

STUDY THE IMPACT OF MONENSIN ,VITAMIN E AND DATE PALM SEEDS EXTRACT ON CERTAIN ARABI RAMS SPERM PARAMETERS.

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ABSTRACT

The aim of this study is to assess the impact of monensin, vitamin E and dates palm seeds extract(DPSE) on some parameters of Arabi rams sperm .Twenty rams were used in this study, randomly distributed in 4 groups, each group contains 5 animals. which treated daily as the following : control (without addition), T1(50 ml of (DPSE)/head), T2 (50 mg vit E / head) and T3 (200 mg monensin /head).The experiment continued for two months. Semen collected weekly by artificial vagina. After collecting the semen the sperm parameters were evaluated in addition to measurement the testosterone level and some antioxidative enzymes and oxidative marker(MDA). The results showed that T1 and T2 exhibited enhancement in semen volume,sperm concentration, individual motility and testosterone levels while MDA levels are reduced and elevation of GPx and SOD as compared to control and T3. It was concluded that DPSE and vit E have advanced effect on sperm parameters of Arabi rams.

INTRODUCTION

Reproductive efficiency is one of the main pillars of the success of animal breeding projects which enhanced through dissemination of high value genetic factors to increase productivity and improve income of breeders(1).

It is possible to increase reproductive efficiency by manipulation with the component of the the ration or supporting it with some additives. Researches have

revealed that date seed contains high nutritional value, especially in term of their content of fibers and antioxidant (2).

Date palm seed comprises various chemical compounds such as saturated fatty acids (stearic and palmitic acid) , un saturated fatty acids(linoleic,oleic acids) which could inhibit the 5 - α reductase enzyme , Zinc(Zn) ,Calcium(Ca) ,potassium(K)(3) , fat ,protein , , dietary fiber of ash . Also date seed contains high level of phenolics , antioxidant(4), ferulic, sinapic, p- coumaric , and acids, procyanidins and flavonoids (5; 6) and lipid, which is either in wax , fat or oil form(7).

Vit E is another additives which is called as anti-sterility since it is required for female reproductive system to function normally. (8), and (9), indicated that vitE is a main chain - breaking anti-oxidant in sperm membrane because it can instantly scavenge free radical such as hydrogen peroxide(H_2O_2), superoxide anion($\bullet O_2^-$), and hydroxyl radicals($OH\bullet$) . Due to its lipid solubility, vitamin E is the main line of protection from peroxidation of the polyunsaturated fatty acid of phospholipids in sperm membrane (10). Monensin belongs to the ionophor group, which increases the plasma membrane permeability of bacteria, causing them to burst and convert to beneficial microbial protein. monensin are reducing the number of methanogenic bacteria (11) which reduce the energy lost due to methane production. (12) and (13) mention that monensen should cause an influx of Ca^{++} , there being abundant Ca^{++} existent to induce capacitation.

MATERIALS AND METHODS

Animals and Collection of semen

These experiments were done in local field in Al-Nasir city, (Thi-qar province) from 5-1-2018 to 5-3-2018 In this study 20 Arabi rams with average 3.5- 4 years old and 56 ± 5 kg body weight were chosen and were held in pens with semi-slatted floorings. Animals were fed a diet based on (14) recommended based on 60:40 forage to concentrate *ad libitum* with free access to water.The animals were divided randomly into 4 groups (5 rams for each group) and they were treated daily as follow: control(without addition), T1(50 ml of date palm seeds extract/head), T2 (50 mg vit E/head) and T3 (200 mg monensin /head). After completion of the preliminary period (two weeks) for adaptation on new diet and training for semen collection, 10

ejaculates (each six days) were collected from each ram every six days by using artificial vagina as described by (15).

Preparation of Date Palm Seed Extract

Date fruits were obtained from the Qalat Sekar orchards in Thi-Qar province; The seeds were picked, rinsed to dry and roasted well afterwards. The dry pits were crushed into a fine powder and added distill water to make a 50 gm/L mixture which was boiled till it becomes brownish in colour and finally filtered.(16).

Semen Processing

After the beginning of the treatments ,semen was collected with an artificial vagina every six days , equipped with graded test tubes, sited in a thermo flask at 37°C, and transferred to the laboratory for assessment. (volume of semen, PH, count of sperm, mass and individual motility, viability and sperm abnormality.

Table(1) Chemical analysis of diets and their content of metabolized energy

Composition	ration
Dry matter	92.05
Crude protein	14.69
Ether extract	5.52
Crude fiber	8.37
Nitrogen free extract	59.57
Organic matter	88.15
ash	3.90
Metabolized energy mega joules /kg dry matter	12.232

Metabolized energy based on values for ruminants(14)

Table(2) Primary feed materials and additives used in experimental diets

diet	control	T1	T2	T3
barley	31	31	31	31
Soybean	15	15	15	15
Wheat flour	10	10	10	10
bran	25	25	25	25
Ground corn cobs	15	15	15	15
Minerals and vitamins	3	3	3	3
salt	1	1	1	1
total	100	100	100	100
additives				
DPSE ml/head			50	
Vit E mg/head		50		
Monensin mg/head				200

based on values for ruminants(14)

Measurement of testicular Malondialdehyde (MDA Concentration):

Testicular lipid peroxidation product(MDA) was done by the way of Yashkochi and Master (17).Reaction of MDA with thio-barbituric acid(TBA) in an acidic media give a colored TBA_complex estimated by spectrophotometer at 520_535 nm in contrast to blank and the value of MDA were stated as n mole MDA/mL semen.

Estimation the activity of testicular Super-oxide Dismutase :

Superoxide dismutase activity(SOD) was assessed rendering to Giannopolitis and Ries(18). The optical absorbance was estimated at waves length (560)nm against blank reagents. SOD = Reading (absorbances) of (SOD)/ mL semen.

Measurement activity of testicular Glutathione peroxidase:

Activity of glutathione peroxidase in semen was determined spectrophotometrically using Ransel reagents from Randox Laboratory Ltd. (Crumlen,UK). Glutathione is oxidized by cumene hydroxyperoxide and is directly reduced to glutathione by glutathione reductase with a simultaneous oxidation of NADPH to NADP⁺. The activities are stated as unit(U) /mL semen.

Measurement of CAT activity:

The method described by (19) was used for the determination of CAT activity in the semen samples.

Statistical analysis:

Data were analyzed using SPSS-Version 19). Two ways ANOVA and least significant differences (LSD) were performed to assess significant difference among means $P \leq 0.05$.

RESULTS

The results in table (3) showed that DPSE and Vit E treatment presented high significant difference($P \leq 0.05$) as compared to control and monensin treatment in semen volume (1.40 ± 0.22 and 1.30 ± 0.26 vs. 1.00 ± 0.34 and 1.00 ± 0.30 respectively) and concentration (3.4 ± 0.33 and 3.2 ± 0.36 vs. 2.8 ± 0.23 and 2.9 ± 0.18 respectively) DPSE exhibited higher significant difference($P \leq 0.05$) in an individual motility(88 ± 3.14)as compared to control group(80 ± 2.22) and non-significant difference as compared to vit E and monensin group (85 ± 3.24 and 82 ± 2.18) respectively. The results also revealed that DPSE and Vit E groups showed lower significant difference($P \leq 0.05$) in an abnormality compared to control and monensin

groups (6.5 ± 0.76 and 6.8 ± 1.16 vs. 8 ± 1.62 and 7.8 ± 0.98 respectively) while there was non-significant difference among treatment in PH, mass motility and viability.

Table (3) *In vivo* Effect of DPSE ,Vitamin E and Monensin on Some Sperm Parameters of Arabi Rams(means \pm SE).

parameters	control	DPSE	vitE	monensin	LSD
volume	1.00 \pm 0.34 b	1.40 \pm 0.22 a	1.30 \pm 0.26 a	1.00 \pm 0.30 b	0.30
PH	6.8 \pm 1.2 a	7.0 \pm 1.3 a	7.0 \pm 0.8 a	6.9 \pm 0.70 a	NS
Concentration mil/ml	2.8 \pm 0.23 b	3.4 \pm 0.33 a	3.2 \pm 0.36 a	2.9 \pm 0.18 b	0.3
Mass motility%	90 \pm 4.5 a	95 \pm 4.4 a	92 \pm 6.4 a	92 \pm 7.20 a	NS
Individual motility%	80 \pm 2.22 b	88 \pm 3.14 a	85 \pm 3.24 ab	82 \pm 2.18 b	6.0
Viability%	90 \pm 4.23 a	95 \pm 3.19 a	95 \pm 6.30 a	92 \pm 4.05 a	NS
Abnormality%	8.0 \pm 1.62 a	6.5 \pm 0.76 b	6.8 \pm 1.16 b	7.8 \pm 0.98 a	1.0

Different small letters vertically denotes significant ($P<0.05$) between groups

Results in Table (4) revealed that DPSE and Vitamin E were significantly ($P < 0.05$) higher than control and monensin group in level of testosterone (16.5 ± 1.24 and 16.0 ± 2.20 vs. 12.3 ± 1.13 and 13.1 ± 2.18 respectively), GPx (3.97 ± 0.88 and 3.76 ± 0.67 vs. 1.97 ± 0.97 and 2.60 ± 0.47 respectively) and SOD (20.65 ± 1.66 and 18.92 ± 1.16 vs. 16.15 ± 2.60 and 17.32 ± 1.24 respectively) while there was a low significant difference ($P\leq 0.05$) in the level of MDA (3.22 ± 0.45 and 3.02 ± 0.32 vs. 5.17 ± 0.78 and 4.32 ± 1.13 respectively), Non-significant differences among treatments in the level of CAT.

Table(4) *In vitro* Effect of DPSE ,Vitamin E and Monensin on Some Sperm Parameters of Arabi Rams(means \pm SE).

parameters	control	DPSE	Vit E	monensin	LSD
Testosterone ng/mL	12.3 \pm 1.13 b	16.5 \pm 1.24 a	16 \pm 2.20 a	13.1 \pm 2.18 b	3.7
MDA(nomL/mL)	5.17 \pm 0.78 a	3.22 \pm 0.45 b	3.02 \pm 0.32 b	4.32 \pm 1.13 a	1.1
GPx(U/mL)	1.97 \pm 0.97 b	3.97 \pm 0.88 a	3.76 \pm 0.67 a	2.60 \pm 0.47 b	1.16
SOD(U/mL)	16.15 \pm 2.60 b	20.65 \pm 1.66a	18.92 \pm 1.16a	17.32 \pm 1.24 b	1.6
CAT(U/mL)	4.11 \pm 0.40 a	3.54 \pm 0.75 a	3.62 \pm 1.42 a	4.50 \pm 1.04 a	NS

Different small letters vertically denotes significant ($P<0.05$) between groups

DISCUSSION

Daily oral treatment with pits extract of DPSE and vit E cause a significant increase in most sperm characteristics such as volume, sperm concentration and individual motility with reduction of abnormalities of Arabi rams spermatozoa. This findings are confirmed by (20) who established that the relative estimation between control and treated groups show that administration of date palm pits suspension enhanced sperm count, motility, morphology . Improvement in sperm parameters in DPSE treatment may be referred to its content of of estrogenic constituents as gonad_stimulating compound that increase male fertility (21) as well, date seed contains a substantial amount of food ingredients such as proteins and mineral that improve sperm quality (22).

These findings of vit E treatment were in agreement with the observations of (23) who studied the impact of dietary vit E on sperm motility and kinetic traits of ram sperm, the positive effect of vitamin E is related to its biological functions that prevent lipoprotein oxidation of sperm membrane(24). vitamin E keeps and decreases gaps in the helix of mitochondria of mid piece of spermatid during late spermatogenesis that may be responsible for improved motility (25). (26) found that vitamin E regulates phospholipase A2 activity that plays a pivotal role in stabilization of viable sperm by interaction with phospholipids. Feeding vitamin E may elicit direct stimulation of steroidogenic enzyme , modification of cAMP second_messenger function and / or interfering with cell membranes properties that could lead to maintain the conformation of cell membrane (27).

Slight enhancement of the semen characteristics in monensin treatment was in agreement with that obtained by (28). A possible mechanisms involve in the monensin_mediated rise in LH liberation was proposed to be an influence at the level of the Golgi apparatus of endocrine cell. *In vitro* researches with pituitary fragments of perfused rat, proposed capability of monensin on redirecting hormone production toward secretion more than storage(29).

The daily oral dealing with pit of date palms produced a noteworthy rise in the level of testosterone in blood serum of Arabi rams, this agrees with (30)who stated that a date palm pollen suspensions increase the level of estradiol and testosterone in plasma which are found at high concentrations in seminal plasma and rat testis.Also, (21) detected that DPSE increase the sperm concentration in guinea pigs and increase

the level of testosterone in rats. DPSE have been involved in animals ration as growth promoter, the effect has been ascribed to an enhancement in the plasma level of testosterone(31). Intra_testicular testosterone levels designates that date palm suspension changed leydig cell steroidogenesis. Though, the particular way of this effect is unknown (32).

Treatment with date palm seeds and vit E cause reduction in the level of MDA and increase GPx and SOD. (33) showed that date palm comprises estradiol and flavonoids and other antioxidant constituents that improve the quality of sperm. The therapeutic use of DPSE is an ancient practice. It has been suggested that its beneficial therapeutic effects referred to their antioxidant constituents. Dates have phenolic compounds mainly(cinnamic acids) and flavonoids(flavones, flavonols, and flavanones) that provided antioxidant activities(34).)Our result was in accordance to that obtained by (35). Oral administration of vitamin E has valuable effects on the male reproductive system. The beneficial effects were apparent in the form of increase of semen quality, antioxidants status and testosterone in mammals. Vit.E is a lipid-soluble antioxidant that neutralize free radical and keeps cellular membrane against O₂ free radicals. It furthermore avoids lipid peroxidation and therefore enhance functions of other antioxidants(36). It was concluded that DPS and vit E enhanced sperm parameters of Arabi rams due to their antioxidant effect.

دراسة تأثير الموننسين وفيتامين هـ و خلاصة نوى التمر في بعض معايير النطف للخراف العربية

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الخلاصة

اجريت هذه الدراسة لتقييم تأثير الموننسين وفيتامين هـ و خلاصة بذور النخيل في بعض معايير النطف لدى الكباش العربية. استخدم عشرون كبشاً عربياً في هذه الدراسة ، وزعت عشوائياً الى 4 مجموعات ، كل مجموعة تحتوي على 5 حيوانات عوملت يوميا على النحو التالي: السيطرة (بدون اضافة)، المعاملة الاولى (50 مل خلاصة بذور النخيل/رأس)، المعاملة الثانية (50 ملغم فيتامين هـ/رأس) والمعاملة الثالثة (200 ملغم موننسين/رأس). جمع السائل المنوي اسبوعيا باستخدام المهبل الاصطناعي. بعد جمع السائل المنوي تم تقييم معايير النطف وكذلك تقدير مستوى هرمون التستسترون وبعض انزيمات الاكسدة. تبين من النتائج ان المعاملتين الاولى والثانية اظهرتا تحسنا معنويا في حجم السائل المنوي وتركيز الحيوانات المنوية والحركة الفردية

ومستوى هرمون التستسترون بينما كان هناك انخفاض في مستوى المالمونيلدهايد وارتفاع مستوى الكلتوتايون
بيروكسيديز والسوبر اوكسايد ديسميوتيز. يستنتج مما سبق بان خلاصة بذور النخيل وفيتامين هـ لهما تأثير
محسن في صفات نطف الكباش العربية.

REFERENCES

- 1-FAO. 2003. Know to move, move to know. Ecological knowledge among the WoDaaBe of south eastern Niger, by N. Schareika. Rome.
- 2-Habib, H.M. and Ibrahim, W.H.2008. Nutritional quality evaluation of eighteen date pit varieties. *Int.J.Food Sci.Nutr.* 16:1–13.
- 3-Shariati, M., Sharifi, E. and Kaveh, M. 2008. The effect of phoenix dactylifera (date-palm) pit powder on testosterone level and germ cells in adult male rats. *J. Zanjan. Uni.Med.Sci. Health Sci.*,15:21-27.
- 4-Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M. and Al-Rawahy, F. 2007. Compositional and functional characteristics of dates, syrups, and their by-products. *Food Chem.* 104(3):943–947.
- 5-Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M. and Shahidi F.2005. Compositional and sensory characteristics of three native sun dried date (Phoenix dactylifera L.) varieties grown in Oman. *J.Agric.Food Chem.*53:7586-7591.
- 6-Gu, L., Kelm, M.A., Hammerstone, J.F., Beecher, G., Holden, J., Haytowitz, D. and Prior, R.L.2003. Screening of foods containing proanthocyanidins and their structural characterization using LCMS/ MS and thiolytic degradation. *J.Agric. Food Chem.* 51(25):7513-7521.
- 7-Abdul Afiq, M.J., Abdul Rahman, R., Che Man, Y.B., AL-Kahtani, H.A. and Mansor, T.S. 2013. Date seed and date seed oil. *IFRJ.*20(5):2035-2043.
- 8-Bansal, B.K., Singh, K.B., Rohan, R., Joshi, D.V., Nauriyal, D.C. and Rajesh, M. 1995. *J. Res. Punjab Agri. Univ, (India)*:32:79-81.
- 9-Balla, B. A., Medinab, V., Gravancea, C. G. and Baumbera, J. 2001. *Theriogenology* .56(4):577-589.
- 10-Bansal, A.K. and Bilaspuri, G. 2009. *Anim Sci Pap and Rep*, 27(1):5-14.
- 11-Broucek, J. (2014). Methods of methane measurement in ruminants. *Slovak J. Anim. Sci.*, 47: 51-60.
- 12-Anassori, E. Naghadeh, B.D., Pirmohammadi, R., Taghizadeh, A., Rezaei, S., Azar, S.F., Besharati, M. and Tahmoozi, M. 2012. In Vitro assessment of the

- digestibility of forage based sheep diet, supplemented with raw garlic, garlic oil and Monensin. *Journal Veterinary Research Forum*, 3: 5 – 11.
- 13-Fraser, L. R., Umar, G. and Sayed, S.** 1993. Na⁺-requiring mechanisms modulate capacitation and acrosomal exocytosis in mouse spermatozoa. *j.rep.fertil.*97;539-549.
- 14-MAFF**, 1975. Ministry of Agriculture, Fisheries and Food. Energy Allowances and Feeding Systems for Ruminants. Technical Bull., 33, H.M.S.O., London.
- 15-Silvestre, M.A., Salvadore, I. and Sanchez, J.P.** 2004. Effect of changing female stimulus on intensive semen collection in young Murciano-Granadina male goats. *Journal of Animal Science*. 82.1641-1645.
- 16-El-Fouhil, A. F.** 2009. Hypoglycemic effect of an extract from date seeds on diabetic rats”. *Deanship of Scientific Research, King Saud University, Riyadh, Kingdom of Saudi Arabia*, (Research Project No.07-610).
- 17-Yashkochi, Y. and Masters, R.S.S.** .1976. Some properties of a detergent. Solubilized NADPA cytochromic (cytochrome P.450) reductase purified by biospecific affinity chromatography”. *J. Biol. Chem.*, 251: 5337-5344.
- 18-Giannopolitis, C.N. and Ries, S.K.**1977. Superoxide dismutases occurrence in higher plants”. *Plant Physiol.*, 59: 309-314.
- 19-Goth, L.** 1991. A simple method for determination of serum catalase activity and revision of reference range. *Clinical Chemistry Acta* **196**: 143–52.
- 20-Bahmanpour, S., Talaei, T., Vojdani, Z., Panjehshahin, M.R., Poostpasand, L.A. and Zareei, S.** 2006. Effect of *Phoenix Dactylifera* pollen on sperm parameters and reproductive system of adult male rats. *Ind. J. Med. Sci.* 31:208-212.
- 21-Zargari, A.** 1999. Medical Plants. *University of Tehran Press*, 3. .33-40.
- 22-Golshan, Tafti, A., Solaimani Dahdivan, N. and Yasini Ardakani, S.A.**2017. Physicochemical properties and applications of date seed and its oil. *International Food Research Journal* 24(4): 1399-1406.
- 23-Kumar, V., Mishra, D.P., Yadav, G.C. and Babu, U.** 2018. Studies on genetic component analysis and gene action for growth, yield and yield attributing traits of pumpkin. *International Journal of Chemical Studies*. 6(2): 2695-2699.
- 24-Sarlos, P., Molnar, A., Kokai, M., Gabor, G. Y. and Rátky, J.** 2002. Comparative evaluation of the effect of antioxidants in the conservation of ram semen. *Acta Veterinaria Hungarica* **50**: 235–45.

- 25-Luo, H. L., Jia, Z. H., Zhu, S. E. and Ding, J. Z. 2004. Effect of vitamin E on the qualities of fresh and frozen thawed ram semen. *China Herbivores* 24: 14–16.
- 26-Jeong, Y., Kim, M., Song, H., Kang, E., Ock, S., Kumar, B. M., Balasubramanian, S. and Rho, G. 2009. Effect of α -tocopherol supplementation during boar semen cryopreservation on sperm characteristics and expression of apoptosis related genes. *Cryobiology* 58: 181–89.
- 27-Chase, C. C., Arshami, J., Ruttle, J. L., Randel, R. D., Bastidas, P. and Long, C. R. 1990. Histological characteristics of testes from Brahman bulls fed diets containing gossypol. *Journal of Animal Science*. 68(Suppl): 14.
- 28-Peters, C.W., Laudert, S.B., Corah, L.R., Krehbiel, C.L. and Nichols, David A. 1994. Effect of monensin on weight gain, growth traits, and semen characteristics in yearling beef bulls," *Kansas Agricultural Experiment Station Research Reports*: Vol. 0: Iss. 1. <https://doi.org/10.4148/2378-5977.2077>
- 29-Farmer, P. K., Tyler, J. M. and Stachura, M. E. 1989. Monensin influences basal and human growth hormone-releasing hormone 44-induced release of stored and new rat growth hormone and prolactin. *Mot. Cell. Endocrinol.* 62: 253-262.
- 30-Kostyuk, V.A., Potapovich, A.I. and Strigunova, E.N. 2004. Experimental evidence that flavonoid metal complexes may act as mimics of superoxide dismutase. *Arch. Biochem. Biophys.* 428:204-208.
- 31-Nayernia, K., Li, M. and Jaroszynski, L. 2004. Stem cell based therapeutically approach of male infertility by teratocarcinoma derived germ cells. *Hum. Mol. Genet.* 13:1451-1460.
- 32-Arfat, Y., Mahmood, N., Ahmad, M., Tayyab, M., Zhao, F., Li, D., Zhihao, C., Yin, C., Shang, P. and Qian, A. 2014. Effect of date palm pollen on serum testosterone and intra-testicular environment in male albino rats. *Afr. J. Pharm. Pharmacol.* 8(31). 793-800.
- 33-Mahran, G.H., Abdel-Wahab, S.M. and Attia, A.M. 1976. A phyto-chemical study of date palm pollen. *Planta Med.* 29:171-175.
- 34-Al-orf, S.M., Ahmed, M.H.M., AL-Atwain., Al-Zaidi, H., Dehwah A. and Dehwah, S. 2012. Review: Nutritional Properties and Benefits of the Date Fruits (*Phoenixdactylifera* L.). *Bull. Nation. Nutri. Instit Arab Repub.Egypt.* 39, 97.

- 35-Saryono, S., Retnani, H. and Santoso, D.** 2015. Date Seeds Steeping (Phoenix Dactylifera) Strengthen Sperm Cells Membrane and Reduce Malondialdehyde Level. *Jurnal Ners* Vol. 10 No. 2 : 355–359.
- 36-Ross, C., Morriss, A., Khairy, M., Khalaf, Y., Braude, P., Coomarasamy A, et al.** 2010.A systematic review of the effect of oral antioxidants on male infertility. *Reprod Biomed Online*; 20: 711-723.