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IMPROVING REPRODUCTIVE BUCK RABBITS BY ADMINISTRATING CITRUS OIL DURING SUMMER CONDITION

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ABSTRACT

Dissimilar natural feed extracts have been used to conserve semen quality individualities of rabbit bucks at heat stress. This reading was carried out to examine the effect of administration orally of vegetable oils on the semen quality of male rabbits. Twenty four adult New Zealand White rabbit male 7-8 months old, with average initial body weight (BW) 3792.40±66.39 kg was erratically spread into four experimental groups (n=6). The control group administered with 1.0 mL of distilled water, the second group received orange oil (1.0 mL kg^{-1} b.wt., (OO)), the third group received lemon oil (1.0 mL kg^{-1} b.wt., (LO)) and the fourth group received 0.5 mL of OO and 0.5 mL of LO kg^{-1} b.wt., mixture. The rabbits were given oils orally once daily for 8 weeks under summer conditions (32.50 C^{o} and 76% relative humidity). The treatments with oils significantly improved semen quality compared to the control group. Conclusion: The oral administration of OO, LO and their mixture improved semen quality characteristics under summer conditions induced heat stress.

Key words: Orange oil, Lemon Oil, semen evaluation, rabbit bucks, motile sperm

INTRODUCTION

The citrus species are a potential source of variable oil which might be utilized for edible and other industrial application (Colecio-Juárez *et al.*, 2012).The 3 main components in citrus oil are D-Limonene, Pulegone and LCarvone (2-cyclohexen-1-one) for orange peel essential oil, D-limonene, 2-cyclohexen-1-ol and β-Pinene for lemon peel essential oil, and β-Pinene, D-limonene and α-Pinene for lime essential oil (Njoku and Evbuomwan, 2014). All of these substances can correct the reproduction of animals during periods of heat stress.

Actually, the good fertility in mammals depends on the respectable semen quality (Dalton, 2011) which affected by inappropriate environmental conditions, (Rasooli *et al.,* 2010). Heat stress is one of the most environmental with adverse effects on the reproductive functions of male rabbits Marai *et al.* (2008) throughout increasing levels of free radicals and disrupting the antioxidant-defense system (Ahmad *et al.,* 2012). Therefore, different natural feed additives have been used to maintain semen quality characteristics of rabbit bucks through the heat stress period (Hashem *et al.,* 2013). Vegetable oils such as; olive oil, rice bran oil, corn germ oil and wheat germ oil have been used as a source of plant antioxidants (Earlier and Ori-Jesu, 2008).

MATERIALS AND METHODS

Animals and experimental design

The experimental work was carried out at the Rabbit Research Laboratory belonging to The El-Nobaria research station, Institute of Animal Production, Dokki, Egypt. This study was carried out during the Egyptian summer (from 21 July-1 September, 2020). The daily mean temperature during the experimental period was 32.5 ºC and average relative humidity was 76%. According to the data obtained from the records of a nearby Meteorological Station, the experimental rabbits exhibited to heat stress. The study investigated the effects of orange, lemon oils and their mixture on semen

evaluation of New Zealand White rabbit bucks. Twenty four adults, fertile NZW rabbit bucks 7-8 months old, with average initial body weight 3792.40±66.39 kg was randomly distributed into 4 experimental groups (n = 6) as follows:
First group served as negative control administered 1.0 mL distilled water orally once daily (T1), second group received orange oil at level 1.0 mL kg^{-1} b.wt. (T2), third group received lemon oil at level 1.0 mL kg^{-1} b.wt. (T3) and forth group received a mixture of 0.5 mL of orange oil and 0.5 mL of lemon oil per kilogram BW (T4).Two local genotypes of oils were obtained from El Naser Factory for natural oil extract at Borg El Arab belong to the governorate of Alexandria, Egypt. Rabbits were given oils once daily via gavage (oral administration). The experimental treatments lasted for 10 consecutive weeks, including 2 weeks for adaptation and the later 8 weeks for semen evaluation and data collection. The basal ration was formulated and pelleted to meet the nutrient requirements of rabbits, according to NRC (1977). The rations were offered to rabbits ad libitum. Rabbits were offered free access to fresh water. All bucks were kept under similar managerial and environmental condition. Bucks were individually housed in galvanized wire cage batteries (50×50×40 cm) provided with feeders and automatic drinkers in a naturally ventilated and lighted Rabbitry.

Semen parameter
Semen collection and evaluation: Semen samples were weekly (weeks 3-10) collected during the experimental trial artificial vagina used to collect semen with exposing four mature females as a teaser handling and collection of semen carried out according to Boiti *et al.* (2005). Ejaculate volume was recorded using a graduated collection tube connected to the end of the artificial vagina after removal of the gel mass. After that, the ejaculate volume (mL) was measured. The pH value was determined by using pH paper (Merck KgaA, 64271 Darmstadt, Germany). The percentage of progressively motile sperm (percentage of forward motility) was immediately performed after semen collection in several microscopic fields for individual semen samples by visual examination under 100 magnifications using a light microscope with heated stage and subjectively assessed from 0-100%. Sperm concentration (10^6 mL^{-1}) was determined after semen dilution (1:100) using the improved Neubauer hemocytometer slide (GmbH and Co, Brandstwiete 4, 2000 Hamburg 11, Germany). Semen mass motility was given a score (0-3) according to Moule (1965). A dried smear of a drop of each semen ejaculates stained with an eosin-nigrosin blue staining mixture was prepared to assess the percentage of sperm viability (live or dead) and sperm abnormality by counting 200 sperm cells. The sperm cells were classified according to the staining pattern into complete or partial purple-stained sperm cells and non-stained sperm cells representing dead and live sperm cell, respectively. All visual semen parameters i.e., sperm progressive motility, sperm viability and sperm morphology were determined by the same trained technician according to Salisbury and Van Demark (1961). The previous measurements were used to calculate the Total Sperm Output (TSO, 10^6 /ejaculate) as a product of semen ejaculate volume (mL) by sperm concentration (10^6 mL^{-1}); total motile sperm (TMS, 10^6 /ejaculate) as a product of TSO by percentage of progressive motility and Total Functional Sperm Fraction (TFSF, 10^6 /ejaculate) as a product of TMS by percentage of normal sperm morphology.

Statistical analysis
Data were statistically analyzed using SPSS 11.0 statistical software (2007). Data were analyzed by using the one-way repeated measures Analysis of Variance (ANOVA). The statistical model included the fixed effect of treatment (control, ornage oil, lemon oil, also the random effect of an individual buck was considered. Differences among means were determined using Duncan (1955) test. Statistical significance was accepted at $p<0.05$.

RESULTS AND DISCUSSION

Table 1 showed that ejaculate volume of rabbit bucks were not increased due to administrating orally of citrus oil as compared to the control group. Semen hydrogen ion concentration (pH) showed a significant effect due to different sources of citrus oil treatments as compared to the control group, individual motility and sperm concentration (SC) of rabbit bucks showed significant increase in the treated groups compared with the control group. Alvariño

(2000) reported that in males, ejaculate volume and sperm motility were significantly decreased as results of exposure to summer heat stress.Also, exposure of sexually fertile Rex rabbits to high THI adversely affected sexual desire, sperm density, testicular cytoarchitecture, and apoptosis in seminiferous tubules (Pei *et al.*, 2012). Additionally, improper environmental conditions lead to reduction in quality and fertility of sperm cells, so HS negatively influences the testicular function (Chen *et al.*, 2015).

In comparison with the control group, administrating orally of orange or lemon oil and their mixture significantly increased the total sperm output and total motile sperm, also the total functional sperm fraction was significantly increased due to different kinds of citrus oil. The best values for total sperm output, total motile sperm and total functional sperm fraction were recorded in the groups of bucks administrated mixture of orange and lemon oil as compared to the control or the other experimental groups. Khaki *et al*. (2010) reported that administration of 400 and 600 mg citrus extract (as a natural source of flavonoids) for thirty consecutive days significantly increased sperm motility *vs* the control group in rats.

A significant increase in normal sperm percentage was observed in all citrus oil as compared with the control group. The same results were observed when the male rabbits supplemented with thyme essential oil caused increased in the sperm viability, sperm motility, and ejaculate volume compared with control at the end of treatments (Abdel-Wareth and Metwally 2020).
Administrating citrus oil to bucks did not affect dead sperm and fructose level.

Table 1. Effect of citrus pulp on bucks semen quality parameters of New Zealand White rabbits.

Treatments	Control	Orange oil	Lemon oil	Mixture	SEM	P value
Ejaculate volume (ml)	0.66	0.74	0.73	0.72	0.10	NS
Hydrogen ion, pH	6.38^{b}	7.79^{a}	7.95^{a}	7.89^{a}	0.03	0.05
Individual motility (%)	71.37^{b}	81.88^{a}	80.91^{a}	80.76^{a}	1.43	0.03
SC ($\times10^{6}$/ml)	208.3^{b}	307.5^{a}	300.4^{a}	326.3^{a}	1.25	0.02
TSO ($\times10^{6}$ ml)	137.48^{d}	171.22^{c}	189.8^{b}	219.29^{a}	0.89	0.005
TMS ($\times10^{6}$ ml)	98.12^{d}	139.52^{c}	152.1^{b}	177.4^{a}	5.66	0.001
TFSF ($\times10^{6}$ ml)	63.17^{c}	103.17^{b}	106.79^{b}	138.02^{a}	5.26	0.0001
Normal sperm (%)	64.38^{c}	73.94^{b}	70.21^{b}	77.79^{a}	1.02	0.03
Dead sperms (%)	16.63	14.33	11.04	11.88	0.15	NS
Initial fructose mg/ 100 ml	249.4	224.5	212.5	276.2	0.2	NS

Values (mean± S.E.) in the same row having the same superscripts are significantly different (P<0.05). SC= Sperm concentration; TSO= Total sperm output;TMS= Total motile sperm;TFSF= Total fraction sperm function.

The increase in sperm motility of the experimental groups in comparison to control group could be due to the protective effect of citrus extract administration. EL Hefnawy *et al.* (2020) suggested that supplementing D-limonene daily to rats, at the old aged ones could protect the sexual organs from aging oxidative stress inducing deterioration of its physiological functions.

CONCLUSION

The oral administration of OO, LO and their mixture improved semen parameters under heat stress conditions. The best values of semen parameters were recorded in the group of bucks received Mixture oil of OO and LO as compared to the control or the other experimental groups.

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