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Testicular characteristics of West African Dwarf (WAD) rams administered aqueous *Aspilia africana* extract

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Abstract

The effects of *Aspilia africana* on testicular consistency and morphometric characteristics of rams were determined. Twenty-four rams were used for the experiment in a completely randomised design. There were 4 treatment groups with 6 rams per group. Each treatment was replicated 3 times with 2 rams per replicate. T_1 (control) received 10 ml of distilled water, T_2, T_3 and T_4 received 1000 mg kg^{-1} Body Weight (BW), 2000 mg kg^{-1} BW and 3000 mg kg^{-1} BW of aqueous *A. africana* extract orally, respectively for 64 days. Testicular consistencies were determined by palpating the testes of experimental animals and scoring them weekly. A day after the administration of the extract, 4 rams in each treatment group were slaughtered and testicular morphometric parameters measured. No significant difference ($P>0.05$) was observed in testicular consistencies of the rams among the various treatment groups, pre-experiment. However, statistical differences ($P<0.05$) existed in testicular consistencies of the rams among the various treatment groups during experiment with rams in T_1 still retaining the same testicular consistencies pre-experiment (2.50), whereas scores for treated rams increased to 3.50, 3.50 and 4.50 for T_2, T_3 and T_4, respectively. Rams in T_4 had the highest mean score while rams in control group (T_1) had the lowest mean values. With exception of Tunica albuginea weight and testes density, testicular morphometric parameters varied significantly. T_1 had the highest significant values in all the measured parameters while T_4 had the lowest values. This indicates that *A. africana* may negatively affect testicular characteristics and might be deleterious to

fertility of rams. Thus, it is recommended that *A. africana* is not suitable for feeding breeding animals.

Key words: *Aspilia africana*, fertility, morphometry, rams, testis

Introduction

Sheep production is crucial and one of the principal activities supporting subsistence, economic and social livelihoods of a large human population (Kosgey, 2004). Sheep are among the most efficient of all domestic animals and have been for thousands of years (Sachse, 2015). Different from cattle and swine, sheep are adapted to the most extreme environmental conditions. Moreover, the importance of sheep production in any nation's economy is tremendous, but such benefits are impaired by certain challenges, one of which is feed shortage caused by high cost of conventional animal feed ingredients and competition between humans and farm animals for the available food sources (Etim *et al*., 2014).

However, when feeding is inadequate, sheep will be deficient in essential nutrients. Consequently, meat, milk and wool production will decline, as well as the overall growth and reproductive performances of the animals (Etim, 2016). This has created the need for sustainable feeding alternatives such as forages. One of such plants is *Aspilia africana* which belongs to the family Asteraceae. It is a weed of field crops in West Africa, and it is grazed by cattle and sheep. It is mostly used in Western States of Nigeria as feed for livestock (Burkil, 1985; Etim and Oguike, 2018). The herb is a good source of minerals such as calcium, potassium, magnesium, sodium, iron and zinc. Its phytochemical analysis revealed that it is rich in saponins, crude protein and terpenes (Okwuonu *et al*., 2008). It was also established that the leaves enclose carbohydrate, especially, monosaccharides (Herb, 2000). Reports by Andersen (2000), Okwu and Josiah (2006), Okwuonu *et al.* (2008), Abii (2011), Ilondu (2013) and Asumeng (2013), stated that phytochemical screening of *A. africana* revealed that it contains alkaloids, flavonoids, glycosides, phenols, phytosterol, saponins, tannins and terpenes, some of which have phytoestrogenic activities. These phytoestrogens could have deleterious effects on fertility. Moreover, Neary (2014) reported that testicles' mass should be firm, failure of which could affect fertility. Ashwood (2009) reported that animals with testicle consistency scores of 2 and 3, generally, produce good quality semen. Teodoro *et al.* (2013) also opined that testicular parameters are related to spermatogenic activities.

Although, *Aspilia africana* has been incorporated into livestock feeding and several experiments have been carried out to examine its effect on growth, little or no research has been conducted to assess its effects or the effects of its phytochemicals on testicular

characteristics and other fertility parameters in rams. Thus, there is a paucity of information on these, whereas, it is imperative to scientifically prove the safety of the plant, with regards to reproduction in sheep. However, even when a plant is usually grazed by animals or fed to them as forage, in some empirical studies, testing their effects could be done through extraction. This is because extraction enables isolation of the desired bioactive compounds or natural products from raw materials (Zhang *et al.*, 2018; Truong *et al*., 2019) with a high level of accuracy (Wong *et al*., 2006; Altemimi *et al*., 2017), for effective testing of their effects. Extraction also makes it possible to maximise the amount of target compounds and to obtain the highest biological activity of the extracts (Chang *et al*., 2002). In addition, there are many factors to control in an in vivo test, for instance, the willingness of the animals to consume the complete doses (Villalba and Provenza, 2010). Hence, offering the plant in the form of extract could provide a more accurate dosage and a faster way to observe any biological; therapeutic or toxic effect caused by the extracted phytochemicals from the plant.

Therefore, this study was conducted using aqueous *Aspilia africana* leaf extract to determine the impacts of the plant on testicular consistencies and testicular morphometric parameters of West African Dwarf rams, to assess the effects of the plant on fertility of rams.

Materials and methods

Location and site of the experiment

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

Obio Akpa is located between latitudes 5°17'N and 5°27'N and between longitudes 7°27'E and 7°58'E. It has an annual rainfall ranging from 3500 – 5000 mm and average monthly temperature of 25 °C. Akwa Ibom State is a coastal State lying between latitudes 4°21'N and 5°3'N and between longitudes 7°27'E and 8°20'E, with a relative humidity between 60 – 90%. It is in the tropical rainforest zone of Nigeria (Etim, 2016).

Collection, preparation and administration of extract

Fresh leaves of *A. africana* were collected from Nung Uyo Idoro village in Uyo Local Government Area of Akwa Ibom State, Nigeria. 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 (1000 g) of the leaf meal was measured into conical flasks and extracted with 600 mL distilled water. The mixture was filtered into 250 mL 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 ^{o}C to produce gel-like aqueous *A. africana* extract. This was weighed and the solution prepared as 100, 200 and 300 mg mL^{-1}, respectively (Etim, 2016).

Experimental animals and management

Twenty-four (24) pubertal West African Dwarf rams of average weight of 4.65 kg, aged 6 – 9 months from farm record, also confirmed by dentition, were sourced from 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 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. The research farm was well ventilated. The sheep were properly identified using plastic neck-tags.

The health of the animals was properly monitored, and adequate treatment was given to unhealthy animals. Routine inspection and regular cleaning were carried out (Etim, 2016).

Experimental diet

The rams were fed 2 kg of forages daily. The forages consisted of: *Panicum maximum* (guinea grass), *Pennisetum purpureum* (elephant grass) and *Cynodon nlemfuensis* (star grass). Each animal also received 500 g 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.

Experimental design

The experiment was in completely randomised design. The treatment consisted of oral administration of aqueous *A. africana* extract at 0 mg kg^{-1} BW (T_1; control), 1000 mg kg^{-1} BW (T_2), 2000 mg kg^{-1} BW (T_3), 3000 mg kg^{-1} BW (T_4). Six rams were randomly assigned to each treatment and balanced for weights. Each treatment was replicated 3 times with two rams per replicate. The experimental model was as follows:

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 = 200 mg, niacin = 15,000 mg, Vit. B_2 = 3,000 mg, Vit. B_{12} = 5mg, Vit. B_1 = 1000 mg, Vit. B_6 = 1000 mg, biotin = 20 mg. antioxidant = 125,000 mg. Mineral Premix: Cobalt = 100 mg, Selenium = 100 mg, Iodine = 100 mg, Iron = 25,000 mg, Manganese = 45,000 mg, Copper = 3,000 mg, Zinc = 35, 000 mg, Choline/chloride = 100,000 mg. Source: Etim (2016)

Table 2. Proximate Composition of Formulated Concentrate Diet

Parameters	Percentages
Dry matter	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

Source: Etim, 2016.

$Yij = \mu + T_1 + Eij$

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).

Administration of aqueous extract to experimental animals

After 2 weeks of quarantine and acclimatisation, the aqueous extract of *A. africana* was administered once a day orally, for 64 days. Ten milliliters (10 mL) syringes were used for the administration of the extract. The control group (T_1) received 10 mL of distilled water, orally, while treatments 2, 3 and 4 received 10 mL of each of the following: 1000, 2000 and 300 mg kg^{-1} BW of aqueous extract of *A. africana*, respectively (Etim, 2016).

Determination of testicular consistencies

After the 2 weeks quarantine, testicular consistency of each ram in all the treatment groups was examined by palpating (squeezing) the testicles of the experimental animal, weekly, before and during the period of administration of the extract and scoring them using the scoring system below (Table 3).

Table 3. Testicular Consistency Scores

Score	Description
1	Very firm
2	Firm
3	Moderate
4	Soft
5	Very soft

Source: Neary, 2014

Testicular evaluation

At the end of the 64 days of extract administration, four rams in each treatment group were fasted overnight and slaughtered by humane method. This consisted of a swift, deep incision of a knife on the throat, cutting the jugular veins and carotid arteries of both sides but leaving the spinal cord intact (Okubanjo, 1997) The testes

were removed and weighed using a sensitive electronic balance. Testes lengths, testicular diameters and testes circumference were measured with the aid of a flexible tape. Testes size was estimated by multiplying testes length by testes circumference. Testicular volumes were measured volumetrically using the Archimedes principles of water displacement in 500 mL measuring cylinders and result recorded. Paired and mean testicular parameters were computed from data obtained for left and right testes as described by Iheukwumere *et al.* (2008). Gonadosomatic index was estimated by dividing gonadal weight by body weight of the rams and multiplied by 100. Testes density was calculated from the testes weight and volume and expressed as gcc^{-1}: Testes density=testes weight (g) divided by testes volume (cc).

Data analysis

Data obtained were subjected to Analysis of Variance (ANOVA) (Steel and Torrie, 1986), using the Statistical Package for Social Sciences (SPSS) software, version 20.0. IBM Corp., Armonk, NY. Statistically significant difference between treatment mean values was determined using Fisher's Least Significant Difference at 5%.

Results

Testicular consistencies of West African dwarf (WAD) rams pre- and during period of administration of aqueous Aspilia africana extract

The result of the testicular consistencies of WAD rams pre-and during experiment is presented in Table 4. No significant differences (P>0.05) existed in the testicular consistencies of the rams among the various treatment groups before administration of aqueous *A. africana* extract as shown in Table 4. The scores obtained for all the treatment groups were between 2 and 3.

Table 4. Testicular consistencies of West African Dwarf Rams pre-and during experiment

Periods	T_1	T_2	T_3	T_4	SEM
Testicular consistencypre-experiment	2.50	2.50	3.00	2.50	0.82
Testicular consistencyduring experiment	2.50^{c}	3.50^{b}	3.50^{b}	4.50^{a}	0.67

$^{a, b, c}$ means in the same row with different superscripts are significantly different (P<0.05); T1 (control): at 0 mg kg^{-1} BW, T2: 1000 mg kg^{-1} BW, T3: 2000 mg kg^{-1} BW, T4: 3000 mg kg^{-1} BW of *A. africana* extract

However, during the period of administration of aqueous *A. africana* extract to the treated rams (experimental period), significant differences (P<0.05) were observed among the various treatment groups. Rams in T_1 (control) still retained the same

testicular consistency score (2.50) as that observed pre-experiment while the testicular consistency scores for the treated rams increased to 3.50, 3.50 and 4.50 for T_2, T_3 and T_4, respectively.

Testicular morphometric characteristics of West African dwarf (WAD) rams administered with aqueous A. africana extract.

The result of the mean testicular morphometric characteristics of the WAD rams administered with aqueous *A. africana* extract is outlined in Table 5. The result of testicular morphometric characteristics revealed significant differences (P<0.05) in most of the parameters measured except Tunica albuginea weight and testes density,

Table 5. Testicular morphometric characteristics of West African Dwarf Rams Administered Aqueous *Aspilia africana* Extract

Parameters	T_1	T_2	T_3	T_4	SEM
Testis weight (right) (g)	51.20[a]	45.90[b]	39.85[c]	34.15[d]	3.36
Testis weight (left) (g)	54.65[a]	46.80[b]	41.80[c]	34.50[d]	3.85
Paired testes weight (g)	105.85[a]	92.70[b]	81.65[c]	68.65[d]	7.06
Testis length (right) (cm)	8.00[a]	7.25[b]	6.50[c]	6.00[c]	0.53
Testis length (left) (cm)	8.25[a]	7.75[a]	6.90[b]	6.50[b]	0.58
Paired testes length (cm)	16.25[a]	15.00[b]	13.0[c]	12.50	0.90
Testis volume (right) (mL)	51.75[a]	43.50[b]	39.00[c]	34.00[d]	2.70
Testis volume (left) (mL)	53.65[a]	47.00[b]	40.00[c]	33.50[d]	3.62
Paired testes volume (mL)	105.50[a]	90.50[b]	79.00[c]	67.50[d]	5.61
Testes density (gcc^{-1})	1.01	1.03	1.04	1.02	0.06
Gonadosomatic index (%)	882.08[a]	686.40[b]	527.3[c]	405.03[d]	51.85
Tunica albuginea weight (right) (g)	4.50	4.40	4.05	3.60	1.07
Tunica albuginea weight (left) (g)	4.80	4.45	4.10	4.65	0.91
Testis size (right) (cm)	116.00[a]	98.00[b]	75.00[c]	66.00[c]	11.38
Testis size (left) (cm)	123.75[a]	108.50[b]	82.80[c]	74.50[d]	5.49
Paired testes size (cm)	239.75[a]	206.50[b]	157.80[c]	140.50[d]	15.77
Paired Tunica albuginea weight (g)	9.30	8.85	8.25	8.15	1.94
Parenchyma weight/volume/(g)	96.55[a]	83.85[b]	73.50[b]	60.40[d]	5.37
Testis diameter (right) (cm)	7.25[a]	6.75[b]	5.75[c]	5.50[c]	0.41
Testis diameter (left) (cm)	7.50[a]	7.00[b]	6.00[c]	5.75[c]	0.24
Paired testes diameter (cm)	14.75[a]	13.75[b]	11.75[c]	11.25[d]	0.47
Testis circumference (right) (cm)	14.50[a]	13.50[b]	11.50[c]	11.00[c]	0.82
Testis circumference (left) (cm)	15.00[a]	14.00[b]	12.00[c]	11.00[d]	0.47
Paired testes circumference (cm)	29.50[a]	27.50[b]	23.50[c]	22.00[d]	0.94

[a, b, c, d] means in same row with different superscripts are significantly different (P<0.05); T1 (control): at 0 mg kg^{-1} BW, T2: 1000 mg kg^{-1} BW, T3: 2000 mg kg^{-1} BW, T4: 3000 mg kg^{-1} BW of *A. africana* extract

with rams in the control group (T_1) recording the highest significant values in all the measured parameters. No significant difference (P>0.05) was observed in the testes density.

Significant differences (P<0.05) were also observed in the result for gonadosomatic index (%) with T_1 having the highest mean value (882.08%) while lower percentages (686.40, 527.13 and 405.03) were recorded for the treated groups (T_2, T_3 and T_4), respectively. There was no significant difference (P>0.05) in the weights of the tunica albuginea among the various treatment groups.

The testes sizes were statistically different among rams (P<0.05) in the various treatment groups. Rams in T_1 (control) had the highest significant mean value (239.75 cm), followed by T_2 (206.50 cm) and T_3 (157.80 cm) and the least value was obtained for T_4 (140.50 cm). The data obtained for the left and right testes size followed similar trend as that of the paired testes size.

Parenchyma weight/volume also differed significantly (P<0.05) among the various treatment groups; 96.55 g, 83.85 g, 73.50 g and 60.0 g were observed for T_1 (control) T_2, T_3 and T_4 respectively. Significant differences (P<0.05) were also observed in testes diameter and circumference. Values obtained for the control rams (T_1) were persistently higher. Significantly (P<0.05) lower values were obtained for the treated groups.

Discussion

The scores obtained for testicular consistencies for rams in the various treatment groups pre-experiment are scores for firm and moderately firm testicles, respectively as was reported by Ashwood (2009). Ashwood (2009) stated that animals with testicle consistency scores of 2 and 3, generally, produce good quality semen.

The scores for the treated rams are indicative of soft and very soft testicles which are of low resilience and are associated with high percentage of abnormal sperm and low reproductive performance. This is in line with the report by Ashwood (2009), that bulls with 4 or 5 score are likely to produce poor quality semen. Neary (2014) stated that testicles mass should be firm, failure of which could affect fertility, but results for rams in T_2, T_3 and T_4 are contrary in terms of firmness. Dose dependent increases in scores were observed among the treated rams for testicular consistencies. The significant differences (P<0.05) in the testicular consistencies of the rams in the various treatment groups might be associated with the test extract, which indicates that the extract may negatively affect fertility, while variation in the dosages of the

extract administered to rams in the treated groups, might be responsible for the significant differences ($P<0.05$) observed among the rams in $T_{2,}$ T_3 and T_4.

Results for all the testicular parameters were larger for the left side of the testes than for the right portion which is consistent with the findings of Oyeyemi *et al.* (2012) but varied with the report by Salheb *et al.* (2001).

The reduction in the testes weight recorded for the treated groups (T_2, T_3 and T_4) compared to the control group (T_1) might be an indication of widespread or diffuse loss of seminiferous epithelial cells as reported by Morton (2006) and Ogbuewu (2008). Results obtained in this study corroborate earlier findings by Etim (2010) who observed reduction in the weight of ovaries of rabbit does fed *A. africana* leaves. Results obtained in the study for testes weight are also in line with the findings of Raji *et al.* (2008) who observed that the left testis was heavier than the right testis. The significantly lower mean values for lengths of the testes for rams in T_2, T_3 and T_4 may be associated with the smaller sizes of the testes.

The significantly higher testes volume of the control group (T_1) is indicative of better spermatogenic activity in the testes of rams in the control group while the lower values of the treated group revealed the degenerative or destructive effect of the aqueous extract of *A. africana* on spermatogenic process in the testes. This agrees with the report by Teodoro *et al.* (2013) that testicular volume is measured in relation to spermatogenic activity. The heavier testes volume observed in T_1 (control) implies that rams in this group could produce more spermatozoa than those in the treated groups which is consistent with the findings of Britto *et al.* (2004), that heavier testes produce more spermatozoa than the smaller testes. It could also be observed that the result for testes volume corroborate the result of testes weight. The testes volume values were about the same as those obtained for testes weight and is consistent with the report by Melo *et al.* (2010).

The result for testes density (gcc^{-1}) obtained in this experiment was within the range of 1.00 ± 0.14, 1.01 ± 0.07 and 1.02 ± 0.13 reported by Ibrahim *et al.* (2012) for Balami, Uda and Yankasa rams, respectively. Testes density obtained in this study corroborates the findings of Melo *et al.* (2010) that testes density in mammals is situated at about 1.

Statistical differences observed in gonadosomatic indices revealed that the aqueous *A. africana* extract administered to the rams in the treated groups at different doses, had diverse levels of suppressive or oppressive effects on the development of the gonads (testes), although it promoted the growth of the animals considerably in terms of body weight. This agrees with the report by Melo *et al.* (2010). The experimental

extract could have also suppressed the maturity of the gonad as was reported by Melo *et al.* (2010) that gonadosomatic index is a tool for measuring sexual maturity of animals in correlation to ovary and testes development.

Significant differences observed in the testes sizes for rams in the different treatment groups imply that rams in the control groups (T_1) had significantly larger seminiferous tubules, which occupied a significantly greater portion of the testicular volume, than rams administered with aqueous *A. africana* extract (T_2, T_3 and T_4). This observation could be associated with the possible effects of the extract which might have atrophied the testes. Thus, posing reproductive risks on the rams in the treated groups. This is consistent with the reports by Oko *et al.* (2011). Results obtained also signifies low number of spermatozoa and volume of ejaculate that the treated rams produced which is in line with the findings of Ashwood (2009). This result could also imply that semen from the treated rams with the smallest testes size and weight will result in reduced fertility. Thus, *A. africana* might have different levels of toxic and anti-fertility effects on the treated rams depending on the dose as is evident in the significant difference ($P<0.05$) and dose dependent decrease observed among rams in the treated groups. The finding of this experiment corroborates the report by Oko *et al.* (2011) that *A. africana* has shown toxic effect on animal physiology and pose some health and reproductive risks to animals. These findings are also synonymous with the reports by Tamura *et al.* (1997) and Oluyemi et al. (2007) that *A. africana* have been found to reduce fertility in animals upon continuous administration.

Significant reduction in parenchyma weight/volume and degenerative and atrophic changes were observed in the testes of the treated rams compared to the control group. This implicates *A. africana* as suppressing fertility in the treated rams while the larger parenchyma weight and volume of the control indicated that the testes of the control rams contained greater number of sustenocytes, seminiferous tubules and thus, support more spermatozoa production.

Significantly lower testes diameter and circumferences in the treated rams indicate that the treated rams had suppressed testicular growth as a result of the extract administered to the animals.

The decrease in mean values of the testicular parameters could lead to infertility in the treated groups as was observed by Oluyemi *et al.* (2007); Eweka (2007); Okwuonu *et al.* (2008); Adewumi *et al*. (2009); Oko *et al.* (2011), who reported on anti-fertility effects of *A. africana* on rats and the present study led to the observation of similar effect on livestock (ram). Moreover, findings of this study aligns with the report by Taziebou *et al*. (2007), that despite the fact that aqueous extract of *A. africana* leaves can been classified as a substance with low toxicity,

prolonged treatment with aqueous extract of the plant at the dosage of 500 mg/kg bodyweight or more could become toxic.

Conclusion

The significantly low mean values for testicular morphometric parameters for rams administered aqueous *A. africana* extract indicates that the plant might have the potential to atrophy testicles and thus, may be deleterious to fertility. Thus, it is recommended that *A. africana* should not be offered to breeding animals.

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