

SEMEN QUALITY, TESTOSTERONE LEVEL, SOME ENZYMES ACTIVITIES AND FERTILITY OF MALE APRI RABBITS TREATED WITH DIFFERENT LEVELS OF OLIVE LEAF EXTRACT

G.E. Younan; Hoda E. El-Gabry and W.A. Morsy

Animal Production Research Institute, Agricultural Research Center, Dokki, Egypt.

SUMMARY

The present study was designed to investigate the efficacy of different dietary levels of ethanolic olive leaf extract (OLE), as a source of natural antioxidants, on quality and production of semen, some enzymes activities and testosterone concentration in blood serum and some seminal plasma characteristics of APRI rabbit bucks. A total of 24 mature rabbit bucks of APRI line (3-3.4 kg live body weight and 9-12 months of age) were divided into four similar groups, 6 bucks in each. Bucks in the 1st group were fed a reference diet (17.75% CP and 2500 DE Kcal/Kg diet) without any supplements (G1, control). While, those in the 2nd (G2), 3rd (G3) and 4th (G4) treatment groups were fed the same diet supplemented with olive leaf extract at levels of 0.5, 1 and 1.5 ml/kg diet, respectively. Bucks were fed *ad libitum* on the experimental diets for a period of 17 wk (8 wk as a treatment period and 9 wk as main semen collection period). Semen was evaluated physically, chemically and by transmission electron microscope (TEM). Results showed that all levels of dietary OLE supplementation increased ($P<0.05$) total semen volume in G2, G3 and G4 as compared to G1, being the highest in G4. Sperm characteristics, including percentages of progressive motility, livability, abnormality and cell concentration of spermatozoa improved ($P<0.05$) in G3 and G4 as compared to G2 and G1. Examination of sperm cells in all groups by TEM cleared enhancing the abnormalities in cell membrane and acrosome, axoneme as well as chromatin of spermatozoa in G4 as compared to other groups. Bucks in G4 showed the lowest ($P<0.05$) peroxidase and fructose testes in seminal plasma as compared to other groups. Activity of LDH in seminal plasma was higher ($P<0.05$) in all treatment groups than in control one, being the highest in G4 and the lowest in G1. Activity of AST and ALT and testosterone concentration in blood serum were not affected by OLE treatment. Activity of ALP in G4 and LDH activity in G3 and G4 increased ($P<0.05$) as compared to other groups. Kindling rate and reproductive index were improved ($P<0.05$) in rabbit does mated by bucks of G3 and G4 as compared to that of G2 and G1, being the highest in G4 (81.67% \pm 6.5). In conclusion, supplementation of the diet with olive leaf extract, in particular at a level of 1.5 ml/kg, as a natural antioxidants source, is essential for improving reproductive performance and consequently semen quality and fertility of breeding rabbit bucks (natural or artificial mating) without adversely effects on healthy status.

Keywords: rabbit, antioxidant, oleuropein, sperm function, axoneme, kindling rate.

INTRODUCTION

Semen quality is the guarantee of successful insemination in breeding rabbits. Artificial Insemination (AI) is widely employed and this diffusion has contributed to the increase in knowledge of spermatozoa metabolism and management of rabbit bucks. There are many endogenous and exogenous factors affecting reproduction of rabbit bucks (Boiti *et al.*, 2005) and thus it is crucial to define suitable protocols to improve sperm characteristics (Brun *et al.*, 2002). Hence, it is possible to produce more doses of semen with higher "expected" fertility and with less variability.

Olive leaf (OL) is one of the potent source of plant polyphenols having antioxidant, antimicrobial, antiviral properties due to its rich phenolic contents. The most phenolic component of this content is oleuropein, which gives high palatability to olive or its olive oil. In order to utilize oleuropein and other bioactive components within OL effectively enough, they should be extracted from olive leaf. Olive leaf extract (OLE) contains compounds with potent antimicrobial activities against bacteria, fungi, and mycoplasma (Huang *et al.*, 2003). Also, Aliabadi *et al.* (2012) indicated that using aqueous OLE had beneficial effects in controlling the microbial infections because it is a potent source of polyphenols having antioxidant, antimicrobial, anti-inflammatory and antiviral properties. Moreover, Sudjana *et al.* (2009)

showed the antimicrobial activity of commercial olive leaf extracts (*Olea europaea*) against campylobacter jejuni (CJ), Helicobacter pylori (HP) and methicillin-resistant *Staphylococcus aureus*. These extracts play a role in regulating the composition of the gastric flora by selectively reducing levels of HP and CJ. The reports describing antimicrobial properties of phenolic compounds in olive products refer to compounds obtained from olive fruit, particularly hydroxyl tyrosol and oleuropein (Periera et al., 2007).

In addition, Juven et al. (1972) observed that oleuropein affected a significant leakage of glutamate, potassium and inorganic phosphate from *Lactobacillus plantarum*. Oleuropein had no effect on the rate of glycolysis when added to resting cells of *L. plantarum* but it caused a decrease in the ATP content of the cells. Shafey et al. (2013) found that dietary addition of 50 g olive leaf/kg altered small intestine measurements and reduced live and carcass weights of broiler chickens. Cayan and Erenner (2015) observed that olive leaf powder (1, 2, or 3%) can be used for reducing egg yolk cholesterol content and egg yolk coloring agent in layer diets.

In rabbits, Dub and Dugani (2013) studied the effect of ethanolic olive leaf extract (EOLE) at levels of 100 and 200 mg/kg per day. They found that EOLE can modify the extrinsic coagulation pathway as evidenced by the prolongation of prothrombin time and changes in thrombus morphology, enough to justify further research to evaluate its possible antithrombotic effects.

The available data on the protective properties of OLE on the function of the testes and accessory sex glands, in terms of semen quality and testosterone production in rabbits are rare. Therefore, the present study was designed to investigate the efficacy of different dietary levels of ethanolic olive leaf extract, as a source of antioxidants, on quality and production of semen, some enzyme activity and testosterone concentration in blood serum, and some characteristics of seminal plasma of APRI rabbit bucks.

MATERIALS AND METHODS

The present study was carried out at rabbit farm of Sakha station, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt during the period from March to June 2017.

Animals and Management:

A total of 24 mature rabbit bucks of APRI line (3-3.4 kg live body weight and 9-12 months of age) were divided into four similar groups, 6 bucks in each. Bucks in the 1st group were fed complete fed diet (17.75% CP and 2500 DE Kcal/Kg diet) without any supplements (G1, control). While, those in the 2nd (G2), 3rd (G3) and 4th (G4) treatment groups were fed the same diet supplemented with olive leaf extract at levels of 0.5, 1 and 1.5 ml /kg diet, respectively. Ingredients and chemical composition of the complete feed diet fed to rabbit bucks in all groups are shown in Table (1).

Table (1): Feed ingredients and calculated chemical analysis of the reference diet.

Ingredient	%	Calculated chemical analysis		
Berseem hay (<i>Trifolium alexandrinum</i>)	30.05	Crude protein	%	17.75
Barley	24.60	Digestible energy	Kcal/Kg diet	2500
Wheat bran	21.50	Crude fiber	%	12.38
Soybean meal (44% CP)	17.50	Ether extract	%	2.27
Molasses	3.00	Calcium	%	1.24
Di-calcium phosphate	1.60	Total phosphorus	%	0.80
Limestone	0.95	Lysine	%	0.98
Sodium chloride (NaCl)	0.30	Methionine	%	0.46
Vitamin & Mineral Mixture*	0.30	Methionine + Cystine	%	0.76
DL-Methionine	0.20	Sodium	%	0.16

*Supplied per kilogram of diet: Vitamin A, 6000 IU; Vitamin D₃, 900 IU; Vitamin E, 40 mg; Vitamin K₃, 2 mg; Vitamin B₁, 2 mg; Vitamin B₂, 4 mg; Vitamin B₆, 2 mg; Pantothenic acid, 10 mg; Vitamin B₁₂, 0.01 mg; Niacin, 50 mg; Folic acid, 3 mg; Biotin, 0.05 mg; Choline, 250 mg; Fe, 50 mg; Mn, 85 mg; Cu, 5 mg; Co, 0.1 mg; Se, 0.1 mg; I, 0.2 mg and Zn, 50 mg.

Bucks were individually housed in wire cages supplied with nipple drinkers and fed *ad libitum* on the diets for an experimental period of 17 weeks, 8 weeks as a treatment period and 9 weeks as main semen collection period.

Preparation of olive leaf extract (OLE):

Olive leaves used in this study were collected in winter (February) from Borg El-Arab region, Alexandria governorate, Egypt. The collected olive leaves were cleaned from extraneous matter, shade-dried at room temperature with passive ventilation and crushed into a fine powder. The air dried plant materials were ground in a blender with a particular size to ensure the plant powders in identical size. The powder (50 g) was macerated in 150 ml ethanol (75%) and allowed to extract for 48 h. The resultant (dark green-brown mixture) was filtered. The crude extracts were kept in refrigerator in glass bottles until the further experiments.

Semen collection:

Semen was collected twice weekly from bucks in all groups using artificial vagina of rabbits. Semen was collected before feeding at 8.00 a.m. Gel plug was removed immediately after ejaculation and semen was kept at 35-37 °C in water bath, then the collected semen was taken immediately to the laboratory.

Semen evaluation:

Immediately after collection, semen pH value was determined by pH meter pen. Semen volume without gel fraction) and gel volume were measured by graduated collection test tube. Percentages of progressive motility, livability and abnormality of spermatozoa were determined in semen diluted with saline solution at a rate of 1:10. Sperm progressive motility was assessed using research microscope with warmed stage (37°C) according to Amman and Hammerstedt (1980). Sperm livability was determined using eosin-nigrosin mixture stain according to Hackett and Macpherson (1965). Live spermatozoa (unstained ones) and dead spermatozoa (stained ones) were counted in field of a total of 200 spermatozoa, then percentage of live spermatozoa was calculated. Sperm abnormality percentage was determined during the examination of live/dead sperm percentage at a high power magnification (400x), according to the classification adopted by Blom (1983). Sperm cell concentration (SCC) was evaluated by Neubauer hemocytometer.

The response of spermatozoa to Hypo-Osmotic Swelling-test (HOS-t) was conducted at osmolarity level of 50 mOsm/l for 30 min, in term of determining curled sperm percentage.

Transmission electron microscope (TEM) evaluation of semen samples

Semen samples were processed for TEM as described in (Oliveira *et al.*, 2011) with some modifications. Briefly, samples (500 µL) were centrifuged and resuspended in a fixative solution composed of 4% glutaraldehyde in Dulbecco's modified phosphate buffered saline for 2h at 4°C. Samples were then washed and post-fixed in 1% osmium tetroxide for 1h at room temperature. Fixed samples were then dehydrated in an ethanol gradient, treated with propylene oxide and embedded in Epon resin (Epon 812; Fluka Chemie, Switzerland) and ultrathin-sectioned (60-70 nm) for TEM. Ultrathin sections were observed at 80 kV using a JEOL 2100 TEM at 80 KV at Faculty of Agriculture, Mansoura University, Egypt. The sperm ultrastructure was examined in 100 sperm cells per sample.

Sperm production:

Sperm production (outputs), in terms of total (TSO), motile (MSO), live (LSO), normal (NSO) and functional (FSO) sperm outputs per ejaculate were calculated as the following:

$$TSO/ejaculate = ejaculate\ volume\ (ml) \times SCC\ (sperm/ml)$$

$$MSO/ejaculate = TSO/ejaculate \times sperm\ motility\ (\%)$$

$$LSO/ejaculate = TSO/ejaculate \times live\ sperm\ (\%)$$

$$NSO = [TSO/ejaculate \times (sperm\ abnormality - 100)] / 100$$

$$FSO = [TSO/ejaculate \times sperm\ motility\ (\%) \times sperm\ livability\ (\%) \times sperm\ normality\ (\%)]$$

Seminal plasma sampling:

At the last collection week, semen samples were taken from each buck in all experimental groups into tubes and incubated for one hour in water bath at 37°C, centrifuged at 1500 rpm for 15 minutes, and then seminal plasma was separated and frozen at -20 °C until assaying peroxidase and fructose tests by determination of peroxidase-positive white blood cells and fructose concentration in seminal plasma according to Aitken (1989) and Foreman *et al.* (1973), respectively.

Blood sampling:

Blood samples were taken from the ear vein of each buck in all experimental groups into tubes. The samples were centrifuged at 3000 rpm for 15 minutes and blood plasma was separated and frozen at -20 °C until assaying the testosterone concentration using a double antibody radioimmunoassay (Diagnostic Products Corporation Kits) according to McCann and Kirkish (1985). However, activity of AST, ALT, ALP and LDH was determined using commercial kits (Diagnostic Products Corporation Kits) according to Young, 1990, Sherwin *et al.* 1984, and Belfield and Goldberg (1971).

Statistical analysis:

Data were analyzed by two-way (treatment x collection week) analysis of variance (ANOVA) using the General Linear Model (GLM) procedure, and also personal correlation coefficients were calculated using computer program of SAS (2004). Significant differences among means were set at P<0.05 using Duncan's multiple range test (Duncan, 1955).

The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages. Data were expressed as mean ± standard error.

RESULTS AND DISCUSSION

Semen characteristics:

Semen volume:

Data in Table (2) show that all levels of dietary OLE supplementation significantly (P<0.05) increased total semen volume in G2, G3 and G4 as compared to control (G1), being the highest in G4 (1.5 ml/kg diet). This increase was associated with significant (P<0.05) increase in net semen volume and insignificant increase in gel volume and semen pH value. It is of interest to note that the effect of collection week or its interaction with OLE treatment was insignificant on volumes and pH value of semen. Such results indicated beneficial effects of dietary addition of OLE at a level of 1.5 ml/kg diet) on semen volume of rabbit bucks.

Table (2). Semen physical characteristics of APRI rabbit bucks fed different olive leaf extract levels at collection weeks.

Variable	Total semen volume (ml)	Semen gel volume (ml)	Net semen volume (ml)	Semen pH value
Effect of olive leaf extract level :				
G1 (control)	0.756 ^c ±0.038	0.133±0.055	0.622 ^b ±0.055	7.208±0.074
G2 (0.5 ml/kg diet)	1.056 ^b ±0.082	0.200±0.069	0.856 ^a ±0.067	7.362±0.071
G3 (1.0 ml/kg diet)	1.356 ^a ±0.097	0.356±0.078	1.000 ^a ±0.044	7.371±0.096
G4 (1.5 ml/kg diet)	1.400 ^a ±0.126	0.333±0.088	1.067 ^a ±0.088	7.491±0.071
Effect of collection week:				
1-3	1.092±0.097	0.250±0.056	0.842±0.077	7.258±0.073
4-6	1.117±0.121	0.242±0.066	0.875±0.076	7.408±0.079
7-9	1.217±0.109	0.275±0.082	0.942±0.071	7.408±0.056
Interaction between olive leaf extract levels and collection week:				
P-value	0.9603	0.9310	0.9999	0.4409

a, b and c: Means in the same column for each factor with different superscripts are significantly different (P<0.05).

The present results revealed that elevation of total semen volume was due to increasing net volume rather than gel volume, which may indicate pronounced effect of OLE treatment, directly on function of accessory sex (seminal vesicles, prostate and Cowper's glands) or indirectly on testosterone secretion, which has vital role on accessory sex gland function in rabbit bucks (Abdel-Khalek *et al.*, 2005). In this way, Awoniyi (2010) stated that, improving semen volume with increasing testosterone concentration may reveal pronounced effect on accessory sex glands and testicular tissues (spermatocytes) within the somniferous tubules of the testis as well as on epididymal spermatozoa. Generally, the present values of net semen volume in all treatment and control groups are within the normal range of rabbits as reported by Abdel-Khalek *et al.* (2005) on NZW bucks and Mangiagalli *et al.* (2012) on hybrid Martini male rabbits, but it was higher than that reported by Sheteifa and Morsy (2014) on APRI bucks. The present study revealed positive relationship between pH values and ejaculate volume. The obtained semen pH values were similar to that obtained on APRI buck semen (Sheteifa and Morsy, 2014), higher than that reported on NZW rabbits (Abdel-Khalek *et al.*, 2005) and lower than that reported on Californian rabbit semen (Ongun *et al.*, 2010).

Physical characteristics of spermatozoa:

Results in Table (3) show that sperm characteristics studied including progressive motility (PM) and sperm livability (SL) of spermatozoa significantly ($P<0.05$) improved in G3 and G4 as compared to G2 and G1. However, sperm abnormality (SA) and sperm cell concentration (SCC) of spermatozoa significantly ($P<0.05$) improved in all treatment groups (G2, G3 and G4) as compared to G1. Generally, bucks in G4 showed significantly ($P<0.05$) the best semen quality as compared to other groups. As affected by collection week, sperm motility significantly ($P<0.05$) improved after three collection weeks. While, both sperm livability and abnormality significantly ($P<0.05$) improved after six collection weeks. Yet, sperm cell concentration showed gradual improvement by advancing collection week, but this change was not significant (Table 3).

Table (3). Sperm characteristics in semen of APRI rabbit bucks fed different olive leaf extract levels at collection weeks.

Variable	Progressive sperm motility (%)	Sperm livability (%)	Sperm abnormality (%)	Sperm cell concentration ($\times 10^6/ml$)
Effect of olive leaf extract level :				
G1 (control)	71.44 ^b ±3.25	80.56 ^b ±1.20	12.22 ^a ±0.57	115.11 ^c ±6.26
G2 (0.5 ml/kg diet)	79.67 ^{ab} ±2.65	82.89 ^b ±1.66	10.11 ^b ±0.98	216.33 ^b ±12.75
G3 (1.0 ml/kg diet)	82.45 ^a ±2.26	85.89 ^a ±1.26	9.22 ^b ±0.78	238.67 ^{ab} ±10.14
G4 (1.5 ml/kg diet)	82.56 ^a ±1.12	86.78 ^a ±1.19	8.56 ^b ±0.82	253.56 ^a ±11.49
Effect of collection week:				
1-3	75.08 ^b ±2.96	81.25 ^b ±1.40	11.50 ^a ±0.76	194.5±17.12
4-6	80.58 ^a ±1.96	83.00 ^b ±0.78	10.42 ^a ±0.54	204.4±16.85
7-9	81.41 ^a ±1.15	87.83 ^a ±1.09	8.17 ^b ±0.72	218.8±20.78
Interaction between olive leaf extract levels and collection week:				
P-value	0.0001	0.8972	0.6381	0.7930

a, b and c: Means in the same column for each factor with different superscripts are significantly different ($P<0.05$).

Analysis of variance revealed that the effect of interaction between treatment and collection week was highly significant ($P<0.0001$) only for sperm motility, reflecting increasing sperm motility in all groups after three collection weeks, being the highest in G2 than in other groups, then G1 showed marked increase versus stability in motility of other groups after 6 collection weeks, but percentage of sperm motility was higher in treatment groups than in control at all collection weeks (Fig. 1).

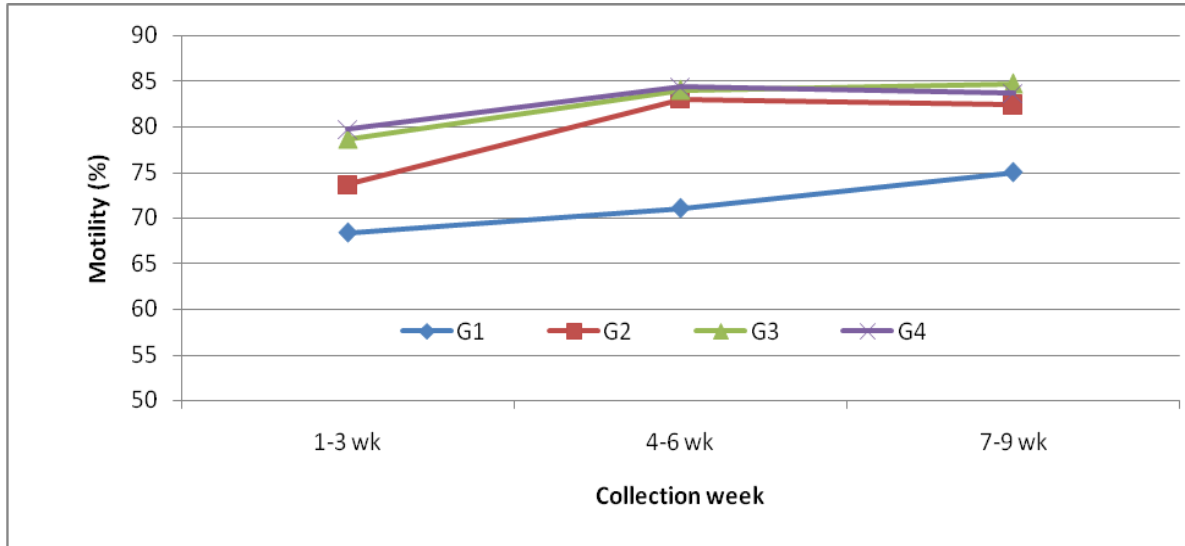


Fig. (1): Change in sperm progressive motility percentage in semen of rabbit bucks in experimental groups at different collection weeks.

Sperm cell membrane integrity (Hypo-osmotic swelling-test, HOS-t):

Results presented in Table (4) revealed that spermatozoa in semen of bucks in all treatment groups at the last collection week showed significantly ($P < 0.05$) higher response to HOS-t in term of increasing percentage of curled spermatozoa than that of the control bucks, being the highest in G4. It is of interest to mention that percentage of curled spermatozoa significantly and positively correlated with each of sperm output parameters, being the strongest with normal and live sperm outputs ($r = 0.828$ and 0.826 , $P < 0.001$), followed by functional, total, and motile sperm outputs ($r = 0.820$, 0.818 and 0.810 , $P < 0.01$). These results indicated impact of all OLE levels on production of spermatozoa with intact membranes.

Table (4). Hypo-osmotic Swelling-test (HOS-t) in semen of APRI rabbit bucks fed different olive leaf extract levels at collection weeks and its correlation coefficients with sperm outputs.

Item	HOS-t (curled spermatozoa %)
Effect of olive leaf extract level :	
G1 (control)	$29.67^c \pm 1.20$
G2 (0.5 ml/kg diet)	$38.67^b \pm 2.40$
G3 (1.0 ml/kg diet)	$43.67^{ab} \pm 2.19$
G4 (1.5 ml/kg diet)	$46.33^a \pm 1.76$
Correlation coefficients of Hypo-osmotic Swelling-test with:	
Total sperm output	0.818^{**}
Motile sperm output	0.810^{**}
Live sperm output	0.826^{***}
Normal sperm output	0.828^{***}
Functional sperm output	0.820^{**}

a, b and c: Means in the same column with different superscripts are significantly different ($P < 0.05$).

*** Significant at $P < 0.01$. *** Significant at $P < 0.001$.*

Ultrastructural examination:

The morphological examination of spermatozoa using electron microscopy indicated the normal membrane integrity and intact acrosome of spermatozoa in semen of all groups, being the best semen of bucks in G4, followed by G3, G2 and G1, respectively. Longitudinal ultrathin sections in sperm cells of semen in all groups showed

intact sperm with normal head, neck and mid piece (A) (Fig. 2a), sperm head showing normal nucleus (N) with homogenous condensed chromatin surrounded by normal acrosomal membrane (AM) and normal axoneme (Fig. 2b), and intact main piece of tail with normal microtubules (MT) and plasma membrane (PM) (Fig. 2c).

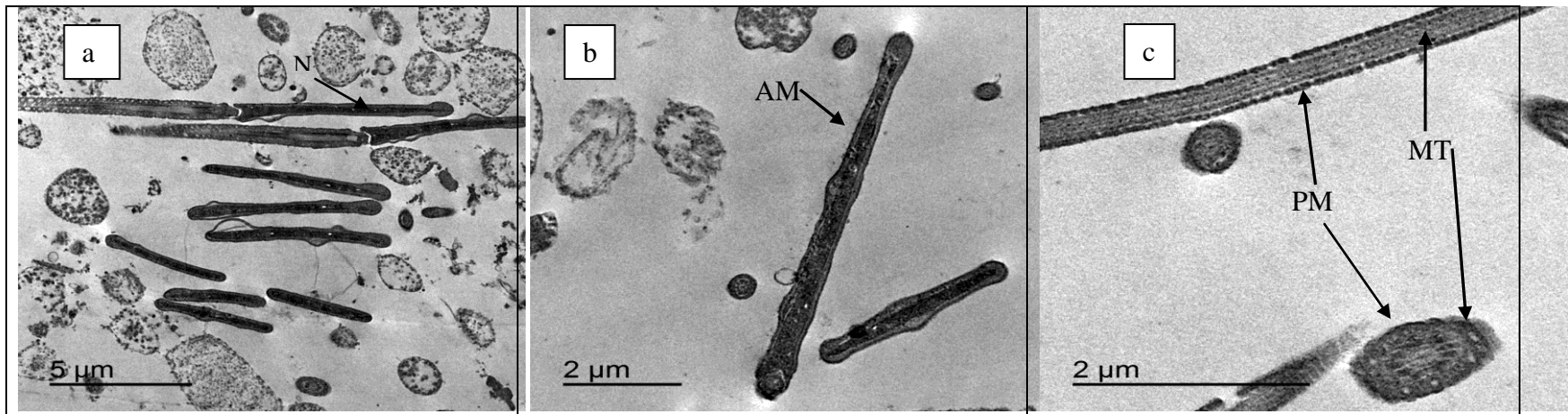
On the other hand, some abnormalities at different levels were observed head of some sperm cells in semen of G3. Longitudinal ultrathin sections in sperm cells of buck semen in G3 showed sperm with head abnormalities, in terms of slightly swollen acrosomal membrane (SAM) and damaged chromatin (DC) (Fig. 3a), swollen head spermatozoa (SH) (Fig. 3b), and damaged chromatin and abnormal acrosomal membrane (Fig. 3c). Other defected spermatozoa were observed in semen of bucks in G2, in terms of normal plasma membrane with unclear arrangement in axoneme (AX) of different segments of the main piece (Fig. 4a), and slight damaged acrosomal membrane (AM) (Fig. 4b), and intact mid piece with normal spiral mitochondria (MI), normal axoneme (AX), and lateral and central microtubules (MT) (Fig. 4c).

It is of interest to not that examination of ultrathin sections in sperm cells of buck semen in G1 (control group) revealed also normal plasma membrane with pronounced swelling in acrosomal membrane (SAM) in some sperm heads versus intact acrosomal membrane (IAM) (Fig. 5a and b), and slight swelling and breakdown in the acrosomal membrane (AM) (Fig. 5c). Similar abnormalities were observed in frozen Friesian semen using electron microscopy Hassan (2015).

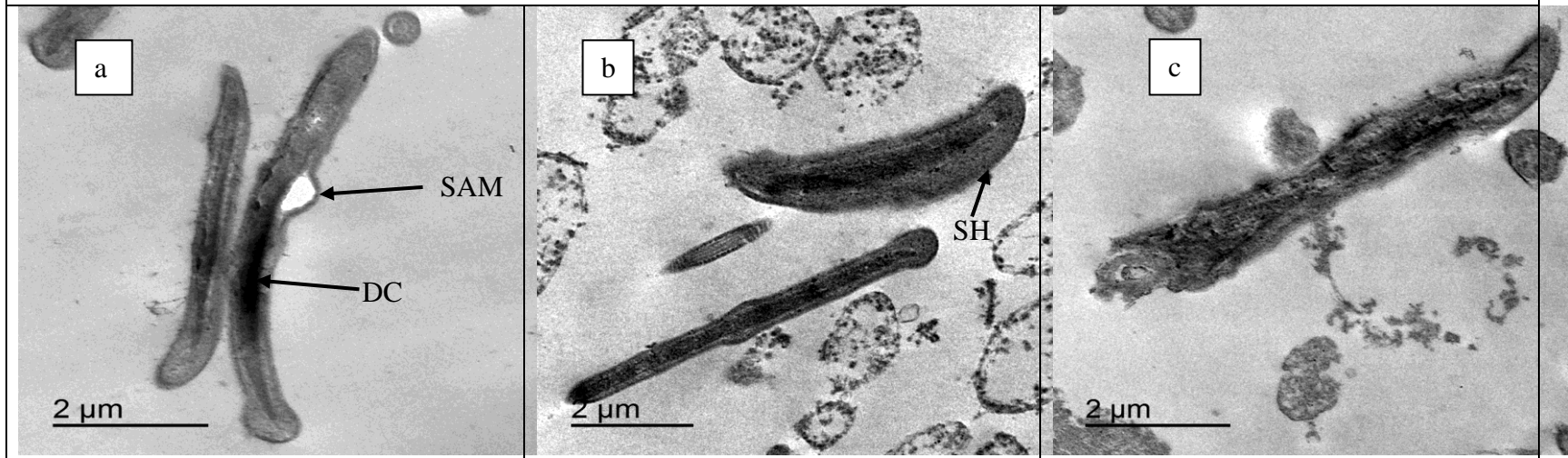
As found in improving semen volume of rabbit bucks as affected by OLE (Table 2), the obtained results (Tables 3 and 4) indicated that OLE had beneficial effects also on function and membrane integrity of spermatozoa in semen of bucks in term of increasing progressive motility, livability and membrane integrity of spermatozoa at levels of 1.0 or 1.5 ml/kg diet, while all OLE levels had impact on reducing sperm abnormality and increasing sperm cell concentration in rabbit semen. The recorded improvement in semen quality was observed after three collection weeks. The highest volume and the best quality of semen were obtained from bucks treated with 1.5 ml/kg diet.

In accordance with the present results, natural antioxidants as *Moringa oleifera* (MO) leaf extract (Khalifa *et al.*, 2016) or MO leaf meal (Oyeyemi *et al.*, 2008) significantly increased the sperm physical characteristics (motility, viability and membrane integrity) of rabbit bucks. The later authors observed a positive correlation between inclusion MO leaf meal and sperm concentration in rabbits. In this respect, Dacheux *et al.* (2003) found that epididymis is known to play a major role in the final development of motility, fertilizing ability and sperm storage. Sperm concentration can increase during epididymal transit with a simultaneous increase in sperm metabolism and the possibility of ROS threatens the survival of these male gametes. These findings may proved the beneficial effects of *in vivo* OLE treatment as antioxidant on improving most sperm characteristics, including motility, abnormality and concentration and may be attributed to the prevention of excessive generation of free radicals produced by sperm by means of the antioxidant properties of olive leaf extract. Also, Purdy *et al.* (2004) demonstrated that flavonoids caused an increase sperm motility. Moreover, Eid *et al.* (2006) found that a higher antioxidant intake was associated with greater sperm numbers and motility. However, Abu *et al.* (2013) results showed that MO leaf meal did not significantly influence sperm motility and concentration of the rabbits. These results supported the obtained results concerning improvement in sperm motility in association with marked increase in sperm cell concentration and reducing sperm abnormality without pronounced effect on sperm livability as affected by *in vivo* OLE treatment.

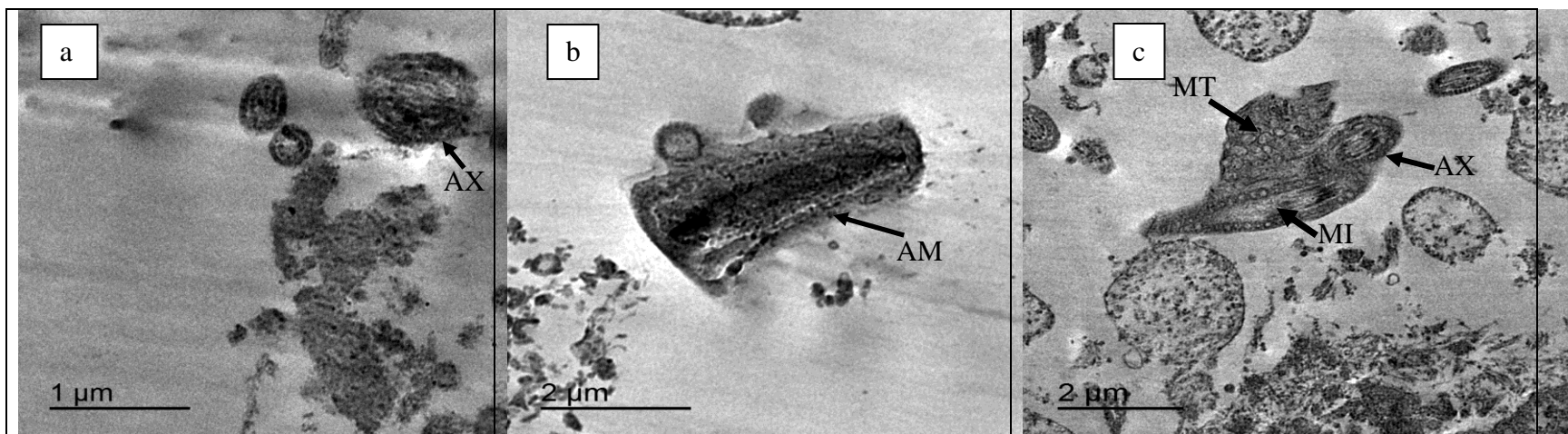
Oxidative stress plays a prominent role in patho-physiology of reproductive malfunction and infertility in animals and humans (Feldman *et al.*, 2000). A shift in pro-oxidant and oxidant status could lead to reactive oxygen species (ROS), but a change in antioxidant activities is frequently used as an important indicator of ROS and the antioxidant defense status determines the extent to which oxidative damage occurs in the sperm (Sikka, 2001; Ong *et al.*, 2002). The generation of ROS by sperm is a normal physiological process, however a shift between ROS production and scavenging activity is deleterious to sperm and it has been shown to be associated with male infertility (Sharma and Agarwal, 1996).



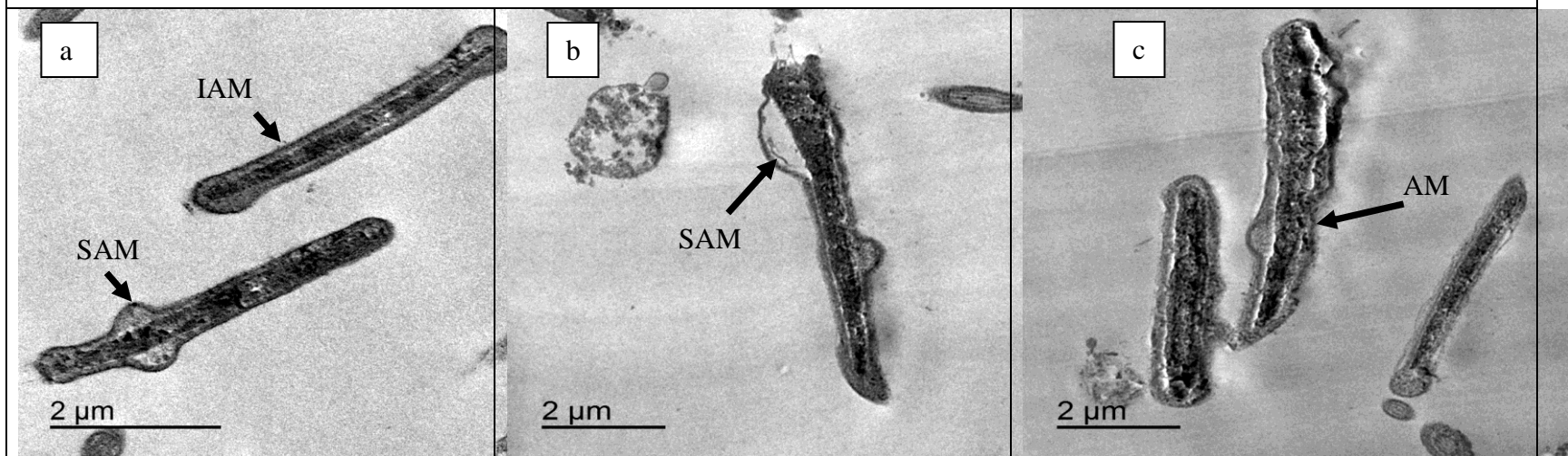
2): Longitudinal ultrathin section in sperm cells in semen of bucks in G4 showing a: intact sperm with normal head, neck and mid piece (a), (b): sperm head showing normal nucleus (N) with homogenous condensed chromatin surrounded by normal acrosomal membrane (AM) and normal axoneme, and c: intact main piece of tail with normal microtubules (MT) and plasma membrane (PM).



Longitudinal ultrathin section in sperm cells of buck semen in G3 showing sperm with head abnormalities, being a: slight swelling in acrosomal membrane (SAM), damaged chromatin (DC), b: sperm cell with swollen head (SH), and c: sperm head with damaged chromatin and acrosomal membrane.



Ultrathin section of sperm in semen of bucks in G2 showing a: normal plasma membrane with unclear arrangement in axoneme (AX) of different segments of the main piece, as well as b: slight damaged acrosomal membrane (AM), and c: intact mid piece with normal spiral mitochondria (MI), normal axoneme (AX), and lateral and central microtubules (MT).



Ultrathin section of sperm in semen of bucks in G1 (control) showing a and b: normal plasma membrane with pronounced swelling in acrosomal membrane (SAM) in some sperm heads versus intact acrosomal membrane (IAM), and c: slight swelling and breakdown in the acrosomal membrane (AM).

Based on the present results in tem of improving semen quality of bucks treated with OLE, several authors indicated that olive leaf or its extract is one of the potent source of plant polyphenols having antioxidant, antimicrobial (against bacteria, fungi, and mycoplasma), antiviral and anti-inflammatory, properties (Suresh *et al.*, 2009; Aliabadi *et al.*, 2012). The most phenolic components of this content is oleuropein, which gives the better taste to olive and its oil (Huang *et al.*, 2003; Periera *et al.*, 2007). Flavonoids and phenolic compounds obtained from olive leaf are known to have diverse biological activities and may also be responsible for the pharmacological actions of olive leaf or, at least synergistically reinforcing those actions (Abaza *et al.*, 2011). Even though, there are several groups, remarkable for determination of antioxidant capacity of flavonoids, it is mainly the o-dihydroxy (catechol) structure which bestows the antioxidant properties to OLE (Benavente-Garcia *et al.*, 2000). Generally, mechanisms of antioxidant action of OLE may include suppressing ROS formation either by inhibition of enzymes or chelating trace elements involved in free radical production, scavenging ROS, and upregulating and protecting antioxidant defenses.

Generally, Saalu *et al.* (2011) reported the antioxidative properties of MO and its ability to elevate a variety of antioxidant enzymes and testicular biomarkers. Improving volume and quality of semen and sperm production may attributed to increasing testosterone concentration, revealing pronounced effect of OLE on accessory sex glands and testicular tissues (spermatocytes) within the somniferous tubules of the testis as well as on epididymal spermatozoa (Awoniyi, 2010).

Total sperm output:

Results in Table (5) showed that sperm outputs (total, motile, live, normal and functional) per ejaculate significantly ($P < 0.01$) increased in G3 and G4 as compared to G2, and in G2 than in G1, being the highest in G4. Such finding was mainly attributed to significant increasing in semen volume along with sperm cell concentration, and percentages of motility, livability and normality of spermatozoa in semen of bucks in G4 and G3 as affected by OLE treatment, respectively. As affected by collection week, sperm outputs gradually increased by advancing collection week, but this increases were insignificant only for total sperm outputs. Analysis of variance revealed that the effect of interaction between treatment and collection week was not significant on all outputs of spermatozoa.

Table (5). Sperm outputs in semen of APRI rabbit bucks fed different olive leaf extract levels at collection weeks.

Treatment	Sperm output ($\times 10^6$) per ejaculate				
	Total sperm (TSO)	Motile sperm (MSO)	Live sperm (LSO)	Normal sperm (NSO)	Functional sperm (FSO)
Effect of olive leaf extract level :					
G1 (control)	73.4 ^c ±9.94	48.7 ^c ±11.49	59.3 ^c ±8.14	64.4 ^c ±8.63	34.9 ^c ±8.20
G2 (0.5 ml/kg diet)	187.4 ^b ±22.04	141.8 ^b ±17.15	156.1 ^b ±19.03	168.8 ^b ±20.08	106.6 ^b ±13.76
G3 (1.0 ml/kg diet)	239.5 ^a ±16.85	193.9 ^a ±14.35	206.3 ^a ±16.13	217.6 ^a ±15.96	152.1 ^a ±13.35
G4 (1.5 ml/kg diet)	265.0 ^a ±17.62	219.8 ^a ±16.18	230.7 ^a ±16.87	242.8 ^a ±17.14	175.6 ^a ±14.80
Effect of collection week:					
1-3	170.8±22.86	122.9 ^b ±22.15	141.0 ^b ±20.15	152.2 ^b ±20.89	91.1 ^b ±17.31
4-6	185.5±23.39	150.2 ^{ab} ±19.86	155.2 ^b ±20.11	166.5 ^{ab} ±21.02	113.0 ^b ±15.47
7-9	217.8±30.73	180.0 ^a ±24.93	193.0 ^a ±27.68	201.6 ^a ±28.93	147.8 ^a ±21.39
Interaction between olive leaf extract levels and collection week:					
P-value	0.9342	0.8867	0.8556	0.8764	0.8161

a, b and c: Means in the same column for each factor with different superscripts are significantly different (P < 0.05).

Sperm cytoplasm contained very low concentrations of scavenging enzymes therefore an increase in the antioxidant enzyme levels by MO treatment could favor the reproductive process (D'cruz and Mathur, 2005). The antioxidants present in the leaves of the plant, acting in concert with the antioxidant system present in the epididymis further preserved and enhanced the process of spermatogenesis (Khalifa *et al.*, 2016). Therefore, improving sperm outputs with increasing testosterone concentration may reveal pronounced effect of OLE on testicular tissues (spermatocytes) within the somniferous tubules of the testis as well as on epididymal

spermatozoa (Awoniyi, 2010). It was reported that administration of aqueous extract from MO seeds (Zade *et al.*, 2013) or MO leaves (Khalifa *et al.*, 2016) has positive effect on spermatogenesis in rats and rabbits, respectively. This impact may be attributing to presence of flavonoids that can ameliorate oxidative stress-related testicular impairments in animal tissues (KujoS, 2004). The presence of flavonoids in OLE had been implicated to have a role in altering androgen levels (Padashetty and Mishra, 2007). Moreover, Shukla and Khanuja (2004) reported that steroid and saponin constituents found in many plants possess fertility potentiating properties, and they are useful in the treatment of impotence. The saponins may boost the level of testosterone in the body as well as trigger libido-enhancing effect (Gauthaman and Adaikan, 2008). Finally, Zade *et al.* (2013) reported that the numbers of mature Leydig cells as well as number of spermatocytes and spermatids were significantly increased in MO treated testes.

Seminal plasma parameters:

Data in Table (6) revealed that bucks in G4 showed significantly ($P<0.05$) the lowest peroxidase and fructose testes in seminal plasma as compared to other groups. However, LDH activity in seminal plasma was significantly ($P<0.05$) higher in all treatment groups than in control group, being the highest in G4. Meanwhile bucks in G1 (control) showed an opposite trend.

Table (6). Peroxidase and fructose tests and LDH activity in seminal plasma of APRI rabbit bucks fed different olive leaf extract levels at the end of collection semen period and their correlation coefficients.

Treatment	Peroxidase test ($U \times 10^3$)	Fructose test (mg/ dl)	LDH activity (U/l)
G1 (control)	1.133 ^a ±0.145	279.00 ^a ±38.35	41.56 ^c ±4.85
G2 (0.5 ml/kg diet)	0.633 ^b ±0.088	137.33 ^b ±6.17	86.86 ^b ±4.40
G3 (1.0 ml/kg diet)	0.566 ^b ±0.088	99.33 ^b ±8.68	106.92 ^a ±6.91
G4 (1.5 ml/kg diet)	0.500 ^b ±0.057	89.00 ^b ±13.07	118.88 ^a ±7.09
Correlation coefficients with sperm outputs:			
TSO	-0.77441**	-0.80265**	0.77724**
MSO	-0.75036**	-0.80606**	0.77407**
LSO	-0.77258**	-0.818**	0.80038**
NSO	-0.77125**	-0.80909**	0.79237**
FSO	-0.74026**	-0.82081**	0.80475**
Correlation coefficients with semen tests:			
Seminal fructose test	0.91725***	-	-
Seminal LDH activity	-0.84008***	-0.89391***	-
HOS-test	-0.81177**	-0.84695***	0.92957***

a, b and c: Means in the same column with different superscripts are significantly different ($P<0.05$).

*** Significant at $P<0.01$. *** Significant at $P<0.001$.*

It is of interest to note that reduction in values of peroxidase and fructose testes in seminal plasma of G4 was attributed to the lowest yield of lipid peroxidation in sperm cells and the highest consumption of fructose by spermatozoa during sperm metabolism in comparing with seminal plasma of bucks in other groups. In this respect, high quality semen should contain higher glycolytic or fructolytic rates than in semen with weak immobile sperms (Mann, 1964). The recorded lowest values of fructose test in G4 were paralleled with the highest progressive motility percentage. Yousef *et al.* (2003) assumed that the increases in sperm motility in semen could in part be attributed to the concomitant induction in semen fructose (Yousef *et al.*, 2003).

Results in the current study as response to OLE as a plant natural antioxidant agreed with that obtained on MO extract as natural antioxidants. In this line, Ghodaiah (2016) reported that total antioxidant activity increased in blood of rabbit bucks orally treated with 60 mg ($P<0.05$) of MOE for 21 days. Also, extracts of MO increased the production of antioxidants in the sperms (Abdou *et al.*, 2012). Moreover, Luqman *et al.* (2012) found that MO extract remove free radicals, activate antioxidant enzymes, and inhibit oxidases.

Results of correlation coefficients (Table 6) revealed that peroxidase or fructose test in seminal plasma correlated negatively ($P<0.01$) with each of total sperm output parameters. However, seminal LDH activity

showed significantly ($P<0.01$) positive correlation with total sperm outputs. It is worthy noting that peroxidase and fructose tests showed negatively highly significant correlations with seminal LDH activity and HOS-t. Also, there were positive correlation between LDH activity and HOS-t ($P<0.001$) as well as between peroxidase test and fructose test ($P<0.001$). Similarly, Mostakhdem *et al.* (2016) found that LDH activity positively ($P<0.0001$) correlated with sperm concentration and total sperm count, while negatively ($P<0.01$) with fructose concentration and true corrected fructose.

Based on the strong correlation in peroxidase and fructose tests in seminal plasma and HOS tests in semen, the present results may suggest conducting only the HOS-t as an important test in semen for evaluation of sperm integrity and hence semen quality of rabbits.

Blood serum parameters:

Data in Table (7) revealed that liver function in term of AST and ALT activity and testosterone concentration in blood serum were not affected by OLE treatment, while ALP activity significantly ($P<0.05$) increased only in G4, while LDH activity significantly ($P<0.05$) increased in both G3 and G4 as compared to other groups.

It is worthy noting that the observed insignificant effect of OLE on AST and ALT activities, and testosterone concentration in blood serum of bucks reflected insignificant and weak correlation coefficients between each of these parameters with semen quality and sperm outputs, except high and positive correlation ($r=0.610$, $P<0.01$) between testosterone concentration and LDH activity in seminal plasma. On the other hand, ALP in blood serum had significant correlation with net semen volume ($r=0.645$, $P<0.05$), sperm outputs (concentration, total and functional spermatozoa), and seminal LDH activity ($r=0.774$, $P<0.01$). Interestingly, LDH in blood serum positively correlated with sperm outputs and seminal LDH activity, being the strongest with sperm concentration and functional sperm outputs ($r=0.842$ and 0.827 , $P<0.001$), followed by total sperm output and seminal LDH activity ($r=0.775$ and 0.774 , $P<0.01$), and sperm livability ($r=0.625$, $P<0.05$, Table 7).

Table (7). Enzyme activity and testosterone concentration in blood serum of APRI rabbit bucks fed different olive leaf extract levels at the end of collection semen period.

Treatment	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	LDH (IU/l)	Testosterone (ng/ml)
Effect of olive leaf extract level :					
G1 (control)	56.00±3.055	60.33±1.45	55.33 ^b ±2.02	1007.0 ^b ±68.7	6.26±0.34
G2 (0.5 ml/kg diet)	58.67±4.630	59.00±3.79	62.00 ^{ab} ±5.5	1207.3 ^{ab} ±79.4	6.53±0.30
G3 (1.0 ml/kg diet)	57.00±8.082	57.67±4.48	65.33 ^{ab} ±4.6	1301.7 ^a ±81.67	6.82±0.24
G4 (1.5 ml/kg diet)	59.50±3.90	59.67±3.28	72.67 ^a ±0.66	1424.0 ^a ±72.3	7.03±0.25
Correlation coefficients with volume and quality of semen:					
Net semen volume	0.296	-0.063	0.645*	0.566	0.514
Sperm motility	0.058	0.276	-0.508	-0.228	-0.518
Sperm livability	0.037	-0.522	0.297	0.625*	0.157
Sperm concentration	0.292	-0.090	0.686*	0.842***	0.425
Total sperm output	0.374	-0.086	0.706*	0.775**	0.506
Functional sperm output	0.36665	-0.13497	0.681*	0.827***	0.448
Seminal LDH activity	-0.025	-0.1489	0.774**	0.774**	0.610**

a, b and c: Means in the same column with different superscripts are significantly different ($P<0.05$).

** Significant at $P<0.05$. ** Significant at $P<0.01$. *** Significant at $P<0.001$.*

Aspartate amino transaminase (AST) is a plentifully found in heart and liver muscles and plays an essential part in amino acid metabolism and pathological changes in these organs raise the activity of AST in the blood. The AST impacts the development of glutamate and oxaloacetate attributable to the transfer of the amino group from aspartate to ketoglutaric corrosive (Abdelhamid *et al.*, 2012). The role of AST activity during the different physiological stages depends on age, breed, sex, pregnancy, natal, breast feeding, feeding systems (Milinković *et al.*, 2005). Therefore, supplementation of the diet with natural antioxidants as OLE may affect

feeding status. Alanine amino transaminase (ALT) is an enzyme found in the highest amount in liver and commonly used to identify injury of liver or muscle (Pratt *et al.*, 2010). It has an effect on glutamate and pyruvate due to the transfer of the amine group from alanine to ketoglutaric acid. Increased activity of the ALT leads to the occurrence of viral hepatitis or liver damage or metabolic disorders. While, diminished ALT activity is reduced due to malnutrition or even starvation (Bagnicka *et al.*, 2014). In agreement with the present results of OLE as a plant natural antioxidant, activity of AST and ALT exhibited non-significant changes in bucks treated with MO leaf extract (Khalifa *et al.*, 2016), leaf and flower of MO (Luqmanet *al.*, 2012) or green tea (GT) leaves and their extracts at different levels in serum of APRI rabbits (Morsy *et al.*, 2013). Also, Abdo *et al.* (2010) found that GT supplementation had no adverse effect on liver and kidney functions for laying hens. The present results of OLE and previous data on MO and GT indicated the save usage of OLE as a natural antioxidant on normal liver function in rabbits.

The obtained results may indicate the vital role of blood ALP and LDH activities in body energy production, in particular, energy required for spermatogenesis within the testes of rabbit bucks as affected by OLE treatment. The LDH is an enzyme that plays an important role in the conversion of lactate to pyruvate, which is one of the important steps in the production of energy in the cell (Perez *et al.*, 2003). Increased blood LDH activity may be important in determining tissue damage and disease within the living body. It converts hydrogen between molecules (Goddard *et al.*, 1997) and stimulates the last step of the glycolic path, which is the conversion from pyruvate to lactate and repetition (Bagnicka *et al.*, 2014). In agreement with the present results, Mostakhdem *et al.* (2016) found that LDH activity had significant ($P < 0.0001$) positive correlation with sperm concentration as well as between total sperm count and LDH activity. Also, Isitua and Ibeh (2013) showed an enhancement in the activities of ALT, AST and ALP in blood as affected by treatment of rabbits with MO leaf extract.

Testosterone is the major secretary hormone of the mature testis and spermatogenesis is controlled directly by testosterone concentration in rabbit bucks (Abdel-Khalek *et al.*, 2005). The present concentration of testosterone in blood serum of APRI bucks in this study is higher than that reported in NZW bucks by some authors (Abd El Elmoty, 1991; Abdel-Khalek *et al.*, 2005). Testosterone supplementation has previously been shown to improve sexual function and libido (Watchoet *al.*, 2005; Monera *et al.*, 2008). Plant extracts might have a role in testosterone secretion allowing better availability of hormone to gonads (Amini and Kamkar, 2005). According to the present results, OLE had beneficial effect on testosterone secretion as the role of MO in enhancing male fertility by inhibiting 6 β -hydroxylation of testosterone and increasing the testosterone level (Khalifa *et al.*, 2016), increasing blood flow to the male reproductive organs and stimulating the nervous system to enhance sexual desires (Monera *et al.*, 2008).

Generally, improving semen quantitatively and qualitatively was associated with tendency of higher testosterone concentration in G4 than in other groups, which may reveal pronounced effect of OLE on testicular tissues (spermatocytogenesis) within the somniferous tubules of the testis as well as on epididymal spermatozoa (Awoniyi, 2010).

Fertility rate:

Results in Table (8) showed that kindling rate and reproductive index significantly ($P < 0.05$) improved in G3 and G4 as compared to G2 and G1, being the highest in G4. This was associated with increasing number of total borns/group and insignificant increase of litter size/doe. These results indicated that doe rabbits mated with bucks receiving high levels of OLE (1.0 and 1.5 ml/ kg diet) recorded higher reproductive performance than in those mated with bucks fed low OLE level or control bucks, reflecting high fertility of semen produced by bucks in G3 and G4.

Motility is critical in enabling the sperm to ascend the female reproductive tract to the site of fertilization which is necessary if fertilization is to be achieved (Aitken, 1990). Free radical-induced oxidative damage to spermatozoa is a condition that has gained favorable attention for its role in inducing poor sperm function and infertility (Russo *et al.*, 2006). Therefore, it is possible that the consumption of OLE may have a reducing or lowering capacity on ROS thereby improving fertility (Roth *et al.*, 2002). Generally, high quality semen should contain a high percentage of vigorous and active sperms (Mann, 1964). It is of interest to observe that improving fertility of semen in bucks treated with OLE, particularly at level of 1.5 ml/kg diet was attributed to exhibiting these bucks the highest percentages of progressive sperm motility and sperm livability, and the lowest sperm abnormality percentage. In this respect, OLE as antioxidant may offer protection against the damaging effect of leukocyte-derived ROS on sperm movement (Baker *et al.*, 1996). It is conceivable that the

increase in sperm concentration might lead to higher fertility, which supported by the findings of Oyeyemi *et al.* (2008).

Table (8). Reproductive performance of rabbit does mated by bucks treated with olive leaf extract at different levels.

OLE (ml/kg diet)	N	Number of does at kindling	Kindling rate (%)	Total borns	Litter size/ doe	Reproductive index
G1 (control)	60	40	66.67 ^c ±2.108	279	6.90±0.258	4.65 ^c ±0.232
G2 (0.5 ml/kg diet)	60	44	73.33 ^{bc} ±2.108	316	7.18±0.307	5.27 ^{bc} ±0.116
G3 (1.0 ml/kg diet)	60	47	78.33 ^{ab} ±3.073	349	7.43±0.428	5.82 ^b ±0.421
G4 (1.5 ml/kg diet)	60	49	81.67 ^a ±3.073	390	7.96±0.365	6.50 ^a ±0.303

a, b and c: Means in the same column with different superscripts are significantly different (P<0.05). N: Number of does at mating.

CONCLUSION

Based on the foregoing results, in briefly, enhancing semen volume, sperm characteristics, seminal plasma tests, serum enzymes, and fertility trails of rabbit does, the current study can conclude that supplementation of the diet with olive leaf extract, in particular at a level of 1.5 ml/kg, as a plant antioxidant source, is essential for improving reproductive performance and consequently semen quality and fertility of breeding rabbit bucks (natural or artificial mating). Further studies are needed in future to detect the effect OLE on histogenesis and histometry of both testes (spermatogenesis) and epididymis (sperm storage ability) of rabbit bucks to indicate impact of OLE on semen quality. Also, freezability of spermatozoa of bucks treated with various levels of OLE should be studied.

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جودة السائل المنوي ومستوى هرمون التستوستيرون وبعض الأنشطة الإنزيمية والخصوبة في ذكور الأرنب الأبري المعاملة بمستويات مختلفة من مستخلص أوراق الزيتون

جورج عزت يونان وهدي السيد الجابري و وائل عوض محمود مرسى

معهد بحوث الإنتاج الحيواني- مركز البحوث الزراعية- الدقي- مصر.

تهدف الدراسة الحالية الي تقييم اضافة مستويات مختلفة من المستخلص الكحولي لأوراق الزيتون (ايثانول) في العليقه، كمصدر لمضادات الأكسدة الطبيعية على نوعية وانتاج السائل المنوي، بعض نشاط الانزيمات، تركيز هرمون التستوستيرون في الدم وبعض خصائص البلازما المنوية لذكور الارانب الابري. تم تقسيم عدد ٢٤ من ذكور الأرنب الأبري الناضجة جنسيا (٣-٤،٣ كجم وزن جسم حي وعمر ٩-١٢ شهر) إلى أربعة مجاميع متماثلة، ٦ ذكور في كل منها. تم تغذية المجموعة الأولى على عليقه متكامله (١٧,٧٥٪ بروتين خام و ٢٥٠٠ كيلو كالوري طاقة مهضومة /كجم علف دون أي اضافات، في حين تم تغذية المجموعات الثانية والثالثة والرابعة على نفس العليقه للمجموعه الأولى مضافا إليها مستخلص أوراق الزيتون على مستويات ١,٠٥، ١,٥، ١,٥ مل/كجم عليقه على التوالي. تم تغذية كل المجموعات على العلائق التجريبيه لمدة ١٧ أسبوع (٨ أسابيع كفترة معاملة و ٩ أسابيع كفترة جمع للسائل المنوي). تم تقييم خصائص طبيعیه وکیمیایه بالإضافة الى الفحص بالميكروسكوب الألكتروني للسائل المنوي لجميع المجموعات. أظهرت الدراسه النتائج الآتیه:

- ١- أظهرت جميع مستويات الاضافة من مستخلص أوراق الزيتون زياده في الحجم الكلى للسائل المنوي ($P < 0.05$) في المجموعة الثانية والثالثة والرابعة مقارنة مع المجموعة الأولى، وكانت أعلاها في المجموعة الرابعة.
- ٢- تحسنت خصائص الحيوانات المنوية، شامله زيادة النسبه المئوية لحركة الحيوانات المنويه التقدمية، الحيوانات المنويه الحيه وتركيز الحيوانات المنويه ($P < 0.05$) وانخفاض النسبه المئوية للحيوانات المنويه الشاذه ($P < 0.05$) في المجموعة الثالثه والرابعه مقارنة بالأولى والثانيه.
- ٣- أظهر الفحص بالميكرو سكوب الألكتروني تحسن واضح في تشوهات الغشاء الخلوي، الأكروسوم، تنظيم الخيوط الليفيه للذيل والماده الوراثيه للحيوانات المنويه للمجموعه الرابعه مقارنة بباقي المجموعات.
- ٤- أظهرت المجموعه الرابعه أدنى مستوى ($P < 0.05$) لأختبارى البيروكسيديز والفرکتوز في البلازما المنويه مقارنة مع المجموعات الأخرى .
- ٥- زاد نشاط أنزيم LDH في البلازما المنويه ($P < 0.05$) في جميع المجموعات المعامله مقارنة بالكنترول وكان أعلاها في المجموعه الرابعه وأقلها في المجموعه الأولى.
- ٦- لم تؤثر المعاملات في نشاط كل من ALT & AST وتركيز التستوستيرون في مصل الدم.
- ٧- زاد نشاط أنزيم ALP في المجموعه الرابعه ونشاط LDH في المجموعه الثالثه والرابعه ($P < 0.05$) مقارنة بالمجموعات الأخرى.
- ٨- تحسنت معدلات الولاده والدليل التناسلى ($P < 0.05$) لأمهات الأرنب الملقحه طبيعيا بواسطة ذكور المجموعه الثالثه والرابعه مقارنة مع المجموعه الأولى والثانية، وكانت أعلاها في المجموعه الرابعه (٦,٥ ، ٨١,٦٧ %)

خلصت الدراسه المقدمه الي أن اضافة المستخلص الكحولي لأوراق الزيتون، وخصوصا عند مستوى ١,٥ مل/كجم علف، كمصدر لمضادات الأكسدة الطبيعيه، أمر ضروري لتحسين الأداء التناسلى وبالتالي نوعية السائل المنوي وخصوبه ذكور الأرنب المستخدمه في التربيه (التلقيح الطبيعي أو الصناعي) دون آثار سلبية على حاله الصحيه.