Impact of *Aspilia africana on* Semen and Testicular Characteristics of Rabbit Bucks

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***Abstract*—A completely randomized design experiment was conducted to study semen characteristics, testicular histology and morphological dimensions of mixed bred rabbit bucks fed *Aspilia africana* Leaf Meal (AALM). The treatments designated treatment one (T_1), treatment 2 (T_2) and treatment 3 (T_3) having 12 rabbits each were replicated 3 times with 4 rabbits per replicate. The age of the rabbits was 3 to 6 months, and they weighed approximately 2.56 kg. Three diets formulated with AALM and supplemented at 0, 10 and 20 g/kg feed were fed to rabbits in the respective treatments. Data were collected on the libido, semen parameters and testicular morphometry and histology. Results showed significant ($P<0.05$) dose-dependent decrease in gross motility (T_1 80.00; T_2 78.67; T_3 70.67 %), total sperm/ejaculate (T_1 57.78; T_2 55.48; T_3 39.97 %), sperm concentration ($T_1$116.8; T_2 110.83; T_3 88.00 x10^6/ml), fructose T_1 0.24; T_2 ; 0.20 T_3, 0.17 mmol/l), zinc (T_1 1.81; $T_2$1.63; T_3 1.51 mmol/l), scrotal circumference (T_1 4.57; T_2 4.43; T_3 4.00 cm), paired testis weight (T_1 3.45; T_2 3.10; T_3 2.94 g) and volume (T_1 3.43; T_2 3.14; 3.14 T_3 3.07 cm^3) following supplementation of AALM. Abnormal sperm significantly ($P<0.05$) increased with increase in the test ingredient. In conclusion, these results indicated that supplementation of AALM even at low levels adversely affected semen characteristics, testicular histology and testicular morphometric parameters.**

Index Terms*—semen, testis, rabbit bucks, *Aspilia africana

I. Introduction

Semen characteristics such as sperm concentration, sperm motility and proportion of live sperm cells are among the parameters used in assessment of semen quality and fertility. Fertility is in turn, an indicator of reproductive capabilities of animals [1]. Poor quality semen and low libido impact negatively on male fertility and reproductive performance of different species of farm animals. The beneficial effects of plant materials are explored with regards to the growth performance and development of reproductive organs in livestock.

The scopes of the biological actions of forage plants are continuously assessed. Among these plants, those implicated as herbal products have received increased attention and some have given satisfactory results [2]. One of such plants is *Aspilia africana (A. africana*). *Aspilia africana* (African marigold plant) is a perennial flowering plant primarily propagated through seed. It grows popularly as herbaceous perennial weed, varying in height from 60 cm to 2 meters depending on rainfall in cultivated lands and fallow [3], [4]. *Aspilia* africana serves as forage for animals, such as rabbits, sheep, goat and for other livestock [5]. Leaf extracts and fractions of *A. africana* effectively arrested bleeding from fresh wounds, inhibited microbial growth of known wound contaminants and accelerated wound healing process [6], [7]. It has also been indicated that *A. africana* possesses antimicrobial, haemostatic, anti-inflammatory, anti-fertility activity [8 unpublished], [9]-[12]. *A. africana* is a good source of protein, vitamins, and minerals such as ascorbic acid, riboflavin and thiamin calcium, phosphorus, potassium, magnesium, sodium, iron and zinc [13], [14].

Although the beneficial effects of *A. africana* have been exploited, but its overall potentials have not been fully elucidated. *A. africana* has been reported to possess the ability to prevent conception, suggesting potential contraceptive and anti-fertility properties [11]; [13]. "Reference [12]" "Reference [14]" corroborate these reports. However, it is not certain whether the levels and methods of administration of *Aspilia* a*fricana* by these researchers contributed to its adverse effects on the reproductive characteristics of the treated animals.

The aim of this study was to investigate the impact of *AALM* administered at lower levels on the semen characteristics and testicular parameters of rabbit bucks.

II. Materials and Methods

A. Experimental Location

This research was conducted in the Rabbitry Unit of the Teaching and Research Farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Umudike lies within the tropical rainforest zone of South Eastern Nigeria. The location is characterized by average annual rainfall of 2,177mm in 148-155 rain days. The average temperature is 25.5^{0}C with minimum and maximum temperature of 22^{0}C and 29^{0}C respectively. Relative humidity ranges from 57-91%. The metrological

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data were collected from the National Root Crop Research Institute, Umudike Abia State, Nigeria.

B. Collection and Preparation of Experimental Materials

Aspillia africana leaves were collected from the demonstration farms of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaves were air-dried under room temperature (26^0C) to a constant weight. The dry *Aspillia africana* leaves were milled using a hammer mill to produce AALM. The dried AALM was used in supplementing diets formulated for the experimental rabbit bucks.

C. Experimental Animals and Management

Thirty-six (36) pre-pubertal mongrel rabbit bucks sourced from the Teaching and Research farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, were used for the study. A quarantine period of 2 weeks pre- experimental trial was allowed during which the animals were vaccinated against ecto and endo-parasite using Ivomectin and Levamisole (0.1ml/kg body weight), respectively. The experimental animals were housed singly in pens of colony hutches for ease of identification throughout the experimental period. They were fed mixed forages and concentrate diet supplemented with AALM. Feed and clean drinking water were provided *ad libitum*. The compositions of the experimental diets are presented in Table I. Routine management practices were also carried out appropriately.

TABLE I. FEED COMPOSITION AND CALCULATED NUTRIENTS OF EXPERIMENTAL DIETS

Ingredients (%)	T_1	T_2	T_3
Maize	44.94	44.94	44.94
Soya bean	17.31	17.31	17.31
Rice husk	32.00	32.00	32.00
Fish meal	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00
Limestone	2.00	2.00	2.00
[1]Vitamin/mineral premix	0.25	0.25	0.25
Salt	0.50	0.50	0.5
Total	100.00	100.00	100.00
Aspilia africana (g/Kg)	0.00	10.00	20.00
Calculated Nutrients			
Protein (%)	17.00	17.00	17.00
Metabolizable energy	2505.42	2505.42	2505.42
(Kcal/kg)	11.36	11.36	11.36
Crude fiber (%)	0.514	0.514	0.514
Lysine (%)	0.199	0.199	0.199
Methionine (%)			

1 Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride, 300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg.

D. Experimental Design

The study was a Completely Randomized Design (CRD) trial with three treatments consisting of T_1, T_2, and T_3. The T_1 served as the control. Twelve (12) rabbits were randomly assigned to each treatment, balanced for weights and replicated 3 times, with 4 rabbits per replicate. The respective experimental diets were AALM included at 0, 10 and 20 g/kg feed.

E. Reaction Time (Libido)

A matured doe (teaser) was introduced to the buck prior to semen collection to monitor their sex drive. The time in seconds it took for the rabbit bucks to sniff, groom and mount the female was recorded with a stop watch. Libido (Reaction time) was determined by observing the time taken (seconds) from exposure of the buck to a doe and the first copulation as recommended by [15].

F. Estimation of Semen Characteristics

Semen evaluation involved the estimation of both the macroscopic and microscopic indices. Semen volume was determined in millimeter directly from a calibrated glass collection tube attached to the Artificial Vagina (AV). Sperm cell concentration was determined using a haemocytometer. Total number of sperm was determined by multiplying semen volume by the sperm cell concentration. Sperm motility was determined subjectively with a drop of fresh semen on a glass slide covered with slip and examined using a microscope. Sperm morphology was determined by performing differential counts of the morphologically normal and abnormal shape of the spermatozoa using eosin-nigrosin stain. Seminal plasma parameters were determined using the methods of [15]

G. Semen Collection and Testicular Measurements

Artificial Vagina (AV) with a calibrated glass collection tube constructed by [16] was used for semen collection. Two weeks prior to semen collection, the bucks were trained to serve an artificial vagina using a teaser rabbit doe. The semen was collected weekly between 09 and 10 hr, local time. During semen collection, the rabbit doe was taken to the buck's cage and held in position for service. The AV was lubricated using glycerol and its temperature adjusted to 36-40^0C. The artificial vagina was strategically placed under the belly of the doe such that the penis of the buck was introduced into the artificial vagina. The scrotal circumference was taken using a measuring tape calibrated in centimeter. At the end of the experiment, three bucks from each replicate was slaughtered using a captive bolt and allowed to bleed. The internal organs were incised and weighed. The testis were incised and used to determine testicular dimensions. Weights of the testicles were recorded after the epididymis has been trimmed off. (All weight measurements were done using electronic weighing scale; Brand - OHAUS Champ II).

The volume of each testis was recorded using Archemedes principle of water displacement.

H. Histological Study

At the end of the experiment, the remaining rabbit from each replicate were slaughtered, their testicles collected and processed for histology. The tissue was embedded, dehydrated in an alcoholic solution of

different concentration. Clearing and impregnation were done using xylene and paraffin wax respectively. The tissue was cut (Sectioned) using a microtome (Brand - Rotary Kepee Model KD 202A), stained with hematoxylin and eosin; and examined using a light microscope of different magnification according to the procedure described by [17] for histological studies. The slides were prepared and examined for histology to observe the testicular tissues using photo micrographic software (Brand - Phoenix Micro Image Analysis - 2003 version 1.33). The photo micrograph was used to project the slides on the computer for clearer assessment. The pictures on the slides were subsequently taken and printed for interpretation and documentation at the Physiology Laboratory of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike.

I. Statistical Analysis

Data generated were subjected to analysis of variance (ANOVA) in accordance with the methods of [18]. Significance means were separated according to Duncan's Multiple Range Test [19].

III. Results and Discussion

The results of semen characteristics of rabbit bucks fed AALM are presented in Table II.

TABLE II. Semen Characteristics of Rabbit Bucks Fed *Aspilia africana* Leaf Meal

Parameter	T_1	T_2	T_3	SEM
Reaction time (sec)	10.71	11.56	12.89	0.62
Semen volume (ml)	0.50	0.50	0.47	0.03
Gross motility (%)	80.00^{a}	78.67^{a}	70.67^{b}	1.85
Live sperm proportion (%)	84.40	83.27	80.40	1.30
Sperm concentration ($x10^{6}$/ml)	116.83	110.83	88.00	6.72
Total sperm/ejaculate (%)	57.78^{a}	55.48^{a}	39.97^{b}	4.08
Total viable sperm ($x10^{9}$)	454.63^{a}	425.85^{a}	276.43^{b}	34.23
Abnormal sperm (%)	1.17^{b}	2.18^{ab}	3.10^{a}	0.35
Normal sperm (%)	98.83^{a}	97.82^{ab}	96.90_{b}	0.35

abcd: means with different superscripts along rows are significantly different (P <0.05) SME=standard error of means

The results of semen characteristics showed that reaction time, semen volume and live sperm proportion were not significantly affected (P>0.05) by AALM at the various levels of supplementation. There were significant (P<0.05) dose dependent decreases on gross motility, sperm concentration, total sperm/ejaculate, total viable sperm and, percentage normal sperm cells. Abnormal sperm cells increases with increase in supplementation of AALM. From these observations, it is clear that AALM have negative effects on semen characteristics of the rabbit bucks. Gross motility, sperm concentration, total sperm/ejaculate, viable sperm and, percentage normal sperm cells are the major traits that are required for the assessment of semen quality of males. Males with questionable attributes in these semen quality parameters are susceptible to sterility or infertility. The results of the current study support the findings of [12], [20] and [21] who reported that *Aspilia africana* leaf has anti-spermatogenic and anti-fertility potentials on the testis of male wistar rats.

The results on semen seminal plasma of rabbit bucks fed AALM are presented in Table III.

No significant differences (P>0.05) were observed on sodium, magnesium, glutamic acid and glucose in the seminal plasma of the groups fed supplemented AALM compared to the control. This means that supplementation of *A. africana* leaf meal did not have any detrimental effects on these seminal parameters. However, the glucose level although not significant (P>0.05), was lower in the group (T_3) fed higher level of AALM. Furthermore, significant decreases (P<0.05) were observed on the seminal fructose and zinc following increased supplementation of AALM. Fructose and glucose are the main energetic components of semen as well as substrate for the sperm cell metabolism. Zinc, on the other hand, is essential for the maintenance of germ cells during spermiation in the progressions of spermatogenesis. It is also essential for ribonuclease activity during spermatogenesis as well as regulation of sperm motility [22]. The decrease in fructose and zinc of the treated groups observed in the present study contributed to the significant decrease obtained in the major semen quality parameters as indicated in Table II. It, therefore, means that fertility could be likely impaired in the groups fed diets supplemented with AALM.

TABLE III. Seminal Plasma of Rabbit Bucks Fed *Aspilia africana* Leaf Meal

Parameters (mmol/l)	T_1	T_2	T_3	SEM
Fructose	0.24^{a}	0.20^{ab}	0.17^{b}	0.01
Sodium	120.83	125.26	123.73	0.98
Zinc	1.81^{a}	1.63^{b}	1.51^{b}	0.05
Magnesium	3.24	2.95	3.16	0.07
Glutamic acid	1.31	1.30	1.35	0.01
Glucose	69.75	72.78	67.96	2.12

abcd: means with different superscripts along rows are significantly different (P <0.05). SEM= Standard error of means

The result of the testicular morphometry of rabbit bucks are presented in the Table IV.

TABLE IV. Testicular Morphometry of Rabbit Bucks Fed *Aspilia africana* Leaf Meal

Parameter	T_1	T_2	T_3	SEM
Scrotal circumference (cm)	4.57^{a}	4.43^{a}	4.00^{b}	0.11
Right testis weight (g)	1.75	1.57	1.42	0.74
Left testis weight (g)	1.70^{a}	1.53^{b}	1.52^{b}	0.65
Paired testis weight (g)	3.45^{a}	3.10^{b}	2.94^{b}	0.15
Right testis volume (cm^3)	1.76	1.58	1.50	0.07
Left testis volume (cm^3)	1.67^{a}	1.56b	1.57^{b}	0.07
Paired testis volume (cm^3)	3.43^{a}	3.14^{b}	3.07^{b}	0.16

abcd: means with different superscripts along rows are significantly different (P <0.05).

The results showed no significant differences (P>0.05) on weight and volume of the right testes of the AALM

supplemented groups although they decreased numerically with increased AALM. The scrotal circumference, left testis weight, paired testis weight, left testis volume and paired testis volume decreased significantly ($P<0.05$) in the treated groups as the level of supplementation increased. This showed that AALM significantly decreased the weight and volume of the testicles and thereby could be detrimental to the development of the testicle of the rabbit bucks. The reduction in the weight and volume of the testicles is detrimental to breeding animals, since spermatogenic cells, Leydig cells, seminiferous tubules and the surface area for spermatogenesis are likely affected by this phenomenon. Thus, the reduction in weights and volumes of testicles following supplementation of AALM in the treated groups is responsible for the reduction in sperm concentration, total sperm per ejaculate, viable sperm cells and normal sperm cells of T_3 in the present study. The decrease in these parameters could also be the reason for the degenerations observed in the histology of T_3 (Fig. 3). "Reference [26]" reported that the higher the weight and volume of a testicle without inflammation, the more the number of sertoli cells then the better the breeding and fertility potentials of a male animal. The results of the current study confirm the reports of these authors [1] [23], [26] who observed that sperm characteristics, seminiferous tubule diameter, epididymis morphometry, testicular weights, sizes, and morphology are good indicators of present and future spermatozoa production, breeding and fertility potentials of a male animal.

The testicular histopathology of bucks fed varying levels of AALM is presented in Fig. 1-Fig. 3.

Fig. 1: Photomicrograph of *testes* of the Control (T_1) shows intact seminiferous tubules and Interstitium with orderly arranged germ cells at varying levels of maturation within the tubule. The mature spermatids density are also variable in tubules.

Fig. 2: Photomicrograph of testes of T_2 (10g/kg AALM) shows slight disruption of seminiferous tubules with few orderly arranged germ cells at varying levels of maturation within the tubule. Compared to the control group, there were observable defects, varying degree of degeneration and slight disruption within the tubules.

Fig. 3: Photomicrograph of testes of T_3 (20g/kg AALM) shows a range of seminiferous tubules degenerative changes of germ cells at varying levels within the tubule. Compared to the control group, there appear to be eroded boundary in the tubules. There are degeneration of spermatocytes and absence of mature spermatozoa to a large extent. However, the interstitial cells are not affected.

The histology of the testes of rabbit bucks fed diets supplemented with AALM appears to have varying degree of degeneration ranging from loss of germinal epithelium, slight disruption of interstitium and cell erosion. These observations were similar to those of [21] on the histology of the testis of wistar rats administered methanolic extracts of *Aspilia africana* leaf.

Having studied the influence of AALM on semen characteristics, histopathology and testicular morphology of rabbit bucks, it is concluded that the semen characteristics, seminal plasma, testicular morphometry and histology of the rabbit bucks were adversely affected by AALM especially as higher levels of supplementation increased. Hence, spermatogenesis and reproductive processes could be impaired with inclusion of AALM in the diets of breeding rabbit bucks.

We recommend that AALM should not be used in feeding breeding rabbit bucks since it has negative effects on reproductive organs and reproductive characteristics.

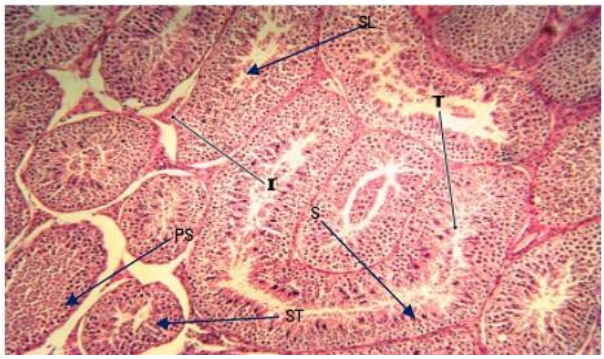

Figure 1. Testicular histology of bucks fed AALM at 0g/kg feed – Control T1

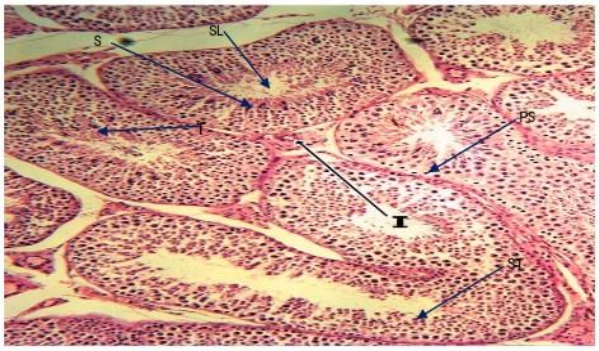

Figure 2. Testicular histology of bucks fed AALM at 10g/kg feed - T2

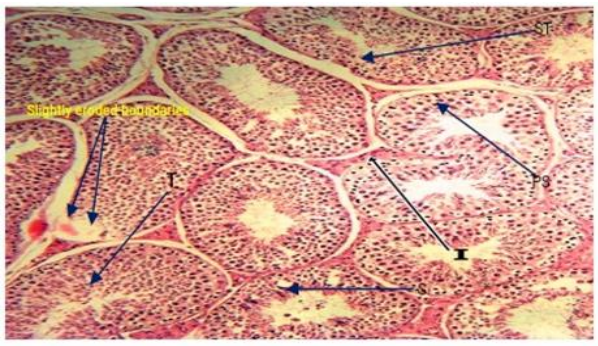

Figure 3. Testicular histology of bucks fed AALM at 20g/kg feed - T3

Note: PS: Primary Spermatocyte, SL: Lumen of Seminiferous Tubule, ST: Sertoli Cell. Interstitium (Lydig Cell), T: Seminiferous Tubule, S: Mature Spermatid

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