J.Animal and Poultry Prod., Mansoura Univ., Vol. 7 (6): 217 - 224, 2016

Influence of Oral Whole Extract from Moringa Oleifera on Semen Characteristics of Rabbits

El-Harairy, M. A.*; Sh. M. Shamiah and A. E. Ghodaia****

*** Anim. Prod. Dept., Fac. Agric., Mansoura University, Egypt.**

**** Biotech. Dept., Anim. Prod. Res. Institute, Agric. Res. Center., Egypt.**

ABSTRACT

The aim of this work was to study the effect of oral administration of whole extract from *Moringa oleifera* (MO) at levels of 0, 60 and 120 mg/head for 21 days on performance and semen characteristics of rabbit bucks. Total of 12 adult New Zealand rabbit bucks having live body weight (LBW) of 2304-2750 g/h kg and at six menthe of age were divided into three similar groups (n = 4 in each). Bucks in the 1st group were given 3 ml sterile distilled water (Control, G1), while those in the 2nd and 3rd groups were given 3 ml distilled water containing 60 (G2) or 120 (G3) mg from the whole extract of *MO*. Bucks in all groups were treated as daily oral administration for 21 days before semen collection. All bucks were fed commercial complete feed diet and kept under the same managerial and climatic conditions. Semen was collected twice a week for 8 weeks. On day of semen collection, reaction time (RT) was calculated and semen was evaluated for volume (SV), pH value, mass (MM) and progressive (PM) motility, sperm livability (SL) and abnormality (SA) percentages, sperm cell concentration (SCC), total sperm output/ejaculate (TSOP), damaged acrosome (DA) and response to osmotic test at osmolaity level of 50 mOsm/l for 30 min at 37°C (curled spermatozoa, CS). The obtained results revealed insignificant effect of MO on LBW of bucks. RT and percentages of MM, PM, SL, SA, SCC, TSO, DA and CS were improved (P<0.05) by MO at both levels. Semen pH value did not differ in G2 or G3 from that in G1, but pH value was higher (P<0.05) in G3 than in G2. SV increased (P<0.05) by about 27% only in G3 as compared to the G1, but did not differ from that in G2. RT and all physical semen characteristics were affected (P<0.05) by collection week, except semen pH value and DA percentage, which showed insignificantly inconsistent trend of changes throughout the collection period. RT and SA decreased (P<0.05), while SV, MM, PM, SCC and TSO increased (P<0.05) by advancing collection week. SV and CS showed inconsistent (P<0.05) trend of change during the collection weeks. The effect of interaction between treatment and collection week was not significant on RT and all semen characteristics studied. Rabbit does mated by bucks in G2 showed the best results reproductive performance, in terms of the highest kindling rate, total number of borns, total and live litter size at birth and viability rate at weaning, but the differences were not significant. Also, does mated by bucks in G2 showed the highest (P<0.05) proportion of females, litter size at weaning, and litter weight at birth and weaning. Rabbit does mated by bucks in G3 showed the highest (P<0.05) average bunny weight at birth. In conclusion, moringa oleifera extract at a level of 60 mg/h as oral administration for 21 days has significant value in improving the antioxidant status and could serve as a supportive treatment in the nutritional management to improve semen production of rabbit bucks, and consequently increasing reproductive performance of rabbit does mated by this semen.

Keywords: Rabbit bucks, semen, *Moringa oleifera extract, fertility.*

INTRODUCTION

In many tropical and subtropical countries, various parts of *Moringa oleifera* (leaves, fruits, immature pods, and flowers) are incorporated into the traditional food of humans (Anhwange *et al*., 2004). A wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers, fruits, and seeds (Anwar *et al*., 2007; Kumar *et al*., 2010). Phytochemical analyses have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids (Bennett *et al*., 2003; Aslam *et al*., 2005; Manguro and Lemmen, 2007; Amaglo *et al*., 2010; Gowrishankar *et al*., 2010).

Polyphenolic compounds which are often abundant in beverages derived from plant origin, such as herbal teas and teas, may contribute to the inhibitory effect of diets on oxidative stress. In this respect, the antioxidant effect of *Moringa oleifera* leaf extract and fruit was explained by Luqman *et al*. (2012) in term of the presence of polyphenols, tannins, anthocyanin, glycosides, and thiocarbamates, which remove free radicals, activate antioxidant enzymes, and inhibit oxidases. In addition, *Moringa oleifera* was reported to prevent effectively, morphological changes and oxidative damage in lens of rats by enhancing the activities of antioxidant enzymes, reducing the intensity of lipid peroxidation and inhibiting generation of free radicals (Sreelatha and Padma, 2009) and suppress formation of reactive oxygen species (ROS) (Sofidiya *et al*., 2006; Ogbunugafor *et al*., 2011).

Semen quality is the guarantee of successful insemination in breeding rabbits. There are many endogenous and exogenous factors affecting reproduction. Administration of antioxidants such as vitamin E, selenium, vitamin C, and carotenoids may reduce the oxidative stress and improve sperm motility (Castellini, 2008). It is important to investigate the protective properties of *Moringa oleifera* on testicular function. Therefore, aim of this paper is to study the effect of oral administration of whole extract from *Moringa oleifera* at levels of 0, 60 and 120 mg/head for 21 days on performance and semen characteristics of rabbit bucks.

MATERIALS AND METHODS

Animals:

Total of thirty adult New Zealand rabbit bucks having live body weight of 2304-2750 g/h and at six months of age were divided into three similar groups (4 animals in each) and allowed to acclimatize for 7 days in their respective cages. Bucks in the 1st group were given 3 ml sterile distilled water (Control, G1) at the same times of treatments in other groups. Bucks in the 2nd and 3rd groups were given 3 ml distilled water containing 60 (G2) or 120 mg (G3) from whole extract of *Moringa oleifera* (each capsule contained 500 mg

whole plant powder, Dynamic Health Co., USA). Bucks in all groups were treated as daily oral administration for 21 days.

All bucks were fed commercial complete feed diet (16% CP and 2850 Kcal/kg diet energy) and kept under the same managerial and climatic conditions.

Semen collection:

Semen was collected twice a week early in the morning (7 a.m.) for 8 weeks as a collection period. Immediately after collection, 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. On day of semen collection, reaction time was calculated in term of time elapsed from introducing female to male up to complete ejaculation

Semen evaluation:

Semen was evaluated for determination of semen volume with gel, semen pH value and mass motility immediately after collection. Percentage of progressive sperm motility in each semen sample was determined using research microscope supplied with hot stage adjusted to 37°C by assessment of degree of movement of spermatozoa in about 0.5 ml diluted semen.

Live sperm percentage was determined by eosin (1.67%) and nigrosin (10%) mixture stain and sperm abnormality percentage was estimated during the examination of live/dead sperm percentage at a high power magnitude (x 400), the morphological abnormalities of spermatozoa were also determined per 200 sperm. Sperm cell concentration was determined by direct cell count using a microscope (x 200) and a Neubauer Heamocytometer. Total sperm output/ejaculate (TSOP) was calculated by multiplying sperm cell concentration/ml (SCC) by ejaculate volume (EV) as the following:

TSOP (x 10^9/ejaculate) = EV (ml) x SCC (x 10^9/ml)

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 minutes and washed by tap water for 15 minutes and stained with Gimsa stain at 37°C for 3 hours. Then the stained slides were washed by tap water for 15 minutes and dried at 37°C. The prepared slides were examined by research microscope at higher magnification (x 100) for determination of spermatozoa with and without intact acrosome per 200 spermatozoa in each field. The acrosome stained light purpledark pink, while sperm remains unstained. Percentage of spermatozoa with intact acrosome was calculated.

Hypo-osmotic swelling test (HOS-test)

The response of buck spermatozoa to osmotic tests was assessed using solution prepared with fructose (1.25%) and Na-citrate (2.9%) in distilled water (3 times) to give osmolarity of 300 mOsm using a freezing-point depression osmometer (Osmett A, Model 5002, Fisher Svientific, Pittsbury, PA, USA). Then, distilled water was added to reach osmolaity level to 50 mOsm using osmometer. One drop of diluted semen was added to one ml of the hypo-osmotic solution with osmolarity of 50 mOsm into glass tube and the mixture was immediately examined in semen incubated for 30 min at 37°C. A semen smear from the mixture was made and dried at the same temperature. The slides were stained with eosin-nigrosine mixture stain.

All prepared slides were examined and numbers of spermatozoa with curled tail were determined using research microscope at (x 400). Two hundred spermatozoa per slide were counted and percentage of spermatozoa having curled tails was calculated.

Statistical analysis:

Data were analyzed by two-way analysis of variance (ANOVA) using the general linear model procedure according to SAS (2004). Values were considered significant at $P<0.05$. The significant differences among groups or sampling times were tested 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

Live body weight of rabbit bucks:

Data in Table 1 revealed insignificant effect of *Moringa oleifera* (MO) treatment on LBW of bucks, although there was a tendency of reduction in LBW of bucks treated with both MO doses (G2 and G3) as compared to control group (G1) during treatment and semen collection periods.

In accordance with the present results, Chumark *et al*. (2008) found that dietary components of MO were reported to increase the body weights of rats significantly with increased MO concentration, while no significant change occurred in rabbits. Contrarily, Nuhu (2010) noticed that offering weaner rabbits a diet containing Moringa leaf meal significantly ($P<0.05$) increased daily weight gain when compared to a control diet.

Table 1. Effect of *Moringa oleifera* (MO) treatment on average live body weight of rabbit bucks during the experimental period.

Live body weight (g/h)	G1 (control)	G2 (60 mg MO/h)	G3 (120 mg MO/h)	±SEM
Initial (start of treatment)	2507.1	2500.0	2550.0	182.04
Final (end of semen collection)	2807.1	2635.7	2721.4	150.48
Changes	300.0	135.7	171.4	-

In addition, Fahey *et al*. (2001) mentioned that the increase in the body weight of rats might be due to the fact that MO is rich in amino acids, vitamins and minerals particularly iron (Subadra *et al*., 1997; Faye, 2011). The reported significant increase in body weights

of rats might also be attributed to captivity, where energy expenditure is minimal (Fadi *et al*., 2010).

Reaction time and physical semen characteristics:

Effect of MO treatment:

Results presented in Table 2 show that effect of MO treatment was significant on reaction time (RT) and all semen characteristics studied. Reaction time, and percentages of mass motility (MM), progressive motility (PM), livability (SL), abnormality (SA), concentration (SCC), total sperm output (TSO), damaged acrosome and curling of spermatozoa were significantly (P<0.05) improved by MO treatment at both levels. However, semen pH value did not differ significantly in G2 or G3 from that in G1, but pH value was significantly (P<0.05) higher in G3 than in G2. Semen volume significantly (P<0.05) increased by about 27% only in G3 as compared to the G1, but did not differ from that in G2.

According to these results, treatment of rabbit bucks with MO at levels of 60 or 120 mg/h had beneficial effects on improving reaction time and all physical semen characteristics of rabbit bucks.

Table 2. Effect of *Moringa oleifera* (MO) treatment on reaction time and semen physical characteristics of rabbit bucks during the collection period (Overall mean).

Item	Experimental group			±SEM
	G1 (Control)	G2 (60 mg MO/h)	G3 (120 mg MO/h)	
Reaction time (s)	20.71^{a}	14.29^{b}	11.88^{b}	2.953
Semen pH value	7.49^{ab}	7.14^{b}	7.59^{a}	0.053
Semen volume (ml)	0.681^{b}	0.756^{ab}	0.867^{a}	0.057
Mass motility (%)	70.42^{b}	82.29^{a}	83.45^{a}	1.958
Progressive sperm motility (%)	74.79^{b}	83.33^{a}	84.79^{a}	0.421
Live sperm (%)	75.45^{b}	87.71^{a}	88.25^{a}	0.582
Sperm abnormality (%)	20.92^{b}	14.37^{ab}	12.08^{a}	2.242
Sperm concentration (x10^{6}/ml)	429.5^{b}	593.7^{a}	558.3^{a}	18.14
Total sperm output (x10^{6}/ejac.)	293.3^{b}	469.3^{a}	485.6^{a}	10.316
Damaged acrosome (%)	15.04^{a}	12.29^{b}	12.25^{b}	0.485
Hypo-osmotic swelling test (%)	26.04^{b}	35.79^{a}	38.88^{a}	1.346

Means denoted within the same row with different superscripts are significantly different at P<0.05.

Generally, reaction time (RT) of NZW rabbit bucks ranged between 13.0 and 24.5 sec (Safaa *et al*., 2008), 26.7- 27.16 sec (Mansour, 2010), 11.49-23.39 sec (El-Tohamy *et al*., 2012). The present values of reaction ime are within these ranges. The present values of physical semen characteristics are within reference values in various rabbit breeds as reported IRRG (2005), being 0.3-0.9 ml for semen volume, 30-90% for progressive motility, 250-600 x 106/ml for sperm cell concentration, and 7.1 for pH value. However, range of live sperm from 69.90 to 89.13% was reported by Mansour (2010) and El-Tohamy *et al*. (2012). Meanwhile, total sperm abnormalities was 15.40-16.79% (El-Tohamy *et al*., 2012); total sperm output was 208.92-289.03 x106/ejaculate as reported by El-Tohamy *et al*. (2012).

Although the present results indicated improvement of sexual desire of bucks in term of reducing the reaction time in G2 and G3 treated with MO as compared to G1 (control), Sudha *et al*. (2010) found that methanolic extract of MO does not affect sexual behavior or serum androgen level but enhances seminiferous tubules, testis and epididymal weight and seminal vesicles in the male rats. This improvement may be due to increasing testicular weight of bucks treated with MO as compared to control (unshown data). Increasing testicular weight of bucks treated with MO may lead to testicular volume and number of leydge cells responsible for testosterone secretion. Improving semen volume may reveal pronounced effect of MO as antioxidant on accessory sex glands and testicular tissues (spermatocytes) within the somniferous tubules of the testis as well as on epididymal spermatozoa (Abdel-Khalek *et al*., 2001).

In this respect, several authors reported that some medicinal plants are extensively used as aphrodisiac to relieve sexual dysfunction, or as fertility enhancing agents. They provide a boost of nutritional value thereby improving sexual performance and libido (Yakubu *et al*, 2007; Sumalatha *et al*., 2010). In addition, Priyadarshani and Varma (2014) found that the administration of MO leaf powder in the treated diabetic male mice with leaf powder of MO leaves showed significantly higher testes and epididymis weight in comparison with diabetic control animals. These effects may be due to that MO leaves are excellent source of vitamin B, calcium, protein and potassium. Beta-carotene and other phytochemicals with known powerful antioxidant ability (Kaempferol, Quercetin, Rutin and Caffeoylquinic acids); powerful antioxidant vitamins (C, E, and A) and essential micronutrients with antioxidant activity (Selenium and Zinc) as explained by several authors (Fuglier, 1999; Jaiswal *et al*., 2009; Vongsak *et al*., 2013). Moreover, MO leaves possess tremendous anti-oxidant properties that ameliorate the deleterious effect of alcohol on pre- pubertal testes of rats (Bassey *et al*., 2013).

As proved in our study, Priyadarshani and Varma (2014) demonstrated that Moringa leaf powder administration for 21 days treatment significantly increased sperm motility, reduced sperm abnormalities including headless sperm, round head sperm and coiled tail sperm in abundance as well as banana head sperm and amorphous head sperm, and significantly increased sperm count. Also, Radwan *et al*. (2015) showed that the rats treated with MO leaf extract (MOLE) significantly lowered DNA fragmentation and morphological sperm abnormalities. However, Obembe *et al*. (2015) reported that the long term exposure of male rats to MO resulted no significant difference in sperm motility and number of normal sperm cells among the groups, while increased sperm count, without affecting the quality of sperm. Awodele *et al*. (2012)

reported that sperm motility and sperm morphology were unaffected following *M. oleifera*. While, sperm count decreased following MO administration.

Effect of collection week:

Reaction time and physical semen characteristics were significantly (P<0.05) affected by collection week, except semen pH value and DA percentage, which showed insignificantly inconsistent trend of changes throughout the collection period (Table 3).

By advancing collection week, RT and SA significantly (P<0.05) decreased, while SV, MM, PM, SCC and TSO significantly (P<0.05) increased. However, SV and CS showed significantly (P<0.05) inconsistent trend of change (Table 3).

Effect of interaction:

Analysis of variance revealed that the effect of interaction between treatment and collection week was not significant on reaction time and semen characteristics studied. According to these effects, results illustrated in Figure 1 indicated marked reduction in RT in all groups, and increasing pH value and SV in G2 and G3 as compared to G1.

Throughout the collection weeks, rabbit bucks in both treatment groups (G2 and G3) showed the best results concerning percentage of MM, PM, SL, SA, SCC and TSO, and the highest response to HOS-t at most collection weeks as compared to G1 (Fig. 2).

The observed impact of MO on semen characteristics may related to anti-bacterial properties of MO due to lipophilic compounds and antibiotic metabolites in MO seed extracts (Jabeen *et al*., 2008; Patel, 2011).

The antioxidant effect of MO leaf extract and fruit was explained by Luqman *et al*. (2012), who noticed that it was due to the presence of polyphenols, tannins, anthocyanin, glycosides, and thiocarbamates, which remove free radicals, activate antioxidant enzymes, and inhibit oxidases. MO leave aqueous extract was observed to have a therapeutic action through reducing of genetic alterations (micronuclei and DNA damage) in irradiated rats by gamma irradiation (Eshak and Osman, 2013).

Table 3. Effect of collection week on reaction time and semen physical characteristics of rabbit bucks (Overall mean).

Collection week	RT (sec)	Semen pH value	SV (ml)	MM (%)	PM (%)	SL (%)	SA (%)	SCC ($x10^6$/ml)	TSO ($x10^6$)	DA (%)	CS (%)
1	26.56^{a}	7.41	0.522^{c}	67.22^{b}	63.89^{c}	85.11^{b}	12.56^{a}	353.3^{f}	183.9^{c}	14.11	35.89^{a}
2	17.67^{b}	7.46	0.911^{a}	80.0^{a}	73.89^{b}	87.33^{a}	12.00^{ab}	425.4^{e}	378.6^{c}	12.22	32.56^{b}
3	24.00^{a}	7.46	0.761^{bc}	81.11^{a}	84.44^{a}	86.22^{ab}	10.67^{c}	477.7^{de}	366.6^{c}	13.33	34.22^{a}
4	14.78^{bc}	7.30	0.722^{bc}	80.0^{a}	87.22^{a}	88.22^{a}	10.67^{c}	532.2^{cd}	389.0^{c}	12.44	30.22^{c}
5	10.89^{c}	7.29	0.839^{ab}	84.44^{a}	90.56^{a}	88.11^{a}	11.11^{bc}	573.3^{bc}	495.6^{b}	13.22	33.67^{a}
6	10.33^{c}	7.51	0.656^{c}	81.11^{a}	83.33^{a}	87.78^{a}	11.78^{ab}	571.0^{bc}	377.9^{c}	13.11	34.56^{a}
7	10.88^{c}	7.46	0.767^{bc}	76.11^{ab}	84.44^{a}	86.67^{ab}	11.11^{bc}	617.7^{ab}	463.6^{b}	12.89	34.44^{a}
8	9.89^{c}	7.38	0.989^{a}	80.00^{a}	$86.60.7^{a}$	87.67^{a}	11.11^{bc}	666.6^{a}	673.2^{a}	14.22	33.00^{b}
±SEM	1.557	0.065	0.093	3.197	3.074	0.654	0.95	10.23	10.516	1.793	0.892

Means denoted within the same column with different superscripts are significantly different at P<0.05. RT: Reaction time. SV: Semen volume. MM: Mass motility. PM: Progressive motility. SL: Sperm livability. SA: Sperm abnormality. SCC: Sperm cell concentration. TSO: Total sperm output. DA: Damaged acrosome. CS: Curled spermatozoa.

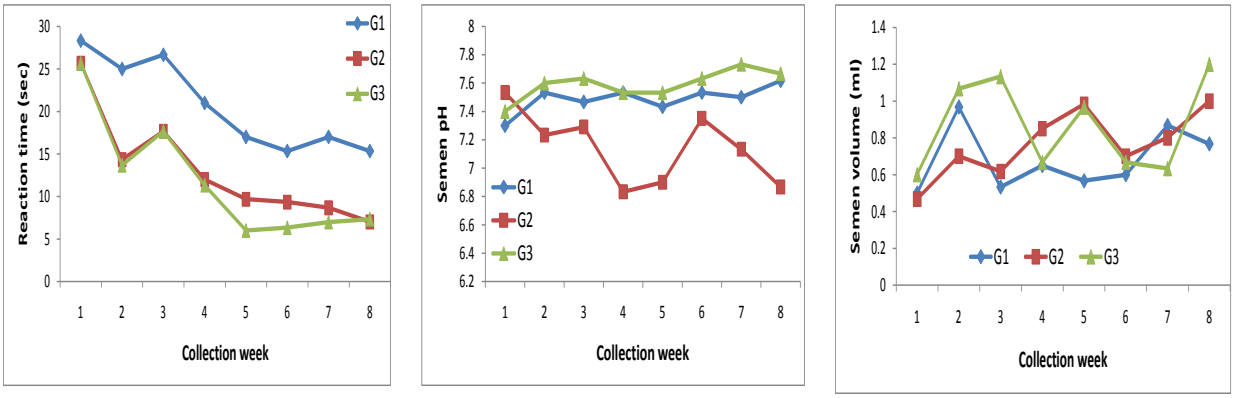

Fig. (1): Changes in reaction time, semen pH value and semen volume during successive collection weeks.

Recently, Radwan *et al*. (2015) showed that ethanolic extracts of MO leaves possessed anti-genotoxic phytoconstituents in mice, the high percentages of micronuclei and DNA damage induced by cyclophosphamide were decreased in animals pre-dosed with the extract (Sathya *et al*., 2010). Many components of MO leave extract such polyphenols and various carotenoids were observed to improve the immune system, scavenge of free radical and reduce the production of DNA mutations in different mammalian cells that were previously exposed to variety of oxidative conditions (Srinivasan *et al*., 2007; Devaraj *et al*., 2008).

Furthermore, polyphenols which are present in MO leave extract were shown in other studies to inhibit a specific protein found in bone marrow and which is responsible for cancer in bone and increased the production of antioxidants in the sperms (Abdou *et al*., 2012). MO was claimed to boost immune systems (Olugbemi *et al*., 2010).

In addition, many of micro- constituents of MO leave extract were considered to be anti-carcinogenesis, they were showed in other studies to reduce the risk of ovarian cancer, lung cancer and prostate cancer in human and mice (Van Breda *et al*., 2005; Gitenay *et al*. 2007). MO has non-toxic properties as previously reported (Lawal *et al*., 2005).

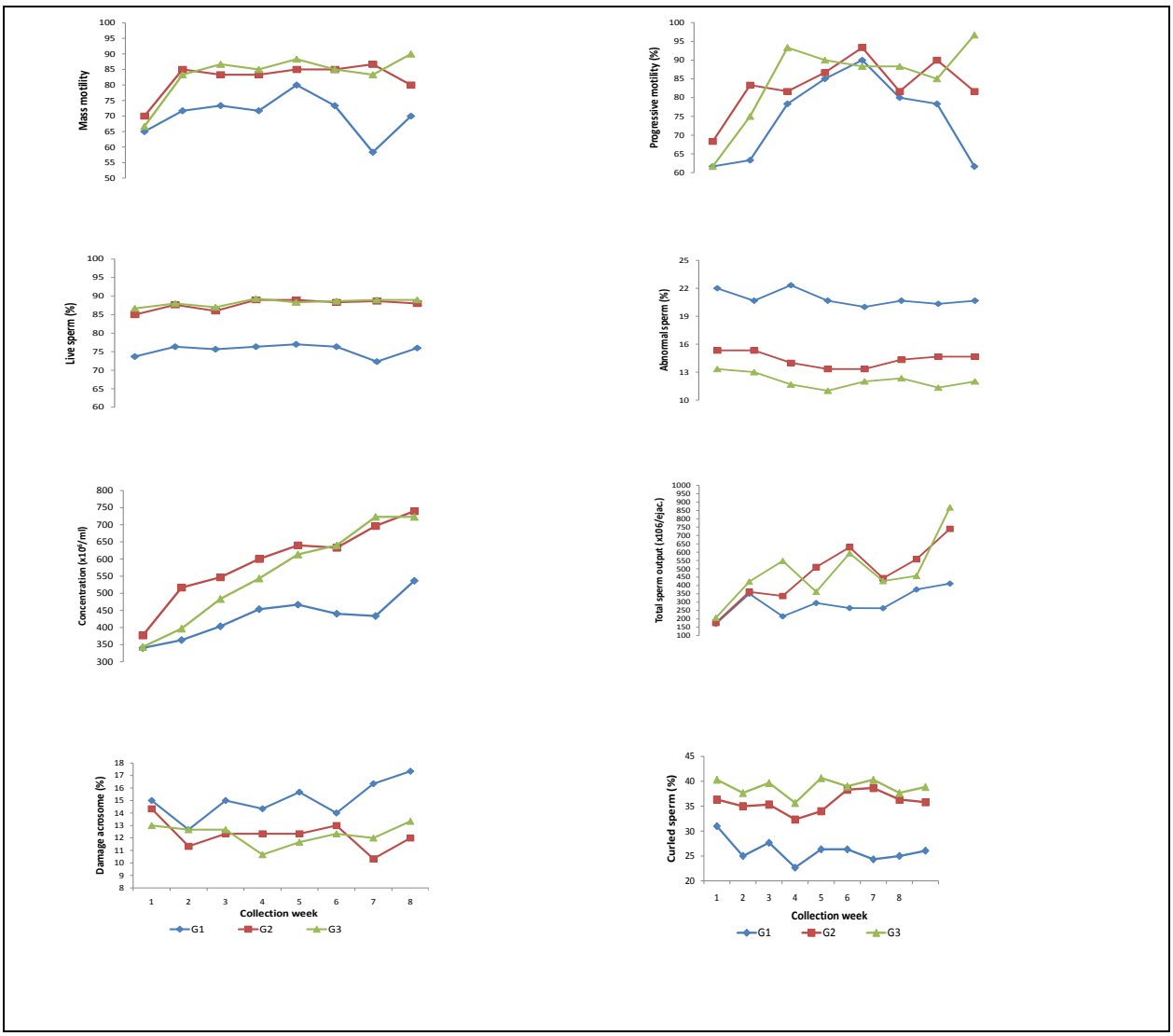

Fig. (2): Changes in semen characteristics during successive collection weeks.

Reproductive performance of mated does:

Data presented in Table 4 revealed that rabbit does mated by bucks in G2 showed the best results regarding the reproductive performance, in terms of the highest kindling rate, total number of borns, total and live litter size at birth and viability rate at weaning, but the differences were not significant. Also, does mated by bucks in G2 showed significantly (P<0.05) the highest proportion of females, litter size at weaning, and litter weight at birth and weaning. However, rabbit does mated by bucks in G3 showed significantly (P<0.05) the highest average bunny weight at birth (g).

Table 4. Reproductive performance of rabbit does mated with bucks in the experimental groups.

Parameter	Experimental group			±SEM
	G1 (Control)	G2 (60 mg/h)	G3 (120 mg/h)	
Kindling rate (%)	66.33	100	100	-
Total number of borns	35	46	35	-
Litter size at birth/doe	5.00	6.57	5.00	0.626
Live borns at birth/doe	5.00	6.57	5.00	0.626
Viability rate at birth (%)	100	100	100	-
Male (%)	54.29^{a}	23.91^{b}	65.71^{a}	10.26
Female (%)	45.71^{b}	76.09^{a}	34.29^{b}	9.465
Litter size at weaning (n)	3.67^{b}	5.67^{a}	4.00^{b}	0.287
Viability rate at weaning (%)	75.56	82.5	79.44	8.912
Average bunny weight at birth (g)	42.6^{b}	51.9^{ab}	55.0^{a}	3.621
Litter weight at birth (g)	213.33^{b}	346.33^{a}	275.0^{b}	20.16
Litter weight at weaning (g)	1476.7^{b}	2150.0^{a}	1373.3^{b}	117.3

Means denoted within the same row with different superscripts are significantly different at P<0.05.

A 20-week feeding trial was conducted by Odeyinka *et al*. (2008) to evaluate the reproductive performance of rabbits fed MO as a replacement for *Centrosema pubescens*. Freshly harvested *C. pubescens* and MO leaves were offered to the animals at 20% of their live weight at the ratios of 100:0 (MO), 75:25 (M25), 50:50 (M50), 25:75 (M75), and 0:100 (M100), in addition to the concentrate feed offered to the animals. There were significant differences in litter size at weaning, and average daily weight gain per kid, on the different treatments ($P<0.05$). However, there was no significant difference in gestation length as well as litter weight at birth. It was concluded that MO can be used to replace *Centrosema pubescens* without adverse effects on the reproductive performance of rabbit does.

CONCLUSION

The current findings suggested that *Moringa oleifera* extract at a level of 60 mg/h as oral administration for 21 days has significant value in improving the antioxidant status and could serve as a supportive treatment in the nutritional management to improve semen production of rabbit bucks, and consequently increasing reproductive performance of rabbit does mated by this semen.

REFERENCES

Abdel-Khalek, A; 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., 26(8): 4773-4786

Abdou, H.S; Salah, S.H; Booles, H.F. and Abdel Rahim, E.A. (2012). Effect of pomegranate pretreatment on genotoxicity and hepatotoxicity induced by carbon tetrachloride (CCl4) in male rats. Journal of Medicinal Plants Research, 6(17): 3370-3380.

Amaglo, N. K; Bennett, R. N; LoCurto, R. B; Rosa, E. A. S; LoTurco, V; Giuffrid, A; LoCurto, A; Crea, F. and Timpo, G.M.(2010).Profiling selected phytochemical sand nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. *Food Chem.* 122:1047–1054.

Anhwange, B.A; Ajibola, V.O. and Oniye, S.J.(2004). Chemical studies of the seeds of *Moringa oleifera* (Lam.) and *Detarium microcarpum* (Guill and Sperr). J. Biol. Sci., 4: 711–715.

Anwar, F; Latif, S; Ashraf, M. and Gilani, A.H. (2007). *Moringa oleifera*: a food plant with multiple bio-chemical and medicinal uses. Phytother Res., 21: 17–25.

Aslam, M; Anwar, F; Nadeem, R; Rashid, U; Kazi, T. G; and Nadeem, M. (2005).Mineral composition of *Moringa oleifera* leaves and pods from dfferent regions of Punjab, Pakistan. *Asian J. Plant .Sci*., 4, 417–421.

Awodele, O; Oreagba, I. A; Odoma, S; da Silva, J. A. T.and Osunkalu, V. O. (2012). Journal of ethnopharmacology, 139(2): 330-336.

Bassey ,R. B; Bala, D. N; Edagha, I.A. and Peter A. I. (2013). The effect of ethanolic extract of Moringa oleifera on alcohol-induced testicular histopathologies in pre-pubertal albino Wistar rats. Biology and Medicine, 5: 40–45.

Bennett, R.N; Mellon, F.A; Foid, l. N; Pratt, J.H; DuPont, M.S; Perkins, L. and Kroon P.A.(2003). Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees Moringa oleifera (Horseradish tree) and Moringa stenopetala. J. Agri. Food Chem: 3546-3553.

Castellini, C. (2008). "Semen production and management of rabbit bucks." 9th World Rabbit Congress, Verona – Italy 265-278.

Chumark, P; Khunawat, P; Sanvarinda, Y; Phornchirasilp, S; Morales, N. P; Phivthong-ngam, L; Ratanachamnong, P; Srisawat, S. and Klai-upsorn ,S. Pongrapeeporn. (2008). The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. Leaves. J.Ethnopharmacol, 1-8.

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. Journal of the American College of Nutrition, 27: 267-273.

Duncan, D. B. (1955). Multiple range and multiple F. Test biometrics, 11: 1-42.

EL-Tohamy, M.M; Kotp, M.S; EL-Nattat, W.S. and Mohamed, A.H. (2012). Semen characteristics and oxidative/antioxidative status in semen and serum of male rabbits supplemented.

Eshak, M.G. and Osman, H. F. (2013). Role of Moringa oleifera Leaves on Biochemicaland Genetical Alterations in Irradiated Male Rats. Middle-East Journal of Scientific Research, 16 (10): 1303-1315.

Fadi, C; Andrzj, P; Ekaterina, M. and Hayes, K.C.(2010). Nutrition correlates and dynamics of diabetes in Nile rats: a novel model for diet induced type 2 diabetes and metabolic syndrome. *J Nutri Metabol*, 7:29.

Fahey, J.W; Zakmann, A.T. and Talalay, P.(2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochem , 56: 5–51.

Faye, B; Bucheton, B. and Banuls, A.L. (2011). Prevalence of leishmania infantum 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.

Fuglier, L.J. (1999). The Miracle Tree: *Moringa oleifera*, Natural Nutrition for the Tropics. Church World Service, Dakkar, Senegal, pp. 68.

Gitenay, D; Lyan, B; Rambeau, M; Mazur, A. and Rock, E. (2007). Comparison of lycopene and tomato effects on biomarkers of oxidative stress in vitamin E deficient rats. Eur J Nutr, 46: 468-475.

Gowrishankar, R; Kumar, M; Menon, V; Divi, S. M; Saravanan, M; Magudapathy, P; Panigrahi, B. K; Nair, K. G. and Venkataramaniah, K. (2010). Trace element studies on *Tinospora cordifolia* (Menispermaceae), *Oci- mumsanctum* (Lamiaceae), *Moringa oleifera* (Moringaceae), and *Phyllan- thus niruri* (Euphorbiaceae) using PIXE. *Biol. Trace Elem. Re,* 133: 357–363.

Hasnaa, A; Radwan, Inas; Ghaly, S; Ibrahim, M. Farag .and Ezzo, M.I. (2015). Protective and Therapeutic Effect of Moringa oleifera Leaf Extract on DNA Damage, Cytogenetic Changes, Sperm Abnormalities and High Level of MDA Induced by CCL4 in Rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences RJPBCS 6(1) :1061-1079.

IRRG, C; Boiti, C; Castellini, M; Theau-Clément, U; Besenfelder, L; Liguori ,T; Renieri. and Pizzi, F. (2005). "Guidelines for the handling of rabbit bucks and semen." World Rabbit Sci. 13:71-91.

Jabeen, R; Shahid, M; Jamil, A. and Ashraf , M. (2008). Microscopic evaluation of the antimicrobial activity of seed extracts of Moringa oleifera. Pak J Bot , 40: 1349–1358.

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(3):392-396.

Kumar, P. S; Mishra, D; Ghosh, G. and Panda, G.S.(2010). Medicinal uses and pharmacological properties of *Moringa oleifera*. *Int. J. Phytomed*. 2: 210–216.

Lawal, M; Wasagu, R. S. U. and Ladan, M. J. (2005). Biological and Environmental Sciences Journal for the Tropics, 2(2):36-38.

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. Evi Bas Compl Alt Med, 2012: 519084.

Manguro, L. O. and Lemmen, P. (2007). Phenolics of *Moringaoleifera* leaves. *Nat.Prod.Res.* 21:6–68.

Mansour, H. (2010). "Manipulation of testis functions and semen characteristics in rabbit." Master Thesis, Ain shams university, cairo, egypt.

Nuhu, F. (2010). Effect of Moringa leaf meal (MOLM) on nutrient digestibility, growth, carcass and blood indices of weaner rabbits. MSc, Faculty of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Obembe, A. O; Urom, S. E; Ofutet, E. O; Ikpi, D. E. and Okpo-Ene A. I. (2015). The effect of aqueous seed extract of Moringa Oleifera on sperm count, motility and morphology in male albino wistar rats. Der Pharmacia Lettre, 7 (3): 129-133.

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.

Ogbunugafor, H.A; Eneh, F.U; Ozumba, A.N; Igwo-Ezikpe, M.N; Okpuzor, J; Igwilo, I.O; Adenekan, S.O. and Onyekwelu, O.A.(2011). Physico-chemical and anti-oxidant properties of *Moringa oleifera* seed oil. In *Pakistan J. Nutri*. 10 (5): 409-414.

Olugbemi, T.S; Mutayoba, S.K. and Lekule, F.P. (2010). Effect of Moringa (*Moringa oleifera*) Inclusion in Cassava based diets to broiler chickens. In *International J. Poultr. Sci*. 9 (4):363-367.

Patel, J.P. (2011). Antibacterial activity of methanolic and acetone extract of some medicinal plants used in India folklore. In *International Journal of Phytomedicine* , 3: 261-269.

Priyadarshani, N. and Varma, M.C. (2014). Effect of Moringa oleifera leaf powder on sperm count, histology of testis and epididymis of hyperglycaemic mice Mus musculus. American International Journal of Research in Formal, Applied & Natural Sciences, 7(1), June-August, 2014 : 7-13.

Safaa, H. M; Emarah, M. E. and Saleh, N. F. A. (2008). "Seasonal effects on semen quality in black baladi and White New Zealand rabbit bucks." World Rabbit Sci. 16:13-20.

SAS. (2004). SAS Institute Inc. SAS User's Guide, Statistics. Cary, NC.

Sathya, T.N; Aadarsh, P; Deepa, V; Balakrishna, Murthy. P.(2010). Moringa oleifera Lam. leaves prevent Cyclophosphamide-induced micronucleus and DNA damage in mice. International Journal of Phytomedicine, 2 : 147-154.

Sofidiya, M.O; Odukoya, O.A; Familoni, O.B. and Inya-Agha, S.I.(2006). Free-radical scavenging activity of some Nigerian medicinal plant extracts. In *Pakistan J. Biolog. Sci*. 9: 1438-1441.

Sreelatha ,S. and Padma, P.R.(2009). Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. *J Plant Food Human Nutri*, 64: 303 – 311.

Srinivasan, M; Sudheer, A. and Menon, V. P.(2007). Ferulic acid: Therapeutic potential through its antioxidant property. J Clin Biochem Nutr, 40: 92- 100.

Subadra, S; Monica, J. and Dhabhai, D. (1997). Retention and storage stability of beta-carotene in dehydrated *M. oleifera*. *Inter J Food Sci. and Nutri,* 48:373 – 379.

Sudha, P. et al.(2010). Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in animals. Indian J Physiol Pharmacol.

Sumalatha, K; Saravana, K.A. and Mohana, L.S. (2010). Review of natural aphrodisiac potentials to treat sexual dysfunction. Int J Pharm Ther., 1: 10- 18.

Van Breda, S.G; Van Agen, E; Van Sanden, S; Burzykowski, T; Kleinjans, J. C. and Van Delft, J. H. (2005). Vegetables affect the expression of genes involved in carcinogenic and anticarcinogenic processes in the lungs of female C57BI/6 mice. J Nutr ,135: 2546-2552.

Vongsak, B; Sithisarn, P. and Gritsanapan, W.(2013). Simultaneous HPLC quantitative analysis of active compounds in leaves of moringa oleifera Lam. J Chromatogr Sci.

Yakubu, M.T; Akanji, M. and Oladiji, A.T. (2007). Male sexaual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. PHCOG Rev., 1(1):49- 52.

تأثير تجريع المستخلص الكامل لنبات المورينجا على صفات السائل المنوي في الأرانب

مصطفى الحرايرى* ، شريف شامية و على السعيد غدية****

*** قسم إنتاج الحيوان- كلية الزراعة – جامعة المنصورة- مصر.**

****قسم التكنولوجيا الحيوية – معهد بحوث الإنتاج الحيواني – مصر.**

تهدف هذه الدراسة إلى معرفة تأثير التجريع بمستخلص نبات المورينجا الكامل عند مستوى صفر و ٦٠ و ١٢٠ مليجرام /ذكر لمدة ٢١ يوم على أداء و صفات السائل المنوي لذكور الأرانب. استخدم في هذه الدراسة 12 من ذكور الأرانب النيوزلاندى بمتوسط وزن 3 كجم وعمر ٦ شهور قسمت إلى ثلاث مجاميع متماثلة (ن =٤) لكل مجموعة. المجموعة الأولى أعطيت ٣ مل ماء مقطر ومعقم (الكنترول) بينما المجموعة الثانية والثالثة أعطيت ٣ مل ماء مقطر يحتوى على ٦٠ و١٢٠ مليجرام /رأس ،على التوالي من مستخلص نبات المورينجا الكامل لمدة ٢١ يوم قبل جمع السائل المنوي. كل الأرانب غذيت على عليقه تجارية وتحت نفس الظروف والرعاية. تم جمع السائل المنوي مرتين أسبوعيا لمدة ٨ أسبوع. في يوم الجمع تم حساب الرغبة الجنسية وتقييم حجم القذفة ودرجة الحموضة والحركة الجماعية والحركة التقدمية ونسبة الحي والشواذ وتركيز الحيوانات المنوية والتركيز الكلى لكل قذفه وشواذ الاكروسوم والاستجابة لاختبار الاسموزية عند مستوى ٣٠ مل اسمول لمدة ٣٠ دقيقة على درجة حرارة ٣٧° م (التفاف الاسبرم). أظهرت النتائج التالى: أن مستخلص نبات المورينجا الكامل ليس له تأثير معنوي على ورن الجسم الحي لذكور الأرانب. الرغبة الجنسية ونسبة الحركة الجماعية والتقدمية والحي والشواذ وتركيز الحيوانات المنوية والتركيز الكلى لكل قذفه وشواذ الاكروسوم والاستجابة لاختبار الاسموزية تحسنت بمعنوية ($P<0.05$) لكل من المستويين من مستخلص المورينجا. لم هناك اختلاف في قيمة درجة الحموضة بين المجموعة الثانية والثالثة والمجموعة الأولى. ولكن قيمة درجة الحموضة في المجموعة والثالثة أعلى بمعنوية ($P<0.05$) عن في المجموعة الثانية. زاد حجم القذفة بنسبة ٢٨ % بمعنوية ($P<0.05$) في المجموعة الثالثة مقارنة بالمجموعة الأولى ولكن لم تختلف مع المجموعة الثانية. الرغبة الجنسية وكل صفات السائل المنوي تأثرت بمعنوية ($P<0.05$) بأسابيع الجمع باستثناء درجة الحموضة ونسبة شواذ الاكروسوم كانت غير معنوية ومتناقضة الاتجاه خلال فترة الجمع. الرغبة الجنسية وشواذ الاسبرم قلت بمعنوية ($P<0.05$) بينما حجم القذفة والحركة الجماعية والتقدمية وتركيز الحيوانات المنوي والتركيز الكلى لكل قذفه زاد بمعنوية ($P<0.05$) بتقدم أسبوع الجمع. كما لوحظ أن اتجاه حجم القذفة والتفاف الاسبرم كان متناقض التغير بمعنوية ($P<0.05$) خلال أسابيع الجمع. التداخل بين المعاملة و أسابيع الجمع لم يكن معنويا سواء في الرغبة الجنسية وكل صفات السائل المنوي تحت الدراسة. إناث الأرانب التي تم تلقيحها من ذكور المجموعة الثانية أعطت أفضل أداء تناسلي، بمعنى أعلى معدل ولادات والعدد الكلى للمواليد والعدد الكلى للبطن وعدد الحي لكل بطن عند الميلاد ومعدل الحي عند الفطام ولكن الاختلافات غير معنوية. أيضا لوحظ أن إناث الأرانب التي تم تلقيحها من ذكور المجموعة الثانية كانت الأعلى بمعنوية ($P<0.05$) في نسبة الإناث وعدد المواليد للبطن عند الفطام ووزن البطن عند الميلاد وعند الفطام. كذلك إناث الأرانب التي تم تلقيحها من ذكور المجموعة الثالثة كانت الأعلى بمعنوية ($P<0.05$) في وزن المواليد عند الميلاد.

الخلاصة:

التجريع بمستخلص نبات المورينجا عند مستوى ٦٠ مليجرام / ذكر لمدة ٢١ يوم له دور فعال في تحسين مضادات الأكسدة وبالتالى تحسين إنتاج السائل المنوي لذكور الأرانب و زيادة الكفاءة التناسلية للإناث التي تلقح بهذا السائل المنوي.

.