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Semen characteristics and libido of rabbit bucks fed diets containing *Garcinia kola* seed meal

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Abstract. The effect of diets containing *Garcinia kola* seed meal on semen and libido of 36 matured rabbit bucks was investigated in an experiment that lasted for 3 months. The animals were randomly assigned to 3 treatments of 3 replicates each; in a completely randomized design (CRD). Three experimental diets, T1 (control; containing 0 % *G. kola* seed meal), T2 (2.5 % *G. kola* seed meal) and T3 (5 % *G. kola* seed meal) were administered ad libitum to the animals. Total sperm count was significantly ($P<0.05$) higher in T3 (115.67+ 33 x10^6) than in T1 (104. 67 + 2.73 x10^6) but not significantly ($P>0.50$) higher than T2 (111.33 + 1.86 x10^6), and no significant ($P>0.05$) difference between T1 and T2 existed. Sperm motility was similar ($P>0.05$) in T2 (76.33 + 0.88 %) and T3 (73.33 + 0.88 %), but significantly ($P<0.05$) higher than T1 (64.33+ 1.86%). Percent live sperm were significantly ($P<0.05$) higher in T2 (93.33+1.67 %) than in T3 (81.67+ 3.33 %) but not significantly higher than T1 (86.67+1.45 %), and T1 not significantly ($P>0.05$) different from T3. Reaction time recorded a dose-dependent difference and were highly significant ($P<0.01$) among the treatments, T1 = 27.57+ 1.90s, T2 = 20.75+ 1.38s and T3 = 11.0+ 0.38s. The results of this study indicate that *G. kola* seed meal improves semen characteristics and sexual drive (libido) in matured rabbit bucks.

Key Words: rabbits, semen, libido, *Garcinia kola*.

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Introduction

Garcinia kola (bitter kola) belongs to the family of plants called *Guttiferae*, the genus is known as *Garcinia* (Iwu 1993). It is a perennial crop growing in the forest, distributed throughout West and Central Africa (Iwu 1993). *G. kola* is also found distributed in the forest zone of Sierra Leone, Ghana, Cameroon and other West African countries; particularly in Nigeria it is common in the South Western States and Edo State of Nigeria (Eka 1971). It is mainly grown on homesteads in Southern Nigeria (Uko *et al* 2001); a detailed description and distribution of the plant has been documented (Iwu 1993). *Garcinia kola* possesses a wide range of potentials and attributes, it is usually called 'bitter kola' or 'male kola' because of its bitter taste, or for its supposed aphrodisiac activity, respectively. It is also popular among the people of Nigeria for nervous alertness and induction of insomnia (Uko *et al* 2001). *Garcinia kola* when chewed in small pieces before any meal have been found to improve digestibility (Adedeji *et al* 2008a), and can also serve as poison antidote (Iwu *et al* 1987). The root and stem of the plant is used as favourite bitter chewing sticks in West Africa (Irvine 1961). The chewing sticks when used without tooth paste is very effective, efficient and reliable in cleaning the teeth of many people in Southern Nigeria (Okwu & Ekeke 2003; Olabanji *et al* 1996). *Garcinia kola* has been of great value for its use in livestock, pharmacology, herbal medicine and brewing industries. It has been found that *Garcinia kola* contains a lot of valuable constituents useful to humans and animals (Adedeji *et al* 2008a).

An important constituent of *G. kola* seed is biflavonoid (kolaviron) having anti-inflammatory properties (Braide 1993) and a natural antioxidant (Olatunde *et al* 2002; Terashima *et al* 2002). Other constituents of *G. kola* seed include 1-3, 8-11 benzophenones, Garcinia biflavonones (GB-1, GB-2) and kolaflavonone (Cotterih *et al* 1978). Apigenin based flavonoids represent 60% of the total flavonoids present in the diethyl ether fraction of *G. kola* seeds (Iwu & Igboko 1982). Phenols, alkaloids, tannins and saponins are other phytochemical constituents of *G. kola* seeds, and they exert various beneficial effects in humans and animals (Okwu 2005).

The biological activities of flavonoids include action against allergies, inflammation, free radicals, hepatoxins (Terashima *et al* 2002). However, excessive ingestion of *G. kola* nuts can result in some adverse effects. Histological alterations in the liver, kidney and duodenum of rats fed diets containing 10% *G. kola* nut have been reported (Braide & Grill 1990). Similarly, Oluwole & Obatomi (1992) observed an increase in both basal and histamine-mediated gastric acid secretion in rats fed *G. kola.*

The inclusion of antibiotics in livestock ration has been discouraged. This is because of the residual effect in livestock products and development of resistant strains of micro-organisms to drug therapy (Oyekunle & Owonikoko 2002). *G. kola* can serve as alternative substance to antibiotics in livestock feeds and be used as a growth promoter (Adedeji *et al* 2008b). It can also be employed in livestock industry to effect some changes in egg quality characteristics of laying hens (Adedeji *et al* 2008a), and

as a contraceptive and fertility control agent in female Sprague-Dawley rats (Akpantah *et al* 2005).
G. kola can also serve as raw material for pharmaceutical industries (Iwu 1989) and also not elucidating its use in herbal medicine (Hertog *et al* 1993; Manimi *et al* 1994; Chairungsrilerd *et al* 1996). *Garcinia kola* possesses anti-bacterial (Madubunyi 1995; Adefule-Ositelu *et al* 2004), anti-hepatoxic (Akintowa & Essien 1990; Braide & Grill 1990), antioxidant (Olatunde *et al* 2004), hypoglycemic (Iwu *et al* 1990; Odeigah *et al* 1999) and aphrodisiac properties (Ajibola & Satake 1992) which makes it highly valued in traditional African medicine for the treatment of various ailments and diseases. The seeds are chewed as an aphrodisiac and also used to cure cough, dysentery, head or chest cold in herbal medicine (Irvine 1961). Among the people of Eastern Nigeria, the raw stem bark of *G. kola* is used as a purgative, and powdered bark is applied to malignant tumours (Iwu 1989). The sap is used to cure parasitic skin diseases and dermatological disorders associated with melanin pigmentation (Okunji *et al* 2007). The latex or gum is used internally against gonorrhea and applied externally on fresh wounds (Iwu 1989). *Garcinia kola* seed is also used in the treatment of cirrhosis and hepatitis (Iwu 1985; Ogu & Agu 1995). Other known medicinal uses include guinea worm remedy (Lewis 1977), anti-atherogenic effects (Adaramoye *et al* 2005), and antilipoperoxidative effects (Emerole *et al* 2005). The plant has been shown to posses even antiviral activity as it halts the replication of the deadly Ebola virus in its tract in laboratory tests and it has been suggested that if the anti-Ebola compound proves successful in animal clinical trials, it will be the first medicine to successfully treat the virus that causes Ebola hemorrhagic fever; an often fatal condition (Tebekeme & Ibiba 2008). This study is however; designed to evaluate the semen characteristics and libido of matured rabbit bucks fed diets containing *G. kola* seed meal.

Materials and Methods

Location of study

This study was carried out at the Michael Okpara University of Agriculture Teaching and Research Farm (Rabbitry Unit) Umudike, Umuahia, Abia State, Nigeria. The University and the farm is located on an elevation of about 120m above sea level at latitude 5°21' North and Longitude 7°29' East. Umudike falls within the rainforest zone of Nigeria which is characterized by hot and humid climate. The mean annual rainfall is about 2177mm, mean annual relative humidity is about 90 % and that of temperature is 22 °C to 36 °C depending on the season.

Management of animals

A total of 36 matured rabbit bucks were used for this study. The hutches for the animals were thoroughly cleaned and disinfected. On arrival, the animals were given Piper dewormer and allowed one week to acclimatize to the environment before administering the experimental treatments. The animals were randomly assigned to 3 experimental diets containing 0 %, 2.5 % and 5 % *Garcinia kola* respectively. Each treatment had 12 rabbits (3 replicates of 4 rabbits each) with feed and water given ad libitum. The feeding troughs were place in such position that spillage was avoided.

Plant material

Nuts of *Garcinia kola* were purchased from 'Afo Enyiogugu' market in Aboh Mbaise LGA, Imo State, Nigeria, and processed by removing the thin layer covering, chopped into pieces, air-dried and ground as described by Uko *et al* (2001).

Experimental diets

The diets were formulated using the feed materials in Table 1. *Garcinia kola* seed meal were included at three different levels in the diets, T1 is the control diet, and contained 0 % level of *Garcinia kola* seed meal, while T2 and T3 contained 2.5 % and 5 % *Garcinia kola* seed meal respectively.

Table 1. Nutrient composition of treatment diets

Component	T_1	T_2	T_3
Ingredients (%)			
Maize	54.9	51.5	47.96
Brewers dried gram	36.6	37.5	38.54
Groundnut cake	1.75	1.75	1.75
Fish Meal	3	3	3
Oyster Shell	2	2	2
Bone Meal	1	1	1
Vitamin/Mineral premix	0.25	0.25	0.25
Salt	0.5	0.5	0.5
Garcinia kola	0	2.5	5
Total	100	100	100
Crude protein content (%)+	17.07	16.77	16.79
Metabolisable energy (Kcal/kg)+	2741.95	2707.16	2706.35

+ Calculated

Semen characteristics and libido

Semen was collected fortnightly from the animals using an artificial vagina (AV) as described by Herbert & Adejumo (1995). Semen volume were read off the collection tube and recorded in milliliters. Sperm concentration ($x10^6$/mL) was determined using the Neubauer haemocytometer and the calculations were made according to Allen & Champion (1955); total sperm per ejaculate ($x10^6$), sperm motility (%), percentage live sperm and abnormal sperm were as described by Zemjanis (1970).
Sexual drive (libido) of the rabbit bucks was determined by introducing the female into the hutch containing the experimental rabbit bucks, and recording the reaction time (seconds) of the rabbit bucks with a stopwatch.

Experimental design and statistical analysis

The experiment was carried out in a completely randomized design (CRD). The data collected were subjected to analysis of variance (ANOVA) to determine significant differences among treatment means according to Steel & Torrie (1980). Where there were significant differences between means, the means were separated using the Duncan's Multiple Range Test.

Results

Table 2 presents the semen characteristics of the experimental animals and their sexual behaviour with particular reference to

their reaction time, following introduction of the female. Semen volume, sperm concentration and percent abnormal sperm of the experimental animals were not significantly ((P>0.05) different among the treatment means. Semen volume exhibited a dose dependent inverse decrease to the level of dietary inclusion of *Garcinia kola* seed meal (T_1 = 0.76±0.10 mL; T_2 = 0.70±0.08 mL; T_3 0.60±0.08 mL). Sperm concentration on the other hand exhibited a dose-dependent increase in the experimental animals (T_1 = 141.87±19.11 x10^6/mL; T_2 = 163±18.71 x10^6/mL; T_3 = 199.14±20.63 x10^6/mL), while percent abnormal sperm among the treatment means followed the trend of 31.67±3.33 % > 26.67±6.01 % > 25.75±1.38 %, for T_1> T_3 > T_2, respectively. Conversely, significant (P<0.05) difference was observed in total sperm count, sperm motility, percentage live sperm and reaction time among treatment means of the experimental animals. Total sperm count was significantly (P<0.05) higher in T_3 (115.67±2.33x10^6) than in T_1 (104.67±2.73x10^6) but not significantly (P<0.05) higher than T_2 (111.33±1.86x10^6), and T_2 not significantly (P<0.05) higher than T_1. Sperm motility of T_2 (76.33±0.08 %) and T_3 (73.33±0.88 %) were similar (P>0.05) but they were significantly (P<0.05) higher than T_1 (64.33±1.86 %). T_3 recorded a significantly (P<0.05) lower percent live sperm of 81.67±3.33 % than T_2 (93.33±1.67 %), but T_1 (86.67±1.45 %) were not significantly (P<0.05) different from T_2 and T_3.

The reaction time of the experimental animals were highly significant (P<0.01) and showed a dose-dependent inverse decrease and followed the trend of 27.57±1.90s > 20.75±1.38 > 11.0±0.38, for T_1 > T_2 > T_3.

Table 2. Semen characteristics and reaction time of rabbit bucks fed diets containing *Garcinia kola* seed meal

Parameters	T1	T2	T3
Semen vol. (mL)	0.76±0.10	0.70±0.08	0.60±0.08
Total sperm (x10^6)	104.67±2.73^b	111.33±1.86^{ab}	115.67±2.33^a
Sperm conc.(x10^6/mL)	141.87±19.11	163.31±18.71	199.14±20.63
Sperm motility (%)	64.33±1.86^b	76.33±0.88a	73.33±0.88^b
Live sperm (%)	86.67±1.45^{ab}	93.33±1.67^a	81.67±3.33^b
Abnormal sperm (%)	23.67±3.33	17.67±2.23	18.67±6.01
Reaction time (s)	27.57±1.90^a	20.75±1.38^b	11.00±0.38^c

a,b,c: Means on the same row bearing different superscripts are significantly different (P<0.05)

Discussion

The significant (P<0.05) increase in total sperm and a dose dependent, but non-significant (P>0.05) increase in sperm concentration induced by *G. kola* is in agreement with results of Adesanya *et al* (2007) in their work with adult Wister rats. Spermatids are produced through the process of spermiogenesis (Guyton & Hall 2000), and *G. kola* has been reported to increase spermatogenic activity through its tissue enhancement and ability to increase peripheral testosterone (Akpantah *et al* 2003; Ofusori *et al* 2008). This ability of *G. kola* to increase peripheral testosterone and tissue enhancement has been attributed to antioxidant compounds present in them (Oluyemi *et al* 2007; Akpantah *et al* 2003).

The non-significant (P>0.05) but dose-dependent decrease in the semen volume of *G. kola* treated animals may be as a result of a mild decrease in the lumen of the accessory sex glands whose products constitutes the seminal plasma, following increase in the volume density of their tissues.

The significant (P<0.05) increase in sperm motility of treated animals was contrary to the result obtained by Adesanya *et al* (2007) with adult Wistar rats. They proposed that their decreased motility result could be due to the rapidity of development of the spermatozoa, which may need a moderate but progressive development for them to have excellent motility, or also due to the presence of some toxic components like benzophenone in the ethanolic extracts of *G. kola*. However, in this study, the animals may not have been affected by any of the above due to species effect, or form of administration of the *G. kola*. Furthermore, other antioxidants like carotenoid present in *Garcinia kola* has been found to protect spermatogenesis in animals exposed to toxicants (Attehsahlin *et al* 2006).

Animals treated with 5 % dietary level of *G. kola* recorded lowest mean value of percentage live sperm. This may be due to competition for space as a result of increased spermatogenesis leading to increase in luminal sperm concentration (Adesanya *et al* 2007). Results of this experiment also demonstrate that at 2.5% dietary level, rabbit bucks achieved maximum percent live sperm.

Reaction time (s), used as an index for libido (sexual drive) recorded a dose dependent inverse decrease (P<0.01) among the experimental animals. This result is in agreement with studies by Uko *et al* (2001) and justifies the use of *G. kola* by the natives as an aphrodisiac (Ajibola & Satake 1992; Iwu 1993), thus minimizing incidence of impotence.

Conclusion

This study has demonstrated that *Garcinia kola* seed meal at 2.5 % dietary level, enhances sexual drive and semen characteristics in matured rabbit bucks. Therefore, breeders may find *Garcinia kola* seed meal as a helpful dietary ingredient to boost reproduction in rabbits.

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