

PHYSIOLOGICAL RESPONSE AND SEMEN QUALITY OF RABBIT BUCKS SUPPLEMENTED WITH SELENIUM AND TRIBULUS TERRESTRIS EXTRACT DURING SUMMER SEASON

M.E. El-Speiy; T.A. Sadaka; M. A. Abd-Elaal and Hanaa. A. Moustafa
Anim. Prod. Res. Instit., Agric. Res. Cent., Egypt, Alexandria, Egypt
e.mail :Mohamed.elspeiy@yahoo.com

ABSTRACT: *The aim of this study was to evaluate the effects of inclusion aqueous extract of Tribulus genuses (ETT) with selenium on semen quality and physiological response of mature buck rabbits during summer season. Thirty six of mature buck rabbits and forty-eight does of Californian breed were randomly divided into six equal treatment groups (6 buck + 8 does per each). The first treatment group was fed on basal diet (unsupplied group) and served as control. The other five treatments were fed the same basal diet and orally supplied with 0.4 mg Se/L (Se), 50 mg ETT/kg BW (ETT1), 100 mg/kg BW (ETT2), 0.4 mg Se/L+ 50 mg ETT/kg BW (ETT1+Se) and 0.4 mg Se/L+100 mg ETT/kg BW (ETT2+Se), respectively. The results indicated that the different supplied groups had a significantly higher follicle stimulating (FSH), luteinizing (LH), testosterone, progesterone and estrogen hormones levels than the control group. The groups supplemented with of ETT and Se showed high levels of FSH, LH, FT and $E_217\beta$ than another groups. However, no significant differences were found among all supplemented groups regarding blood biochemical parameters. Administrations of ETT and Se significantly increased plasma total antioxidant capacity and immunoglobulin M levels. On the other hand, the level of malondialdehyde was significantly decreased for the treated groups compared with the control group. Also, ejaculate volume, sperm motility, sperm concentration, cell integrity, fertility rate and litter size at birth were significant increases, while reaction time, abnormal sperm and dead sperm were significantly decreased. The groups ETT1+Se and ETT2+Se recorded height rate of fertility and increase number of litter size at birth. Treated groups showed statistically heaviest final body weight, body weight gain and lowest feed conversion compared with the control group. Conclusively, extract Tribulus Terrestris (al-Hasakah) plant with selenium led to improve the activity of sex hormones, oxidative status and immunity, and the additives improved the quality of semen and increased*

fertility and Litter size at birth **Key word:** *Tribulus Terrestris, semen abdominal size at birth during summer condition.* *quality, sex hormones, immune status.*

INTRODUCTION

In rabbits, the quantity and quality of semen plays an important role in the fertility and reproduction of rabbits bucks (Oseni and Lukefahr, 2014). So, optimal reproductive results are achieved with artificial insemination or natural mating primarily because the procedures aim to maximize efficiency the probability of oocytes being fertilized; while quality of semen are highly changing between buck rabbits (Tusell *et al.*, 2012). Semen evaluation must provide information on the fertilizing ability of spermatozoa. The most pertinent parameters correlated with the fertility rate are the number of spermatozoa inseminated and their motility, in spite of the use of a single attribute is not sufficiently rigorous to prognosticate the fertilizing ability of the semen (Lavara *et al.*, 2005).

Tribulus terrestris L. (TT) is a member of the Zygophyllaceae family, aqueous extract contains many compounds such as alkaloids, flavonoids oil, saponins, resins and nitrates (Abdul-Wahed, 2002). The biological properties of *Tribulus* extracts increased release of nitric oxide from endothelium and nerve termination it relaxes smooth muscles and increases angiogenesis converting enzyme inhibition (Sharifi *et al.*, 2003). Another theory, it is believed that TT strongly affect the androgen metabolism, significantly increasing levels of testosterone or its precursors, and some studies indicate that this effect is due to the dominant component of the TT as protodioscin (Natasha *et al.*, 2018). *Tribulus terrestris* have been extensively used as male sexual stimulants treatment of infertility, low sex drive and erectile dysfunction (Zhang and Chan, 2015). Abeer *et al.* (2012) reported that the beneficial effect of orally *T. terrestris* (150 mg / kg / day of an aqueous extract of TT) on testes can be attributed to antioxidant and metal chelating effect of *T. terrestris*.

Furthermore, the fruit of *Tribulus terrestris* has been used as aphrodisiac and to treated sexual dysfunction (Chhatre *et al.*, 2014), possibility to promote hormone levels of testosterone and enhance premature ejaculation (Ghosian *et al.*, 2013) and improved both sperm motility and count (Khoradmehr *et al.*, 2010).

Selenium (Se) as a trace mineral is play pointedly involves in numerous biological functions like growth, immunity, antioxidant status (Murray *et al.*,

2000), production and reproductive performance, necessary component of selenoproteins (Kryukov *et al.*, 2003 and Pappas and Zoidis, 2012).

Selenium plays major roles for spermatogenesis, and maintaining sufficient viability of spermatozoa and had ability to diminishing abnormalities of spermatozoa through direct effect on raise antioxidant status (Ebeid, 2009). Selenium is essential for spermatogenesis and its deficiency results in deterioration sperm motility and morphological abnormalities in rodents (Kehr *et al.*, 2009).

Therefore, the objective of this study was to evaluate the effect aqueous extract of *Tribulus terrestris* (TT) and selenium (Se) on semen quality, antioxidant capacity, blood biochemical parameters and fertility of buck rabbits.

MATERIALS AND METHODS

Housing and management:

The present study was carried out at a City of Scientific Research and Technological Applications Alexandria, Egypt, from the medal of July to the medal of October. The work aimed to study the effect of *Tribulus Terrestris* (TT) and selenium dioxide supplementations on semen quality and fertility rate of Californian buck rabbits. The ambient temperature and relative humidity were recorded daily and daily photoperiod (daylight length) was obtained from a nearby Meteorological station as shown in Table (1).

The temperature–humidity index (THI) was calculated using the equation: $THI = db^{\circ}C - [(0.31 - 0.31 (RH)) (db^{\circ}C - 14.4)]$, where db °C is the dry bulb temperature and RH the relative humidity percentage/100. The obtained THI values were then classified as follows: < 27.8 = absence of heat stress, 27.8 to < 28.9 = moderated heat stress, 28.9 to < 30.0 = sever heat stress and 30 and over = very sever heat stress (Marai *et al.*, 2001). Animals were kept in clean, separate wire-floor metal cages (50 cm length × 45 cm width × 40 cm high), maintained under standard laboratory conditions and kept the managerial condition, healthy, hygienic and clinically free of external and internal parasites.

Aqueous extraction of tribulus terrestris

Tribulus terrestris (TT) was collected from the local commercial in Alexandria city. Then, the dried material was ground with a blender. The powder was kept in glass bottle inside a refrigerator (20 °C) until starting the experiments. The plant powder was thoroughly mixed with boiled distilled water for 24 hours. Then, the mixture was filtered through a Whatman No.1

Table: 1: Ambient temperature (°C), relative humidity (%), temperature humidity index (THI) and photoperiod (h) during the experiment period

Parameters	Mid-July	August	September	Mid-October	Overall	SEM	P-Value
Ambient temperature(°C)	31 ^b	32 ^a	31 ^b	28 ^c	30.5	0.4	0.001
Relative humidity (%)	76 ^a	73 ^b	76 ^a	76 ^a	75.3	0.08	0.001
THI	29 ^b	31 ^a	30 ^b	27 ^c	29.3	1.2	0.001
Photoperiod(h)	14	13	12	11	12.5	0.8	0.001

^{a,b,c} Means within a row with different superscript letters are significantly.

THI values were then classified as follows: < 27.8 = Absence of heat stress, 27.8 to < 28.9 = Moderated heat stress, 28.9 to < 30.0 = Sever heat stress and 30 and over = Very sever heat stress (Marai *et al.* , 2001 and 2008).

filter paper and centrifuged for 15 minutes at 5000 rpm. The supernatant was collected and evaporated under reduced pressure at 37 °C (Sara *et al.*, 2017).

The major component in Tribulus terrestris extract (TTE) is methyl linolenate (18.56%), Also, the higher percentage of fatty acid is the heptadecanoic acid (33.56%). Meanwhile, carbohydrate and amino acid revealed the presence of inulin (5.61%) and glutamic acid (2.85%), respectively, Saponin yield is 7.38% and phenolic and flavonoid contents (11.16 and 6.076%), respectively (Nagwa *et al.*, 2018).

Selenium:

Selenium dioxide (SeO₂) used as source of Se, which reacts with water particularly hot water, to give selenious acid, a weak acid that is corrosive stable to light molecular weight is 110.97g/mol.

Experimental design:

The experiment was dune offer tow stage:

First stage: a total number of 36 un mature Californian rabbits buck (aged 4 month) with initial body weight of 2150 ±10.33g were randomly divided into six treatments (each one consisting from 6 individual replicate with one buck) to study the effect of orally supplementation of ETT with selenium (selenium dioxide used as source of Se) in buck rabbit's semen quality. During this stage, rabbit buck was administered orally via drinking water daily with tow dose of 50-100 mg/kg BW/day/rabbi and 0.4mg Se /litter drinking water for 8 weeks (from 4 -6 month, spermatogenesis period approximately). Also, the experimental dosages were continually used for bucks during the second stage.

Experimental treatments as follows:

Group1: unsupplied group used as a control group (Control).

Group2: supplied with 0.4 mg Se / litter/day drinking water (Se).

Group3: supplied with 50 mg ETT /kg BW / day/rabbit (ETT1).

Group4: supplied with 100 mg ETT /kg BW/ day/rabbit (ETT2).

Group5: supplied with 50 mg ETT/kg BW/ day/rabbit + 0.4mg Se / litter/day (ETT1+Se).

Group6: supplied with 100 mg ETT/kg BW/day/rabbit + 0.4mg Se/ litter/day (ETT2+Se).

Second stage: the previous bucks were used for fertilize dose to study the effect of experimental supplementation agent on fertility rate and dose litter size at birth of the different treatments. These animals represented progeny of pool semen and artificial 8 does in each group (Lopez and Alvariño, 2000). Forty-eight mature untreated does (aged 6 months) with initial body weight 3050 ± 10.7 g of witch were randomly divided into six treatments with 8 individual replicate. Only receptive females (red color of vulvae lips) were inseminated with about 40 million spermatozoa in three sequence parities. Does were artificially inseminated with the control and the other tested supplementations. Does were injected with 0.8 mg (0.2 ml) of gonadotropin-releasing hormone analogue (Buserelin, Suprefact®, Hoechst-Roussel, Germany (Receptal)) immediately at the time of insemination according to (Boussin, 1989). Does were inseminated artificially with semen extenders, which described previously. The insemination procedure was as described by (Adams, 1981).

Feed and drinking water were offered on *ad libitum* basis. All rabbits were fed the same basal diet formulated according to the nutritional requirements of the National Research Council (NRC, 1977), which ingredients and calculated chemical composition are displayed in Table 2.

Semen collection:

Semen was collected twice weekly (during the second stage) from each buck by artificial vagina using a female teaser rabbit with an interval of 3–4 days between successive ejaculation. The temperature of the inner rubber sleeve of the artificial vagina was adjusted to 41- 43°C and the lubrication of the inner sleeve was performed using white Vaseline. At four month of bucks age, started the training period with artificial vagina for 2 months by collected two ejaculate per buck per week. Will the does be 8 months old, semen collection was began

Table 2. The composition and chemical analysis of the basal experimental diet

Ingredients	%	Calculated analysis			
		Crude protein, %	Crude fiber, %	Ether extract, %	
Yellow corn	6.22	18.8			
Soybean meal, 44%	22.33	13.0			
Wheat bran	23.33	3.0			
Barley	15.00	2680			
Alfalfa hay	30.12	0.3			
Ground limestone	1.00				
Dicalcium phosphate	1.20				
Common salt	0.50				
Vit. + min. premix*	0.30				
Total	100.0				
<i>Determined analysis (g/kg)</i>					
		Dry matter	897.1	Crude fiber	138.5
		Organic matter	801.4	Ether extract	26.2
		Crude protein	169.8	Nitrogen-free extract	575.0
				Ash	87.9

*Each 3 kg of premix contains: Vit. A: 12,000,000 IU; Vit. D3: 3,000,000 IU; Vit. E: 10.0 mg; Vit. K3: 3.0 mg; Vit. B1: 200 mg; Vit. B2: 5.0 mg; Vit. B6: 3.0 mg; Vit. B12: 15.0 mg; Biotin: 50.0 mg; Folic acid: 1.0 mg; Nicotinic acid: 35.0 mg; Pantothenic acid: 10.0 mg; Mn: 80 g; Cu: 8.8 g; Zn: 70 g; Fe: 35 g; I: 1 g; Co: 0.15 g and Se: 0.3 g.

for semen examination. During collection period (8 weeks), two ejaculates per buck per week were collected. All ejaculates (average 560-576 samples for all treatment during semen collection period) were stored at 37 °C in a water bath until evaluation, not later than 10 minutes after collection.

Semen characteristic:

Physical semen characteristics *i.e.* reaction time (RT) determined by (Chibundu, 2005).

Ejaculate volume (Ej V, ml), determined by using graduated tube. **Spermatozoa concentration (SC)**, number of sperms per ml) was counted using a haemocytometer according to Smith and Mayer (1955). For evaluation of percentage of sperm advanced motility (AdM, drop of semen was examined under the low power of microscope using a hot stage at 37 °C.

Percentage of motile sperm (Sd) was estimated by a phase-contrast microscope according to Melrose and Laing (1970).

Spermatozoa concentration percentage of live and abnormal sperms (SAb) were determined after staining with eosin and nigrosine (Blom, 1950) and then calculated as a percentage out of randomly chosen 100 sperm counted, total sperms in the ejaculate (10^6 /ejaculate), number of motile sperms per ejaculate were estimated in residual semen samples.

Rabbit libido:

Libido (sexual desire) was estimated by observation of the reaction time (in seconds) from the time of introducing the doe to the buck until the buck start to mount and ejaculate the first copulation.

Blood biochemical constituents:

Blood samples (from bucks) were withdrawn at morning (8.00:9.00 h) from marginal ear veins under vacuum in clean tubes without heparin for each treatment group before access feed and water. Serum was obtained by centrifugation the blood 3500 rpm for 20 minutes and stored at -20°C for later analysis. The blood biochemical parameters total plasma protein(TP), albumin(Alb) were measured by the methods Doumas *et al.* (1981), globulin(GLo) was calculated, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG) (Fasati and Prencipe, 1982), high-density lipoprotein (HDL), low-density lipoprotein (LDL) using the method of Stein (1986). Serum samples were subjected to biochemical analysis of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (Reitman and Frankel, 1957). All biochemical parameters were

analyzed by commercially available kit methods. GNW-Model: SM-721 Spectrophotometers, Absorbance Microplate Reader and other laboratory equipment aids were used for biochemical analysis.

Determination hormones, immune response and serum Antioxidant:

Bucks serum concentrations of estrogen (E_2 17 β), progesterone (P_4), free testosterone (FT), follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were determined by using enzyme-linked immunosorbent assay (ELISA) kits according to Odell and Parlow (1981).

Different types of immunoglobulins in blood serum (IgG and IgM) were determined using commercial ELISA kits (Kamiya Biomedical Company, USA). Biochemical analyses of serum total antioxidant capacity (TAC) and malonaldehyde (MAD) were determined using commercially available kits methods using spectrophotometers, (GNW-Model: SM-721) according to Ippoushi *et al.*, (2005).

Statistical analysis:

Using SAS (2002), the data were statistically evaluated using Snedecor and Cochran (1982) fully randomized design. The following model was used:

$$Y_{ij} = \mu + \text{Tri} + e_{ij}$$

Where, Y_{ij} = Observations, μ = Overall mean, Tri = Effect of i^{th} treatment (i: 1-6), e_{ij} = Experimental error.

The significant means differences among groups were separated by Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Temperature degrees ($^{\circ}C$) and relative humidity (%):

In Table 1 are shown the change of temperature during the experimental period (summer season). The mean of ambient temperature, relative humidity and THI and photoperiod during the experimental interval were 30.5, 75.3, 29.3 and 12.5h, respectively.

The data demonstrated that the ambient temperature during July, Agust, September and October are 31, 32, 31 and 28.0 $^{\circ}C$ with overall average 30.5. These results indicated that the rabbits exposed to sever HS during summer session. These observations are in agreement with that reported by Marai *et al.* (2001 and 2008).

Effect of ETT on physiological response:***Hormonal profile:***

Table 3 illustrated that the group supplied with 50 mg ETT (ETT1) had a significantly higher FSH, LH, FT and E₂17 β concentrations compared with control group and the other experimental groups except for the concentration of E₂17 β for the group supplied with 0.4 mg SE/ l of water. While the group supplemented with 100 mg ETT + Se (ETT2+Se) showed the lowest levels of FSH, LH and E₂17 β than the control and the other experimental groups. Also, the groups supplied with 100 mg ETT (ETT2) or 100 mg ETT + Se (ETT2+Se) showed the lowest levels of E₂17 β than the control and the other experimental groups. These results are in agreement with Mohammad *et al.* (2013) who recorded that treatment with TT can increase the hormones level of FT, P4, E₂17 β and LH. Antonio *et al.* (2000) recorded that the upswing in LH impact to a signal for testosterone to increase. Also, Kazim *et al.* (2016) recorded that highest effectiveness of *Tribulus* on Serum testosterone levels. The same result recorded by Georgiev *et al.* (1988) it was shown that TT extract administration improved LH also sperm production and testosterone levels in ram. Also, Karimi *et al.* (2012) who mentioned that dioscin is a important component of *Tribulus terrestris* that increases male sexual ability by increasing free testosterone concentration and modulating estrogen, progesterone and pregnenolone levels.

Regarding the role of Se, Table (3) showed that serum FT concentration for the group supplied with only Se was increased compare with that recorded on the control group. That result are in agreement with similar response detected by El-Sisy *et al.* (2008) and Ibrahim and Mohamed, (2018) who indicated that supplemented male Baladi goats fed a diet with selenium yeast led to improved their reproductive efficiency and increase serum testosterone compared to control group. Also, Abdel-Waretha *et al.* (2019) recorded the same result in rabbit bucks supplemented nano-selenium.

Interestingly, Gauthaman and Adaikan (2005) examined the hormonal effects by TT extracts in rabbits and they detected an increase in androgenic hormone levels and erectile dysfunction improvement.

They all illustrated that TT had aphrodisiac activity, due to its androgen rising property. Also, Nasroallah *et al.* (2013) showed that TT raising testosterone by increasing gonadotropin releasing hormone which in modify stimulates the production of LH and follicle stimulating hormone, testosterone and that reflected on raising fertility and libido.

Table 3: Effect of selenium dioxide and *Tribulus terrestris* aqueous extract and their combination on hormonal profile measured in blood plasma of Californian buck rabbits

Parameters	control	Se	ETT1	ETT2	ETT1+Se	ET T2+Se	SEM	P- Value
FSH(ug/ml)	1.02 ^{ab}	0.77 ^c	1.10 ^a	0.96 ^b	0.44 ^d	0.16 ^e	0.021	0.001
LH(ug/ml)	0.35 ^e	0.44 ^d	0.79 ^a	0.65 ^b	0.57 ^c	0.19 ^f	0.323	0.001
FT(ug/ml)	1.77 ^d	2.78 ^c	4.99 ^a	3.64 ^b	2.87 ^c	3.77 ^b	0.532	0.001
P₄(ug/ml)	0.09 ^c	0.13 ^c	0.24 ^b	0.35 ^a	0.27 ^b	0.11 ^c	0.053	0.001
E₁17β(pg/ml)	22.9 ^b	28.3 ^a	29.9 ^a	16.6 ^c	23.8 ^b	17.7 ^c	1.235	0.001

*Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at P<0.05 Se: 0.4mg/liter water Selenium dioxide, ETT1: Aqueous extract *TribulusTerrestris* 50mg/kg BW, ETT2: Aqueous extract *TribulusTerrestris* 100 mg/kg BW. ETT1+Se= Aqueous extract *TribulusTerrestris* 50 mg/kg BW+ 0.4mg/liter water Selenium dioxide. ETT+Se= Aqueous extract *TribulusTerrestris* 100 mg/kg BW+Se0.4mg/liter water Selenium dioxide. FSH: follicle stimulating hormone, LH: luteinizing hormone, FT: FreeTestosterone, P₄: Progesterone, E₂17β: Estrogen.

Blood biochemical parameters:

Serum-biochemical blood data were summarized in Table 4. There were no significant differences in activity of AST, ALT, ALP or concentration of TP, ALb and GLO between the control and experimental groups during tested period. Furthermore, no significant changes were observed in the concentrations of creatinine compared to the control group. Also, lipid profile (TG, HDL and LDL) were not significant differences for all experimental groups compared with control group.

However, No significant changes were observed in the biological parameters alterations in components synthesized by liver like enzymes and proteins. Even the combination between ETT and Se doesn't affected or significantly changed parameters. In addition, ETT plant, which medicine had no toxic effects on kidney functions so it's likely to have a protective effect on kidney. These results are in agreement with a previous study by Abdel-Kader *et al.* (2016) who confirmed the positive effect of the plant on the kidney tissues and function. These results may be due to presence of abundant bioactive phytochemicals as glycosides, saponins, flavonoids, phytosteroids, alkaloids, glycosides, and numerous constituents in TT (Shama *et al.*, 2019). Disagreement with our results, Grigorova *et al.* (2008) and Mohsen and Mehran (2016) showed that hens serum total cholesterol tended to be lower in TT treated group relative to control birds. On the other hand, Haytham (2016) recorded that a significant decrease in total serum cholesterol, blood glucose. Also, the previous researchers indicated that the level of serum alkaline phosphatase is no difference among the hens of the all groups. However, the carnet results indicated that Se had no effect on biochemical blood parameters, while, Emará *et al.* (2019) reported that feed rabbit different source of Se (Nano-selenium and sodium selenite) led to significantly decrease TC, LDL, HLD, VLDL, triglycerides, and phospholipids. Also, El-Kholy *et al.* (2019) revealed that rabbits supplemented with different source of Se caused significant increases in total protein (TP), albumin (Alb) and globulin (Glb) but these increases were still within normal range.

Serum antioxidant and immunoglobulin status:

Results Table 5 indicated that there were no significant differences on TAC, MAD and IgG activity among the control and experimental groups during tested period. Furthermore, significant decreasing in IgM for treated groups supplied with ETT1+Se and ETT2+Se compare to control. Our date indicated that the combinations between ETT and Se had appositve results than supplementation of each of them individuals. These results are in agreement

Table 4: Effect of Selenium dioxide and *Tribulus Terrestris* aqueous extract and their combination on biochemical parameters measured in the blood plasma of Californian buck rabbits

Parameters	Control	Se	EIT1	EIT2	EIT1 +Se	EIT2 +Se	SEM	P-Value
TP(g/dl)	7.39	7.44	7.19	7.26	7.37	7.26	0.123	0.056
Alb(g/dl)	4.43	4.30	4.06	4.27	4.36	4.03	0.130	0.085
Glb(g/dl)	2.96	3.09	3.13	2.99	2.89	3.03	0.155	0.085
AST (U/l)	25.0	24.7	26.0	24.9	27.2	25.5	0.625	0.452
ALT(U/l)	32.3	30.7	28.7	27.3	30.4	32.7	0.845	0.541
ALP (U/l)	171	161	174	171	166	173	4.054	0.689
Cre (mg/dl)	0.99	1.07	1.10	1.08	0.96	0.98	0.235	0.066
TAC(mg/dL)	114	122	112	118	122	108	6.541	0.942
HDL(mg/dL)	12.9	12.5	12.6	11.9	12.1	12.8	0.691	0.653
LDL(mg/dL)	88.8	89.9	90.3	88.6	89.1	94.4	2.659	0.084
TG(mg/dL)	123	122	124	116	121	127	5.46	0.562
TL(mg/dl)	321	288	290	289	287	288	12.561	0.452

*Means \pm Standard error which superscripts with different small letters (a-c) within the same row differ significantly at $P < 0.05$. Se: 0.4mg/litter water Selenium dioxide, EIT1: Aqueous extract *TribulusTerrestris* 50mg/kg BW, EIT2: Aqueous extract *TribulusTerrestris* 100 mg/kg BW. EIT1+Se= Aqueous extract *TribulusTerrestris* 50 mg/kg BW+ 0.4mg/litter water Selenium dioxide. EIT+Se= Aqueous extract *TribulusTerrestris* 100 mg/kg BW+Se0.4mg/litter water Selenium dioxide. TP: Total protein, Alb: Albumin, Glb: Globulin, ALP: Alkalinephosphatase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, Cre: Creatinine, TAC: Total cholesterol, LDL: low density lipoprotein, HDL: High density lipoprotein, TG: triglycende, TL: Total lipid.

Table 5: Effect of Selenium dioxide and *Tribulus Terrestris* aqueous extract and their combination on antioxidant and immunoglobulin status measured in the blood plasma of Californian buck rabbits

Parameters	Control	Se	ETT1	ETT2	ETT1+Se	ETT2+Se	SEM	P Value
TAC($\mu\text{mol/ml}$)	2.04	2.49	2.17	2.55	2.38	2.82	0.104	0.424
MAD($\mu\text{mol/ml}$)	2.27	1.97	1.69	1.97	1.88	1.76	0.062	0.0558
IgG, (mg/dl)	23.66	26.2	27.67	28.63	31.33	33.37	1.567	0.2898
IgM, (mg/dl)	3.62 ^b	4.23 ^b	4.58 ^b	4.55 ^b	5.51 ^a	5.65 ^a	0.550	0.0013

*Means \pm Standard error which superscripts with different small letters (a-c) within the same row differ significantly at $P < 0.05$. Se: 0.4mg/liter water Selenium dioxide, ETT1: Aqueous extract *Tribulus Terrestris* 50mg/kg BW, ETT2: Aqueous extract *Tribulus Terrestris* 100 mg/kg BW. ETT1+Se= Aqueous extract *Tribulus Terrestris* 50 mg/kg BW+ 0.4mg/liter water Selenium dioxide. ETT2+Se= Aqueous extract *Tribulus Terrestris* 100 mg/kg BW+Se0.4mg/liter water Selenium dioxide.

TAC: Total antioxidant capacity, MAD: Malonylaldehyde, IgG: Immunoglobulin G, IgM: Immunoglobulin M.

with Emara *et al.* (2019) who recorded that the supplemented diet with nano-selenium and sodium selenite cause significantly improving on the status of the estimated antioxidants. Another study by Dokoupilová *et al.* (2007) which recorded that supplemented Se in organic or inorganic forms to provide a margin of safety of immunity against deficiency and to maintain productive performance. Same result obtained by El-Kholy *et al.* (2019) who mentioned that supplemented rabbit diet with organic or inorganic Se form increased in immunoglobulin concentrations with Se addition could be an indication of Se either in organic or inorganic form induces earlier maturation of the humeral indication of Se either in organic or inorganic form induces earlier maturation of the humoral immune responses. Also, higher serum IgG levels in rabbits supplemented with Se indicate that Se is effective in improving humeral immunity. Since, Meissonnier *et al.* (2008) demonstrated that limitation of the levels of serum immunoglobulin such as IgA, IgG and IgM are the most common methods of testing humeral immune responses. On the other hand, Dutt-Roy *et al.* (2017) observed that an *in vivo* study that the treatment rat with TT extracts raised the activities of TAC as catalase, superoxide dismutase and decline the malondialdehyde (MDA) level.

Productive performance:

In Table 6 are shown the values of final body weight, body weight gain, feed intake and feed conversion at the end of the experiment. The growth rabbit performances from all treated groups supplied with both levels of ETT with or without Se had a significantly heaviest final body weight, body weight gain and the best feed conversion comparison with the control and Se groups. However FI was not significantly change among experimental groups. The results obtained are in accordance with other study, who confirmed a significant increase of body weight in rats supplemented with different doses of TT (Gauthaman *et al.*, 2003). This effect can be explained by the androgenic role of the effective product, which perhaps stimulates the appetite. Androgens play an important role in the growth and development of reproductive tissue and in the differentiation of other tissues (Desislava *et al.*, 2019). On the other hand, Dimitrov *et al.* (1987) and Çek *et al.* (2007) both researcher illustrated an increase in body weight at the same trend Valchev *et al.* (2009) documented that the rabbit supplemented with TT extract increase in the average daily gain and improved feed efficiency as compared to the control. Finally, El-Badry *et al.* (2019) are concordant with our study results that live body weight and body weight gain and lowest feed conversion were significantly with rabbits received

Table 6: Effect of Selenium dioxide and *Tribulus Terrestris* aqueous extract and their combination on body weight feed intake and feed conversion ratio in the blood plasma of Californian buck rabbits

Parameters	Control	Se	ETT1	ETT2	ETT1 +Se	ETT2 +Se	SEM	P Value
Initial BW, g	2150	2157	2159	2153	2249	2158	18.37	0.9998
Final BW, g	3083 ^c	3220 ^c	3384 ^a	3421 ^a	3442 ^a	3428 ^a	29.37	0.0038
BWG, g	933 ^c	1062 ^c	1225 ^a	1260 ^a	1293 ^a	1260 ^a	31.63	0.0072
FI, g/ buck	5841	5111	5361	5434	5562	5466	92.95	0.1643
FCR ratio	6.32 ^a	4.81 ^b	4.39 ^b	4.32 ^b	4.34 ^b	4.35 ^b	0.168	0.0056

* Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at P<0.05. Se: 0.4mg/liter water Selenium dioxide; ETT1: Aqueous extract *TribulusTerrestris* 50mg/kg BW; ETT2: Aqueous extract *TribulusTerrestris* 100 mg/kg BW. ETT1+Se= Aqueous extract *TribulusTerrestris* 50 mg/kg BW+ 0.4mg/liter water Selenium dioxide. ETT+Se= Aqueous extract *TribulusTerrestris* 100 mg/kg BW+Se0.4mg/liter water Selenium dioxide.

nano-Se. Regarding the role of selenium on productive performance, our results are in agreement with Ebeid *et al.* (2012) who illustrated that consumed rabbits a diet supplemented with organic Se had heaviest live body weight and daily weight gain compared with control group. However, are in contrast the present results Dokoupilová *et al.* (2007) who showed that body weight gain of growing rabbits did not significantly affect by adding dietary selenium.

Semen quality:

Values for measured sperm parameters are illustrated in Table 7. There were significantly improving on rabbit semen quality (EjV, AdM, SC, CI, FR and LSB) for the all supplemented groups with different agents compared with the values recorded for control group. However, groups supplemented with ETT+ Se recorded the significantly or numerically values and completely archives the adverse effect of HS of the previous parameter. Excellent semen quality is desired for achieving adequate fertility in mammals (Dalton, 2011) inappropriate environmental cues lead to decreases in semen quality and fertility of farm animals (Rasooli *et al.*, 2010).

Exposing to heat stimulates the hypothalamo–pituitary–adrenal axis activity evoking the sympathetic system functions, which increase levels of free radicals, cortisol level and imbalances in the antioxidant-defense status (Ahmad *et al.*, 2012) causes decline the fertility by effects on ejaculate volume, sperm motility, number of motile sperm per ejaculate, sperm-cell concentration and total sperm-output of the buck rabbits (Alsaied *et al.*, 2008). The present study are in agreement with those of Kazim *et al.* (2016) who documented that supplemented rat with TT extracts showed highest total sperm motility percentage, increase in sperm counts. Oliveira *et al.* (2015) explained the mechanism of TT extract, it has been concluded that the ethanol extract of *T. terrestris* influences spermatogenesis, as shown by the clear changes in the tubular compartment of the testes, such as increases in the total tube length, tubular volume and height of the seminiferous tubules epithelium. The hexane and aqueous soluble fraction in the methanol fractions promoted changes in the intratubular compartment because they increased the nuclear volume, cytoplasmic volume and individual volume of Leydig cells in male Wistar rats. Bitzer *et al.* (2013) recommended that in vitro addition of TT extract to human sperm could affect male fertility capacity. The incubation of human semen with TT extract significantly enhanced the total sperm motility, number of progressive motile spermatozoa, and curvilinear velocity.

Regarding the role of Se on semen quality, our results agree with result obtained by Ewuola and Akinyemi (2017) who reported that buck rabbits

Table 7. Effect of Selenium dioxide and *Tribulus Terrestris* aqueous extract and their combination on semen quality and reproductive performance of Californian buck rabbits

Parameters	Control	Se	ETT1	ETT2	ETT1+Se	ETT2+ Se	SEM	P Value
RT (Sec)	6.21 ^a	4.05 ^b	3.98 ^b	3.21 ^b	3.00	2.98 ^c	0.235	0.001
Ej(ml)	0.43 ^d	0.79 ^{ab}	0.82 ^b	0.71 ^b	0.85 ^a	0.83 ^a	0.084	0.001
AdM %	62.2 ^c	78.4 ^a	76.3 ^a	71.3 ^b	79.4 ^a	78.4 ^a	1.365	0.001
Sperm conc. x10 ⁶	180 ^c	287 ^{ab}	298 ^a	278	300 ^a	285 ^{ab}	25.233	0.001
SAb %	17.8 ^a	12.8 ^b	13.0 ^b	14.2 ^c	11.9 ^c	12.1 ^c	1.254	0.001
Dead sperm %	24.1 ^a	15.9 ^b	16.3 ^{cb}	15.8 ^{cb}	13.6 ^c	13.3 ^c	3.182	0.001
Cell integrity %	50.2 ^c	73.3 ^b	74.1 ^b	73.5 ^b	78.9 ^a	81.3 ^a	6.241	0.001
Fertility rate %	55.2 ^d	72.3 ^c	76.2 ^b	77.8 ^b	81.1 ^a	81.5 ^a	4.56	0.001
L.S.B, n	5.6 ^c	7.1 ^b	7.5 ^b	8.2 ^a	8.4 ^a	9.1 ^a	0.361	0.001

*Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at P<0.05.

Se: 0.4mg/liter water Selenium dioxide, ETT1: Aqueous extract *TribulusTerrestris* 50mg/kg BW, ETT2: Aqueous extract *TribulusTerrestris* 100 mg/kg BW. ETT1+Se= Aqueous extract *TribulusTerrestris* 50 mg/kg BW+ 0.4mg/liter water Selenium dioxide. ETT+Se= Aqueous extract *TribulusTerrestris* 100 mg/kg BW+Se0.4mg/liter water Selenium dioxide. RT: Reaction time, EjV: Ejaculate volume, AdM: advanced motility, SAb, sperm: abnormal sperm, L.S.B: litter size at birth.

supplemented with organic selenium increased significant volume, mass activity, progressive motility, and live sperm cells and sperm concentration, also, decline percentage dead and abnormality sperm. Another study by Nourhan *et al.* (2020) recorded that supplemented diet bucks rabbits with organic selenium had lower reaction time, higher ejaculate volumes, sperm concentration and percentages of sperm progressive motility, live sperm, sperm with integrated membranes, and total functional sperm compared to the values obtained with the control diet. Also, they indicated that, the kindling rates, litter size, and weight at birth of females mated with males fed the organic selenium diet were significantly higher than those of females mated with males fed the control diet.

Conclusively, from these results it could be concluded that extract *Tribulus terrestris* (al-Hasakah) plant with selenium led to improve the activity of sex hormones, oxidative status and immunity, and the additives improved the quality of semen and increased fertility and Litter size at birth abdominal size at birth during summer condition.

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الاستجابة الفسيولوجية وجودة المنوى الذكوري لذكور الأرانب لاضافة السيلينيوم ومستخلص الحسكة خلال موسم الصيف

محمد السيد محمد السبيعي- طارق امين صداق- محمد على عبد العال - هناء مصطفى
معهد بحوث الانتاج الحيواني - مركز البحوث الزراعية - وزارة الزراعة - جيزة - مصر

الهدف من هذه الدراسة هو تقييم تأثير اضافة المستخلص المائي لنبات تريبولوس تيريستريس (الحسكة) (ETT) مع السيلينيوم على جودة السائل المنوي والاستجابة الفسيولوجية لذكور لأرانب الناضجة خلال موسم الصيف. تم تقسيم ستة وثلاثين من الأرانب الناضجة وثمانية وأربعين من اناث سلالة الكاليفورنيا بشكل عشوائي إلى ست مجموعات معاملة متساوية (٦ ذكور + ٨ اناث لكل منها). تم تغذية المجموعة الأولى على النظام الغذائي الأساسي (المجموعة غير معاملة، الضابطة) واستخدمت كمجموعة تحكم. تم تغذية المجموع الخمسة الأخرى على نفس النظام الغذائي الأساسي وتمت المعاملات عن طريق الاضافة الى مياة الشرب بـ ٠.٤ مجم Se / L (Se)، 50 مجم ETT / كجم من وزن الجسم (ETT1)، 100 مجم / كجم من وزن الجسم (ETT2)، 0.4 مجم Se / L + 50 Se / L + 50 ETT / كجم من وزن الجسم (ETT1 + Se) و ٠.٤ مجم سي / لتر + ١٠٠ مجم من ETT / كجم من وزن الجسم (ETT2 + Se) على التوالي.

أشارت النتائج إلى أن المعاملات المختلفة السابقة كان لديها مستويات أعلى بكثير من تحفيز الجريب (FSH) واللوئين (LH) والتستوستيرون والبروجسترون وهرمونات الاستروجين مقارنة بالمجموعة الضابطة. أظهرت المجموعات المكمل بـ ETT و Se مستويات عالية من FSH و LH و FT و E217 β مقارنة بالمجموعات الأخرى. ومع ذلك ، لم توجد

فروق ذات دلالة إحصائية بين جميع المجموعات المكملة فيما يتعلق بمكونات الدم البيوكيميائية. ادى تناول ETT و Se إلى زيادة كبيرة في كفاءة مضادات الأكسدة الكلية للبلازما ومستويات الغلوبولين المناعي M. من ناحية أخرى ، انخفض مستوى malondialdehyde بشكل معنوي في المجموعات المعاملة مقارنة مع مجموعة التحكم. كما أن حجم قذفة السائل المنوي ، وحركة الحيوانات المنوية ، وتركيز الحيوانات المنوية ، وسلامة الخلايا ، ومعدل الخصوبة ، وعدد الخلفة عند الولادة كانت هناك فروق إحصائية كبيرة ، بينما انخفض وقت الاستجابة للوثب، والحيوانات المنوية غير الطبيعية والحيوانات المنوية الميتة بشكل كبير. سجلت المجموعتان ETT1 + Se و ETT2 + Se معدل ارتفاع في الخصوبة وزيادة عدد المواليد عند الولادة. أظهرت المجموعات المعاملة فروق إحصائية في وزن الجسم النهائى ، زيادة في معدل الزيادة فى وزن الجسم وأدنى تحويل غذائي مقارنة بمجموعة التحكم.

التوصية : أدى مستخلص نبات الحسكة (تريبولوس تيريستريس) مع السيلينيوم إلى تحسين نشاط الهرمونات الجنسية ، وحالة الأكسدة والمناعة ، كما أدت الإضافات إلى تحسين جودة السائل المنوي وزيادة الخصوبة والخلفات عند الولادة خلال فصل الصيف.