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Haemogram and hormonal profile of WAD bucks treated with leaf ethanolic extract of *Spondias mombin*

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Abstract

Twelve post pubertal West African Dwarf (WAD) bucks of average age of 1.8 ± 0.19 years and average weight of 8.76 ± 0.72 kg were experimentally treated orally with 800mg/kgBW of ethanol extract of *Spondias mombin*. Experiment was carried out in the month of August at Abeokuta, South West Nigeria with temperature range of 24^{o}C and 33^{o}C on Latitude 07^{o} 10'N and Longitude 03^{o} 2'E. The goats had well-formed pendulous scrotum with well descended free bilateral testicles. The goats were kept in a standard goat pen and fed with fresh green centrosema leaves in the morning and commercially prepared ration in the evening. Water was served *ad libitum*. Pre-treatment haematological and hormonal profiling after aseptical collection of blood from the bucks was followed with 14 days of oral treatment with ethanol extract of *Spondias mombin* at the dosage rate of 800mg/kg BW. Post-treatment haematological and hormonal studies followed after. The hormones assayed were follicle stimulating hormone (FSH), luteinising hormone (LH), testosterone, progesterone, estrogen and prolactin. The haemogram revealed significantly ($p < 0.05$) higher PCV (24.33±0.6 %) in pre-treatment compared to 19.92± 1.1 % recorded for post-treatment. However, both were lower in values than normal range. WBC and RBC did not change significantly but while RBC values for pre-treatment and post-treatment fell within normal range, only the WBC value of post-treatment did. Assay of FSH, progesterone, estrogen and prolactin revealed no significant (p . 0.05) difference between pre-treatment and post-treatment; however, noticeable higher pre-treatment LH concentration and elevated post-treatment testosterone concentration were observed. The work showed that *Spondias mombin* at 800mg/kgBW has profertility tendencies in the buck and can be considered as an aphrodisiac in goat breeding in developing countries probably at a higher dosage.

Keywords: Buck, Ethanol, Extract, Leaf, *Spondias mombin*
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Introduction

The importance of goat in the livestock economy of people living in subsistence agriculture in the humid zone of West Africa has made them the species of choice for peasant farmers and for the supply of animal protein and other products in the sub-region. FAO (1991) estimated the goat population of Africa in 1990 to be 171 million, out of which 22 million were found in Nigeria. The 2011 National Agricultural Sample Survey indicated that Nigeria was endowed with an estimated 72.5 million goats (Premium Times, 2016). In Nigeria, the most popular genotype, i.e. the West African Dwarf (WAD) goat which supplies excellent quality meat, milk, skin and other products, has been adjudged as one of the

most prolific in the world, with a remarkably high reproductive potential (Wilson, 1989; Gall *et al*., 1992). The improvement of the tropical breeds of goats generally and the WAD goat in particular for higher productivity has been successfully done (Leboeuf *et al*., 1998). Specifically, studies on semen characteristics and hormonal profiles of adult and pubertal WAD bucks have been done by some authors (Akusu *et al*., 1984; Ugwu & Orji, 1984; Bitto *et al*., 2000). Also, haematology, testosterone concentation, testicular morphometry and biochemical characteristics of spermatozoa and seminal plasma have been evaluated under different conditions (Bitto *et al.,* 2000; Bitto & Egbunike, 2006, 2007 and 2012).The WAD goat, however, still needs to be largely improved. The use of medicinal plants, such as *Spondias mombin* (a fructiferous plant found in the tropics), has been advocated in order to achieve higher productivity in livestock (Mussarat *et al.,* 2014). Phytochemical studies on *Spondias mombin* revealed that the leaves contain saponins, tannins, alkaloids, flavonoids, phytates, oxalates and anthraquinones and the therapeutic effects of leaf extracts of the plant on reproduction in female animals have been linked to its constituents (Offiah, & Anyanwu, 1989; Njoku & Akumefula, 2007; Taylor, 2012). Oloye *et al*. (2011) and Oloye *et al.* (2012) established a fertility enhancement property by the plant's leaf aqueous extract administered at the dosage rates of 600mg/kg and 800mg/kg body weight in male wistar rats. The fertility rating was based on semen characteristics evaluation and testicular morphometry and histology. This work was aimed at studying the effect of leaf ethanol extract of *Spondias mombin* on haemogram and major reproductive hormones of post pubertal bucks.

Materials and Methods

Plant collection and extract preparation

Plant leaves were collected within the premises of the Federal University of Agriculture Abeokuta and authenticated at the National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan, Oyo state, Nigeria and identified with herbarium number 473 on number 121 of Flora of West Tropical Africa families and numbers. The sum of 4.5kg of the powdered leaves was soaked in hexane to reduce the fat. Air dried residue was treated with ethanol for 3 days. Resultant filtrate was then concentrated with the use of rota-evaporator. Dark brownish paste recovered was kept in fume hood to solidify for five days at 25°C. A yield of 68g of dried extract was obtained from which a stock solution of 8000mg of extract in 1ml of propylene glycol was constituted.

Dose was calculated using the formula:

$$\text{Dose} = \text{Weight of animal(mg) x Dosage (mg/kg)} / \text{Concentration(mg/ml)}$$

Experimental animals

Twelve postpubertal West African Dwarf bucks of average age of 1.83±0.18 years and average weight of 8.76± 0.7 kg were used. Weighing was done by balancing the goats in a sac and suspending on a spring balance for measurement. Age estimation was through dentition (NARI, 2003). Experiment was done in the month of August at College of Veterinary Medicine Federal University of Agriculture Abeokuta with temperature range of 24^{o}C and 33^{o}C on Latitude 07^{o}10'N and Longitude 03^{o} 2'E. South West Nigeria. All goats had well-formed pendulous scrotum containing bilaterally well descended free testicles. The goats were kept in a standard goat pen and fed with fresh green centrosema leaves in the morning and commercially prepared ration in the evening *ad libitum*. Water was served *ad libitum*. They were given albendazole tablets at 10mg/kg *per os* once and 20% oxytetracycline injection given once intramuscularly at 10mg/kg for prophylactic antihelminthic and anti-protozoan treatment respectively during two weeks of quarantine.

Leaf extract administration

After two weeks acclimatisation at average ambient temperature of 27^{o}C, blood was collected for haematological studies and hormonal concentration evaluation. After, animals were treated orally with ethanol extract of *Spondias mombin* at the dosage rate of 800mg/kg BW for 14 days after which hematology and hormonal concentration evaluation were repeated.

Haematology

Blood was collected through the Jugular vein of the animals into heparinized tubes. They samples were analysis for Packed cell volume (PCV) and White blood cell (WBC) following the methods described by Schalm *et al*. (2010).

Reproductive hormonal profiling

Testosterone, follicle stimulating hormone and luteinizing hormone were profiled using serum that was collected before and after 14 days treatment with 800mg/kgBw of ethanol extract of *Spondias*

mombin. Blood was collected from the Jugular vein of the animals into non-heparinized tubes.

Serum from the blood was refrigerated at 5^{o}C. Using appropriate hormonal kit, and procedures prescribed, the sera were assayed for Testosterone (LOT numberRN42852, TECO diagnostics, USA), FSH (BXE0631A), LH (Kit number BXE0651A), Estradiol (BXEO86OA); Prolactin (BXEO671A); Progesterone (BXEO661A).

Statistical analysis

Pre-treatment and post-treatment data was presented as mean ± standard deviation (SD). Means were compared using Student's *t* test and mean differences were considered significant at p≤0.05. Statistical package used was Graphpad prism (version 6.0).

Results

The haemogram revealed significantly higher PCV from pre-treatment to post-treatment (p<0.05). While pre-treatment PCV fell within values considered normal, post-treatment PCV did not (Table 1). WBC and RBC did not change significantly (p>0.05) but while RBC values for pre-treatment and post-treatment fell within normal range, post-treatment WBC slightly fell short and pre-treatment was quite lower than normal (Table 1). Comparing pre-treatment steroidal hormonal concentrations with post-treatment concentrations, no significant difference was observed in all (Table 2). However, it may be worth pointing out that at p value of 0.056 and 0.27 there were elevated post-treatment testosterone (Table 2) and reduced LH concentrations respectively.

Discussion

The mean packed cell volume (PCV) of the bucks before treatment fell within the 22-38% normal range for adult bucks (Goat-Link, 2009) while post-treatment PCV fell short of the range. Both were lower than the recordings of 28.9 ± 2.1% for WAD adult bucks and 25.6 ± 0.5%for kid bucks by Opara *et al*. (2010). Both pre-treatment and post-treatment WBC values fell short of the normal 4.0-13.0x10^{3} range but RBC for both were within 8.0-18.0 x10^{6} normal range (Goats-Link, 2009). The significant decrease in PCV from pre-treatment to post-treatment is at variance with the finding of Eniolorunda *et al*. (2006) who opined that *Spondias mombin* leaves fed to growing bucks for 63 days had no significant effect on PCV but their work was in agreement with the non-significant effect on WBC recorded in this work. At variance with this work, RBC was reported by Eniolorunda *et al*. (2006) to be significantly affected by treatment with *Spondias mombin* in the growing buck. Also, working with lactating West African Dwarf ewes, Oguike & Udeh (2008) reported that *Spondias mombin* did not significantly influence PCV and WBC values.

Reduction in PCV observed in this work might be the direct effect of the alkaloid constituent of the plant's leaves as opined by Ajibade & Egbebi (2011). The

Table 1: Mean ± SD values of haemogram before and after treatment of post pubertal bucks with 800mg/kg leaf ethanol extract of *Spondias mombin*

	Pre-treatment	Post-treatment	Normal value (*)
PCV (%)	24.33 ± 2.19^{a}	19.92 ± 3.70^{b}	22.0 - 38.0
RBC (x10^{6})	10.79 ± 2.56	11.6 ± 3.50	8.0 - 18.0
WBC (x10^{6})	2.90 ± 0.69	3.73 ± 2.12	4.0 - 13.0

Values with different superscripts within row differ significantly (p<0.05)

* Source: Goat-Link, 2009

Table 2: Mean (±SD) values of hormone concentrations before and after treatment of post pubertal bucks with 800mg/kg leaf ethanol extract of *Spondias mombin*

	Pre-treatment	Post-treatment	P value
Progesterone (ng/ml)	0.23 ± 0.38	0.16 ± 0.36	0.81
Estrogen (pg/ml)	61.3 ± 40.86	34.2 ± 38.33	0.45
Follicle stimulating hormone (mIU/ml)	11.7 ± 6.26	11.8 ± 3.80	0.96
Luteinising hormone (mIU/ml)	31.38 ± 37.75	8.06 ± 7.37	0.27
Prolactin (ng/ml)	10.74 ± 4.77	25.8 ± 41.50	0.49
Testosterone (ng/ml)	1.0 ± 1.21	5.56 ±4.72	0.056

pronounced post-treatment testosterone concentration of 5.56 ± 4.72 ng/ml in this work was slightly higher than 5.00ng/ml recorded by Howland *et al*. (1985) who worked around similar period of the year (August) on intact normal pygmy goats while still kept away from mating. These workers also recorded 40ng/ml as prolactin concentration for the pygmy goats which was higher than 10.74 ± 4.77ng/ml reported in this work for pre-treatment and 25.80 ± 41.50ng/ml for post-treatment. Sanni *et al.* (2012) had also reported a 6.22 ± 0.70 nmol/ml testosterone concentration for the goat and 7.17 ± 0.72nmol/ml for rabbit.

Increase in mean testosterone concentration supports the submission of Qin *et al*. (2000) that testosterone secretion increased after oral treatment of immature wistar rats and *in vitro* treatment of cultured leydig cells with flavonoids. Zhang & Yang (2006) had also reported increased level of circulating testosterone in Sprague rats treated with a flavonoid-containing Icariin. One can therefore safely link the flavonoid constituent in *Spondias mombin* to the increase observed.

The findings in this work agrees with the report of Asuquo *et al*. (2012) working with 800mg/kg aqueous *Spondias mombin* in Wistar rat, where it caused reduction in LH, although their report contradicts our observation of increased testosterone concentration after treatment with *Spondias mombin*. The reason for reduction in LH levels could be as a result of the regressing effect of the plant extract on the anterior pituitary cells as opined by Asuquo *et al*. (2012). It was thought that the plant acted directly on the anterior pituitary to inhibit synthesis of gonadotropins. But this present study did not report reduced level of FSH which should have been affected too if the anterior pituitary cells were affected. Hence LH reduction might not be as a result of effect on anterior pituitary. A more likely explanation is the negative feedback on LH synthesis and release resulting from increase testosterone that this work reported (Mayer, 2011). Mayer (2011) had explained that as testosterone levels rise, they have a direct negative feedback on the hypothalamus and anterior pituitary gland. In the arcuate and preoptic nuclei of the hypothalamus, testosterone will decrease the release of gonadotropin releasing hormone (GnRH) hence less GnRH is released into the hypothalamo-pituitary portal system and because of this the anterior pituitary will release less luteinizing hormone (LH).

In conclusion, the study showed that *Spondias mombin* at 800mg/kgBW has profertility properties in the buck and can be considered as an aphrodisiac in goat breeding in developing countries probably at a higher dosage. However, PCV should be monitored with administration.

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