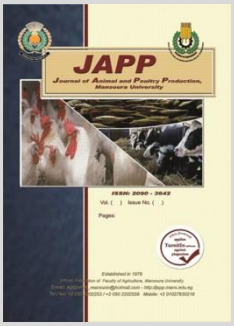
Journal of Animal and Poultry Production

Journal homepage: www.japp.mans.edu.eg
Available online at: www.jappmu.journals.ekb.eg

Improving Reproductive Hormones, Semen Quality, and Antioxidative Capacity of Rabbit Bucks Administrated with Moringa Seeds Extract

Omnia M. D. Agwa; Khalil El. Sherif and Sara F. Fouda*

Department of Poultry Production, Faculty of Agriculture, Mansoura University, Mansoura, Egypt



ABSTRACT

Effects of daily oral administration with ethanolic *Moringa oleifera* seeds extract (EMS), as an improving factor, were investigated on physiological and semen quality traits of NZW rabbit bucks. Adult bucks (n=40) were randomly divided into four groups of 10 bucks in each. Bucks were daily treated with oral dose of EMS (0, 900, 1200, and 1500 mg/buck) for 16 weeks; 8 weeks as a preliminary interval, and 8 weeks as a main semen collection interval. Results showed that all levels of EMS administrations mass motility, progressive motility, livability, membrane integrity of spermatozoa, sperm cell concentration, total sperm output, motility index, semen initial fructose, blood plasma FSH and LH, hemoglobin, red blood cells and globulin as well as testosterone, total proteins, albumin, and antioxidants capacity in blood plasma and the seminal plasma, epididymal weights, and spleen-somatic index were increased (P<0.05), while semen pH value, abnormality, acrosomal damage of spermatozoa, platelets, lipid profile, the activity of aspartate and alanine aminotransferase, and malondialdehyde were decreased (P<0.05). Administration of EMS (900 mg/buck) improved (P<0.05) only white blood cells. Pregnancy rate and litter size at birth (live) and weaning, and kit viability rate at birth were (P<0.05) improved. Practically, it could be suggested that oral supplementation of EMS (900, 1200, and 1500 mg/buck) is a suitable tool for improving semen quality, antioxidant status, and reproductive traits of rabbit bucks, particularly EMS at a level of 900 mg/buck.

Keywords: Rabbit, Moringa extract, hormones, semen quality, antioxidant

INTRODUCTION

Improved reproductive efficiency of rabbit buck's dependent on many factors such as feeding, genetic strain, health status, management condition, season and age (Morsy *et al*., 2017). These factors are contributing to the great variation in semen qualities and infertility (El-Deeb *et al*., 2015). In mammals, male fertility depends on semen quality, in terms of count of functional sperm output and proportion of membrane integrity of sperm cells (Morshedy *et al*., 2020). In rabbit farms, the artificial insemination (AI) is an essential method for enhancing the reproductive efficiency of bucks (Rriad *et al*., 2016). In comparing with the natural mating, AI reduces number of bucks for genetically used for breeding programs. Also, AI control spread of disease and decrease the insemination cost (Vasicek *et al*., 2014).

Rabbit spermatozoa are rich in polyunsaturated fatty acids (PUSFA) in their plasma membranes display high metabolic activity, which may cause increasing lipid peroxidation (Attia *et al*., 2017), so they are sensitive to free radicals attacks. This may result in reducing sperm motility, fragmentation of DNA and reducing sperm fertilizing ability (Attia *et al*., 2019). Also, an excessive free radicals production exceeds the antioxidant capacity of the seminal plasma, leads to damaged mitochondria and membranes (acrosomal and plasma) of spermatozoa (Mizeraa *et al*., 2019).

Semen quality of bucks is the main factor for determining the reproductive efficiency of rabbit does (El-Ratel and Gabr, 2019). The DNA and damage of other molecules caused by oxidative process can be protected safely and economically by various natural antioxidants treatments (El-Desoky *et al*., 2017; El-Ratel *et al*., 2020a). It is essential to explore the sources of antioxidants with high quality.In this line, *Moringa oleifera* (*M. oleifera*) belonging to family *Moringaceae* is a medicinal plant (Dafaalla *et al*., 2017). The seeds of *M. oleifera* have several chemical components, such as crude fiber, reducing sugars, resins, alkaloids, flavnoids, organic acids, sterols, tannins, saponins, and proteins (Dafaalla *et al*., 2017). In this respect, Mishra *et al.* (2011) indicated that *M. oleifera* is a good source of polyphenols and antioxidants, because it is rich in vanillin, carotenoids, ascorbates, tocopherols, β-sitosterol, moringine, kaempferol, and quercetin. Also, it contains unsaturated fatty acids (linoleic, oleic and palmitic), amino acids and minerals (Faye *et al*., 2011). Moreover, it contains a number of important vitamins, including vitamins A, B complex (B1, B3, B6 and B7), C, D, E and K (Dafaalla *et al*., 2017). The *M. oleifera* has protective effects by decreasing liver lipid peroxides, and acts as an antimicrobial, antitumor, antinflammatory, antiulcerous, antihyperlipidaemic, antidiabetic, anticancereous and cholesterol lowering agents (Paul *et al*., 2018; Faizi *et al*., 1998). It was reported that most minerals and vitamins present in *M. oleifera* are crucial nutrients in the reproductive system for the normal hormone functioning, sperm production (Ogunlesi *et al*., 2009), and vitamin C in moringa prevents sperm agglutination thereby causing progressive motility (Glenville, 2008).

The growing interest for more knowledge concerning reproductive functions and improvement using natural plants to enhance fertility for successful application of AI in rabbits. Therefore, the current study was conducted to investigate the beneficial effect of different levels of ethanolic extract of *M. oleifera* seeds (EMS) on reproductive performance of rabbit bucks, and biochemical properties and oxidative stress in their blood and seminal plasma.

* **Corresponding author.**
E-mail address: sarafikry@mans.edu.eg
DOI: 10.21608/jappmu.2020.130063

MATERIALS AND METHODS

The experimental work of this study was done at a private commercial farm of rabbit production located in Mansoura City, Dakahlia Governorate, Egypt. The analytical procedures were conducted at Physiology and Biotechnology Laboratory, belonging to Animal Production Department, Mansoura University, Egypt.

The EMS (National Research Center, Dokki, Egypt) was prepared in ethanol (85%) by Soxhlet apparatus according to Dafaalla *et al*. (2017). Then the ethanol extract was dried in Rotary Evaporator apparatus, weighed as a powder and dissolved in distilled water to give final concentrations of 300, 400 and 500 mg extract/ml.

A total of 40 sexually adult New Zealand White rabbit bucks aged 16 weeks were used in this study. Animals were held under observation for approximately 2 weeks prior the beginning of the experiment to eliminate any latent infections. Rabbits were housed individually in stainless steel cages (40 × 50 × 35 cm) within a natural and ventilated building with artificial light. Rabbit cages were accommodated with feeders and automatic fresh-water nipples. Bucks in all groups were under *ad libitum* feeding with a pelleted diet containing 16% crude protein, 14% crude fiber and 2850 Kcal digestible energy/kg to cover the requirements of adult bucks according to NRC (1977).

Rabbits were randomly divided into four experimental groups (10 bucks/group). The 1st group was served as control group (G1), while bucks in the 2nd, 3rd and 4th groups were daily treated with oral dose of EMS at levels of 900 (G2), 1200 (G3) or 1500 (G3) mg/buck, respectively. Rabbit bucks were treated with EMS during an experimental period of 16 weeks, 8 weeks as a preliminary interval and 8 weeks as a main semen collection interval.

Semen was collected twice/week from all bucks in each group. Rabbit bucks were transported into cage of a teaser doe. Semen was collected early before morning feeding by using an artificial vagina. The collected ejaculates were taken in a water bath (37°C) to the laboratory, immediately after complete semen collection.

In the lab., net semen volume (semen without gel) and pH values estimated by pH meter. Then semen was evaluated for mass motility (score 1-5) in fresh semen. However, percentage of progressive motility was determined by research microscope supplied with hot stage (37ºC) in diluted semen. The sperm livability was determined by eosin and nigrosin stain. The sperm abnormality was estimated during the livability test at a high-power magnitude (×400). Sperm cell concentration (SCC) was determined by direct cell count (×200) and a Neubauer Heamocytometer. However, motility index and total sperm output/ejaculate (TSO) was calculated as the following:

Motility index = mass motility (score) x progressive motility (%).

$$\textbf{TSO = EV (ml) x SCC (× } 10^6\textbf{/ml).}$$

Semen sample (10 µl) was gently mixed with sucrose solution (2 ml) with osmolarity level (50 mOsm/kg) in a water bath (37 °C) for 30 min to asses' sperm membrane integrity by hypo-osmotic swelling test (HOS-t). For acrosome integrity, fresh semen was extended (1:5) with normal saline, then smeared on glass slides, air-dried, fixed (10% neutral formal saline for 15 min), washed (running water for 20 min), stained with Giemsa solution overnight. The stained smear was rinsed in two changes of distilled water and air-dried. Percentage of sperm cells with damaged acrosome for 100 sperm cells per 5 fields were examined and counted at higher magnification (1000 ×).

During the last week of semen collection, blood samples were taken before morning feeding via the ear vein of five bucks in each group. Blood samples were collected in clean test tubes with EDTA for blood hematology [hemoglobin (Hb) concentration, hematocrit value (Ht), red blood cells (RBCs) and white blood cells (WBCs) and platelets counts in the whole blood. Both blood plasma and seminal plasma were isolated by blood centrifugation (3000 rpm for 20 min.) and both stored till analysis (-20 ºC). In blood and seminal plasma, concentration of total proteins (TP), albumin (Alb) and glucose (Obour City industrial area, Cairo, Egypt); total lipids and total cholesterol (Boehringer Ingelheim GmbH, Ingelheim, France); and activity of aspartate (AST) and alanine (ALT) aminotransferases (Diamond Diagnostics, Egypt) were determined using spectrophotometer. Globulin (Glb) concentration was calculated by subtracting Alb from TP.

In blood plasma and seminal plasma, level of total antioxidant capacity (TAC), glutathione content (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and malondialdehyde (MDA) were assayed using commercial kits (Bio-diagnostic Co., Recycling Crusher-SBM®) and spectrophotometer. After the semen collection in the last week, concentration of initial semen fructose was assayed immediately according to Mann (1948). Testosterone profile in blood plasma and seminal plasma was estimated by radio-immuno-assay (RIA) using commercial kits (Biosource-Europe S.A. 8, rue de L'Lndustrie.B-1400 Nivelles, Belgium). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) profiles in blood plasma were determined by RIA using specified kits (Radim, Italy).

At the end of semen collection, bucks (n=5) in each group were weighed and slaughtered. After slaughtering, testes were isolated, trimmed of adhering connective tissue and fats, then epididymal tissue was isolated from each testis and weighed to calculate their relative weights. Also, length, width and thickness of each testis were estimated. In the same time, weight of liver, kidney, spleen and abdominal fat was recorded, and their somatic indices were calculated.

Total of 80 receptive NZW nulliparous rabbit does were naturally mated by randomly five treated bucks in each group. Pregnancy was diagnosed abdominally to on day 10-12 post-mating to calculate pregnancy rate. Doe cages were prepared by nest boxes pre-expected date of parturition. Kindling rate was computed, and total live (after 12 h of kindling) litter size at birth and litter size at weaning were recorded. Also, viability rate of kits was calculated at birth and weaning.

Data were subjected to one-way ANOVA using General Linear Model Procedure (GLM) of statistical analysis system SAS (2012) Cary, NC, USA. The following statistical model was applied for analysis of all measurements: $Y_{ij}=\mu+ G_i+ e_{ij}$. Where, Y_{ij}= Observations, μ= Overall mean, G_i = Effect of group (i: 1-4), e_{ij}= Random error. The percentage values were subjected arcsine transformation before performing the analysis of variance. The significant differences were assessed by using Duncan's multiple range test (Duncan, 1955). The statistical significance was accepted at $P< 0.05$.

RESULTS AND DISCUSSION

Results

Semen characteristic parameters:

According to the results of Table 1, administration of EMS at levels of 900, 1200 and 1500 mg/buck improved

(P<0.05) the semen production, quantitatively and qualitatively as compared to the control group. Mass motility, percentages of progressive motility, livability and membrane integrity of spermatozoa, SCC, TOS, motility index and semen initial fructose concentration increased (P<0.05) in EMS treatment groups as compared to the control one. On the other hand, semen pH value, percentage of abnormality and acrosomal damage of spermatozoa decreased (P<0.05) in EMS treatment, while net semen volume was not affected significantly (P>0.05) in comparing with the control group.

Table 1. Effect of ethanolic moringa seeds extract on semen quality parameters of rabbit bucks.

Item	Ethanolic moringa seeds extract levels				±SEM	P-Value
	Control	900 mg/buck	1200 mg/buck	1500 mg/buck		
Net semen volume (ml)	0.77	0.80	0.79	0.80	0.0165	0.7419
Semen pH value	7.49^{a}	7.31^{b}	7.35^{b}	7.36^{b}	0.0218	0.0001
Mass motility (Score: 1-5)	3.20^{b}	4.40^{a}	4.30^{a}	4.10^{a}	0.2934	0.0256
Progressive sperm motility (%)	63.50^{b}	77.50^{a}	76.00^{a}	74.50^{a}	1.6478	0.0001
Motility index	203.20^{b}	341.00^{a}	326.80^{a}	305.45^{a}	22.283	0.0003
Livability (%)	66.10^{c}	85.50^{a}	82.00^{ab}	80.50^{b}	1.2844	0.0001
Abnormal sperm (%)	22.30^{a}	11.80^{b}	11.90^{b}	13.70^{b}	0.8176	0.0001
SCC (×10^{6}/ml)	289.50^{b}	372.90^{a}	365.50^{a}	360.60^{a}	4.4460	0.0001
Acrosomal damage (%)	23.50^{a}	10.40^{b}	10.70^{b}	12.40^{b}	1.0211	0.0001
Sperm membrane integrity (%)	25.60^{b}	36.20^{a}	34.50^{a}	33.70^{a}	1.2456	0.0001
TSO/ejaculate (×10^{6})	222.92^{b}	298.32^{a}	288.75^{a}	288.48^{a}	6.1066	0.0001
Initial semen fructose (mg/dl)	97.00^{b}	128.70^{a}	128.00^{a}	126.60^{a}	1.6077	0.0001

SCC: Sperm cell concentration; TSO: Total sperm output.

^{a,b} Means within a row with different superscript letters are significantly different (P<0.05).

Gonadotrophins and testosterone profiles:

Effect of EMS on average concentrations of blood plasma FSH and LH, and testosterone in blood and seminal plasma of rabbit bucks is presented in Table 2. Treatment with EMS increased (P<0.05) concentrations of FSH and LH in blood, and testosterone concentrations in blood and the seminal plasma as compared to the control group (Table 2).

Table 2. Effect of ethanolic moringa seeds extract on profile of FSH, LH and testosterone of rabbit bucks.

Item	Ethanolic moringa seeds extract levels				±SEM	P-Value
	Control	900 mg/buck	1200 mg/buck	1500 mg/buck		
		Blood plasma				
FSH (mIU/ml)	10.40^{b}	15.02^{a}	16.50^{a}	16.22^{a}	0.8856	0.0001
LH (mIU/ml)	12.10^{b}	13.25^{a}	13.90^{a}	14.10^{a}	0.0421	0.0125
Testosterone (ng/ml)	2.30^{b}	3.50^{a}	3.65^{a}	3.62^{a}	0.2527	0.0356
		Seminal plasma				
Testosterone (ng/ml)	10.33^{b}	15.10^{a}	15.80^{a}	16.02^{a}	0.7562	0.0001

FSH: follicle-stimulating hormone; LH: luteinizing hormone.

^{a,b} Means within a row with different superscript letters are significantly different (P<0.05).

Testicular and epididymal characteristics:

Data in Table 3 revealed non-significant differences among groups in the testicular characteristics. However, absolute and relative epididymal weights increased (P<0.05) in treatment groups as compared to the control group.

Table 3. Effect of ethanolic moringa seeds extract on testicular and epididymal characteristics of rabbit bucks.

Item	Ethanolic moringa seeds extract levels				±SEM	P-Value
	Control	900 mg/buck	1200 mg/buck	1500 mg/buck		
Pre-slaughtered weight of bucks (g)	4100.80	4098.20	4096.40	4104.80	-	-
Absolute testicular weight (g)	10.00	10.80	10.60	10.40	0.5831	0.7945
Relative testicular weight/kg BW (%)	0.24	0.26	0.25	0.25	0.0143	0.7915
Average testicular length (cm)	3.24	3.27	3.25	3.24	0.0176	0.5591
Average testicular width (cm)	1.25	1.27	1.267	1.257	0.0187	0.8523
Average testicular thickness (cm)	0.98	0.93	0.95	0.94	0.0357	0.8226
Absolute epididymal weight (g)	2.50^{b}	2.91^{a}	2.92^{a}	2.94^{a}	0.0338	0.0001
Relative epididymal weight/kg BW (%)	0.06^{b}	0.07^{a}	0.07^{a}	0.07^{a}	0.0008	0.0001

^{a,b} Means within a row with different superscript letters are significantly different (P<0.05).

Blood hematological parameters:

The influences of different level of EMS administration on some hematological parameters of bucks are shown in Table 4. The Hb concentration and RBCs count significantly (P<0.05) increased, while platelets significantly (P<0.05) decreased in treatment groups compared with the control group. However, administration of EMS at levels of 900mg/buck improved (P<0.05) only WBCs count as compared to control group. Hematocrit value was not affected (P>0.05) by treatment.

Table 4. Effect of ethanolic moringa seeds extract on hematological parameters of rabbit bucks.

Item	Ethanolic moringa seeds extract levels				±SEM	P-Value
	Control	900 mg/buck	1200 mg/buck	1500 mg/buck		
Hb (mg/dl)	9.49^{b}	11.01^{a}	10.94^{a}	10.99^{a}	0.2782	0.0029
RBCs (×10^{6}/mm^{3})	4.86^{b}	5.48^{a}	5.40 ^{a}	5.44^{a}	0.1205	0.0067
WBCs (×10^{6}/mm^{3})	6.35^{b}	7.20^{a}	6.39^{b}	6.4^{b}	0.0448	0.0001
Hematocrit value (%),	44.80	45.20	48.00	47.80	2.4701	0.5088
Platelets (×10^{3}/mm^{3})	243.40^{a}	210.00^{b}	239.20^{b}	242.40^{b}	3.4914	0.0001

Hemoglobin: Hb; RBCs: red blood cells; WBCs: white blood cells

^{a,b} Means within a row with different superscript letters are significantly different (P<0.05).

Biochemical constituents and enzymatic activity in blood and seminal plasma:

The effect of different levels of EMS administration on biochemical constitutes and enzyme activity in blood and seminal plasma of rabbit bucks is presented in Table 5. In blood plasma, concentration of TP and Alb increased (P<0.05) in treatment groups as compared to the control group. Total lipids, total cholesterol, urea, and creatinine concentrations in treatment groups, as well as activity of AST and ALT only in EMS at a level of 900 mg/buck decreased (P<0.05) in comparing with the control group. However, Glb and glucose concentrations were not affected (P>0.05) by treatment.

In seminal plasma, concentrations of TP, Alb and Glb were significantly (P<0.05) increased, while concentration of total lipids and total cholesterol, and activity of AST and ALT significantly (P<0.05) decreased in bucks treated with EMS in comparing with the control group (Table 5).

Table 5. Effect of ethanolic moringa seeds extract on biochemical constituents and enzymatic activity in blood and seminal plasma of rabbit bucks.

Item	Ethanolic moringa seeds extract levels				±SEM	P-Value
	Control	900 mg/buck	1200 mg/buck	1500 mg/buck		
			Blood plasma			
Total proteins (TP, g/dl)	5.31^{b}	6.27^{a}	6.20^{a}	6.25^{a}	0.0603	0.0001
Albumin (Alb, g/dl)	3.18^{b}	4.14^{a}	4.08^{a}	4.12^{a}	0.0489	0.0001
Globulin (Glb, g/dl)	2.13	2.13	2.12	2.13	0.0673	0.9979
Total lipids (g/l)	2.72^{a}	2.10^{b}	2.25^{b}	2.00^{b}	0.3752	0.0324
Total cholesterol (mg/dl)	120.00^{a}	95.60 ^{b}	98.80^{b}	98.50 ^{b}	3.8392	0.0012
Glucose (mg/dl)	76.40	82.20	80.60	84.40	3.3194	0.5227
Urea (mg/dl)	40.20^{a}	29.40^{b}	31.80^{b}	30.60^{b}	2.0493	0.0074
Creatinine (mg/dl)	2.13^{a}	1.82^{b}	1.86^{b}	1.87^{b}	0.0389	0.0001
AST (IU/l)	52.20^{a}	41.40^{b}	45.20^{ab}	45.40^{ab}	2.6552	0.0694
ALT (IU/l)	32.40^{a}	21.80^{b}	24.60^{ab}	25.00^{ab}	2.8398	0.0927
			Seminal plasma			
Total proteins (TP, g/dl)	4.25^{b}	5.16^{a}	5.12^{a}	5.09^{a}	0.0532	0.0001
Albumin (Alb, g/dl)	2.99 ^{b}	3.14^{a}	3.19^{a}	3.17^{a}	0.0498	0.0455
Globulin (Glb, g/dl)	1.26^{b}	2.02^{a}	1.93^{a}	1.92^{a}	0.0830	0.0001
Total lipids (g/l)	1.83^{a}	1.30^{b}	1.33^{b}	1.20^{b}	0.0925	0.001
Total cholesterol (mg/dl)	100.20^{a}	81.21^{b}	84.12^{b}	80.50^{b}	2.7858	0.0352
AST (IU/l)	39.20^{a}	30.20^{b}	33.40^{ab}	32.80^{ab}	2.1083	0.0498
ALT (IU/l)	27.60^{a}	15.40 ^{b}	16.00 ^{b}	16.60^{b}	1.8493	0.0006

AST: Aspartate aminotransferase; ALT: alanine aminotransferase

^{a,b} Means within a row with different superscript letters are significantly different (P<0.05).

Antioxidants capacity in blood and seminal plasma:

The effects of EMS administration on antioxidants capacity and lipid peroxidation in blood and seminal plasma are shown in Table 6. Results revealed that all levels of EMS increased (P<0.05) levels of TAC, GPx, GST, and SOD in blood and seminal plasma. Level of GSH in blood and seminal plasma were not affected significantly by EMS treatment. Although level of MDA was not altered in blood plasma by treatment, EMS treatment decreased (P<0.05) MDA level in the seminal plasma (Table 6).

Table 6. Effect of ethanolic moringa seeds extract on antioxidants status and lipid peroxidation in blood and seminal plasma of rabbit bucks.

Item	Ethanolic moringa seeds extract levels				±SEM	P-Value
	Control	900 mg/buck	1200 mg/buck	1500 mg/buck		
			Blood plasma			
TAC (mmol/l)	1.14^{b}	1.31^{a}	1.33^{a}	1.27^{a}	0.0377	0.0110
GSH (mg/dl)	19.80	24.80	25.00	23.20	2.2869	0.3755
GPx (mg/dl)	5.28^{b}	6.11^{a}	5.96^{a}	6.06^{a}	0.0615	0.0001
GST (IU)	1.30^{b}	1.68^{a}	1.64^{a}	1.66^{a}	0.0273	0.0001
SOD (IU)	6.89^{b}	7.55^{a}	7.57^{a}	7.52^{a}	0.0381	0.0001
MDA (nmol/ml)	15.04	10.80	11.80	11.40	1.51175	0.2340
			Seminal plasma			
TAC (mmol/l)	1.10^{b}	1.22^{a}	1.21^{a}	1.21^{a}	0.0323	0.0546
GSH (mg/dl)	18.20	23.40	23.80	23.60	2.1035	0.2169
GPx (mg/dl)	5.14^{b}	5.88^{a}	5.83^{a}	5.80^{a}	0.0384	0.0001
GST (IU)	1.19^{b}	1.30^{a}	1.28^{a}	1.29^{b}	0.0268	0.0285
SOD (IU)	5.97^{a}	6.45^{b}	6.48^{b}	6.46^{b}	0.1199	0.0311
MDA(nmol/ml)	21.00^{a}	13.20^{b}	14.40^{b}	14.80^{b}	1.6613	0.0188

TAC: total antioxidant capacity, GSH: glutathione content, GPx: glutathione peroxidase, GST: glutathione S transferase, SOD: superoxide dismutase; MDA: malondialdehyde.

^{a,b} Means within a row with different superscript letters are significantly different (P<0.05).

Health indices:

Data in Table 7 showed pronounced effect on relative weight of abdominal fat and spleen, being lower (P<0.05) in treatment groups than in the control group. However, hepato- and renal-somatic indices were not affected (P>0.05) by EMS treatment.

Fertility trails:

Reproductive performance parameters and kit performance of rabbit does naturally mated with bucks of the experimental groups are shown in Table 8. Pregnancy rate, litter size at birth (live) and at weaning, and kit viability rate at birth were higher (P<0.05) for does mated with bucks in treatment groups than in the control group. However, kindling rate and viability rate at weaning were not affected (P≥0.05) by EMS treatment (Table 8).

Table 7. Effect of ethanolic moringa seeds extract levels on absolute organs weights and health indices of rabbit bucks

Item	Ethanolic moringa seeds extract levels				±SEM	P-Value
	Control	900 mg/buck	1200 mg/buck	1500 mg/buck		
Live body weight (g)	4100.80	4098.20	4096.40	4104.80	-	-
Liver weight (g)	86.60	83.00	83.20	84.20	2.1295	0.6231
Hepato- somatic index	2.11	2.03	2.03	2.05	0.0511	0.6231
Kidney weight (g)	20.80	20.20	21.00	20.40	0.9274	0.9249
Renal- somatic index	0.51	0.49	0.51	0.4970	0.0223	0.9188
Abdominal fat weight (g)	76.20^{a}	51.40^{b}	57.40^{b}	57.20^{b}	2.3769	0.0001
Relative weight of abdominal fat/kg	1.89^{a}	1.25^{b}	1.40^{b}	1.39^{b}	0.0594	0.0001
Spleen weight (g)	1.67^{b}	1.91^{a}	1.90^{a}	1.92^{a}	0.0297	0.0001
Spleen- somatic index	0.04^{b}	0.05^{a}	0.05^{a}	0.05^{a}	0.0007	0.0001

^{a,b} Means within a row with different superscript letters are significantly different (P<0.05).

Table 8. Reproductive performance of rabbit does naturally mated by rabbit bucks treated with different levels of ethanolic moringa seeds extract

Item	Ethanolic moringa seeds extract levels				±SEM	P-Value
	Control	900 mg/buck	1200 mg/buck	1500 mg/buck		
Pregnancy rate	65% (13/20)	90% (18/20)	85% (17/20)	90% (18/20)	-	-
Kindling rate	84.62 % (11/13)	94.44 (17/18)	94.12% (16/17)	94.44% (17/18)	-	-
Total litter size at birth (n)	7.73	8.76	8.31	8.44	0.2685	0.1087
Live litter size at birth (n)	5.73^{b}	7.94^{a}	7.38^{a}	7.56^{a}	0.2811	0.0001
Viability rate at birth	74.13^{b}	90.64^{a}	88.81^{a}	89.57^{a}	2.3173	0.0001
Litter size at weaning (n)	5.09^{b}	7.12^{a}	6.75^{a}	6.69^{a}	0.2568	0.0001
Viability rate at weaning	88.83	89.67	91.46	88.49	2.6478	0.8738

^{a,b} Means within a row with different superscript letters are significantly different (P<0.05).

Discussion

The present study aimed to evaluate the effect of different levels of EMS on semen quality, hematological parameters, and biochemical properties, oxidative stress in blood and seminal plasma, and testicular, epididymal characteristics, and somatic indices of rabbit bucks. According to the obtained results, oral administration of different levels of EMS showed significantly marked improvement in all semen parameters studied in raw semen of bucks. In agreement with the present results, Dalton (2011) found that EMS supplementation improved motility, viability, and sperm cell membrane integrity of cattle spermatozoa, which are in association with sperm fertilizing ability. These findings were supported by many authors in rabbits (El-Desoky *et al.,* 2017) and in rats (Akunna *et al.,* 2012; Dafaalla *et al.,* 2017). The improvement observed in semen quality in our study as affected by EMS was associated with increasing concentration of semen initial fructose. In this respect, El-Ratel and Gabr (2019) recently found a positive relationship of semen initial fructose level with most sperm characteristics. Improving semen quality of bucks in treatment groups in our study was in parallel directly with increasing testosterone level in blood and seminal plasma, and relative epididymal weight, and indirectly with increasing levels of gonadotrophins (FSH and LH) and improving antioxidant status. The spermatogenesis completion is controlled by testosterone hormone (Walker, 2011), which was increased in blood by oral administration of *M. oleifera* at a level of 50 mg/kg for 100 days in rats (Akunna *et al.,* 2012). The noticeable effect of EMS on improving levels of FSH and LH may be the main reason of increasing testosterone secretion from Leydig cells. It was reported that semen characteristic parameters in mammals are regulated by FSH and LH. It is well known that testosterone secretion is stimulated by LH. Testosterone affects the seminiferous tubules (Sertoli cells) to stimulate spermatogenesis (Singh *et al.,* 1995). The biologically effective role of EMS on increasing epididymal weight may be related to the action of steroids on increasing the sexual organ weight as mentioned by Thakur and Dixit (2006). In accordance with the present results, testosterone and androgens directly affect the structure and physiology of epididymis. In this respect, EMS treatment (Dafaalla *et al.,* 2017) and methanolic *M. oleifera* extract (Sudha *et al.,* 2010) increased epididyml weight of rats. Generally, growth and secretion activity of the sexual organs is controlled by testosterone (Dafaalla *et al.,* 2017). Based on these findings, the role of EMO on increasing FSH, LH, testosterone, and consequently the weight of epididymis of treated bucks were supported in our study.In addition, PUSFA are found to be important for maintaining the sperm cell membranes. These fatty acids provide the plasma membrane of sperm cells with fluidity, ion exchange and motility, which are essential for sperm fertilizing ability (El-Desoky *et al.,* 2017). Fatty acid profile in the membrane of sperm cells was affected by types of fatty acids in the diet. The dietary supplementation alters quality and functional ability of sperm cells (Alizadeh *et al.,* 2014), and high containing of PUSFA in *M. oleifera* could be explained the improving the sperm quality observed in the present study.

As such, semen quality enhancement of treated bucks may be also explained by improving the antioxidant components in blood and seminal plasma, which has the ability to prevent the cell damage through increasing of the enzymes of the sperm antioxidant defense system (Dafaalla *et al.,* 2017). Also, the properties of EMS as an antioxidant cause protection of spermatocytes from apoptosis at different spermatogenesis stages, which lead to improving production of sperm cells (El-Desoky *et al.,* 2017). Seminal plasma contains SOD, catalase and some reductases, as endogenous antioxidants, for inactivating generation of ROS, leading to protecting the spermatozoa (Faustini *et al*., 2004).

The *M. oleifera* contains several phytogenic components which act as antioxidant, antibacterial and anti-inflammatory activity (Dafaalla *et al.,* 2017). In this concern, MDA level was decreased in mice treated with aqueous extract of *M. oleifera* (Luqman *et al.,* 2012). In our study, bucks treated with EMS showed improved oxidative status and reduced lipid peroxidation in blood and seminal plasma as previously reported by using different types of antioxidants on rabbits (Ojo and Adetoyi, 2017; El-Ratel and Gabr, 2019; El-Ratel *et al*., 2020b). Polyphenols and carotenoids present in *M. oleifera* seem to improve the antioxidants capacity and decreased mutations of DNA in cells of mammals (Devaraj *et al*., 2008). So, antioxidants

may eliminate the negative impacts of the oxidative stress in plasma membrane of spermatozoa (El-Desoky *et al.,* 2017).

It is worthy noting that increasing semen quality of treated bucks may be attributed to the hematological and biochemical parameters by indicating higher protein utilization and reducing the lipid profile with normal liver and kidney functions. Similar results were reported on rabbits by Khalifa *et al.* (2016), El-Desoky *et al.* (2017) and Ghadhban *et al*. (2019), who found that EMS increased the hematological parameters especially Hb, RBCs and WBCs, which may indicate the role of *M. oleifera* in supporting the immune system of the body against different infection (Jaiswal *et al.*, 2009). The *M. oleifera* have high protein, amino acids, vitamins and minerals (Faye, *et al.*, 2011). The bioactive component of *M. oleifera* (sitosterol) may in part be responsible for the hypocholesterolemic effect (Mbikay, 2012). The hypolipidemia of *M. oleifera* may be due two mechanism actions: HMG-Co-A reductase catalyzes rate limiting process of cholesterol biosynthesis and reduced the absorption of dietary cholesterol and liver cholesterol by biliary secretion (Hassarajani *et al.,* 2007). The EMS has positive effect on protection of liver from hepatocellular injury via stopping the increase of AST and ALT activity, leading to normal liver function and good health conditions.

The obtained results indicated higher fertilizing ability of bucks treated with EMS. Similar results were reported by Odeyinka *et al.* (2008) and El-Harairy *et al.* (2016) on semen of bucks treated with *M. oleifera*. These results are associated with improving immunity, and pronounced reduction in the oxidative stress, which led to decreasing injury sperm damage, consequently an enhancement in male fertility (Calogero *et al*., 2017). Increasing sperm cell concentration in semen of treated bucks may be the major signal to a possible high fertility rate (Oyeyemi and Okediran, 2007).

CONCLUSION

Moringa oleifera seeds as an ethanolic extract at a level of 900 mg/buck given as an oral administration for 16 weeks could improve semen quality traits, antioxidant capacity, health status, and fertility of rabbit bucks.

REFERENCES

Akunna, G.G., Ogunmodede, O.S., Saalu, C.L., Ogunlade, B., Bello, A.J. and Salawu, E.O. (2012). Ameliorative effect of *Moringa oleifera* (drumstick) leaf extracts on chromium-induced testicular toxicity in rat testes. World J. Life Sci. Med. Res. 2:20-26.

Alizadeh, A., Esmaeili, V., Shahverdi, A. and Rashidi, L. (2014). Dietary fish oil can change sperm parameters and fatty acid profiles of ram sperm during oil consumption period and after removal of oil source. Cell Journal. 16: 289–298.

Attia, Y.A., Asmaa, Sh., Bahaa, M. and Abdella, A. (2019). Effect of supplementation with trimethylglycine (betaine) and/or vitamins on semen quality, fertility, antioxidant status, DNA repair and welfare of roosters exposed to chronic heat stress. Animals. 8: 547- 561.

Attia, Y.A., Hamed, R.S., Bovera, F., Abd El-Hamid, A.E., Al-Harthi, M.A. and Shahba, H.A. (2017). Semen quality, antioxidant status and reproductive performance of rabbits bucks fed milk thistle seeds and rosemary leaves. Anim Reprod Sci. 184: 178-186.

Calogero, A.E., Condorelli, R.A., Russo, G.I. and La Vignera, S. (2017). Conservative non-hormonal options for the treatment of male infertility: antibiotics, anti-Inflammatory drugs, and antioxidants. Biomed Res. Int. 1-17.

Dafaalla, M. M., Salah, A., Salah, A., Abdel Wahab, H. and Omer, F. Idris (2017). Effect of ethanol extract of Moringa oleifera seeds on fertility hormone and sperm quality of male Albino Rats. Int. J. Multidiscip. Res. Dev., 4:222-226.

Dalton, J.C. (2011). Semen quality factors associated with fertility. In Proceedings of Applied Reproductive Strategies in Beef Cattle, Institute of Agriculture and Natural Resources, 30 September–1 October 2011, Boise, ID, USA.

Devaraj, S., Mathur, S., Basu, A., Aung, H. H., Vasu, V. T., Meyers, S. and Jialal, I. (2008). A dose-response study on the effects of purified lycopene supplementation on biomarkers of oxidative stress. J. Am. Coll. Nutr. 27: 267-273.

Duncan D.B. (1955). Multiple range and Multiple F test. Biometrics, 11: 1-42.

El Deeb, Mariam A., Afifi, O.S., Mahmoud, H.A. and Refay, M.S. (2015). Effect of nutritional and functional properties of Moringa oleifera leaves (MOL) on: I. Semen quality and offspring performance of New Zealand White (NZW) bucks. Assiut J. Agric. Sci., 46: 120-134.

El-Desoky, N. I., Hashem, N. M., Elkomy, A. and Abo-elezz, Z. R. (2017). Physiological response and semen quality of rabbit bucks supplemented with Moringa leaves ethanolic extract during summer season. Animal. 11:1549–1557.

El-Harairy, M.A., Shamiah, Sh. M. and Ghodaia, A. A. (2016). Influence of oral whole extract from Moringa oleifera on semen characteristics of rabbits. J. Animal and Poultry Prod., Mansoura Univ. 6; 217-224.

El-Ratel, I.T., Abdel-Khalek, A.E, Gabr Sh. A., Hammad M.E., El-Morsy H.I. (2020a). Influence of allicin administration on reproductive efficiency, immunity and lipid peroxidation of rabbit does under high ambient temperature. J. Anim. Physiol. Anim. Nutr. 104:539–548.

El-Ratel, I.T. and Gabr, A. A. (2019). Potential impact of *Spirulina alga* as an antioxidant on improving semen production and oxidative stress in blood and seminal plasma of rabbit bucks. Egypt. Poult. Sci. 40:209-224.

El-Ratel, I.T., K.A. Attia, El-Raghi and Sara F. Fouda (2020b). Relieve the negative effects of heat stress on semen quality, reproductive efficiency and oxidative capacity of rabbit bucks using different natural antioxidants. Asian-Australas J. Anim. Sci. Published online. DOI: https://doi.org/10.5713/ajas.20.0258.

Faizi, S., Siddiqui, B.S., Saleem, R., Aftab, K., Shaheen, F. and Gilani, A.H. (1998). Hypotensive constituents from the pods of Moringa oleifera. Planta. Med. 64:225-228.

Faustini, M., Torreb, M., Stacchezzini, S., Norberti, R., Consiglioc, A., Porcelli, F., Conteb, U., Munari, E., Russoa, V. and Vigoa, D. (2004). Boar spermatozoa encapsulated in barium alginate membranes: Na microdensitometric evaluation of some enzymatic activities during storage at 18ºC. Theriogenology. 61: 173-184.

Faye, B., Bucheton, B. and Banuls, A.L. (2011). Prevalence of leishmaniainfantum in a rural area of Senegal: analysis of risk factors involved in transmission to humans. J. Trans. R. Sco. Trop. Med.Hyg. 105:333-340.

Ghadhban, R. F., Eisa, A. H., Shegab, Zainab A. (2019). Physiological effects of *Moringa olifera* seed extract on some hematological parameters, thyroid hormones and liver enzymes in laboratory rats. Bas.J.Vet.Res.18: 278-287.

Glenville, M. (2008). The nutritional approach to male factor infertility. Dragons Tale.18:4-5.

Hassarajani, S., Souza, T. D., Mengi, S. A. and Chattopadhayay (2007). Efficacy study of the bioactive fraction (F-3) of Acoruscalamus in hyperlipidemia. Indian J. Pharmacol. 39: 196-200.

Jaiswal, D., Kumar Rai, P., Kumar, A., Mehta, S. and Watal, G. (2009). Effect of *Moringa oleifera Lam.* leaves aqueous extract therapy on hyperglycemic rats. J Ethnopharmacol. 123:392-396.

Khalifa Walaa H., Faten M. Ibrahim, Aida I. El makawy, Hafiza A. Sharaf, Wagdy K. B. Khalil and Nagwa A. Maghraby (2016). Safety and fertility enhancing role of moringa oleifera leaves aqueous extract in New Zealand rabbit bucks. Int. J. Pharm. 6: 156-168.

Luqman, S., Srivastava, S., Kumar, R., Maurya, A.K. and Chanda, D. (2012). Experimental assessment of Moringa oleifera leaf and fruit for its antistress, antioxidant, and scavenging potential using in vitro and in vivo assays. Evid. Based Complement. Alternat. Med. Article ID 519084, 12 p.

Mann, T. (1948). Fructose content and fructolysis in semen: practical application in the evaluation of semen quality. J. Agric. Sci. (Camb.) 38: 323–331.

Mbikay, M. (2012). Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: A review. Frontier Phamacol. 3: 1-12.

Mishra, G., Singh, P. and Verma, R. (2011). Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: an overview, Der Pharmacia Lettre. 3:141-164.

Mizeraa A., Marian, K. and Anna, S. (2019). Impact of the spirulina maxima extract addition to semen extender on bovine sperm quality. Ital. J. Anim. Sci. 18, 601–607.

Morshedy Sabrin A., Mohamed, B., Ahmed, A., Soliman A. Zahran and Mohamed, A. H. (2020). Effect of oral administration of Wheat (*Triticum aestivum L.*) Germ, Eruca sativa Mill. seed oils and their mixture on semen evaluation of V-line rabbit bucks. Asian J. Anim. Sci. 14: 44-53.

Morsy, A.S., Ashgan, M. Ellamie and Ibrahim, N.H. (2017). Effect of moringa peregrine seeds and leaves additives on semen quality, pre-weaning offspring performance and blood constituents of rabbits. Res. J. Anim. and Vet. Sci. 9: 1-7.

NRC. 1977. Nutrient requirements of rabbits, second revised edition, 1977. Washington, DC: The National Academies Press.

Odeyinka, S. M., Oyedele, O. J., Adeleke, T. O. and Odedire, J. A. (2008). Reproductive performance of rabbits fed Moringa oleifera as a replacement for Centrosema pubescens. 9th World Rabbit Congress Verona-Italy, June 10-13: 411-416.

Ogunlesi, M., Okoiel, W., Ofor, E. and Awonuga, O. (2009). Determination of the concentrations of Zinc and Vitamin C in oysters and some medical plants used to correct male factor infertility. J. Nat. Prod. 2: 89-97.

Ojo, O. A. and Adetoyi, S. A. (2017). Effect of *Moringa oleifera* leaf extract on the haematological and serum biochemistry of rabbits reared in a semi-humid environment. Afr. J. Biotechnol. 16:1386-1390.

Oyeyemi, M.O. and B.S. Okediran (2007). Testicular parameters and sperm morphology of chinchilla rabbits fed with different planes of soy meal. Int. J. Morphol., 25: 139-144.

Paul, T.K., Yousuf, A.S., Moniruzzaman, T., Asaduzzaman, R., Anowarul, H. and Mahmudul, H.S. (2018). Water supplementation of *Moringa oleifera* as a substitute for antibiotics on performance and blood parameters of broiler chickens. J. Bangladesh Agril. Univ. 16: 266–270.

Rriad, R, M., Seleem, T.S.T., El-Kholy, K.H. and Rawash, Z.M. (2016). Effect of sperm concentration and site of insemination on conception rate of rabbits. Alex. J. Vet. Sci. 50: 49- 56.

SAS Institute. (2012). SAS Statistics User's Guide. Statistical Analytical System, 5th rev. ed. SAS. Institute Inc., Cary, NC, USA.

Singh, J., O'Neill, C. and Handelsman, D.J. (1995). Induction of spermatogenesis byandrogens in gonadotropindeficient (hpg) mice. Endocrinology. 136:5311-5321.

Sudha, P., Asdaq, S.M., Dhamingi, S.S. and Chandrakala, G.K. (2010). Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in animals. Indian. J. Physiol. Pharmacol. 54: 133–140.

Thakur, M. and Dixit, V.K. (2006). Effect of Chlorophytum borivilianum on androgenic sexual behaviour of male rats. Indian Drugs. 43:300- 306.

Vasicek, J., Pivko, J. and Chrenek, P. (2014). Reproductive Performance of New Zealand White rabbits after depletion of apoptotic spermatozoa. Folia Biologica (Kraków), 62: 109-117.

Walker, W. H. (2011). Testosterone signaling and the regulation of spermatogenesis. Spermatogenesis. 1: 116–120.

تحسين هرمونات التناسل، جودة السائل المنوى والقدرة التأكسدية لذكور الارانب المعاملة بمستخلص بذور المورينجا

أمنية محمد درويش عجوة، خليل الشحات شريف وسارة فكرى فودة*

قسم انتاج الدواجن، كلية الزراعة، جامعة المنصورة

تهدف هذه الدراسة الى تقييم دراسة تأثيرات التجريع بمستخلص بذو المورينجا أوليفيرا الإيثانولي كعوامل محسنة على الصفات الفسيولوجية وجودة السائل المنوي لذكور الأرانب النيوزيلاندى البيضاء. استخدم فى هذه الدراسة ٤٠ ذكر ناضج جنسيا تم تقسيمهم الى ٤ مجاميع (١٠ ذكور/مجموعة). جرعت الذكور يوميا بمستويات مختلفة من مستخلص بذو المورينجا أوليفيرا الإيثانولي (صفر، ٩٠٠، ١٢٠٠ و ١٥٠٠ ملجم/ ذكر لمدة ١٦ اسبوع ، ٨ اسابيع قبل جمع السائل المنوى و ٨ اسابيع اخرى خلال مدة جمع السائل المنوى. اظهرت النتائج المتحصل عليها أن المعاملة بالمستويات المختلفة من مستخلص بذو المورينجا أوليفيرا الإيثانولي أدت الى زيادة معنوية في الحركة الكلية، النسبة المئوية للحركة التقدمية، الحيوية، سلامة غشاء الحيوانات المنوية، التركيز، العدد الكلى للحيوانات الكلية/قذفة، مؤشر الحيوية، الفركتوز الاولى فى السائل المنوى، وكذلك الهرمون المنبه للحويصلات المبيضية وهرمون التبويض، هيموجلوبين الدم، كرات الدم الحمراء و الجلوبيولين فى بلازما الدم ، كما أدت ايضا الى زيادة معنوية فى تركيزات هرمون التستستيرون والبروتين الكلى ، الالبيومين، ونشاط مضادات الأكسدة فى بلازما الدم والبلازما المنوية، وزيادة وزن البربخ ومؤشر وزن الطحال. كما أظهرت النتائج انخفاض معنوى فى قيمة الأس الهيدروجينى للسائل المنوى، الحيوانات المنوية الشاذة و ضرر الاكروسوم و وكذلك الصفائح الدموية و صورة الدهن ونشاط إنزيمات الكبد والمالونديالديهيد مقارنة بمجموعة الكنترول. ادت المعاملة بـ ٩٠٠ ملجم/ ذكر من مستخلص بذو المورينجا أوليفيرا الإيثانولي الى زيادة معنوية فقط فى عدد كرات الدم البيضاء. كما لوحظ تحسن معنوى فى معدل الحمل وعدد البطن الحية عند الميلاد وعند الفطام ومعدل الحيوية للخفات عند الميلاد. من الناحية العملية ، يمكن اقتراح استخدام مستخلص بذو المورينجا أوليفيرا الإيثانولي بمستوى ٩٠٠ ملجم/ذكر، فى تحسين جودة السائل المنوي، حالة مضادات الأكسدة، والصفات التناسلية لذكور الأرانب.