermatozoa percentages and total sperm output. The highest values of wave motion, sperm motility, sperm concentrations and live spermatozoa percentages were obtained with NZW buck rabbits fed basal diet and treated with Nano Se and Vit. E when compared with other treatment groups. Values of abnormal spermatozoa percentages were the highest with NZW buck rabbits fed basal diet with Vit. E when compared with other treatment groups. These results agreed with those reported by Yaseen *et al*., (2016) who found that semen quality was significantly higher in rabbit bucks received Vit. E and selenium than control rabbit bucks.

Biochemical components of seminal plasma of NZW buck rabbit.

Table 3 shows that the mean values of total protein and globulin were significantly (P<0.05) increased in rabbits fed the basal diet and orally treated with organic selenium and Nano selenium than those fed the control diet, but cholesterol and ALT were significantly (P < 0.01) increased in rabbits fed basal diet (control) compared with the other treatment groups. However, albumin and AST levels were not significantly affected by Org-Se or Nano-Se treatment. Ghodaia (2016) reported that seminal plasma

Table 3: Effect of selenium sources and vitamin E administration and their interaction on biochemical components of seminal plasma of NZW buck rabbits (mean± SE).

Items	Traits					
	Total protein (g/dl)	Albumin(g/dl)	Globulin (g/dl)	Cholesterol (mg/dl)	Alanine amino transferase (U/I)	Aspartate amino transferase (U/I)
Selenium sources:						
Control	3.92 ± o.17[b]	1.92 ± 0.06	1.99 ± 0.13[b]	40.33 ± 0.71[a]	18.71 ±0.26 [a]	40.83 ±0.45
Organ – Se	3.97 ± 0.04[b]	1.89 ± 0.11	2.08 ± 0.11[b]	27.16 ± 1.97[c]	17.43 ±0.43 [b]	41.91 ±0.91
Nano – Se	4.98 ± 0.50[a]	2.02 ± 0.04	2.97 ± 0.51[a]	33.17 ±0.83 [b]	17.05±0.14 [b]	39.63 ±0.78
Significant test	*	NS	*	**	**	NS
Vit. E supplementation:						
Without Vit. E	4.54±0.39	1.93±0.06	2.61±0.37	33.22±2.51	17.57±0.36	40.34±0.64
With Vit. E	4.03±0.08	1.95±0.07	2.08±0.08	33.89±1.74	17.89±0.32	41.24±0.66
Significant test	NS	NS	NS	NS	NS	NS
Interaction effects:						
Control without Vit. E	3.81±0.28[b]	1.88±0.12	1.92±0.17[b]	41.00±1.15[a]	18.73±0.45[a]	41.10±0.81
Control With vit. E	4.03±0.23[b]	1.96±0.03	2.07±0.22[b]	39.66±0.88[a]	18.68±0.36[a]	40.57±0.53
Org. Se without Vit. E	3.97±0.07[b]	1.93±0.16	2.04±0.19[b]	24.33±1.76[c]	16.77±0.52[c]	41.17±1.09
Org. S with With vit. E	3.96±0.04[b]	1.84±0.19	2.12±0.15[b]	30.00±2.88[b]	18.10±0.44[ab]	42.67±1.54
Nano Se without Vit. E	5.85±0.70[a]	1.98±0.05	3.87±0.67[a]	34.33±0.88[b]	17.20±0.15[bc]	38.76±1.07
Nano Se with Vit. E	4.11±0.11[b]	2.05±0.06	2.05±0.07[b]	32.00±1.15[b]	16.90±0.23[bc]	40.50±1.07
Significant test	**	NS	**	**	**	NS

a ,b, c Means are bearing different letters in each classification, differ significantly at P< 0.05.
NS = Not significant, * = P< 0.05 and ** = P< 0.01.
Means ±SE of three samples for each buck group.

proved to be of great biochemical interest as it contains many organic compounds. These substances produced by various accessory glands in response to testosterone hormone (El-Sherbieny, 2004).The biochemical components in seminal plasma play a biovital role in providing substrate energy forming essential link in the energy generating cycles in sperm metabolism, during the process of fertilization and in the maintenance of constant osmotic pressure during semen preservation (Dhami and Kodagali, 1987). Estimation of these biochemical components in the ejaculated semen or directly in the glands can be used as an index of accessory glands function (White, 1976).

The mean values of total protein, globulin, cholesterol, albumin, ALT and AST were not significantly affected by vitamin E treatment. The interaction effects between selenium source and vitamin E were significant ($P<0.05$, $P < 0.01$) in total protein, globulin, cholesterol and ALT. However, albumin and AST levels were not significantly affected by selenium source and vitamin E. The highest values of total protein and globulin were obtained with NZW buck rabbits fed basal diet and treated with Nano-Se when compared with other treatment groups. The highest values of cholesterol and ALT were obtained with NZW buck rabbits fed basal diet with Vit E. On the other hand, the lowest values of cholesterol and ALT were recorded with NZW buck rabbits fed basal diet and treated with Org-Se and Vit E when compared with other treatment groups (Tables 3).

Blood parameters of rabbits:

Hematological blood parameters:

The present results in Table 4 show that means of RBCs, WBCs, blood platelets, hematocrit, MCV and MCHC, were within the normal physiological range (Hewitt *et al.,* 1989) and the differences were not significantly affected by selenium sources administration. However, Hb, MCH and lymphocytes percentage were significantly increased ($P < 0.05$ and $P < 0.01$) for buck rabbit's fed basal diet with Nano Se. On the other hand Neutrophils percentage was significantly decreased ($P < 0.01$) for buck rabbit's fed basal diet with Nano selenium. The mean values of RBCs, Hb, hematocrit, MCHC and lymphocytes percentage were significantly higher ($P < 0.05$ and $< P$ 0.01) in buck rabbits fed basal diet with vitamin E administration than un-treated rabbits. However, the mean values of blood platelets and neutrophils percentage were significantly higher ($P <0.05$ and $P<0.01$) in buck rabbits fed basal diet without vitamin E than those fed basal diet and treated with vitamin E

Table 4: Effect of selenium sources and vitamin E administration and their interaction on haematological blood parameters of NWZ buck rabbits (mean± SE).

Items	Haematological blood parameters									
	RBC	WBC	HB	PLT	HCT	MCV	MCH	MCHC	Neutrophils (%)	Lymphocyte (%)
Selenium sources:										
Control	5.36± 0.06	22.13± 1.56	9.43± 0.23 ^{b}	436.17±29.97	28.92±0.61	53.93±1.32	17.42±0.53^{b}	32.37±0.24	61.00±1.44^{b}	34.00±1.67^{a}
Org – Se	4.94± 0.17	23.52± 1.54	9.25± 0.45 ^{b}	384.67±50.98	28.27±1.42	56.97±1.28	18.92±0.50^{a}	32.82±0.27	64.00±1.24^{a}	31.17±1.40^{b}
Nano – Se	5.25± 0.38	19.45±1.66	10.55± 0.69 ^{a}	368.17± 65.43	30.58±1.98	56.50±0.75	18.98±0.24^{a}	33.12±0.18	58.67±2.59^{c}	35.67±2.56^{a}
Significant test	NS	NS	*	NS	NS	NS	*	NS	**	**
Vit. E supplementation:										
Without Vit. E	4.88± 0.17	22.37± 1.22	8.96± 0.24	459.44±25.14	27.34±0.82	54.56±0.81	18.07±0.29	32.47±0.15	64.89±0.63	29.67±0.67
With Vit. E	5.49± 0.17	21.03± 1.51	10.53± 0.42	333.22±42.69	31.17±1.13	57.04±1.02	18.81±0.51	33.07±0.22	57.56±1.31	37.56±1.12
Significant test	**	NS	**	*	**	NS	NS	*	**	**
Interaction effects:										
Control without Vit. E	5.43±0.03^{ab}	24.70± 1.29	9.27±0.15b^{c}	485.00± 44.80	29.27± 0.33^{b}	53.00±1.00	17.40±0.26	32.03±0.23	64.00± 1.00 ^{ab}	30.67± 1.45^{d}
Control With vit. E	5.30±0.11^{abc}	19.57± 1.96	9.60±0.46b^{c}	387.33±9.91	28.56± 1.27^{b}	54.87±2.61	17.43±1.16	32.70±0.35	58.00±0.58 ^{c}	37.33± 0.88^{b}
Org. Se without Vit. E	4.70±0.27^{cd}	23.30± 2.63	8.53±0.65^{c}	493.00± 34.44	26.00± 2.20^{b}	54.63±1.52	18.00±0.49	32.57±0.19	66.33±1.33 ^{a}	28.33± 1.20^{d}
Org. S with With vit. E	5.18± 0.07^{bcd}	23.73± 2.22	9.97± 0.26^{b}	276.33± 8.41	30.53± 0.32^{ab}	59.30±0.70	19.83±0.43	33.07±0.52	61.67±0.67 ^{b}	34.00± 0.58^{c}
Nano Se without Vit. E	4.52±0.16^{d}	19.10± 0.83	9.07±0.32b^{c}	400.33± 41.87	26.77± 0.54^{b}	56.03±1.52	18.80±0.46	32.80± 0.10	64.33± 0.67 ^{ab}	30.00±0.58^{d}
Nano Se with Vit. E	5.98±0.40^{a}	19.80± 3.61	12.03±0.28^{a}	336.00±136.43	34.40± 2.17^{a}	56.97±0.55	19.17±0.22	33.43± 0.24	53.00±1.00 ^{d}	41.33±0.67^{a}
Significant test	**	NS	*	NS	*	NS	NS	NS	**	**
Normal range	3.7-7.5	5.2-16.5	8.9-15.6	112-715	26.7-47.2	58-79.6	19.2- 29.5	31.1-37		

a ,b; c,d Means are bearing different Letters in each classification, differ significantly at P< 0.05.

NS = Not significant, * = P< 0.05, ** = P< 0.01.

Means±SE of three samples for each buck group.

administration. On the other hand, the mean values of WBCs, MCV and MCH were not significantly affected by vitamin E, as shown in Table 4.

These results are in agreement with those obtained by Hashem *et al.* (2013) and Okachi and Ani (2016) who showed that Hb and RBC were significantly (P<0.05) higher by supplemented diets with Vit. E, while MCV were not significantly (P <0.05) affected by Vit. E supplementation. Ayyat *et al.* (2018) reported that blood HB significantly increased in rabbits fed diet supplemented with 0.3 mg org-Se/ kg diet.

The interaction effects between administration of selenium source and vitamin E were significant (P<0.05 and P < 0. 01) in RBCs, Hb, hematocrit, neutrophils and lymphocytes percentage. However, WBCs, MCV, MCH, MCHC and blood platelets levels were not significantly affected by administration of selenium source and vitamin E. Highest values of RBCs, Hb, Hematocrits and lymphocytes percentage were obtained with NZW buck rabbits fed basal diet supplemented diet with Nano Se and Vit E when compared with other treatment groups. However, the highest values of neutrophils were obtained with NZW buck rabbits fed basal diet and treated with organic selenium when compared with other treatment groups, as shown in Table 4. These results are in agreement with those obtained by Hashem *et al.* (2013) and Okachi and Ani (2016).

Biochemical blood parameters:

Protein fractions*:*

Total protein and globulin were significantly increased (P<0.05 and 0.01), while, albumin and AL/GL ratio, were not significantly affected by selenium administration sources. Total protein was significantly improved (P<0.01) in buck rabbits fed basal diet and treated with vitamin E when compared with un-treated with vitamin E (Table 5). These results are in agreement with those obtained by El-Badry *et al.* (2019) who found that the values of total protein contents in blood was significantly (P< 0.01) increased in rabbits fed Nano-Se followed by those fed the Org-Se as compared with those fed the control diet. Ebeid, (2012) observed no significant effect on albumen level, among rabbits fed diets treated with vitamin E or selenium diets for 6 weeks.

The interaction effects between selenium sources and vitamin E were significant (P < 0.01) in total protein and globulin, however, insignificantly differences were observed in albumin and AL/GL ratio. The highest values of globulin were obtained with NZW buck rabbits fed basal diet and treated with Nano Se alone. On the other hand, the lowest values of total protein and globulin were obtained with NZW buck rabbits fed basal diet with Org-

Table 5: Effect of selenium sources and vitamin E administration and their interaction on biochemical blood parameters of NWZ buck rabbits(mean±SE).

Items	Biochemical blood parameters***									
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin /Globulin ratio (%)	Triglycerides (g/L)	Blood urea nitrogen (mg/dl)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Alanine Amino transferase (U/l)	Aspartate amino transferase (U/l)
Selenium sources:										
Control	7.12±0.25 ^{b}	2.83± 0.12	4.29± 0.14 ^{b}	0.67±0.02	139.00±8.55 ^{a}	28.50±0.56	1.28±0.06	118.16±5.49 ^{a}	19.88±2.07^{b}	85.33±3.60^{a}
Org – Se	7.03± 0.21 ^{b}	2.55± 0.11	4.49±0.32 ^{b}	0.59±0.08	109.83± 4.28 ^{b}	28.83±0.79	1.20±0.09	99.16 ±5.64 ^{b}	15.33±1.28^{c}	68.00±5.63^{b}
Nano – Se	8.09± 0.09 ^{a}	3.06±0.38	5.03± 0.31 ^{a}	0.65± 0.14	113.33±2.20 ^{b}	32.33±2.17	1.20±0.07	104.00 ±1.23 ^{ab}	32.83±2.01^{a}	78.67±3.92^{ab}
Significant test	**	NS	*	NS	**	NS	NS	*	**	*
Vit. E supplementation:										
Without Vit. E	7.13±0.25	2.63±0.08	4.50±0.29	0.61±0.05	127.88±7.78	30.33±1.33	1.17 ±0.06	106.88 ±4.77	20.14±2.53	80.44±2.84
With Vit. E	7.69±0.15	2.98±0.26	4.71±0.17	0.66±0.09	113.55±2.98	29.44±1.13	1.27 ±0.05	107.33 ±4.46	25.22±3.14	74.22±5.22
Significant test	**	NS	NS	NS	**	NS	NS	NS	*	NS
Interaction effects:										
Control without Vit. E	6.70± 0.25^{b}	2.65± 0.18	4.05±0.08^{c}	0.65±0.03	157.00±5.86^{a}	28.33±0.88	1.20±0.06	117.33±6.69	16.09±1.98^{c}	81.00±1.15^{a}
Control With vit. E	7.53±0.30^{a}	3.00± 0.12	4.53±0.19^{bc}	0.66±0.02	121.00±2.65^{b}	28.67±0.88	1.37±0.09	119.00±10.26	23.67±1.76^{b}	89.67±6.69^{a}
Org. Se without Vit. E	6.63±0.23^{b}	2.77± 0.11	3.87±0.34^{c}	0.73±0.09	114.33±6.94 ^{b}	27.33±0.88	1.06±0.16	99.33±11.78	14.67±1.86^{c}	78.33±5.81^{a}
Org. S with With vit. E	7.43±0.09^{a}	2.32± 0.05	5.11±0.09^{ab}	0.46±0.01	105.33±4.84 ^{b}	30.33±0.33	1.33±0.03	99.00 ±10.97	16.00±2.08^{c}	57.67±4.26^{b}
Nano Se without Vit. E	8.07±0.04^{a}	2.49± 0.08	5.58±0.05^{a}	0.45± 0.02	112.33±2.90^{b}	35.33±0.88	1.26 ±0.08	104.00 ±1.52	29.67±0.88 ^{b}	82.00±7.64^{a}
Nano Se with Vit. E	8.10±0.21^{a}	3.62± 0.63	4.48±0.43^{bc}	0.86± 0.25	114.33±3.84 ^{b}	29.33±3.71	1.13±0.12	104.00 ±2.30	36.00± 3.06^{a}	75.33±2.73^{a}
Significant test	**	NS	**	NS	**	NS	NS	NS	**	*
Normal range	5.3-7.5	2.5-4.5	1.9-3.5			10-28	0.8-2.5		17-67	16-108

a ,b, c,d Means are bearing different Letters in each classification, differ significantly at P< 0.05.

NS = Not significant, * = P< 0.05, ** = P< 0.01.

Means±SE of three samples for each buck group.

Se plus vitamin E. These results are in agreement with those obtained by El-Kholy *et al.* (2012) who reported that the improvements of blood components as a result of dietary supplementation with selenium sources was due to the improvement in the immune response. In this concern, Tietz (1986) reported that increase of globulin concentration is important for immunologic responses. Also, Ismail *et al.* (2003) stated that albumin/globulin ratio is a good indicator for improved immunity. Fortun-Lamothe and Drouet-Viard, (2002) indicated that treatment with Se sources or Vit E supplementation resulted in increase of the immune system.

Lipid fractions:

Data in Table 5 show that the total cholesterol concentrations and triglycerides were significantly lower ($P<0.05$ or $P < 0.01$) for rabbits fed basal died and orally treated with selenium sources than the control group. The reason of this reduction may be due to the vital role of Se in dominant effects of thyroid (T_3) hormone on fat metabolism (Masukawa *et al*., 1983). Hypercholesterolemia has been found to be related with Se, which is related to the improvement in 3- hydroxy-3-methylglutary CoA reductase, which stimulates the microsomes of liver activity (Nassier *et al*., 1997). Selenium is a component of activity of the glutathione peroxidase (GSH-Px), which plays a vital role as an anti-oxidant enzyme affects on lowering cholesterol syntheses.

The mean values of triglycerides were significantly higher ($P<0.01$) in buck rabbits fed basal diet without vitamin E than diet with vitamin E administration, However, the mean values of total cholesterol were insignificantly affected by Vit. E treatment (Table 5). Triglycerides for buck rabbits fed basal diets supplemented with selenium source and vitamin E were significantly lower ($P < 0.01$) than those control group. However, total cholesterol concentrations were not significantly affected by selenium sources and vitamin E treatment (Table 5).

Liver function:

Alanine amino transferase (ALT) was increased ($P < 0.01$) in rabbits fed basal diet with Nano selenium when compared with the other groups. However, aspartate amino transferase was increased ($P < 0. 05$) in rabbits fed basal diet (control) as compared with rabbits fed basal diet with selenium sources. These results are in agreement with those obtained by Abdel-Wareth *et al.* (2019) who observed that rabbits fed diets with Nano-Se showed lower ($P < 0.01$) serum levels of aspartate aminotransferase (AST) activities than those fed the un-supplemented diet. El-Deep *et al*. (2017) reported that

birds fed either Nano-Se or Se enriched yeast (Sel-Plex) recorded lower values of AST enzyme than control group.

The mean values of ALT were significantly higher ($P<0.05$) in buck rabbits fed basal diet and orally treated with vitamin E than un-treated diet, while the mean values of AST were not significantly affected by Vit E treatment (Table 5).

ALT and AST for buck rabbits fed basal diets and orally treated with selenium source and vitamin E were significant ($P < 0.05$ or $P < 0.01$). The highest values of ALT and the lowest values of AST were recorded with NZW buck rabbits fed basal diet and treated with Nano selenium plus Vit E.

Kidney function:

Results in Table 5 show insignificant differences were obtained in blood urea-N and creatinine for buck rabbits fed basal diet and treated with selenium sources as compared with bucks fed basal diet (control) only. Abdel-Wareth *et al.* (2019) found that serum urea- nitrogen, and creatinine significantly decreased ($P < 0.05$) in rabbits fed diet and treated with Nano-Se when compared with those fed un-treatd rabbits.

The mean values of creatinine and blood urea-N were not significantly affected by Vit E administration. Creatinine and blood urea-N concentrations were not significantly affected by the interaction between selenium source and vitamin E (Table 5).

Testosterone concentration:

Table 6 show that testosterone concentrations in seminal plasma and blood serum were significantly increased ($P < 0.05$ and $P < 0.05$, respectively) in buck rabbits fed basal diet with Nano selenium administration as compared with the other groups. These results are in agreement with those obtained by Rezvanfar *et al*., (2013) who reported that feeding Nano- selenium increased testosterone concentration in semen.

The mean values of testosterone concentration in seminal plasma and blood serum were insignificantly affected by Vit E treatment. Testosterone concentrations in seminal plasma and blood serum for buck rabbits fed basal diets supplemented with selenium source and vitamin E were significant ($P < 0.05$ or $P < 0.01$). The highest values of testosterone concentration in seminal plasma and blood serum were recorded with NZW buck rabbits fed basal diet and orally treated with Nano-Se plus Vit E.

In conclusion, orally treatment of buck rabbits with selenium sources (Nano-Se or Org-Se) without or with vitamin E had improved semen quality and hematological and biochemical blood parameters. From the result of present experiment, it is recommended to support the commercial basal diet of

Table 6: Effect of selenium sources and vitamin E administration and their interaction on Testosterone concentration in seminal plasma and blood serum of buck rabbits (mean ± SE).

Items	Testosterone concentration	
	Seminal plasma	**Serum**
Selenium source		
Control	2.41 ±0.11 [b]	0.05± 0.004 [b]
Org – Se	2.73 ±0.17 [ab]	0.13± 0.02 [b]
Nano – Se	3.07 ±0.13 [a]	1.20± 0.05 [a]
Significant test	*	**
Vit. E supplementation		
Without Vit. E	2.75±0.15	0.44± 0.18
With Vit. E	2.72±0.14	0.48± 0.18
Significant test	NS	NS
Interaction effect		
Control without Vit. E	2.62±0.02[ab]	0.05± 0.003 [b]
Control With vit. E	2.20±0.11[b]	0.05± 0.008 [b]
Org. Se without Vit. E	2.55±0.31[ab]	0.08±0.005 [b]
Org. S with With vit. E	2.91±0.13[a]	0.17± 0.01 [b]
Nano Se without Vit. E	3.07±0.29[a]	1.17± 0.08 [a]
Nano Se with Vit. E	3.06±0.04[a]	1.23± 0.07 [a]
Significant test	*	**

a ,b, c Means are bearing different letters in each classification, differ significantly at P< 0.05.
NS = Not significant, * = P< 0.05, ** = P< 0.01.
Mean of three samples for each buck group.

buck NZW rabbits by orally treatment of rabbits with 0.5 mg Organic- Se / kg BW to maintain their health status, protect them from heat stress and improve their semen quality in hot climate of Egypt in summer season.

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تأثير المعاملة بالنانو سيلينيوم وفيتامين هـ على جودة السائل المنوي وبعض خصائص الدم فى ذكور الارانب

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تهدف الدراسة إلى معرفة تأثير المعاملة بالنانو سيلينيوم (Nano-Se) ، والسيلينيوم العضوي (Se) ، وفيتامين هـ على جودة السائل المنوي وبعض مقاييس الدم لذكور الأرانب النيوزيلندية البيضاء. تم استخدام عدد 24 من ذكور الأرانب النيوزيلندية البيضاء بمتوسط وزن جسم حي أبتدائي 185 ±2671 جرام عمر 24 أسبوعا. تم توزيع الذكور بشكل عشوائي لثلاث مجموعات تجريبية. المجموعة الأولى(كنترول) تم تغذيتها على عليقه قاعدية, المجموعة الثانية غذيت على عليقة قاعدية + 0.5 ملجم سيلينيوم عضوى/ كجم من وزن الجسم . المجموعة الثالثة غذيت على عليقة قاعدية + 0.5 ملجم نانو سيلينيوم / كجم من وزن الجسم. تم تقسيم المجموعات التجريبية وفقًا للمعاملة بفيتامين هـ إلى مجموعتين فرعيتين. المجموعة الفرعية الأولى (بدون معاملة بفيتامين هـ) والمجموعة الفرعية الثانية تم معاملتها عن طريق الفم بـ 250 مجم فيتامين. هـ / كجم وزن الجسم.

أظهرت النتائج مايلي:-

1- تحسين معنوى (عند مستوى احتمال 01.) فى صفات جودة السائل المنوى فى ذكور الارانب المعاملة عن طريق الفم بمصادر السلنيوم المختلفة وفيتامين هـ مقارنة بذكور مجموعة الكنترول.
2- كانت تركيزات كرات الدم الحمراء ,كرات الدم البيضاء, الصفائح الدموية, الهيماتوكريت, MCV وMCHC فى مستوى التركيزات الفسيولوجية الطبيعية ولم تتاثر باضافة مصادر السلنيوم , فى حين ارتفعت نسبة الهيموجلوبين , MCH و الخلايا الليمفاوية معنويا (عند مستوى احتمال 05. و 01.) فى ذكور الارانب المعاملة بالنانوسلينيوم و انخفضت نسبة Netrophils معنويا (عند مستوى احتمال 01.) فى ذكور الارانب المعاملة بالنانوسلينيوم.
3- ارتفعت مستويات كرات الدم الحمراء والهيموجلوبين والهيماتوكريت وMCHC و الخلايا الليمفاوية معنويا (عند مستوى احتمال05. و 01.) فى ذكور الارانب المعاملة بفيتامين هـ مقارنة بتلك التى لم تعامل بفيتامين هـ.
4- ارتفاع معنوى فى تركيز الصفائح الدموية ونسبة Netrophils (عند مستوى احتمال05. و 01.) فى ذكور الارانب التى لم تعامل بفيتامين هـ مقارنه بالمجموعة

المعاملة بفيتامين هـ , فى حين لم تتاثر كلا من MCV, MCHبالمعاملة بفيتامين هـ.

5- وجود فروق معنوية (عند مستوى احتمال05. و 01.) فى تاثير التداخل بين المعاملة بمصادر السلنيوم وفيتامن هـ بالنسبة لكرات الدم الحمراء والهيموجلوبين والهيماتوكريت والخلايا الليمفاوية و Netrophils , فى حين لم تتاثر كرات الدم البيضاء و MCV,MCH, MCHC و مستويات الصفائح الدموية بشكل كبير بمصادر السلنيوم وفيتامين هـ.

6- ذادت نسبة البروتين الكلي والجلوبيولين معنويا (عند مستوى احتمال05. و 01.) بينما لم تتاثر نسبة الالبيومين والالبيومين الى الجلوبيولين بمصادر السلنيوم, فى حين حدوث تحسن معنوى فى مستوى البروتين الكلي (عند مستوى احتمال 01.) فى ذكور الارانب المعاملة بفيتامين هـ عند مقارنتها بالتى لم تعامل بفيتامين هـ.

7- انخفاض مستوى الكوليسترول والدهون الثلاثية (عند مستوى احتمال05. و 01.) فى ذكور الارانب المعاملة بمصادر السلنيوم المختلفة مقارنة بتلك الغير معاملة, بينما لم تتاثر قيمة الكوليسترول فى ذكور الارانب التى عوملت بفيتامين هـ.

8- كانت جميع مؤشرات سيرم الدم البيوكيميائية فى الحدود الطبيعية.

9- حدوث زيادة معنوية فى تركيزات التستستيرون فى السائل المنوى والدم (عند مستوى احتمال05.) فى ذكور الارانب المعاملة بالنانوسيلينيوم مقارنة مع المجموعات الاخرى, فى حين لم تتاثر تركيزات هرمون التستستيرون فى السائل المنوى والدم بشكل معنوى بالمعاملة بفيتامين هـ (عند مستوى احتمال01.).

10- سجل هرمون التستستيرون فى السائل المنوى والدم اعلى مستوى فى ذكور الارانب المعاملة بالنانوسيلينيوم مع فيتامين هـ مقارنة بالمجموعات الاخرى.

التوصية:

من نتائج التجربة ومن وجه النظر الاقتصادية نوصى بمعاملة ذكور الارانب النيوزلاندية البيضاء التامة النضج فى فصل الصيف عن طريق الفم بـ 0.5 مجم سيلنيوم عضوي- / كجم من وزن الجسم لتحسين صفات السائل المنوى والمحافظة على حالتها الصحية وحمايتها من الاجهاد الحرارى فى الطقس الحار فى فصل الصيف فى مصر .

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