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Scrotal Morphometric Characteristics of West African Dwarf Rams Administered Aqueous African Marigold Plant (*Aspilia africana*) Extract

NseAbasi NsikakAbasi Etim[1, *], Mary Anthony Oguike[2], Udo Herbert[2]

[1]Department of Animal Science, Akwa Ibom State University, Uyo, Nigeria

[2]Department of Animal Breeding and Physiology, Michael Okpara University of Agriculture, Umudike, Nigeria

Email address

etimbobo@yahoo.com (N. N. Etim)

[*]Corresponding author

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Abstract

Twenty-four West African Dwarf (WAD) rams aged 6-9 months with average weight of 4.65 kg were used in this study. Scrotal morphometric characteristics of rams administered aqueous *Aspilia africana* extract were examined. The experiment was in a Completely Randomized Design (CRD). The rams were randomly assigned to four (4) treatment groups, designated T_1, T_2, T_3 and T_4. There were six rams per treatment; each treatment was replicated 3 times with 2 rams per replicate. Rams in T_1 (control) received 10 ml of distilled water whereas T_2, T_3 and T_4 were administered aqueous *Aspilia africana* extract at 1000, 2000, and 3000 mg-^{1}kg body weight, respectively. Rams in all the treatment groups were fed 2 kg of mixed forages, 500 g of the same concentrate diet daily and water were supplied *ad libitum*. Scrotal circumferences, scrotal lengths and scrotal volumes of the rams were measured weekly pre-, during and post-experiment. Results of the study revealed no significant differences ($P>0.05$) in the scrotal morphometric characteristics of the rams pre-experiment. Whereas, significant differences ($P<0.05$) were observed in the scrotal circumferences and scrotal volumes of the rams among the various treatment groups from the sixth and fifth weeks of administration of the extract, respectively. There were dose-dependent decreases in scrotal circumferences (SC) and scrotal volumes (SV) of rams in the treated groups. While, rams in the control group (T_1) had the highest mean values for scrotal circumference and scrotal volume. Similar trend was observed post-experiment where rams in T_2, T_3 and T_4, had scrotal circumferences of 19.67cm, 18.83cm and 16.67cm and scrotal volumes of 175.00ml, 100.00ml, 50.00ml, respectively. Rams in the control group had SC of 21.83cm and SV of 383.33ml. The findings of this study showed that *Aspilia africana* might have deleterious effects on fertility of rams administered with it. Thus, it is recommended that *Aspilia africana* be fed to animals not meant for breeding until an anti-dote which can suppress its anti-fertility effect is discovered.

Keywords

Aspilia africana, Extract, Fertility, Rams, Scrotum

1. Introduction

Reproductive organs are the dynamic organs in an animal as it reflects very sensitively various changes in the environment, nutrition, among others. Many times, they are the only organs which at low toxicity show structural and functional changes (Lukes *et al.*, 2000). Reproductive efficiency and increased production can only be achieved by understanding the histology and morphometric characteristics of these very important and sensitive organs of reproduction (Masanyi *et al.,* 2000). This is because information on organ measurement helps in evaluating the possibility of improving fertility of animals generally (Akpa *et al.*, 2012). Therefore, any quantifiable physical parameter that directly correlates with the fertilization capacity of semen could be potentially

used as a measure of semen quality. As sperm production and quality can be affected by animal reproductive organ size and physiological status of the animal (Akpa *et al.*, 2012).

If the basic morphometric characteristics of the reproductive organs in animals are not assessed, farmers may lack valuable information necessary for the evaluation of the breeding ability or breeding soundness and potential fertility of animals (Ogbuewu, 2008).

The plant *Aspilia africana* is a common weed of field crops in West Africa and sometimes found in fallow land, especially, in the forest zone (Akobundu, 1987; Etim and Oguike, 2015). It has a somewhat carroty smell. It is ligneous at the base; it fruits quadrangular and leaves opposite and hairy. The plant is a weed grazed by cattle and sheep (Burkil, 1985; Etim and Oguike, 2014).

This study was, therefore, conducted to examine the effect of administration of *Aspilia africana* on scrotal morphometric characteristics of West African Dwarf rams.

2. Materials and Methods

2.1. Location and Site of the Experiment

The research was conducted in the Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture, Akwa Ibom State University, Obio Akpa Campus, Akwa Ibom State Nigeria.

Obio Akpa is located between latitudes $5^{o}17^{I}$N and $5^{o}27^{I}$N and between longitudes $7^{o}27^{I}$E and $7^{o}58^{I}$E. It has an annual rainfall ranging from 3500mm – 5000mm and average monthly temperature of 25°C. Akwa Ibom State is a coastal State lying between latitudes $4^{o}28^{I}$N and $5^{o}3^{I}$N and between longitudes $7^{o}27^{I}$E and $8^{o}20^{I}$E, with a relative humidity between 60 – 90%. It is in the tropical rainforest zone of Nigeria.

2.2. Collection and Identification of *A. africana*

Fresh leaves of *A. africana* were collected from Nung Uyo Idoro village in Uyo Local Government Area of Akwa Ibom State and authenticated by Botany Department of the University of Uyo, Uyo, Akwa Ibom State.

2.3. Preparation and Administration of Extract

The leaves were sorted to remove contaminants, dead matter and sand particles. They were prepared fresh to prevent loss of bioactive ingredients which can take place during drying. The leaves were chopped into tiny pieces with chopping stick and sharp knife and ground using hand blender to produce *A. africana leaf* meal. One thousand grams (1000g) of the leaf meal was measured into conical flasks and extracted with 600ml distilled water. The mixture was filtered into 250ml conical flasks with Whatman paper no. 1. The solution was filtered while the filtrate was concentrated to a semi-solid form using a rotary evaporator at 40°C to produce gel-like aqueous *A. africana* extract. This was weighed and the solution prepared as 100mg/ml, 200mg/ml and 300mg/ml respectively.

2.4. Experimental Animals and Management

Twenty-four (24) pubertal West African Dwarf rams of average weight of 4.65kg, aged 6 – 9 months from farm record and confirmed by the dentition, were sourced from four (4) Local Government Areas (Uyo, Abak, Oruk Anam and Etim Ekpo) of Akwa Ibom State and used for the study. The flock was managed intensively. The sheep were quarantined for two (2) weeks before the commencement of the experiment. Routine medications against endo and ectoparasites as well as suitable vaccination, together with fumigation were performed during the pre-experimental period. The animals were randomly assigned to 4 treatment groups, with one (1) ram per pen. The pens were constructed with concrete halved walls and iron doors in the research farm that was well ventilated. The sheep were properly identified using plastic neck-tags.

During the period of the experiment, the animals were periodically washed (dipped) with Prectosol® against ticks and other ectoparasites. The health of the animals was properly monitored and adequate treatment was given to unhealthy animals. Routine inspection and regular cleaning were carried out.

2.5. Experimental Diet

The rams were fed 2kg of forages daily. The forages included: *Panicum maximum* (guinea grass), *Pennisetum purpureum* (elephant grass) and *Cynodon nlemfuensis* (star grass). Each animal also received 0.5kg (500g) of concentrate daily. Water was provided ad-libitum throughout the study. The quantity of forage and concentrate diet offered to the animals were weighed daily and the left-over feeds were weighed every morning using a sensitive electronic balance. Tables 1 and 2 show the composition of the concentrate diet given to the experimental animals.

Table 1. *Gross composition of concentrate.*

Ingredients	%
Maize	40.01
Soybean meal	4.31
Rice bran	41.30
Palm kernel cake	11.38
Bone meal	2.00
*Vitamin/mineral premixes	0.50
Salt	0.50
Total	100

Vitamin/mineral premixes (Growers) produced by Animal Care Product/Care Services Konsult (Nig) Ltd, Iperu Road-Ibadan Express way, Ogera Remo, Ogun State. *Vitamin Premix: Vit. A=8,000,000 I. U, Vit D_3 = 1,700,000 I. U, Vit. E = 5,000mg, Vit K_3 = 150mg, Folic acid = 200mg, niacin = 15,000mg, Vit. B_2 = 3,000mg, Vit. B_{12} = 5mg, Vit. B_1 = 1000mg, Vit. B_6 = 1000mg, biotin = 20mg. antioxidant = 125,000mg. Mineral Premix: Cobalt = 100mg, Selenium = 100mg, iodine = 100mg, Iron = 25,000mg, Manganese = 45,000mg, Copper = 3,000mg Zinc = 35, 000mg, Choline/chloride = 100,000mg.

Table 2. Proximate Composition of Formulated Concentrate Diet.

Parameters	Percentages
Drymatter	86.26
Crude protein	12.71
Ether Extract	7.59
Crude fibre	7.6
Ash	5.46
Nitrogen free extract	52.9
Metabolizable energy (Kcal/kg)	2529.57

2.6. Experimental Design

The experiment was in a Completely Randomized Design (CRD). The treatment consisted of administration of aqueous *A. africana* extract at 0mg/kg body weight (control), T_1), 1000mg/kg weight (T_2), 2000mg/kg body weight (T_3), 3000mg/kg (T_4). Six (6) rams were randomly assigned to each treatment and balanced for weights. Each treatment was replicated three (3) times with two (2) rams per replicate. The experimental model was as follows:

$$Yij = \mu + T_1 + Eij$$

Where:
Yij = Individual observation
μ = Overall mean
Ti = Treatment effect
eij = Random errors, which is assumed to be independently, identically and normally distributed with zero mean and constant variance (iind) (P=0.05).

2.7. Administration of Aqueous Extract to Experimental Animals

After two weeks of quarantine and acclimatization, and eight weeks which were used for collection of pre-experimental data, the aqueous extract of *A. africana* was administered once a day orally for 64 days. Ten milliliters (10mls) syringes were used for the administration of the extract. The control group (T_1) received 10mls of distilled water while treatments 2, 3 and 4 received 10mls of each of the following 100mg/kg, 200mg/kg and 300mg/kg body weight of aqueous extract of *Aspilia africana*, respectively.

2.8. Measurement of Scrotal Circumference and Scrotal Length

Scrotal circumferences (SC) and Scrotal Lengths (SL) of the rams were measured weekly for 8 weeks pre-experiment, for 9 weeks during experimental period and a week post-experiment. Scrotal Length was measured with the help of flexible tape which was held vertically above the head of the epididymis to the tip of the scrotum. The tape was also passed round the broad part of the scrotum; the widest point that is equidistant from the scrotal poles to measure scrotal circumference (Oyeyemi *et al.,* 2012) while the rams were restrained in a sitting position. The testes were pulled fully into the scrotum before measurement. Pressure was applied with a hand above the head of the epididymides, thereby, gently forcing the testes into the scrotum (Jibril *et al*., 2013). Two fingers were placed on either side of the scrotum and not below or above it during measurement.

2.9. Scrotal Volume

Scrotal volume was estimated by the volume of liquid displaced, by immersing the whole scrotal sac of a standing ram in 1 litre container filled with warm water according to Archimedes Law of Bouyancy (Piperelis *et al.,* 2008; Azawi *et al.,* 2012).

2.10. Data Analysis

Data obtained were subjected to Analysis of Variance (ANOVA) (Steel and Torrie, 1986). Significant means were separated using Fisher's Least Significant Difference (LSD) as described by Akindele (2004).

3. Results and Discussion

3.1. Scrotal Lengths of Rams Before and During Period of Administration of Aqueous *A. africana* Extract

The result of the scrotal length of rams before and during the period of administration of aqueous *A. africana* extract is presented in Table 3.

Table 3. Scrotal Lengths of Rams before and during Period of Administration of Aqueous A. africana Extract (cm).

Scrotal Length of Rams before Administration of Extract					Scrotal Length of Rams during Administration of Extract				
Weeks T_1	T_2	T_3	T_4	SEM	T_1	T_2	T_3	T_4.	SEM
Week 1 8.50	8.50	8.50	8.67	1.66	9.33	9.17	9.66	9.33	1.63
Week 2 9.33	9.17	9.17	9.17	1.71	9.50	9.17	9.66	9.50.	1.45
Week 3 9.50	9.17	9.50	9.50	1.85	9.50	9.17	9.66	9.50	1.45
Week 4 9.50	9.17	9.50	9.50	1.85	9.50	9.17	9.66	9.50	1.45
Week 5 9.33	9.00	9.33	9.33	1.57	9.83	9.33	9.67	9.50	1.68
Week 6 9.33	9.00	9.33	9.33	1.57	9.83	9.33	9.67	9.50	1.68
Week 7 9.33	9.00	9.33	9.33.	1.57	10.00	9.33	9.67	9.67	1.87
Week 8 9.33	9.00	9.33	9.33	1.57	10.00	9.33	9.67	9.67	1.83
Week 9 -	-	-	-	-	10.00	9.33	9.67	9.67	1.83

No significant differences (P>0.05) were observed in the scrotal lengths of the rams among various treatment groups pre-experiment.

During the experimental period, no significant differences in scrotal lengths were still observed among the various treatment groups.

3.2. Scrotal Circumferences (SC) of Rams Before and During the Period of Administration of Aqueous *Aspilia africana* Extract

The result of the scrotal circumferences of rams before and during the period of administration of *A. africana* extract is presented in Table 4.

Table 4. *Scrotal Circumferences (SC) (cm) of Rams before and during the Period of Administration of Aqueous Aspilia africana Extract.*

Scrotal Circumferences before Extract Administration					Scrotal Circumferences during Extract Administration				
Weeks T_1	**T_2**	**T_3**	**T_4**	**SEM**	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**
Week 1 20.17	20.17	20.50	21.00	1.43	20. 83	20.50	21.00	21.33	1.52
Week 2 20.17	20.17	20.50	21.00	1.40	20. 83	20.67	21.00	21.67.	1.45
Week 3 20.17	20.17	20.50	21.00	1.35	20. 83	20.50	20.67	20.67	1.40
Week 4 20.17	20.33	20.50	21.00	1.47	20. 83	20.83	20.67	20.67	1.50
Week 5 20.17	20.33	20.50	21.00	1.47	21. 83	20.67	20.67	20.67	1.50
Week 6 20.17	20.33	20.50	21.00	1.47	21.83^{a}	20.33^{b}	20.00^{b}	19.83^{b}	1.23
Week 7 20.83	20.50	21.00	21.33	1.52	21.83^{a}	20.00^{b}	18.67^{bc}	17.83^{c}	1.40
Week 8 20.83	20.50	21.00	21.33	1.58	21.83^{a}	20.00^{b}	18.67^{bc}	17.83^{c}	1.40
Week 9 -	-	-	-	-	21.83^{a}	20.00^{b}	18.67^{bc}	17.83^{c}	1.40

a, b, c, means in same row with different superscripts are significantly different (P<0.05).

There was no significant difference (P>0.05) in the scrotal circumferences of the rams among the various treatment groups in the eight weeks that preceded the period of administration of aqueous *A. africana* extract to the rams.

On administration of aqueous *A. africana* extract to the rams, no significant difference (P>0.05) was observed among the rams in all the treatment groups during the first 5 weeks. From 6 to 9 weeks, significant differences (P<0.05) were observed in the scrotal circumferences (SC) of the rams in the various treatment groups. The highest scrotal circumferences were recorded for rams in the control group (T_1) while those in the treated groups were lower. Among the treated groups, T_2, T_3 and T_4; T_4 had the lowest SC. The values obtained for the treated rams during that period agree with the 15-19.17cm reported by Osasanya *et al.* (2014). They were lower than 21.50cm reported by Ahemen and Bitto (2007) for West African Dwarf rams. The SC of this study were also lower than 31.25cm, 38cm and 35.25cm reported by Ibrahim *et al.* (2012) for natured Balami, Uda and Yankasa rams respectively. This was due to species differences. The lower SC values for the treated groups observed in this experiment could be due to the effects of the experimental aqueous *A. africana* extract on testes size which might have reduced the scrotal circumferences of the rams. This might likely affect fertility as was posited by Ashwood (2009) that SC is the most accurate indicator of testes size and its measurement is directly related to the mass of sperm producing tissue, sperm cell normality, the onset of puberty and fertility of female progeny. Thus, the lower SC values for the treated rams suggested that the experimental extract negatively affected the semen characteristics of the treated rams.

Furthermore, the result for SC in this study of the treated rams is contrary to the findings of Ali *et al.* (2014) that rams with larger scrotal size might possess larger body size and good reproductive trait but as evident in this study. Though, the control group had larger scrotal sizes, hence, good reproductive traits, their body sizes were lower whereas, the treated rams had larger body size and lower scrotal size. Moreover, data obtained for SC in this study agrees with the report by Ibrahim *et al.* (2012) that SC of Balami (31.25 ±3.18), Uda (38.00±0.00) and Yankasa rams (35.25 ± 1.77) were significantly higher than 21.50 reported for WAD rams. The significant differences observed between the treated groups could be due to differences in doses of extract administered to rams in those groups.

3.3. Scrotal Volumes (SV) (ml) of Rams Before and During the Period of Administration of Aqueous *A. africana* Extract

Table 5 presents the result of scrotal volumes of rams before and during the period of administration of the experimental extract.

No significant difference (P>0.05) was observed among the treatments for scrotal volumes throughout the eight weeks that preceded the period of administration of *A. africana*.

During the period of administration of *A. africana* extract, no significant difference (P>0.05) was observed in the first 4 weeks. Significant differences occurred in the SV from 5^{th} to 9^{th} week. Animals in the control group recorded the highest SV values (383.33ml) throughout the period of administration of the extract. The SV of the treated groups; T_2, T_3 and T_4 significantly decreased with increasing weeks and dosages of administration of *A. africana* as shown in Table 5. The reduction in scrotal volume of the rams in the treated groups may be as a result of the anti-reproductive potentials of the aqueous *A. africana* extract which might have atrophied the testicle of the rams in the treated groups compared to the progressive growth and increase in scrotal volumes observed in the control group (T_1). The aqueous *A. africana* extract might have caused reduction in the quantity of the testicular parenchyma as stated by Teodoro *et al.* (2013) that scrotal volume is a good indicator of the quantity of testicular parenchyma. The significant differences observed in values for SV in the treated groups (T_2, T_3 and T_4) could be associated with the variation in dosage of extract

administered to rams in the different treatment groups. While the difference observed between the scrotal volumes of the control rams in relation to the values of 484.3-466.8 ml reported by Teodoro *et al.* (2013) could be due to differences in breed, age and weight of the rams.

Table 5. *Scrotal Volume (SV) (ml) of Rams before and during the Period of Administration of Aqueous A. africana Extract.*

Scrotal Volumes Before Administration of Extract					Scrotal Volumes During the Period of Administration of Extract				
Weeks T_1	**T_2**	**T_3**	**T_4**	**SEM**	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**
Week 1 258.33	260.00	258.33	260.00	23.11	321.67	320.00	316.67	323.33.	55.68
Week 2 258.33	260.00	258.33	260.00	23.11	321.67	308.33	316.67	321.67	55.08
Week 3 258.33	260.00	258.33	260.00	23.11	321.67	306.67	305.00	300.00	46.77
Week 4 258.33	261.67	258.33	260.00.	22.93	321.17	320.00	305.00	300.00.	46.77
Week 5 258.33	261.67	258.33	260.0	22.93	383.33^{a}	300.00^{b}	305.00^{b}	300.00^{b}	50.68
Week 6 258.33	261.67	258.33	260.00	22.93	383.33^{a}	266.67^{b}	283.33^{b}	266.67^{b}	57.13
Week 7 321.67	320.00	316.67	323.33	55.68	383.33^{a}	266.67^{b}	250.00^{bc}	191.67^{c}	67.99
Week 8 321.67	320.00	316.67	323.33.	55.68	383.33^{a}	266.67^{b}	250.00^{bc}	$191.67^{c.}$	67.99
Week 9 -	-	-	-	-	383.33^{a}	266.67^{b}	250.00^{bc}	$191.67^{c.}$	67.99

a, b, c means in same row with different superscripts are significantly different (P<0.05)

3.4. Scrotal Morphometric Characteristics of Rams Post-experiment

Table 6 shows the scrotal morphometric characteristics of rams a week after the period of administration of aqueous *A. africana* extract.

Table 6. *Scrotal Morphometric Characteristics of Rams Post-experiment.*

Parameters	T_1	T_2	T_3	T_4	SEM
Scrotal Circumferences (cm)	21.83^{a}	19.67^{b}	18.83^{b}	16.67^{c}	1.99
Scrotal Volumes (ml)	383.33^{a}	175.00^{b}	100.00^{c}	50.00^{c}	61.46
Scrotal Lengths (cm)	10.17	10.00	10.17	9.67	1.83

a, b, c, means in same row with different superscript are significantly different (P<0.05)

The result of the scrotal morphometric characteristics of the rams a week post-experiment revealed that there were significant differences (P<0.05) in the scrotal circumferences of the rams in the different treatment groups with rams in the control group (T_1) recording the highest SC value (21.83cm) while treated groups recorded lower values; 19.67cm, 18.83cm and 16.67cm were recorded in T_2, T_3 and T_4 respectively. The values in T_1 were within the range reported by Osansanya *et al.* (2014) for West African Dwarf rams while those values obtained in the treated groups (T_2, T_3, T_4) persistently reduced below the value recorded by Osasanya *et al.* (2014) for WAD rams. The lower SC and SV suggest that the aqueous *A. africana* extract administered to the treated groups at various doses could have adversely affected the testes size of these rams in the treated groups; atrophying the testes and thereby reducing the quantity of testicular parenchyma of the treated rams and possibly may affect their fertility. The significant higher value of the T_1 in SC and SV suggests that the experimental extract might have had deteriorating effects on the fertility of the treated animals.

No significant differences (P>0.05) were observed for the scrotal length (SL) of rams among the various treatment groups. All the values obtained were within the range (8.83-12.75cm) reported by Osasanya *et al.* (2014).

4. Conclusion

The study revealed that the administration of aqueous *Aspilia africana* leaf extract to rams can cause significant reduction in scrotal circumferences and scrotal volumes but has no significant effect on scrotal lengths of WAD rams. This might be attributed to deleterious effects of *Aspilia africana* on the testes as observed in the study. This indicates that even 1000mg/kg BW of *Aspilia africana* administered over 12 weeks may negatively affect scrotal volume and if administered beyond 13 weeks may reduce scrotal circumferences of rams. This shows that *Aspilia africana* has anti-fertility properties.

Thus, it is recommended that *Aspilia africana* should not be administered to rams meant for breeding.

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