Semen characteristics and scrotal size of pubertal West African dwarf rams fed diets containing *Garcinia kola* (Bitter kola) Seed Meal

¹Jinadu, K. B., ¹Akingbade, A. O., ¹Olona, J. F., ¹Adekanbi, A. O., ¹Popoola, M. A. ²Agboola, T. B., ³Olagbaju, O. T., ⁴Olufayo, O. O. and ⁵Oladele-Bukola, M. O.

¹Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan

²Department of Animal Science, University of Ibadan, Ibadan.

³Real people concept Africa, Adekunle Fajuyi Road, Ibadan Office Ibadan

⁴Department of nutrition and dietetics, Federal Polytechnics Ilaro, Ogun state

⁵Institute of Agricultural Research and Training, Moor Plantation, Ibadan.

Corresponding author: abdkabirj@yahoo.com

Abstract

*The effect of the Garcinia Kola Seed Meal (GKSM) on the spermatozoa attributes and scrotal characteristics of West African dwarf rams was assessed in a 16 week study. Twelve West Africa dwarf rams weighing between 12.50 and 13.80kg were randomly allotted to four dietary treatments in a completely randomized design. Rams were fed experimental diets for 16 weeks. The diets included; 0% GKSM, 2.5 % GKSM, 5.0% GKSM and 7.5% GKSM inclusion levels as treatments 1, 2, 3 and 4 respectively. Each treatment had three replicates while semen was collected once from all replicates in the treatments using electro ejaculator and assessed for semen colour, semen pH, semen temperature, sperm concentration, mass activity, sperm motility and live sperm cells. The results showed that scrotal circumference ranged from 18.16 to 21.00cm and scrotal length from 8.60 to 10.30cm but did not differ significantly ($p > 0.05$) across treatments. Sperm motility (80.00 – 84.16 %) and sperm volume (0.29 – 0.43 mL) were not significantly different ($p > 0.05$). Rams on treatment I had the highest sperm concentration of 2.59×10^9 . Despite the reproductive potential benefits of *Garcinia kola*, the inclusion of GKSM up to 7.5% in the diets of rams used in this study did not have any direct or adverse effects on the sperm motility, live sperm cells, sperm volume, and scrotal characteristics of West African dwarf rams.*

Keywords: Sperm motility, *Garcinia kola*, Scrotal size, rams,

Introduction

The efficiency of sperm production, libido and quality of sperm tends to remain uniform throughout the productive life of an animal but may be significantly altered by age, nutrition, environment health status, drugs and chemicals (Togun and Egbunike, 2006). Medicinal plants like *Garcinia kola* are of great importance to the health of individuals and communities. It constitutes an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depends on them as primary healthcare (Adebisi, 2004). The vitamin E

analogue garcionic acids and kolaviron (a bioflavonoid) contained in *Garcinia kola* seeds may have influence on the renal and reproductive functioning of animals (Adaramoye, 2009). Vansoom (2006) reported that the potential fertility of breeding males can be evaluated in the field by assessment of mating ability, physical examination and a genital tract examination of both external and internal genitalia (including a scrotal circumference measurement). Therefore, this study was designed to determine the effects of *Garcinia kola* seed meal on the semen characteristics and scrotal size of pubertal West African dwarf rams.

Semen characteristics and scrotal size of pubertal West African dwarf rams

Materials and methods

The study was conducted at the small ruminant unit of the Institute of Agricultural Research and Training Moor Plantation, Ibadan. The unit is located in south western part of Nigeria. The area lies within the rain forest ecological zone and fall within longitude and latitude 7°-27°N and 3°-25°E respectively and attitude of 200-300m above sea level with an average rainfall of about 1250mm. The temperature and relative humidity ranges from 30°-35°C and 76-84%, respectively. Twelve (12)

growing West African dwarf rams between 6-7 months of age and weighing between 12.50 and 13.80kg were used for the experiment. *Garcinia kola* seeds were freshly weighed and dry matter weight was determined. The seeds were sliced into pieces to increase the surface area and they were air dried for four (4) consecutive days. The dried seeds were ground into fine powder and incorporated into the diet at inclusion levels of 0%, 2.5%, 5.0%, and 7.5% GKSM, respectively.

Table 1: Gross composition of experimental diets (g/100g) fed to WAD rams

Ingredients	T1(kg)	T2(kg)	T3(kg)	T4(kg)
Dried cassava peel	40.00	37.50	35.00	32.50
Palm kernel cake	10.00	10.00	10.00	10.00
Brewer dried grains	10.00	10.00	10.00	10.00
Groundnut haulms	20.00	20.00	20.00	20.00
Cowpea husk	15.00	15.00	15.00	15.00
<i>Garcinia kola</i> seed meal	0.00	2.50	5.00	7.50
Dicalcium phosphate	2.00	2.00	2.00	2.00
Urea	1.00	1.00	1.00	1.00
Premix	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00
Total	100	100	100	100

Treatment1 - 100% control diets

Treatment 2 – 97.5% control diet + 2.5% *Garcinia kola* seed meal

Treatment 3 – 95% control diet + 5% *Garcinia kola* seed meal

Treatment 4 – 92.5% control diet + 7.5% *Garcinia kola* seed meal

Table 2: Proximate composition (%) for experimental diets fed to WAD rams

Parameters	T ₁	T ₂	T ₃	T ₄
Dry matter	85.09	84.74	82.09	80.34
Crude protein	13.84	13.45	12.72	12.68
Crude fibre	16.07	15.64	15.32	14.71
Ether extract	9.07	9.24	9.35	9.74
Ash	7.36	7.03	6.42	5.10
Nitrogen free extract	53.66	54.64	56.19	57.77
Acid detergent fibre	31.47	36.23	38.14	38.04
Neutral detergent fibre	43.44	47.74	53.46	52.98
Acid detergent lignin	8.53	7.68	7.14	6.38
Hemicelluloses	11.97	11.51	15.32	14.94
Cellulose	22.94	28.35	31.02	31.66

The rams were randomly allotted to the dietary treatments consisting of four (4) treatments with three (3) replicates in a completely randomized design. Testicular size was estimated by measuring the scrotal circumference with a flexible measuring tape at the point of maximum circumference of the paired testes (Notter *et al.*, 1981). Both testes were gently pushed into the posterior region of the scrotal sac and the skin of the scrotum was stretched taut to get more accurate measurements of the scrotal circumference. Testis length was measured from the top of the testis to the bottom of the epididymis with a pair of vernier calipers. Semen was collected once from the rams using electro-ejaculation (EE) method. The electro-ejaculator was used with a rectal probe of about 22cm long, 2.5cm in diameter and with two electrodes. The rectal probe was lubricated and gently inserted into rectum, and oriented so that the electrodes were positioned ventrally. The electro-ejaculator was used in automatic setting, applied for few seconds with rest at 2-seconds intervals between stimuli, increasing the voltage stimuli by one volt at a time. The penis was prolapsed beyond the prepuce, and semen was collected into a graduated tube. The probe was then inserted up to about 12 inches and held in a position of rectal floor. Alternative current increasing in voltage gradually from 0-5 volts and returning again to zero within 5 to 10s was initially passed. The subsequent stimulation made progressively higher so that at about fifth stimulus a maximum of 10-15 volts was reached. Erection and ejaculation was obtained. The source of electric current was AC/220-250volts/single phase/50 cycles. After collection by electro-ejaculator, the volume of each ejaculate was measured in a graduated tube. The proportion of spermatozoa with an intact apical ridge was evaluated. After fixation in a buffered 2%

glutaraldehyde solution and examined under differential interference contrast microscopy at magnification of 400, total number of spermatozoa per concentration and volume of the ejaculate and percentage of abnormal spermatozoa (considering all normal forms in sperm head, intermediate piece and tail) was estimated. The sperm colour was determined using a standard colour charts. The volume of the ejaculate was measured with a graduated cylinder. The sample volume can also be determined directly in the collection tube by weighing; assuming 1ml equals 1g. Thereby loss of volume associated with transfer from the collection tube to either another tube or a pipette can be avoided (Jergensen *et al.*, 1997). Sperm motility was assessed by the method described by Zemjanis (1997) and was evaluated microscopically within 2 to 4 min of their isolation from the caudal epididymis and later expressed as percentages. A fixed volume of semen (not more than 10mL) was delivered onto a clean warm glass slide with a few drops of 2.9% sodium citrate and covered with a 22x22mm cover slip. The preparation was then examined at a magnification of x400 under a light microscope. The percentage livability was assessed with a drop of semen which was mixed with 1% eosin and 5% nigrosine in 3% sodium citrate dehydrates solution for the live/dead ratio as described by Wells and Awa (1977). The morphology was determined by placing on a clean, warm glass slide, a drop of semen as well as two drops of Wells and Awa stain. The semen and stain were thoroughly mixed together with a smear made on another clean and warm slide. The smear was air-dried and observed using the light microscope starting with low power to high magnification. The presence of abnormal cells out of at least 400 sperm cells from several fields on the slide was counted and their total percentage estimated (Wells and

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Awa, 1977).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) in a completely randomized

design and significant differences between treatment means were separated using Duncan Multiple range test of the same package (SAS, 2000).

Table 3: Scrotal and semen characteristics of WAD rams fed diets containing *Garcinia kola* seed meal

Parameter	T 1	T 2	T3	T4	★Range	SEM±
Scrotal circumference (cm)	18.16	19.16	21.00	20.00	18-25	0.79
Scrotal length (cm)	9.56	8.60	10.30	8.94	10-15	0.40
Sperm motility (%)	81.33	80.00	84.16	83.86	50-95	2.32
Sperm volume (mL)	0.29	0.31	0.43	0.40	0.2-0.6	0.02
Sperm concentration (x10 ⁹)	2.57 ^a	2.22 ^{ab}	1.99 ^b	2.19 ^{ab}	2.5-3.0	0.08
Sperm Colour	Milky	Milky	Milky	Milky	Creamy-milky	
Semen temperature (°C)	31.06	31.36	30.89	31.38	30-32	0.12
Live sperm cells (%)	87.50	90.00	84.17	87.66	95	0.25
Semen P ^H	7.08 ^a	6.77 ^{ab}	6.44 ^b	6.67 ^b	6.5-7.5	0.08

★Maule (1996)

ab means in the same row with different superscripts are different at p<0.05.

Table 4: Correlation of scrotal and semen characteristics of wad rams fed diets containing *Garcinia kola* seedmeal

	SCR	S.Temp	S.Mort	S.Vol.	S.pH	S.Conc	S.length
SCR		-0.715	-0.018	-0.009	0.115	0.046	0.807
S.Temp	-0.715		-0.229	-0.191	-0.062	-0.034	-0.672
S.Mort.	-0.018	-0.229		-0.140	-0.427	-0.084	-0.123
S.vol	-0.009	-0.191	-0.140		0.281	0.335	0.334
S.P ^H	0.115	-0.062	-0.427	0.281		0.827	0.048
S.conc.	0.046	-0.034	-0.084	0.335	0.827		-0.098
S.length	0.807	-0.672	-0.123	0.334	0.048	-0.097	

Keys:

SCR = Scrotal circumference

S. Temp = Semen temperature

S. Mort = Sperm motility

S. Vol = Semen volume

S. pH = Semen pH

S. Conc = Semen concentration

S. Length = Semen length

Result and discussion

The semen characteristics of rams fed (GKSM) *Garcinia kola* seed meal are presented in Table 3. There was no significant difference (p>0.05) in the scrotal diameter among the treatments. Scrotal diameter however ranged from 18.16cm and 21.00cm. This finding was in line with earlier report by Land and Carr (1995) on Indigenous Malin rams with scrotal diameter of 16cm to 21.40cm. Though the authors concluded that testis sizes are related to body size. Scrotal

circumference is an important indicator when observing animals for breeding soundness. It is favorably correlated to testes mass, sperm production and semen quality, age at puberty, body weight and age in young bulls (Puglisi *et al.*, 2016). The scrotal characteristics in terms of scrotal diameter increased with increasing crude protein level in this study. This agreed with Smith and Akinbamijo (2000) that nutritional factors if well manipulated could ensure positive outcomes in terms of testicular size. Bielli *et al.* (1999) also

reported that there was no significant difference in testicular dimension of rams fed improved pasture or high dietary protein. Thompson *et al.* (1992) however reported that scrotal circumference was not an accurate predictor of semen concentration or motility when scrotal circumference of 32cm was used to predict the recommended animal standards for semen quality. The scrotal length of the rams fed GKSM varied from 8.60-10.30cm with negative correlation between semen output and testicular length. This result was contrary to the findings of Yarney *et al.* (1990) of high positive correlation between semen output and testicular size. The result obtained in this study was in line with Fernandez *et al.* (2004) and Hotzel *et al.* (2003) that testicular growth can be affected when animals were fed above maintenance requirement. Similarly, Masters and Fels (1984) reported that testicular growth is controlled by nutrition. A number of studies have demonstrated that the spermatogenesis in rams is sensitive to increase in protein intake as a result of increase in testicular tubules. (Oyeyemi *et al.*, 2011). The mass motility observed in this study ranged between 80.00 and 84.16% with no significant difference ($p>0.05$) although the values were higher than the minimum value of 50% required for satisfactory fertility. This showed that *Garcinia kola* fed to the rams did not have adverse effect on the sperm motility of the animal. The range of values obtained was in line with report by Oyeyemi *et al.* (2000) on the effects of Pumpkin plant on sperm motility of WAD bucks. The semen volume (0.29-0.43 mL) observed with T₃ with 7.5% GKSM inclusion level had the highest value. However, this difference was not statistically significant between groups throughout the experiment. Semen volume is a major factor in semen evaluation and reproductive performance in the males (Ax

et al., 2000). The difference observed in quantity of semen collected could be due to degree of stimulation received by the rams on each semen collection day (Naoman and Taha, 2010). Sperm concentration was significantly influenced by the inclusion levels of the GKSM. Sperm concentration in this study (1.99 to 2.57×10^9) was higher than (46.32 to 66.9×10^7) reported by Ososanya *et al.* (2013). This is an indication that the influence of GKSM in increasing testosterone production in WAD rams positively influenced semen quality and quantity. However, good semen quality with high cell concentration of ram is essential in sheep production. Semen quality and quantity assessment is very important and useful especially for diagnosing fertility problems. Same case was reported by Adeyemi *et al.* (2014) that sperm concentration was significantly influenced by the inclusion levels of *Moringa oleifera* in the diets rabbit bucks. All values obtained were within the range reported by Maule (1996) as indicated in Table 4. In this study, semen pH varied between 6.44 and 7.01 and was influenced by the inclusion levels of GKSM. Rams on Treatment 1 had the highest semen pH which showed they were significantly different ($p<0.05$) across the treatment groups but were still within the normal range as reported by Maule (1996). Semen pH is an indicator of semen acidity or alkalinity. It has been reported that normal semen p^H in breeds of rams vary between 6.5 and 7.5 and delay in analysis could lead to more acidity of semen due to degradation of fructose by the sperm cells (Sumalatha, 2010).

There was no significant difference ($p>0.05$) in the percentage livability of semen in this study. Value however ranged from 87.50-90.00% which showed that inclusion of GKSM up to 7.5% did not adversely affect sperm livability. The

increased proportion of livability (Live sperm cells) may be that the dietary treatment did not adversely affect the process of spermatogenesis as reported by (Sumalatha, 2010). There was a negative correlation of scrotal and semen characteristics (except between scrotal circumference and semen concentration, semen pH) of WAD rams fed *Garcinia kola* seed meal as shown in Table 4 shows negative correlation. This indicated that scrotal circumference and scrotal length might have positive influence on semen concentration. This result confirmed the findings by Yarney *et al.* (1990) that there was high positive correlation between semen output and testicular size.

Conclusion

From the results of this study, it can be concluded that despite the acclaimed reproductive potential benefits of *Garicinia kola*, the inclusion of GKSM up to 7.5% in the diets of rams used in this study did not have any direct or adverse effects on the sperm motility, live sperm cells, sperm volume, and scrotal characteristics of West African dwarf rams.

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