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Research Article
Semen Quality, Fertility and Testicular Histology of Rabbit Bucks Orally Administrated with Ethanolic Grape Seed Extract

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Abstract

Objective: Effect of daily oral administration with ethanolic grape seed extract (EGSE) as enhancing factors on reproductive performance of APRI line rabbit bucks was investigated in this study. **Materials and Methods:** Total of 18 rabbit bucks (6 in each group) were treated with 3 mL distilled water containing 0 as control group, 75 and 125 mg kg^{-1} live body weight (LBW) from EGSE, for 28 days as a treatment period. Semen was collected twice a week for 8 wks as a collection period, then the average of physical semen characteristics were calculated for three semen collection intervals. Aspartate (AST) and alanine (ALT) transaminases activity, total antioxidant capacity (TAC) and testosterone concentrations were determined in blood serum. **Results:** Administrated with 125 mg EGSE kg^{-1} LBW showed a significant improved ($p<0.05$) testicular weights, semen quality (progressive motility, livability, abnormality, acrosomal damage, cell concentration and outputs/ejaculate of spermatozoa), except semen volume and by advancing semen collection interval, testosterone and TAC concentrations, while, ALT activity ($p<0.05$) reduced compared with the control group. **Conclusion:** Practically, using natural antioxidant such as grape seed extract for 28 days, may increase the antioxidative capacity of testicular tissues of rabbit bucks to improve semen quality and reproductive traits.

Key words: Fertility, grape seed extract, rabbit, semen quality, testosterone hormone

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The grape is a popular fruit has a significant role as a source of natural antioxidants. It contains polyphenols, anthocyanins and other biologically active components[1]. Grape seed extract (GSE) is a natural extract from the seed of *Vitis vinifera*. It is a rich source of one of the most beneficial groups of plant flavonoids, pro-anthocyanidins oligomers[2]. The grape seed extract (GSE) (aqueous or alcoholic) has a high antioxidant potential[3]; its beneficial effects include the modulation of antioxidant enzyme expression, protective effect on oxidant-induced production and deposition of extracellular matrix components[4]. The flavonoids in GSE involve in ameliorating the oxidative stress *in vitro* and *in vivo*[5] and inhibit enzyme systems that are responsible for the production of free radicals[6]. Also, this compound increases intracellular vitamin C levels and scavenge reactive oxygen species (ROS) and free radicals. In fact, GSE has a greater antioxidative activity than vitamin C and vitamin E[7,8].

Mono-(catechin and epicatechin), di-, tri- and poly-meric flavanols, procyanidins and phenolic acids, as flavonoid compounds with a phenolic nature, are presented in grape by-products[9]. The GSE have been reported for anti-inflammatory, anti-microbial and anti-carcinogenic activity. It has cardioprotective, hepatoprotective and neuroprotective effects as well[6,10].

Containing the sperm cell membrane higher polyunsaturated fatty acids (PUSFA) concentration leads to damage of mature spermatozoa[2]. Oxidative damage to PUSFA of sperm cell membranes impairs fluidity and permeability of sperm cell membrane. The reduction of sperm motility[11], sperm-oocyte fusion inhibition[12], and reduction in fertility[13], may be caused through modification in cytoskeleton and axoneme of sperm cells by ROS. Attacking the DNA of the sperm nucleus by ROS damage the genome leading to infertility and reproductive performance[14].

The technology of artificial insemination (AI) in rabbit production has now been applied for a long time[15]. In this respect, reproductive performance of doe rabbits, including kindling rate and litter size, is mainly depending on quality and quantity of buck semen[16].

There are several environmental factors affecting semen quality, involving ambient temperature, age, breed, nutrition, etc but semen production was more sensitive to the dietary components[17]. In rabbits, semen oxidative stability has a relationship with vitamins C and E. Antioxidant as dietary antioxidant supplementation, because antioxidant defense system against ROS was found to be heavily affected by nutritional factors[18,19]. Also, antioxidants can prevent sperm damage by the effect of leukocyte-derived ROS on motility of spermatozoa[20].

Therefore, the current study aimed to investigate the effect of oral administration of grape seed execrate, as a natural antioxidant, on physical semen characteristics, testosterone level, total antioxidant capacity, enzyme activity, fertility and testicular histology of APRI rabbit bucks.

MATERIALS AND METHODS

Animals: A total of 18 APRI line rabbit bucks (Egyptian line selected for litter weight at weaning according to Abou Khadiga *et al.*[21]) were used in this study. All bucks were fed *ad libitum* on commercial complete feed diet (17.26% CP and 2518.7 kcal kg^{-1} digestible energy) and kept under the same managerial and climatic conditions.

Experimental bucks having live body weight (LBW) of 2.188±0.07 kg and at 4.33±0.12 months of age were divided into three similar groups (n = 6) and allowed to acclimatize for 7 days in their respective cages. Bucks in the 1st group were orally given 3 mL sterile distilled water (Control, G1), while bucks in the 2nd and 3rd groups were orally given 3 mL distilled water containing 75 (G2) or 125 (G3) mg from grape seed extract per kg LBW. Bucks in all groups were treated as daily oral administration for 28 days as a treatment period prior to semen. Bucks were weighed at the start of treatment (initial weight) and at the end of treatment period.

Preparation of grape seed extract: Grape fruits were purchased from local market, then grape seeds (GS) were isolated, air dried in shade for one week and mild to obtain fin powder. Powder of GS macerated in 75% ethanol for 72 h at room temperature. The ethanolic extract was evaporated (Rotary evaporated) to eliminate ethanol and obtain GS extract (GSE) as lyophilized powder (yield 25-30%) according to Ewuola and Egbunike[22].

Collection and evaluation of semen: Semen was collected twice a week early in the morning (7 a.m.) for 9 weeks as a collection period, then average of physical semen characteristic were calculated for three semen collection intervals. Immediately after collection of semen, the ejaculates were transferred to the laboratory and were placed in a water bath at 30°C and care was taken to avoid exposure of the semen to any unfavorable conditions during or after collection.

Immediately after collection, semen was evaluated for determination of semen volume using graduated test tubes and the value recorded with gel mass. Also, percentage of

sperm progressive motility in each semen sample was determined using research microscope (Nikon E 200, China) by assessment of degree of movement of spermatozoa in about 0.5 mL diluted semen according to the method of Ewuola and Egbunike[22]. Live sperm percentage was determined by eosin (1.67%) and nigrosin (10%) mixture stain. Sperm abnormality percentage was estimated during the examination of live/dead sperm at a high power magnitude (×400). The morphological abnormalities of spermatozoa were determined per 200 sperm according to classification adopted by Bolm[23]. Sperm cell concentration (SCC) was evaluated by direct cell count using the New Improved Neubauer haemocytometer in aliquots under microscope (Avishkar AVI-504 Advance Research Binocular Microscope, Avishkar International, India) according to Ewuola and Egbunike[22]. Total sperm output/ejaculate (TSOP) was calculated by multiplying sperm cell concentration/ml (SCC) by ejaculate volume (EV) using the following equation:

$$TSOP\ (\times 10^6/ejaculate) = EV\ (mL) \times SCC\ (\times 10^6\ mL^{-1})$$[24]

Acrosome status: Examination of the acrosome status was carried out by adding one drop of diluted semen incubated at 37°C to one drop of sodium citrate (2.9%) at the same temperature, then the mixture was placed on a slide to make a smear, which was dried at 37°C. The dried slides were fixed in 10% formal solution for 15 min and washed by tap water for 15 min and stained with Gimsa stain at 37°C for 3 h. Then, the stained slides were washed by tap water for 15 min and dried at 37°C. The prepared slides were examined by research microscope at higher magnification (×100) for determination of spermatozoa with and without intact acrosome per 200 spermatozoa in each field. The acrosome stained light purple dark pink, while sperm remains unstained.

Sperm membrane integrity: Water test was performed by mixing 10 µL of semen and 40 µL of distilled water (0 mOsm) on a microscope slide and covered with a thin 24×30 mm coverslip. The mixture was incubated for 5 min in a moist chamber at 37°C before it was examined with a microscope at higher magnification (×400). At least 100 sperm cells were examined and the percentage of sperm that showed coiled tails was calculated according to Lomeo and Giambersio[25].

Blood sampling: Blood samples were collected from three bucks in each group at the end of treatment. The blood samples were taken from the ear vain of bucks. On the day of sampling, blood was taken into vacutainer tubes, left to clot for 2-3 h and then centrifuged at 3000 rpm for 20 min in order to separate blood serum using serological pipettes. Serum was carefully decanted into labeled tubes and stored at -20°C until analysis of aspartate (AST) and alanine (ALT) transaminases activity[26] and testosterone and total antioxidant capacity (TAC) concentrations.

Testosterone concentration: Testosterone concentration was determined using special kits according to the procedures outlined by the manufacturers. The total serum testosterone assay was conducted by radioimmunoassay method (RIA). Determination- Pontex 335 kit (I125) was used to measure the levels of testosterone. Types of testosterone assayed were (A) total testosterone (direct extraction-coated tubes) and (B) free testosterone. It is well known that total testosterone in serum include free testosterone and that bound to 1 pound to sex steroid hormone binding globulin (SHBG) albumin, corticosteroid binding globulin (CBG). According to the instructions of the producing company (Pantex Santa Monica), t+9he solvents used in this assay break the protein binding during extraction process. The standard curve of testosterone ranged between 0.1 and 25.6 ng mL^{-1}.

Slaughtering and histological study: The testicular characteristics of the animals were taken immediately after slaughtering three animals from each group. The testes were immediately removed after slaughter, trimmed of adhering connective tissue and fats. Weight, length, width and thickness of testis were measured.

Representative samples were taken from the median part of each testis, fixed in Bouin's solution (24 h), washed, dehydrated in ascending grades of ethyl alcohol, cleared and embedded in paraffin wax. Thereafter, the samples were sectioned at 7-8 microns, stained by hematoxylin and eosin stains (H and E) and histologically examined using the routine method of Bancroft and Stevens[27]. Largest, smallest and average diameter, thickness of spermatogenic layer and density/mm^2 of seminiferous tubules in different experimental groups were measured.

Fertility trial: Total of 9 APRI rabbit does were naturally mated by 3 rabbit bucks from each experimental group (3 does per buck). Fertility was determined as conception and kindling rates, total and live litter size at birth and average live weight of bunnies at birth.

Statistical analysis: Collection and evaluation of semen were subjected to analysis of variance using factorial pattern (3 treatments×3 semen collection interval). However, other data were analyzed by one-way ANOVA to study the effect of treatment. All data were analyzed using computer program of SAS[28]. The significant differences among means were tested using Duncan's multiple range test[29] and differences of $p<0.05$ were considered statistically significant.

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

Live body weight and testicular characteristics of rabbit bucks: Data in Table 1 revealed insignificant effect of ethanolic grape seed extract (EGSE) treatment on LBW of bucks, although there was a tendency of heavier bucks in groups treated with both grape seed extract doses (G2 and G3) as compared to control group (G1) at the end of treatment periods. These results reflected the highest increase in LBW of bucks in G3, followed by G2 and the lowest in G1 but the differences were not significant.

In accordance with the present results, feeding grape seed oil improved body weight of pigs[30]. However grape residue inclusion up to 30 g kg^{-1} diet did not have any negative effects on growth performance of the broilers[31]. In this respect, it was reported that grape products rich in polyphenols, which are effective in increasing feed conversion ratio of growing pigs[32] and polyphenols are able to cause a shift in the microbial population in the intestinal tract in rats and broilers[33]. In the later study, feeding broilers on grape pomace extract or grape seed extract, increased counts of beneficial ileal bacteria populations such as *Enterococcus* and decreased counts of potential pathogens such as *Clostridium*.

Testicular weights as absolute or relative to LBW were significantly ($p<0.05$) higher in G3 than in G1, while did not differ significantly in G2 from that in G1. However, testicular measurements, including length, width and circumference of bucks were not affected significantly by grape seed extract treatment Table 1.

These results indicated that increasing level of EGSE treatment from 75-125 mg significantly ($p<0.05$) improved testicular weight of rabbit bucks, without any effects on testicular volume, which may suggest an impact on semen production of rabbit bucks. In contrast to the present results, Salma *et al.*[34] found no significant effect of GSE on liver, kidney, heart and testes relative weights in rabbit bucks.

Physical semen characteristics

Effect of grape seed extract treatment: All physical semen characteristics was affected significantly ($p<0.05$) by both EGSE treatments, except semen volume. Percentage of progressive motility (PM), live sperm (LS) and membrane integrity (MI) as well as sperm cell concentration (SCC) and total sperm output (TSO) significantly ($p<0.05$) increased, while percentage of sperm abnormality and acrosomal damage significantly ($P<0.05$) decreased in G2 and G3 as compared to G1, being the highest in G3 (Table 2).

Table 1: The effect of grape seed extract treatment on live body weight and testicular characteristics of rabbit bucks during treatment period of 28 days

	Grape seed extract level		
Items	G1 (control)	G2 (75 mg kg^{-1})	G3 (125 mg kg^{-1})
Initial live body weight (kg)	2.16±0.150	2.28±0.100	2.13±0.140
Final live body weight (kg)	2.74±0.080	2.91±0.059	2.95±0.049
Change in live body weight (kg)	0.58±0.071	0.64±0.048	0.80±0.068
Absolute testicular weight (g)	6.33±0.35^{b}	8.00±0.58^{ab}	9.83±1.167^{a}
Relative testicular weight (g kg^{-1} LBW)	0.23±0.008^{b}	0.27±0.02^{ab}	0.34±0.05^{a}
Testicular measurements (cm)			
Length	2.77±0.15	2.63±0.12	2.40±0.12
Width	1.00±0.15	1.01±0.15	1.03±0.07
Circumference	3.10±0.17	3.01±0.06	3.27±0.39

$^{a, b}$Means with different superscripts in each row are different significantly ($p<0.05$)

Table 2: Physical semen characteristics of rabbit bucks as affected by grape seeds extract treatment

	Grape seed extract level		
Items	G1 (control)	G2 (75 mg kg^{-1})	G3 (125 mg kg^{-1})
Semen volume with gel (mL)	0.75±0.07	0.81±0.04	0.88±0.08
Sperm progressive motility (%)	68.90±1.82^{b}	86.10±2.13^{a}	85.60±1.56^{a}
Live sperm (%)	71.60±1.79^{b}	86.80±1.08^{a}	87.70±1.73^{a}
Sperm abnormality (%)	20.00±0.87^{a}	10.10±0.54^{b}	8.60±0.34^{b}
Acrosomal damage (%)	18.90±1.48^{a}	11.10±0.35^{b}	9.40±0.38^{b}
Sperm concentration ($\times 10^{6}$ mL^{-1})	52.80±3.03^{b}	68.40±4.52^{a}	69.90±2.14^{a}
Sperm membrane integrity (%)	20.10±0.87^{c}	28.60±1.3^{b}	41.10±1.61^{a}
Total sperm output ($\times 10^{6}$/ejaculate)	38.30±2.28^{b}	55.60±4.69^{a}	62.00±6.56^{a}

$^{a, b}$Means with different superscripts in each row are different significantly ($p<0.05$), (Overall Mean±SE)

Table 3: The physical semen characteristics of rabbit bucks as affected by collection interval

	Collection interval		
Items	1-3 week	4-6 week	7-9 week
Semen volume (mL)	0.73±0.03[b]	0.77±0.06[ab]	0.95±0.08[a]
Sperm progressive motility (%)	77.20±2.48[b]	80.00±3.0[ab]	83.30±4.08[a]
Live sperm (%)	80.00±2.26[b]	81.10±2.81[ab]	84.90±3.63[a]
Sperm abnormality (%)	13.10±1.6	13.00±2.1	12.60±1.96
Acrosomal damage (%)	14.30±2.18	12.60±1.42	12.60±1.32
Sperm membrane integrity (%)	26.90±2.67[b]	30.10±3.57[a]	32.80±3.3[a]
Sperm concentration ($\times 10^6$ mL^{-1})	61.00±3.12	67.40±5.1	62.70±4.26
Total sperm output ($\times 10^6$/ejaculate)	44.10±2.66	54.50±6.21	57.30±7.1

[a, b]Means with different superscripts in each row are different significantly ($p<0.05$), (Overall Mean±SE)

Table 4: The effect of grape seeds extract treatment on testosterone concentration, enzyme activity and total antioxidant capacity in blood serum of bucks at the end of treatment period

	Grape seed extract level		
Items	G1 (control)	G2 (75 mg kg^{-1})	G3 (125 mg kg^{-1})
ALT (IU L^{-1})	16.670±1.20[a]	14.500±0.76[ab]	13.400±0.40[b]
AST (IU L^{-1})	35.670±0.88	32.330±1.86	32.170±0.44
TAC (nmol mL^{-1})	0.409±0.005[c]	0.481±0.045[b]	0.552±0.013[a]
Testosterone (ng mL^{-1})	1.900±0.02[b]	2.370±0.25[ab]	2.800±0.07[a]

[a, b, c]Means with different superscripts in each row are different significantly ($p<0.05$), ALT: Alanine transaminases. AST: Aspartate transaminases, TAC: Total, (Mean±SE)

According to these results, treatment of rabbit bucks with GSE at levels of 75 or 125 mg kg^{-1} LBW had beneficial effects on improving semen quality of rabbit bucks. This improvement may be due to the significant increase in testicular weight of bucks treated with EGSE, which may be associated with increasing seminiferous tubules and number of Leydge cells responsible for testosterone secretion[35] and increasing testicular tissues (spermatocytes) within the somniferous tubules of the testis as well as on epididymal spermatozoa[36].

The observed improvement in all characteristics of spermatozoa in treatment groups (G2 and G3) the current study may be in relation with the protective action of GSE against cisplatin-induced testicular toxicity. This finding was evidenced by decreasing lipid peroxidation and increasing the enzymatic and non-enzymatic antioxidants in the testicular tissues[37]. In rats, GSE administration as a daily long-term, elevates potentiality of antioxidant and reduces lipid peroxidation and protein oxidation of cell membrane[38]. Moreover, extracts of grape leaves has protective effect via reducing the lipid peroxidation and improving status of antioxidants[39]. Moreover, polyphenolic compounds present in grape have powerful antioxidant properties as free radical scavenging activity[40].

Sperm motility parameters increased in this study by oral of GSE due to the protection effect of GSE against the harmful effect of leukocyte-derived ROS on movement of spermatozoa[19]. It could be assumed that the observed increases in sperm motility after GSE treatment could partly be attributed to the concomitant induction in semen fructose[19]. In the same way, Murthy *et al.*[41] detected positive effects of dietary grape supplementation on count, motility and livability of spermatozoa. These findings were indicated by several authors[42,43].

Effect of semen collection interval: Physical semen characteristics, including semen volume and percentage of PM, LS and MI were affected significantly ($p<0.05$) by collection interval, being the best at the interval from 7-9 weeks of semen collection. However, percentage of sperm abnormality and acrosomal damage as well as SCC and TSO were not affected by collection interval (Table 3).

Effect of interaction (treatment x collection interval): The effect of interaction between EGSE treatment and collection interval on semen volume, sperm abnormality, acrosomal damage, sperm abnormality and membrane integrity was not significant, reflecting the best characteristics of bucks, in G3 than in G1 and G2 at all collection intervals and during the interval of 7-9 week than in other intervals. On the other hand, this effect was significant ($p<0.05$) on progressive motility, livability, concentration and total output of spermatozoa, reflected better characteristics of bucks in G2 than in G3 during some intervals, while the opposite was observed during other intervals (Fig. 1a-h).

Testosterone concentration, enzyme activity and total antioxidant capacity: Activity of ALT was significantly ($p<0.05$) the lowest, while testosterone and total antioxidant concentrations was significantly ($p<0.05$) the highest in G3 as compared to G1 and G2 but no significant differences in AST activity were observed among groups (Table 4).

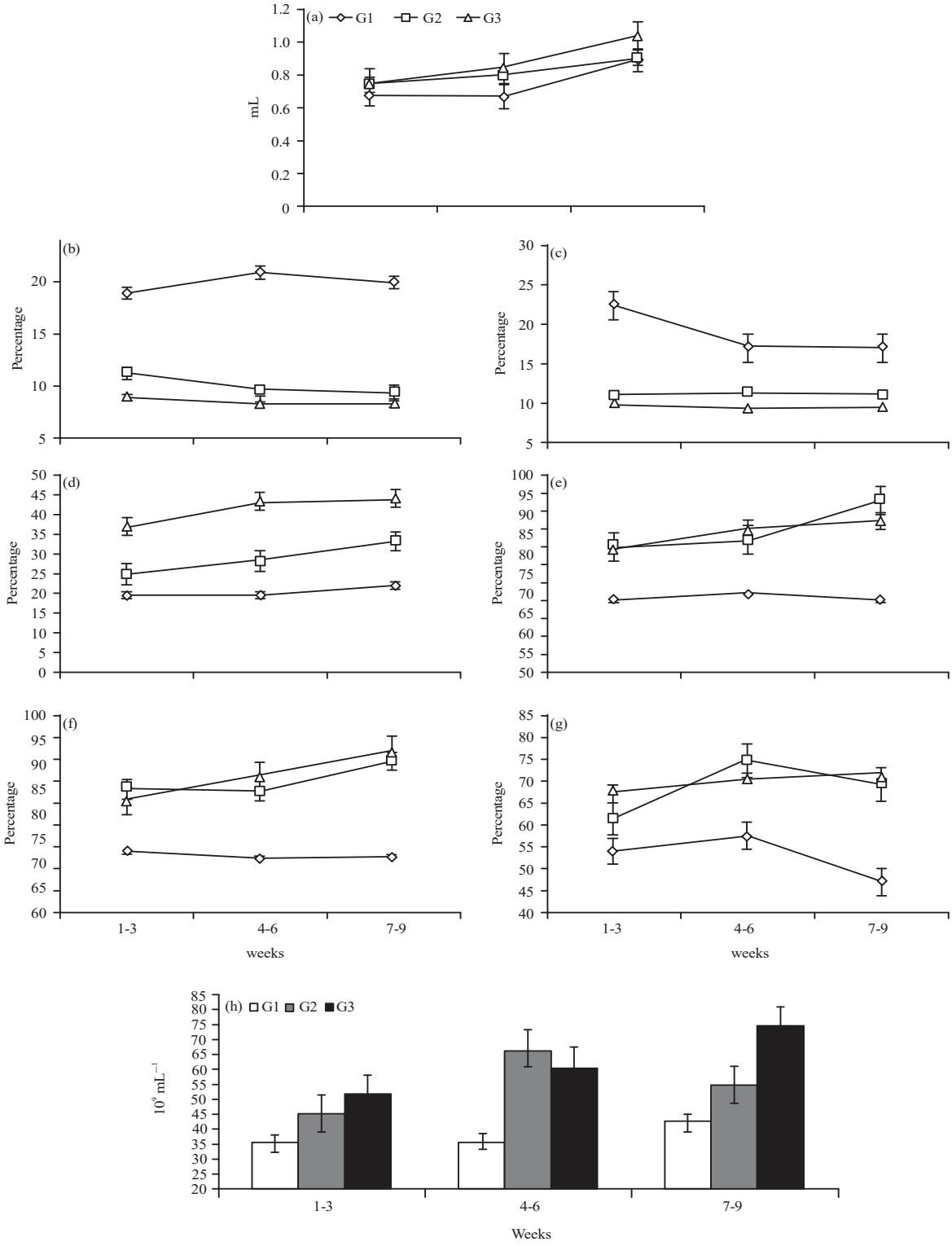

Fig. 1(a-h): Change in physical semen characteristics and total sperm output of rabbit bucks in experimental groups at different week intervals of semen collection. (a) Semen volume (mL), (b) Sperm abnormality (%), (c) Sperm with acrosomal damage (%), (d) Sperm responded to water test (%), (e) Sperm progressive motility (%), (f) Live sperm (%), (g) Sperm concentration ($\times 10^9$ mL^{-1}) and (h) Total sperm output (10^9 mL^{-1})

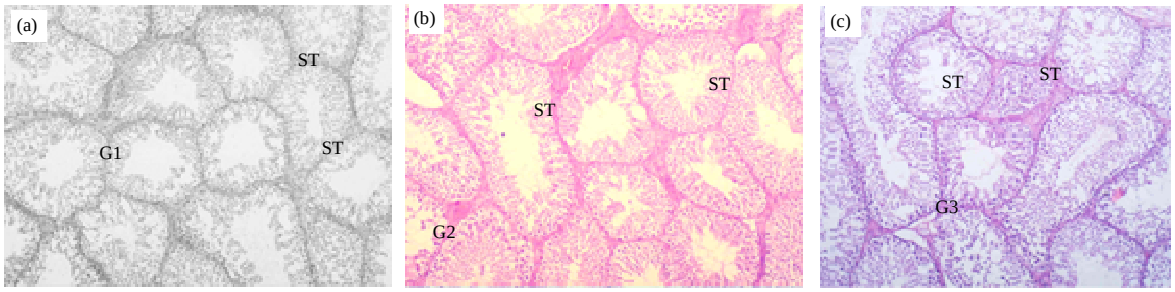

Plate 1(a-c): Section in testicular tissue of rabbits showing compacted seminiferous tubules (ST) with various shapes, size, lumens and spermatogenic layers in G1, G2 and G3. (H and E, ×200)

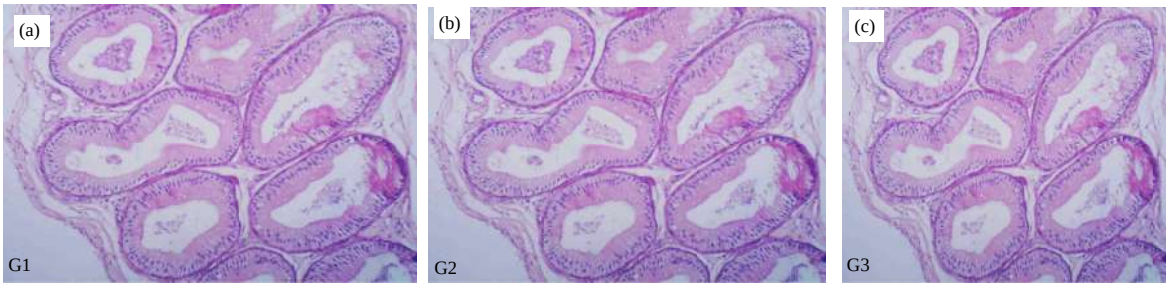

Plate 2(a-c): Section in epididymal tissue (cauda) of rabbits showing epididymal tubule with single layer of ciliated columnar epithelial tissue and mass of spermatozoa are seen within the lumens in G1, G2 and G3. (H and E, ×200)

It is of interest to note that the obtained improvement in semen characteristics of bucks in G3 was associated with the highest testosterone and TAC concentration, the lowest ALT activity in the same group. Also, In accordance with the present results, GSE significantly increased testosterone in blood plasma of rabbit bucks[34].

Presence of high levels of PUSFA results in sperm cell membrane results in damaging the mature spermatozoa. Therefore, lipid oxidative damage of cell membranes has impairs fluidity and permeability of sperm cell membrane[2]. Free radical scavenging enzymes such as SOD, CAT, GSH-Px and GST are the first line of defense against oxidative injury. The inhibition of antioxidant system may cause the accumulation of H_2O_2 or products of its decomposition[44].

Flavonoids, which are polyphenolic antioxidants possess several physiological properties: antioxidant, antibacterial, antiviral, anti-inflammatory, anti-mutagenic and anti-tumoral activity[45]. These flavonoids exerts many-health-promoting effects including the ability to increase intra-cellular vitamin C levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals[2].

Histological study: The histological examination of testicular tissues in rabbit bucks of different experimental groups revealed normal architecture of the testicular tissues with compacted seminiferous tubules with various shapes, size, lumens and spermatogenic layers in G1, G2 and G3, without any advisable effects on testes function in all groups (Plate 1). Also, epididymal tissues, in term of shape, diameter, lumen and thickness of epithelial layer of the epididymal tubules were intact and similar in all groups (Plate 2).

Histological examination cleared that largest (LDST), smallest (SDST) and average (ADST) diameter of the seminiferous tubules (ST) as well as ST density insignificantly improved in both treatment groups as compared to control one. However, thickness of spermatogenic layer (TSGL) was significantly ($p<0.05$) higher in G3 than in G1 and G2 (Fig. 2).

Based on these findings, treatment with GSE at a level of 125 mg kg^{-1} LBW enhanced the production and quality of spermatozoa, without adversely effects of sperm storage ability. In supporting these findings, the dietary inclusion of grape seeds ameliorated the harmful effects of toxicity[34]. Grape seed addition to diet of rabbits showed noticeable

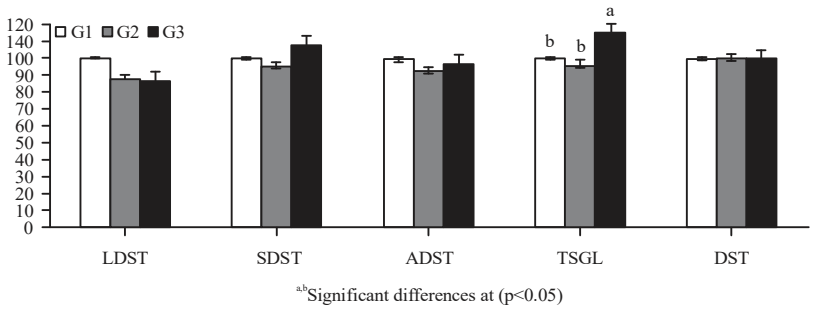

Fig. 2: Largest (LDST), smallest (SDST) and average (ADST) diameter, thickness of spermatogenic layer (TSGL) and density/mm^2 of seminiferous tubules in G2 and G3 relative to that in G1

Table 5: Conception rate and litter size of rabbit does naturally mated with bucks in different experimental groups at the end of collection interval. (mean±SE)

	Grape seed extract level		
Items	G1 (control)	G2 (75 mg kg^{-1})	G3 (125 mg kg^{-1})
Number of mated does	9	9	9
Number of conceived does	6	7	8
Conception rate[1]	66.67^{b}	77.78^{ab}	88.89^{a}
Kindling rate	100	100	100
Total litter size/doe at birth	7.17±0.43^{b}	8.29±0.43^{ab}	9.63±0.49^{a}
Live litter size/doe at birth	6.50±0.21^{b}	8.00±0.26^{a}	8.75±0.33^{a}
Reproductive index of does[2]	4.33	6.22	7.78
Average bunny weight at birth (g)	52.23±2.09^{a}	47.32±0.82^{b}	46.48±0.95^{b}

$^{a, b}$Means with different superscripts in each row are different significantly (p<0.05), [1]Statistically analyzed by Chi-square test. [2]Reproductive index = Live litter size×conception rate/100

improvement in the testicular tissues, leading to increase in quality of the rabbit semen probably due to the physiological and antioxidant effects of grape seeds.

Fertility trail and reproductive performance of mated does: Data presented in Table 5 revealed that rabbit does mated by bucks in G3 showed the best results regarding their reproductive performance, in terms of the highest conception rate (88.89%), kindling rate (100%) and total and live litter size at birth (9.63 and 8.75/doe), reflecting the highest reproductive index (7.78). Does mated with bucks of G2 ranked the second showing corresponding values of 77.78%, 100%, 8.29 and 8.0/doe, respectively but did not differ significantly from those in G3, reflecting moderate reproductive index (6.22). However, does in G1 (control) showed the lowest values. Yet, average bunny weight at birth was significantly (p<0.05) lower in G2 and G3 than in G1 (47.32 and 46.48 vs. 52.23 g), indicating a negative relationship with litter size.

Generally, increasing conception rate and litter size at birth in treatment groups (G2 and G3) as compared to control (G1) was mainly due to improving semen quality and higher fertilizing ability of spermatozoa produced by bucks treated with GSE at a level of 125 mg kg^{-1} as compared to control bucks or those treated with GSE at low level (75 mg kg^{-1}). The role of GSE in male fertility is very well documented by Sallam *et al.*[46], who reported that supplementing GS has ability to restore the spermatogenic process and thus fertility damage caused by toxic heavy metals.

CONCLUSION

In male rabbit testes, grape seeds may be a natural therapeutic agent used to impair reproductive performance of toxic rabbit bucks, therefore using natural antioxidant such as grape seed extract (125 mg kg^{-1} LBW) for 28 days may increase the antioxidative capacity of testicular tissues of rabbit bucks to improve semen quality.

SIGNIFICANCE STATEMENT

Grape seed extract administration of breeding bucks can have synergistic beneficial effects on reproductive performance. The use of natural antioxidants can improve semen characteristics and more efficient testosterone and total antioxidants capacity that enhances the fertility of rabbit bucks.

REFERENCES

1. Ma, Z.F. and H. Zhang, 2017. Phytochemical constituents, health benefits and industrial applications of grape seeds: A mini-review. Antioxidants, Vol. 6, No. 3. 10.3390/antiox 6030071
2. El-Ashmawy, I.M., A. Saleh and O.M. Salama, 2007. Effects of marjoram volatile oil and grape seed extract on ethanol toxicity in male rats. Basic Clin. Pharmacol. Toxicol., 101: 320-327.
3. Garavaglia, J., M.M. Markoski, A. Oliveira and A. Marcadenti, 2016. Grape seed oil compounds: Biological and chemical actions for health. Nutr. Metab. Insights, 9: 59-64.
4. Dulundu, E., Y. Ozel, U. Topaloglu, H. Toklu, F. Ercan, N. Gedik and G. Sener, 2007. Grape seed extract reduces oxidative stress and fibrosis in experimental biliary obstruction. J. Gastroenterol. Hepatol., 22: 885-892.
5. Martínez-Flórez, S., J. González-Gallego, J.M. Culebras and M.J. Tuñón, 2002. Flavonoids: Properties and anti-oxidizing action. Nutr. Hosp., 17: 271-278, (In Spanish).
6. Maier, T., A. Schieber, D.R. Kammerer and R. Carle, 2009. Residues of grape (*Vitis vinifera* L.) seed oil production as a valuable source of phenolic antioxidants. Food Chem., 112: 551-559.
7. Shi, J., J. Yu, J.E. Pohorly and Y. Kakuda, 2003. Polyphenolics in grape seeds-biochemistry and functionality. J. Med. Food, 6: 291-299.
8. Singh, R.P., A.K. Tyagi, S. Dhanalakshmi, R. Agarwal and C. Agarwal, 2004. Grape seed extract inhibits advanced human prostate tumor growth and angiogenesis and upregulates insulin like growth factor binding protein 3. Int. J. Cancer, 108: 733-740.
9. Yilmaz, Y. and R.T. Toledo, 2004. Health aspects of functional grape seed constituents. Trends Food Sci. Technol., 15: 422-433.
10. Shenoy, S.F., C.L. Keen, S. Kalgaonkar and J.A. Polagruto, 2007. Effects of grape seed extract consumption on platelet function in postmenopausal women. Thromb. Res., 121: 431-432.
11. De Lamirande, E. and C. Gagnon, 1992. Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. J. Androl., 13: 379-386.
12. Aitken, R.J., J.S. Clarkson and S. Fishel, 1989. Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biol. Reprod., 41: 183-187.
13. Wishart, G.J., 1984. Effects of lipid peroxide formation in fowl semen on sperm motility, ATP content and fertilizing ability. J. Reprod. Fertil., 71: 113-118.
14. Roberts, K.P., 1998. Y-chromosome deletions and male infertility: State of the art and clinical implications. J. Androl., 19: 255-259.
15. Theau-Clément, M., 2007. Preparation of the rabbit doe to insemination: A review. World Rabbit Sci., 15: 61-80.
16. Brun, J.M., M. Theau-Clement and G. Bolet, 2002. The relationship between rabbit semen characteristics and reproductive performance after artificial insemination. Anim. Reprod. Sci., 70: 139-149.
17. Nizza, A., C. Di-Meo and S. Taranto, 2000. Influence of dietary protein content on libido and semen characteristics bucks. Proceedings of 7th World Rabbit Congress, July 2000, Valencia, Spain, pp: 217-224.
18. Castellini, C., P. Lattaioli, A. Dal Bosco, A. Minelli and C. Mugnai, 2003. Oxidative status and semen characteristics of rabbit buck as affected by dietary vitamin E, C and n-3 fatty acids. Reprod. Nutr. Dev., 43: 91-103.
19. Yousef, M.I., G.A. Abdallah and K.I. Kamel, 2003. Effect of ascorbic acid and Vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim. Reprod. Sci., 76: 99-111.
20. Baker, H.W.G., J. Brindle, D.S. Irvine and R.J. Aitken, 1996. Protective effect of antioxidants on the impairment of sperm motility by activated polymorphonuclear leukocytes Fertil. Steril., 65: 411-419.
21. Abou Khadiga, G., Y.M.K. Youssef, K. Saleh, R.Y. Nofal and M. Baselga, 2010. Genetic trend in selection for litter weight in two maternal lines of rabbits in Egypt. World Rabbit Sci., 18: 27-32.
22. Ewuola, E.O. and G.N. Egbunike, 2010. Effects of dietary fumonisin B_1 on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits Reproduction, 139: 439-445.
23. Blom, E., 1983. Sperm morphology with reference to bull infertility. Proceedings of the 1st All-India Symposium on Animal Reproduction, (AR'83), Ludhiana, India, pp: 61-81.
24. El-Ratel, I.T., 2017. Impact of lycopene or folic acid treatment on semen quality, blood constituents and fertility of rabbit bucks. Egypt. J. Nutr. Feeds, 20: 213-223.
25. Lomeo, A.M. and A.M. Giambersio, 1991. 'Water-test': A simple method to assess sperm-membrane integrity. Int. J. Androl., 14: 278-282.
26. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
27. Bancroft, D. and A. Stevens, 1982. Theory and Practice of Histological Techniques. 2nd Edn., Churchill Livingstone, Edinburgh, London, Melaborne, Pages: 262.
28. SAS., 2004. SAS user's guide, statistical. Version 9.1, SAS Institute Inc., Cary, NC, USA.
29. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
30. Best, P., 2006. Warning against rapeseed oil for pigs. Feed International, September 11-13, 2006
31. Goni, I., A. Brenes, C. Centeno, A. Viveros and F. Saura-Calixto *et al*., 2007. Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility and susceptibility to meat lipid oxidation in chickens. Poult. Sci., 86: 508-516.

32. Fiesel, A., D.K. Gessner, E. Most and K. Eder, 2014. Effects of dietary polyphenol-rich plant products from grape or hop on pro-inflammatory gene expression in the intestine, nutrient digestibility and faecal microbiota of weaned pigs. BMC Vet. Res., Vol. 10. 10.1186/s12917-014-0196-5
33. Viveros, A., S. Chamorro, M. Pizarro, I. Arija, C. Centeno and A. Brenes, 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. Poult. Sci., 90: 566-578.
34. Hafsa, S.H.A., H.A. Ayman, A.S. Elham, A. Popescu and D. Mocuta, 2016. Grape seed (*Vitis vinifera* L.) alleviate reproductive toxicity caused by lindane in New Zeeland white male rabbits. Sci. Pap. Ser. Manage. Econ. Eng. Agric. Rural Dev., 16: 17-28.
35. Abdel-Khalek, A.E., S.A. Darwish, A.F. Mehrez and M.B. Aboul-Ela, 2001. Morpho-histmetric study on testis, epididymis and ampulla of fertile and sub-fertile Egyptian buffaloes. J. Agric. Sci. Mansoura Univ., 8: 4773-4786.
36. Awoniyi, D.O., 2010. The role of rooibos (*Aspalathus* Linearis), green tea (*Camellia sinensis*) and commercially available rooibos and green tea antioxidant supplements on rat testicular and epididymal function. M.Sc. Thesis, Faculty of Health and Wellness Science, Cape Peninsula University of Technology, South Africa.
37. Yildirim, N.C., F.M. Kandemir and F. Benzer, 2011. Beneficial effects of grape seed extract against Cisplatin-induced testiculer damage in rabbits. Digest J. Nanomat. Biostruct., 6: 155-159.
38. Chis, I.C., M.I. Ungureanu, A. Marton, R. Simedrea, A. Muresan, I.D. Postescu and N. Decea, 2009. Antioxidant effects of a grape seed extract in a rat model of diabetes mellitus. Diab. Vasc. Dis. Res., 6: 200-204.
39. Pari, L. and A. Suresh, 2008. Effect of grape (*Vitis vinifera* L.) leaf extract on alcohol induced oxidative stress in rats. Food Chem. Toxicol., 46: 1627-1634.
40. Monagas, M., B. Hernandez-Ledesma, C. Gomez-Cordoves and B. Bartalome, 2006. Commercial dietary ingredients from *Vitis vinifera* L. leaves and grape skins: Antioxidant and chemical characterization. J. Agric. Food Chem., 54: 319-327.
41. Murthy, K.N.C., R.P. Singh and G.K. Jayaprakasha, 2002. Antioxidant activities of grape (*Vitis vinifera*) pomace extracts. J. Agric. Food Chem., 50: 5909-5914.
42. Marin-Guzman, J., D.C. Mahan and J.L. Pate, 2000. Effect of dietary selenium and vitamin E on spermatogenic development in boars. J. Anim. Sci., 78: 1537-1543.
43. Eid, Y., T. Ebeid and H. Younis, 2006. Vitamin E supplementation reduces dexamethasone-induced oxidative stress in chicken semen. Br. Poult. Sci., 47: 350-356.
44. Halliwell, B., 1996. Antioxidants in human health and disease. Ann. Rev. Nutr., 16: 33-50.
45. Rice-Evans, C. and L. Packer, 1998. Flavonoids in Health and Disease. Marcel Dekker, New York, USA., ISBN: 9780824700966, Pages: 541.
46. Sallam, S.M.A., M.E.A. Nasser, M.S.H. Yousef, A.M. El-Morsy, S.A.S. Mahmoud and M.I. Yousef, 2005. Influence of aluminum chloride and ascorbic acid on performance, digestibility, caecal microbial activity and biochemical parameters of rabbits. Res. J. Agric. Biol. Sci., 1: 10-16.