



Enhancing the Reproductive Performance of Male Rabbits by Supplementing their Diet with Tangerine Peel Extract During the Summer Months

Mohamed Basyony Mohamed Mahmoud

Poultry Nutrition Research Department, Animal Production Research Institute, Egypt

Abstract

Various natural feed extracts were tested to preserve semen quality in male rabbits under heat stress conditions. This study investigated the impact of orally administering tangerine waste extract (TWE) on the semen quality of male rabbits. Twenty-four male California rabbits, aged 7-8 months and weighing an average of 3579.40±61.39 kg, were divided into four experimental groups with six rabbits in each group. The control group was administered 3.0 mL of distilled water, the second group received 2.0 mL/kg of body weight of TWE, the third group received 4.0 mL/kg of body weight of TWE, and the fourth group received 6.0 mL/kg of body weight of TWE. The rabbits were given the extract orally once daily for 8 weeks under summer conditions (32.50°C and 76% relative humidity). The administration of TWE significantly enhanced semen quality compared to the control group. In conclusion, oral TWE supplementation improved semen quality characteristics in the presence of heat stress during summer conditions.

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

Introduction

The citrus species are a valuable source of versatile oil that can be used for both edible and industrial purposes (Maria et al., 2012). The main components found in citrus oil include D-Limonene, Pulegone, and L-Carvone 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 Ebuomwan, 2014). These components have been shown to improve animal reproduction during periods of heat stress.

The fertility of mammals relies on the quality of semen (Dalton, 2011), which can be affected by unfavorable environmental conditions (Rasooli et al., 2010). Heat stress is a significant environmental factor that can have negative effects on the reproductive functions of male rabbits, leading to increased levels of free radicals and disruption of the antioxidant-defense system (Marai et al., 2008; Ahmad et al., 2012). To maintain the semen quality of rabbit bucks during heat stress, various natural feed additives, such as vegetable oils like olive oil, rice bran oil, corn germ oil, and wheat

germ oil, have been used as a source of plant antioxidants (Eleroglu and Ori-Jesu, 2008; Hashem et al., 2013).

Materials and Methods

The experimental research was conducted at the Rabbit Research Laboratory located at The El-Nobaria research station, Institute of Animal Production, Dokki, Egypt. The study took place during the Egyptian summer from July 21 to September 1, 2023. Throughout the experimental period, the daily average temperature was 32.50 degrees Celsius, and the average relative humidity was 76%. Based on data from a nearby Meteorological Station, the experimental rabbits experienced heat stress. The study examined how tangerine waste extract affects the semen evaluation of California rabbit bucks. Twenty-four adult, fertile NZW rabbit bucks aged 7-8 months, with an average initial body weight of 3579.40±61.39 kg, were randomly divided into 4 experimental groups (n = 6) as follows:

The first group served as the negative control and was given 3.0 mL of distilled water orally twice daily (T1). The second group received tangerine waste extract at a dosage of 2.0 mL per kg of body weight (T2). The third group received tangerine waste extract at a dosage of 4.0 mL per kg of body weight (T3). The fourth group received 6.0 mL of tangerine waste extract per kilogram of body weight (T4). These doses were divided into two daily doses, one in the morning and one in the afternoon. Two local oil genotypes were sourced from El Naser Factory for natural oil extract in Borg El Arab, Alexandria, Egypt. The oils were administered to rabbits twice daily via gavage. The experimental period lasted for 10 weeks, with 2 weeks for adaptation and the remaining 8 weeks for semen evaluation and data collection. The basal diet was prepared and pelleted to meet the nutritional needs of rabbits, as outlined in NRC (1977). The diets were provided to the rabbits freely. Fresh water was available to the rabbits at all times. All male rabbits were kept in the same management and environmental conditions. Each buck was housed individually in galvanized wire cage batteries (50×50×40 cm) equipped with feeders and automatic drinkers in a Rabbitry that was naturally ventilated and lit. Semen collection and evaluation: Semen samples were collected weekly (weeks 3-10) during the experimental trial using an artificial vagina. Four

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***Corresponding author:** Mohamed Basyony Mohamed Mahmoud, Poultry Nutrition Research Department, Animal Production Research Institute, 12618, Giza, Egypt

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mature females were exposed as teasers, and semen collection was carried out following the method described by Boiti et al (2005). The ejaculate volume was measured using a graduated collection tube attached to the artificial vagina after removing the gel mass. The pH value was determined using pH paper from Merck KgaA, 64271 Darmstadt, Germany. The forward motility percentage of sperm was assessed immediately after semen collection by visually examining several microscopic fields in individual samples under 100x magnification using a light microscope with a heated stage. The motility was subjectively assessed on a scale of 0-100%. Sperm concentration was determined after diluting the semen 1:100 and using the improved Neubauer hemocytometer slide. Semen mass motility was scored on a scale of 0-3 following the method described by Moule (1965). A dried smear of a drop of semen stained with an eosin-nigrosin blue staining mixture was prepared to assess sperm viability and abnormality by counting 200 sperm cells. The sperm cells were classified as complete or partial purple-stained (dead) and non-stained (live). Packed sperm volume was measured using Micro-AID® microhematocrit tubes and centrifuged for 5 min at 4000 rpm. Sperm progressive motility, viability, and morphology were determined by a trained technician following Salisbury and Van Demark (1961). Total Sperm Output (TSO), Total Motile Sperm (TMS), and Total Functional Sperm Fraction (TFSF) were calculated based on these measurements. Statistical analysis: The data were analyzed using SPSS 11.0 statistical software (2007) through one-way repeated measures Analysis of Variance (ANOVA). The statistical model included the fixed effect of treatments, as well as the random effect of an individual buck. Differences among means were assessed using the Duncan (1955) test, with statistical significance set at $p < 0.05$.

Results and Discussion

Table 1 indicated that the average ejaculate volume of rabbit bucks showed a numerical increase when orally administered tangerine waste extract compared to the control group (T1). The pH of semen showed a significant effect ($P=0.05$) due to different doses of TWE treatments compared to the control group (T1). There was a significant increase in individual motility and sperm concentration (SC) of rabbit bucks in the treated groups compared to the control group ($P=0.03$ and 0.02). Alvariano (2000) found that exposure to summer heat stress led to a significant decrease in ejaculate volume and sperm motility in males. High THI exposure in sexually fertile Rex rabbits negatively affected sexual desire, sperm density, testicular cytoarchitecture, and apoptosis in seminiferous tubules (Pei et al., 2012). Improper environmental conditions can reduce the quality and fertility of sperm cells, with heat stress negatively impacting testicular function (Chen et al., 2015).

In comparison with the control group, administrating orally of orange or lemon oil and their mixture significantly ($P=0.005$ and 0.001) increased the total sperm output and total motile sperm, also the total functional sperm fraction was significantly ($P=0.0001$) increased due to different doses of TWE. The groups of bucks that received 6.0 mL of tangerine waste extract per kilogram of body weight (T4) showed the highest values for total sperm output, total motile sperm, and total functional sperm fraction compared to the control group and other experimental groups. Khaki et al. (2010) found that giving rats 400 and 600 mg of citrus extract (a natural source of flavonoids) daily for thirty days significantly improved sperm motility compared to the control group. Administering tangerine waste extract to male deer resulted in a numerical decrease in dead sperm percentage compared to the control group. Initial fructose levels were not significantly affected by the different treatments, but there was a numerical increase in initial fructose in the T4 group compared to the control group.

A statistically significant increase ($P=0.03$) in normal sperm percentage was noted in all treatment groups compared to the control group. Similar findings were seen in male rabbits supplemented with thyme essential oil, which led to improvements in sperm livability, motility, and ejaculate volume compared to the control group by the end

of the treatment period (Abdel-Wareth and Metwally 2020). The higher sperm motility observed in the experimental groups compared to the control group may be attributed to the protective impact of tangerine waste extract administration. EL Hefnawy et al (2020) proposed that daily supplementation of D-limonene to older rats could help safeguard their sexual organs from the effects of aging-induced oxidative stress, which can lead to a decline in physiological functions.

Table 1: Effect of citrus pulp on bucks semen quality parameters of California rabbits.

Treatments	T1	T2	T3	T4	SEM	P value
Ejaculate volume (ml)	0.69	0.72	0.73	0.74	0.10	NS
Hydrogen ion, pH	6.18 ^b	7.79 ^a	7.87 ^a	7.89 ^a	0.03	0.05
Individual motility (%)	70.37 ^b	81.88 ^a	80.91 ^a	81.76 ^a	1.43	0.03
SC ($\times 10^6$ /ml)	208.3 ^b	307.5 ^a	300.4 ^a	326.3 ^a	1.25	0.02
TSO ($\times 10^6$ ml)	136.48 ^d	171.23 ^c	189.84 ^b	219.39 ^a	0.89	0.005
TMS ($\times 10^6$ ml)	98.11 ^d	139.92 ^c	153.1 ^b	177.4 ^a	5.66	0.001
TFSF ($\times 10^6$ ml)	63.15 ^c	104.17 ^b	106.78 ^b	138.12 ^a	5.26	0.0001
Normal sperm (%)	64.38 ^c	73.95 ^b	70.21 ^b	77.78 ^a	1.02	0.03
Dead sperms (%)	16.64	14.40	11.14	11.78	0.15	NS
Initial fructose mg/ 100 ml	249.5	224.15	212.45	276.14	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.

Conclusion

The semen evaluation parameters were enhanced when tangerine waste extract was orally administered to male rabbits under heat stress conditions. The group of bucks that received 6.0 ml/kg of body weight of tangerine waste extract showed the highest semen evaluation values compared to the control group and other experimental groups.

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