Assessment of semen characteristics of rabbit bucks administered hydro-alcoholic extract of *Phoenix dactylifera* fruit

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Abstract

The semen characteristics of 24 rabbit bucks of age 28-32 weeks weighing between 1.0-1.5kg was assessed after the administration of hydroalcoholic extract of phoenix dactylifera (date palm) fruit. Phoenix dactylifera hydroalcoholic extract (HAEPD) was administered to four treatment groups at varied levels having T1 (no extract), T2 (300mg), T3 (600mg) and T4 (900mg) in a completely randomized design. The following semen parameters were assessed in the bucks after the administration of HAEDP for 56 days (i.e volume (mL), massal motility (%), individual motility (%), viability (%), morphology (%), libido (sec), liquefaction (min) and semen pH). The results obtained showed that semen volume (mL) was highest at 600mg dose of the extract (77mL) being significantly ($P<0.05$) higher than other treatment groups between which were not different from each other. No significant differences ($p>0.05$) were observed across treatment groups for mass motility (%). Individual motility of T3 (600mg) and T4 (900mg) were significantly ($P<0.05$) higher than T1(0mg) and T2(300mg). Sperm viability (%) and concentration ($\times 10^6/\text{ml}$) were higher in treatment groups receiving date palm extract compared to the control. There was no significant difference ($p>0.05$) in morphology (%) and liquefaction (min). reaction time (sec) was lower in T3 and T4 compared to control and T2. PH ranged between 6.10-6.33 (slightly acidic) across treatment groups. In conclusion, date palm extract positively influenced sperm volume and sperm concentration in rabbit bucks.

Keywords: Rabbit buck, date palm, semen characteristics

Introduction

Rabbits have been shown to possess potentials to bridge the gap in the supply of animal protein to humans, especially in the developing and underdeveloped world. They are efficient converter of feed to meat and can utilize up to 30% crude fibre as against 10% by most poultry species Egbo *et al.* (2001).

Male breeder rabbits have enormous potential with regard to stock population and sustainable reproductive capacity in livestock production. For an animal to successfully reproduce, puberty must be attained, leading to sexual maturity which eventually serves as a gateway to procreation of the animal's species

(Osinowo, 2006). Spermatogenesis commences at puberty, approximately 15-18 weeks of age, with subsequent sexual maturity attained by 20-24 weeks of age, depending on breed, body size and nutritional status of the animal. Male rabbits can breed throughout the year in tropical and subtropical environments, even though its reproductive capacity may decline during hot periods. This is unlike seasonal breeders in which reproductive capacity is affected by changes in day length (Marai *et al.*, 2007). The importance of the male rabbits is well recognized, even at small scale subsistence production in the sub Saharan region of Africa, where exchange of male rabbits for breeding has

been observed in order to exploit their genetic potential (Abu *et al.*, 2008). The ejaculate volume of an adult buck ranges from 0.5 to 1.5mL, with a sperm concentration ranging between 100-300 x 10⁶/mL. However, several authors have implicated a number of genetic, management and physiological factors affecting male fertility (Nwoko and Ibe, 2005; Garcia *et al.*, 2008). In addition, other studies revealed a reduction in male fertility in hot tropical and subtropical climates which subsequently led to impairment of production and reproduction (Marai *et al.*, 2008). Research has indicated the importance of nutritional modifications of male rabbit diets in order to alleviate the negative effects of temperature, with respect to seminal characteristics (Herbert *et al.*, 2005), sperm production rate and testicular sperm reserves (Bitto and Olokpo, 2005). The adoption of good husbandry practices that could lead to improvements in male reproductive potential is of paramount importance in order to ensure all services result in conception for sustainable production.

Some plant-derived chemicals are used to relieve sexual dysfunction and they have sex enhancing potentials. These phytochemicals increase libido, sexual potency and sexual pleasure as some of them have been found to interfere with synthesis and stimulation of reproductive hormones. Modern scientific studies in experimental animals have confirmed the effect of some of these herbs on the reproductive system without producing apparent toxic effects (Prakash *et al.*, 1974). The influence of date fruits on reproductive health and their performance enhancement attributes has been reported by Lesile (2003) to be due to the following phytochemicals; alkaloids, lignins, flavonoids, lipids, benzenoids, steroids,

alkanes, tannin and saponins. On the aerial parts, some crystalline lignins including phyllantive and phypopyllanthine revealed wonderful overall increased reproductive performance in human and animals (Lesile, 2003). In the light of the foregoing, this study seeks to evaluate the potential of *Phoenix dactylifera* fruit extracts in enhancing the quality of rabbit semen for increased reproductive efficiency.

Materials and methods

Management

The experiment was conducted using 24 composite rabbit bucks of age 28-32 weeks weighing between 1.0-1.5kg were allocated randomly across treatment groups. Water and feed were served *ad libitum* throughout the period of the experiment. The rabbits were also fed with mixture of guinea grasses and legumes (*Panicum maximum* and *Centrosema molle*). Medications like anti stress/ Multivitamin, Embazin Forte (Coccidiostat), Neocryl Plus (Antibiotics) were administered during the acclimatization period of two weeks. Routine management practices of daily cleaning of the rabbit unit, and washing of the drinkers were strictly observed.

Preparation of fruit extracts

Phoenix dactylifera fruits were sourced from Benin City, Edo State. The acquired fruits were split, air-dried, and finely ground and stored in an air tight container. The ground *phoenix dactylifera* fruits were weighed and extraction was carried out with 70% methanol in a soxhlet apparatus. The extract obtained was concentrated using a water bath and the concentrated extract was preserved in an air tight bottle.

Experimental design

Treatment 1 (0mg extract) which were not administered the date- fruit extract; Treatment 2 (300mg of the date fruit extract were administered); Treatment 3

(600mg of the date fruit extract were administered) and Treatment 4 (900mg of the date fruit extract were administered). Date palm hydroalcoholic extract (HAEPD) was administered for 56 days at 24 hours interval. The twenty-four rabbit bucks were assigned to four treatment groups consisting of 6 bucks per treatment (having 3 replicates of 2 bucks per replicate) in a completely randomized design.

Data collection and evaluation

Semen collection

Two weeks prior to semen collection, the rabbit bucks were trained to serve an Artificial vagina (AV) using a teaser rabbit doe. On the 57th day following the administration of the experimental diets, the rabbit buck under study was placed on a semen collection schedule of three times per week. The rabbit does were taken to the buck's cage and the doe was held in position for service. When the male attempts to mount, the AV was strategically placed below the belly of the doe in such a way that the penis of the males was introduced into the AV. The temperature of the inner rubber sleeve of the AV was adjusted to 40-42°C at the time of semen collection. Lubrication of the inner sleeve was performed using glycerin.

Estimation of semen characteristics

Semen volume was estimated directly from the AV by use of a graduated measuring cylinder inserted into the AV.

The pH was evaluated by the use of a pH meter.

Liquefaction was estimated by observing the time (minutes) which elapsed between the times of ejaculation to the collection vessel and when semen became homogenous (becomes thinner and quite watery with only small areas of coagulation remaining).

Progressive sperm motility percentage score was assessed according to the procedure outlined by Arrebola and Fernandez (2011). At least, six widely spaced fields were examined to provide an estimate of the percentage of the progressively motile sperm cells.

Percentage of morphologically normal sperm cells was estimated by the method previously described by Darszon *et al.* (1999). The principle is based on the ability morphologically normal sperm cells to appear white in color as the plasma membrane will prevent the dye to enter, while abnormal sperm cells take up the dye and stain dark color and observing the microscope at 100X objective, the cells were examined. At least five fields will be viewed covering the whole slide. Example of morphological abnormalities that were considered will include; double-headed, elongated head, pyriform head, bent head, bent tail, bent mid-piece, coiled tail, double tail, headless, tailless etc.

Sperm cell concentration ($\times 10^6$ mL) was determined using improved Neubauer counting chamber (haematocytometer) in a dilution of 1:100 in a solution of 45ml normal saline and 5ml formalin. Total sperm ($\times 10^6$) was determined by multiplying the semen ejaculate volume by the sperm cell concentration.

Libido was estimated by observing the reaction time (seconds) which elapsed between exposure of a buck to a doe and the first copulation (serving the AV).

Statistical analysis

The data generated from the test procedures was subjected to statistical analysis of variance (ANOVA) procedure of Genstat 12th edition at 5% probability level. Occurrence of significant means was separated using Duncan Multiple Range Test (DMRT) of the same statistical

software (Duncan, 1955).

administered hydro-alcoholic extract of *Phoenix dactylifera* fruit are shown in Table 1

Results

The Semen characteristics of rabbit bucks

Table 1 : Semen characteristics of rabbit bucks administered hydro -alcoholic extract of *Phoenix dactylifera* (HAEPD) fruit

Parameters	TREATMENTS (HAEPD)				SEM
	0mg	300mg	600mg	900mg	
Volume (mL)	0.13 ^b	0.27 ^b	0.77 ^a	0.27 ^b	0.05
Massal motility (%)	2.00	2.00	3.00	3.00	0.00
Individual motility (%)	66.00 ^b	70.00 ^b	76.67 ^a	77.00 ^a	1.64
Viability (%)	69.33 ^d	74.67 ^c	80.33 ^b	88.33 ^a	1.48
Morphology (%)	71.33 ^a	70.33 ^a	73.67 ^a	75.00 ^a	2.10
Libido (sec)	14.02 ^a	14.04 ^a	11.35 ^b	10.78 ^b	0.52
Liquefaction (min)	13.00 ^a	13.33 ^a	14.67 ^a	13.00 ^a	0.69
pH	6.17 ^b	6.33 ^a	6.17 ^b	6.10 ^b	0.04
Conc.($\times 10^6$ /mL)	195.30 ^d	219.00 ^c	405.30 ^b	490.10 ^a	6.54

^{a, b, c, d} Means bearing different superscripts within the same row differ significantly ($P < 0.05$)

The semen volume (mL) was highest at 600mg dose of the extract (0.77mL) being significantly ($P < 0.05$) higher than other treatment groups between which were not different from each other. No significant differences ($p > 0.05$) were observed across treatment groups for mass motility (%). Individual motility of T3 (600mg) and T4 (900mg) were significantly ($P < 0.05$) higher than T1 (0mg) and T2 (300mg).

Sperm viability (%) and concentration ($\times 10^6$ /ml) were higher in treatment groups receiving date palm extract compared to the control. There was no significant difference ($p > 0.05$) in morphology (%) and liquefaction (min) across treatment groups. Reaction time (sec) was lower in T3 and T4 compared to control and T2. PH ranged between 6.10-6.33 (slightly acidic) across treatment groups.

Stained slides of semen from experimental rabbit bucks

Figure A (control-0mg of extract)

Figure B (300mg of extract)

Figure A has a mean concentration of 1.95×10^6 /mL while Figure B has a mean concentration value of 219.0×10^6 /mL (Table 1),

Figure C (600mg of extract)

Figure D (900mg of extract)

Figure C has a mean concentration value of $405.3.0 \times 10^6/\text{mL}$ while Figure D had the highest concentration as shown in Table 1 with a mean concentration value of $490.1.0 \times 10^6/\text{mL}$.

Discussion

Date extracts have been shown to improve sperm counts in Guinea pigs, enhance spermatogenesis and increase the concentration of testosterone, estrogen, follicle stimulating hormone and Luteinizing hormone in rats Wafaa *et al.*, (2012). Aqueous extracts of *Phoenix dactylifera* was found to enhance testosterone, estradiol and the orientation of males towards female rats at a maximum dose of 140mg/kg (Abedi *et al.*, 2012). Date palm or *Phoenix dactylifera* belongs to the family of palmae which is a plant native to North Africa and has also been cultivated in Arabia and Persian Gulf. Date palm pollen has been used extensively in traditional medicine for treating male infertility (Mehraban, *et al.*, 2014). This is because date palm contains estradiol and flavonoid components that could potentially have positive effects on the sperm quality. Estradiol has been reported to improve sperm concentration and libido in pre-pubertal rabbit bucks (Uwaeziozi, 2017). The result obtained from this study shows that the administration of HAEPD up to levels of 600mg and 900mg significantly increased the individual motility of rabbit

sperm cells from 66.00% in the control group to 76.67% in those receiving 600mg and 77.00% in those receiving 900mg. Spermatozoa viability was significantly increased from 69.33% in the control group to 88.33% in T4(900mg). There was also a significant improvement in libido (reaction time) from 14.02sec in the control group and 14.04sec in T2 (300mg) to 10.78sec in those receiving 900mg. The administration of HAEPD to levels of 900mg also increased semen concentration from $1.95 \times 10^6/\text{mL}$ in the control group to $490.1 \times 10^6/\text{mL}$ in T4 (900mg). These findings agreed with Bahmanpour *et al.*, (2006), who reported that there were improvement in sperm parameters such as motility, concentration and morphology of Sprague-Dawley rats fed on *Phoenix dactylifera* especially with levels of 120mg/kg body weight and also it agreed with Sadiq and Bawazir, (2010) who observed that there were positive improvements in sperm parameters as well as in testicular morphology after examining the effects of date fruit extracts on testicular dysfunction induced by Cadmium (Cd) which has gonado-toxic and spermato-toxic potentials.

Conclusion

The study showed that the administration of Hydro-alcoholic extracts of *Phoenix dactylifera* fruits to levels of 600mg and 900mg in rabbit bucks with an average weight of 1.2 kg improved semen (motility, concentration, viability and semen volume per ejaculate) as well as the libido of rabbit bucks.

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