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Testicular Histology, Gonadal and Extragonadal Sperm Reserve of Sheep (rams) Fed Tigernut (*Cyperus esculentum*) Chaff and Cowpea (*Vigna unguiculata* L. Walp) Husk in a Mixed Ration.

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Target audience: small ruminant farmers, animal reproductive physiologists, nutritionists.

Abstract

Gonadal, extra-gonadal sperm reserves and testicular histology of Yankasa rams fed tiger nut chaff and cowpea husk in a mixed ration were studied. Thirty growing and apparently healthy Yankasa rams were randomly allotted five dietary treatments with six animals per treatment. Treatments compared were T_1 (Cowpea husk 40% + Tiger nuts chaff 0%), T_2 (Cowpea husk 30% + Tiger nuts chaff 10%), T_3 (Cowpea husk 20% + Tiger nuts chaff 20%), T_4 (Cowpea husk 10% + Tiger nuts chaff 30%), and T_5 (Cowpea husk 0% + Tiger nuts chaff 40%). The testicular morphometry and gonadal and extra-gonadal sperm reserves showed significant differences ($p<0.05$) in all the parameters observed. For testicular morphometry, the weight and length of the right testes for T_1 were highest (180 g) and (13 cm) and least for T_5 (38 g) and (7cm) respectively. A similar trend was observed for the weight and length of the left testes as 175 g and 12cm, and 39 g and 7cm respectively. Based on the result of this investigation, testicular morphometry, gonadal, and extra-gonadal sperm reserves of rams fed 40% cowpea husk and 0% tiger nuts chaff mixed ration were of the best value. However, semen from animals fed a mixed ration of cowpea husk should be further investigated to determine the impact on the fertility of breeder rams.

Keywords: Testicular histology, gonadal and extra-gonadal sperm reserve, tiger nut, cowpea husk

Description of Problem

Nutrition is a major factor in the effectiveness of reproductive function. It may affect the efficiency of related hormone production and the growth of reproductive organs. Studies have demonstrated that luteinizing hormone (LH) secretion during early gonadotropin rise is elevated and can be sustained for a longer period when pubertal cattle are fed with improved nutrition during calf-hood (1). There is a serious shortage in the supply of conventional feed ingredients such as maize, and groundnut cake for a concentrated diet for ruminants. In addition, the production of grains in developing countries is mostly for human consumption, leading to very high prices for the ingredients (2).

Supplementation with unconventional feeds is therefore helpful to reduce the harsh feed scarcity that occurs mostly during the dry season. This to a very large extent will help forestall the consequences of inadequate feed supply experienced during such periods. The utilization of unconventional feed materials that are viable, cheap, and not in high demand by humans (3) is of utmost importance in animal production.

Tiger nut (*Cyperus esculentus*) is one of the underutilized crops. Tiger nut is cultivated for its nutritive, edible nut and has a high content of soluble glucose of about 21% (4). Tiger nut has been reported to have a lot of medicinal benefits (5). Tiger nut is rich in minerals such as potassium 267.18 mg, phosphorus 158.86 mg, calcium 43.36 mg, magnesium 118.14 mg; sodium 17.02 mg; copper 0.54 mg; iron 2.82 mg, and zinc 1.39 mg (6). Tiger nut chaff is obtained after milk extraction from the nut and is discarded as waste. The tiger nut chaff is reported to have a high energy content (7). Despite the numerous nutritional, and health benefits, potentials, and prospects, tiger nut residue as a nutrient source for livestock has not been studied extensively.

Cowpea husk is another unconventional feedstuff. Cowpea (*Vigna unguiculata*) is one of the most popular legume grains in Africa. It is a member of the Vigna (peas and beans) genius. *Unguiculatais* Latin for "with a small claw", which reflects the small stalks on the flower petals (8). Most cowpeas are grown on the African continent, particularly in Nigeria and Niger, which account for 66% of world cowpea production (6). Cowpea husk: a by-product of cowpea production, is obtained after the seeds have been removed by threshing the pods and winnowing out the husks to collect the seeds. The husk is most of the time left on the farm to rot or they are burnt off. Cowpea husk contains 95.49% DM, 12.79% CP, 3.40% CF, 7.00% EE, 10.70% Ash and 41.63% NFE (9). Cowpea husk is characterized by a high level of crude fiber (about 31%) and a relatively low level of protein (12-13%) (10). Cowpea husk has been used in the diets of sheep (11) without adverse effects on the animals. Feeding a mix ration of cowpea husk and tiger nuts chaff is to ascertain if the mix has any deleterious effect on some reproductive indices of rams.

For increasing and long-term improvements in animal productivity and profitability, effective reproduction is crucial (12). Examining testicular morphometry is important to forecast not only sperm production but also the storage of sperm. Knowledge about gonadal and extragonadal sperm reserves seems essential for a careful assessment of male fertility (13). Extragonadal sperm reserves (ESR) represent sperm stored in the caput, corpus, and cauda epididymis, and the number of spermatozoa stored in the epididymis has been said to be related to sperm production by the testes (14). This study was designed to investigate the testicular morphometry, gonadal and epididymal sperm reserves and histology of the testes of Yankasa rams fed a mixed ration containing cowpea husk and tiger nuts chaff.

Materials and Methods

Experimental site

The study was conducted at the small ruminant experimental unit of the National Open University of Nigeria (NOUN), Kaduna farm located at Latitude 10.61362 N$10^0 36^1 47.692$”

Longitude 7.46852 E $7^0 28^1 23.667$” at an altitude of 612.65 meters. The climate is tropical as classified by Koppen. The wet season lasts from April through to mid-October with a peak in August; while the dry season extends from mid-October of one calendar year to April the next year (15). The spatial and temporal distribution varies, decreasing from an average of about 1,203 mm. The highest average air temperature occurs in April (28.9^{0}C) and the lowest in December (22.9^{0}C) through January (23.1^{0}C) (16). The mean atmospheric relative humidity ranges between 70% – 90% and 25% – 30 % for the rainy and dry seasons respectively (15).

Experimental Animals and Diet

Thirty (30) growing Yankasa rams aged six to eight months with an initial weight of 15.50 ± 0.67 kg were used for this study. The animals were purchased from an open livestock market in Makarfi LGA of Kaduna State. The pens and the surroundings were cleaned and disinfected with a strong antiseptic (Morigad) two weeks before the arrival of the animals. Upon arrival, the animals were administered a prophylactic treatment consisting of a long-acting antibiotic (Oxytetracycline 20% LA at 1.0 ml/10kg body weight) intramuscularly, and Ivomec injection at 200 µg/kg (0.3ml/10kg) to control endo and ectoparasites (subcutaneous). The animals were housed in their cages and allowed to adapt to the environment for 14 days before the commencement of the experiment. The cowpea husks used for the study were from the previous harvest from nearby farms that have been allowed to dry in the sun while the tiger nuts chaff used was obtained from a local processor who produces local drink, and the chaff was dried under shade.

Experimental Design and Treatment

The experimental animals were allocated to five dietary treatments with six animals per treatment in a complete randomized design:

T_1 = Cowpea husk 40% + Tiger nuts chaff 0%

T_2 = Cowpea husk 30% + Tiger nuts chaff 10%

T_3 = Cowpea husk 20% + Tiger nuts chaff 20%

T_4 = Cowpea husk 10% + Tiger nuts chaff 30%

T_5 = Cowpea husk 0% + Tiger nuts chaff 40%

Feeding and Management

The study comprised 14 days of the feed adaptation period and 13 weeks (91 days) of the measurement period. Feed was weighed and fed twice daily at 8 a.m. and 2 p.m. Water was provided *ad libitum*. The quantity of feed provided, and the residue of the previous day were weighed to determine the feed intake of each animal. The goats were weighed at the beginning of the experiment and at weekly intervals in the morning before feeding.

Chemical analysis

The feed samples of experimental diets in triplicates were collected and dried in an air-draft oven at 60^0C for 96 hours, ground separately to pass through a 1 mm sieve in a Wiley mill and sampled for chemical analysis using the standard methods of the Association of Official Analytical Chemists (17). Dry matter was determined by drying at 100^0C for 24 hours, ash concentration was determined after ignition at 550^0C for 4 hours in a muffle furnace and used to calculate organic matter (OM). Fiber fraction analysis was done by the methods of (18). Hemicellulose and cellulose were estimated as differences between neutral detergent fiber (NDF) and acid detergent fiber (ADF) and lignin, respectively.

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) in a completely randomized design using the SAS software (19) where significant differences were observed, the means were separated using the Duncan Multiple Range Test (DMRT). Polynomial (linear, quadratic, and cubic) contrasts were used to determine responses to varying levels of the mixed ration of cowpea husk and tiger nuts chaff.

Determination of Gonadal and Extra Gonadal Sperm Reserves

At the end of the experiment, three rams from each treatment were orchidectomised and the testes and epididymis were used to determine the gonadal and extragonadal (epididymal) sperm reserves, respectively, as described by (20) and (21). Six (6) testes per group were harvested and used for gonadal sperm reserves. Right, and left testes were labelled and appropriately used to determine the gonadal sperm reserves. The length and weight of each testis were determined using a measuring tape and digital weighing balance, respectively. The tunica albuginea was removed from each testis, thereafter, the left and right testes were homogenized separately using a high-speed blender operated for two minutes in 50 ml normal saline that contained 100 IU/ml of Sodium Penicillin G and 1.0 g/ml Streptomycin. The homogenate volume was measured after rinsing the blender with 20 ml of effluent. 5 ml of the homogenate was further diluted with 80 ml of normal saline in a conical flask. The diluted testicular homogenate sample

Table 1: Composition of the experimental diets (%)

Ingredient	Treatment Diets				
	T_1	T_2	T_3	T_4	T_5
Tiger nut	0.00	10.00	20.00	30.00	40.00
Cowpea husk	40.00	30.00	20.00	10.00	0.00
Sorghum stover	25.00	25.00	25.00	25.00	25.00
Wheat bran	24.00	24.00	24.00	24.00	24.00
Groundnut cake	10.00	10.00	10.00	10.00	10.00
Salt	0.50	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50	0.50
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

was stored overnight at 5°C and filtered through a gauze then filtrate volume was measured, and sperm concentration was determined using a hemocytometer as described by (20). The *caput*, *corpus*, and *cauda* epididymis were separated using sharp scissors, minced separately in 20 ml of saline, stored overnight at 5°C, and filtered through gauze, then the filtrate volume was measured. One milliliter (ml) of the filtrate was placed in a test tube and further diluted with 2 ml of normal saline. The epididymal sperm reserves were determined using a hemocytometer. Sperm reserves were expressed in billions using the procedures of (22) and (23). Testicular and epididymal weight in grams (g) was obtained using an electronic weighing scale while length was measured using a meter rule in centimeters (cm).

Results

Chemical composition of experimental diets

The chemical composition of the experimental diets is shown in Table 2. From the Table, the dry matter content of the experimental diets ranged from 895.7 g kg^{-1} DM in T_4 (10% Cowpea husk and 30% Tiger nuts chaff ration mix) to 920.1 g kg^{-1} DM in T_3 (20% Cowpea husk and 20% Tiger nuts chaff ration mix). T_1 was observed to have the lowest ash content compared to other treatments. The pattern of differences ($p<0.05$) for lignin and NDF were similar with the highest values (122 g kg^{-1} DM and 453.7 g kg^{-1} DM respectively) recorded in T_1 and lowest values (113 g kg^{-1} DM and 420.6 g kg^{-1} DM respectively) in T_5. ADF was significantly different with T_1 recording the highest value of 265.1 g kg^{-1} DM and lowest in T_5 232.0 g kg^{-1} DM. Crude protein values for dietary treatments were significant ($p <0.05$) with T_1 higher (155.1 g kg^{-1} DM), lowest in T_5 (145.1 g kg^{-1} DM), and 150.3 g kg^{-1} DM, 149.2 g kg^{-1} DM, 148.0 g kg^{-1} DM respectively compared to other treatments. A similar trend was observed with the energy content of feeds with T_1 having the highest energy of 10.68MJ and the lowest for T_5 (10.13MJ).

Effect of Tiger nuts chaff and Cowpea husk in a mixed ration on testicular morphometry of Yankasa rams

The result of Tiger nuts chaff and cowpea husk on the testicular morphometry of Yankasa rams is shown in Table 3. Significant differences ($p <0.05$) were observed among treatments. T_1 had the highest values for testicular weight, and length of the right and left testes. The weight of the right testes for T_1 was highest (180 g) and least for T_5 (38 g). A similar trend was observed for the weight of the left testes as 175 g and 39 g for T_1 and T_5, respectively. The length for the right testes was highest for T_1 (13 cm) and lowest for T_5 (7 cm), also the length for the left testicle was highest for T_1 (12 cm) and lowest for T_5 (7 cm).

Effect of Tiger Nuts Chaff and Cowpea Husk in a mixed ration on Gonadal sperm reserves ($x10^9$) of Yankasa rams

There were significant cubic effects ($p<0.005$, $p<0.044$) on the gonadal sperm reserve observed among treatments for both the left and right testes as presented in Table

Table 2: Chemical Composition of experimental diets on dry matter (DM) (g/kg DM)

Parameter	T_1	T_2	T_3	T_4	T_5	SEM
DM	904.70^{ab}	908.00^{a}	920.10^{a}	895.70^{b}	910.10^{a}	1.80
CP	155.10^{a}	150.30^{ab}	149.20^{ab}	148.00^{ab}	145.10^{b}	0.70
EE	41.00^{a}	39.40^{a}	36.70^{ab}	35.40^{ab}	34.00^{b}	0.60
Ash	68.50^{b}	71.10^{a}	71.80^{a}	72.60^{a}	73.30^{a}	0.40
ADF	265.10^{a}	244.70^{b}	240.60^{bc}	235.90^{bc}	232.00^{c}	2.60
NDF	453.70^{a}	433.60^{b}	429.00^{b}	422.00^{b}	420.60^{b}	2.70
Lignin	122.00^{a}	117.70^{b}	115.00^{b}	114.20^{b}	113.30^{b}	0.70
Energy (MJ)	10.68^{a}	10.44^{ab}	10.26^{b}	10.14^{c}	10.13^{c}	0.05

a,b,c means in the same row with different superscripts are significantly ($p< 0.05$) different, DM=Dry matter, CP=Crude Protein; EE=Ether Extract; ADF=Acid Detergent Fiber; NDF=Neutral Detergent Fiber; SEM= Standard error of mean; T_1 = 40% Cowpea husk, 0% Tiger nut residue; T_2 = 30% Cowpea husk, 10% Tiger nut residue; T_3 = 20% Cowpea husk, 20% Tiger nut residue; T_4 = 10% Cowpea husk, 30% Tiger nut residue; T_5 = 0% Cowpea husk, 40% Tiger nut residue

Table 3: Effect of Tiger nuts chaff and Cowpea husk in a mixed ration on testicular morphometry of Yankasa rams

	Treatments						P-value		
Parameter	T_1	T_2	T_3	T_4	T_5	SEM	Linear	Quadratic	Cubic
Right testicular length (cm)	13.00^{a}	10.00^{b}	7.00^{c}	8.00^{c}	7.00^{c}	1.14	0.613	0.910	0.702
Left testicular length (cm)	12.00^{a}	9.00^{b}	6.00^{c}	7.00^{c}	7.00^{c}	1.07	0.252	0.946	0.853
Right testicular weight (g)	180.00^{a}	100.00^{b}	40.00^{c}	40.00^{c}	38.00^{c}	27.72	0.691	0.413	0.425
Left testicular weight (g)	175.00^{a}	150.00^{a}	35.00^{b}	40.00^{b}	39.00^{b}	30.76	0.215	0.731	0.287

^{a,b,c} means in the same row with different superscripts are significantly ($p<0.05$) different, SEM=Standard error of mean; T_1 = 40% Cowpea husk, 0% Tiger nut residue; T_2 = 30% Cowpea husk, 10% Tiger nut residue; T_3 = 20% Cowpea husk, 20% Tiger nut residue; T_4 = 10% Cowpea husk, 30% Tiger nut residue; T_5 = 0% Cowpea husk, 40% Tiger nut residue

4. Rams in T_1 had the highest value of 2.84 x 10^9 sperms/testes and T_5 recorded the lowest value of 1.41 x 10^9 sperms/testes (right testes), and for the left testes, T_1 had a value of 2.83 x 10^9/testes and T_5 with 1.24 x 10^9/testes.

Effect of Tiger nuts chaff and Cowpea husk in a mixed ration on Extra gonadal sperm reserves of Yankasa rams

The result of extragonadal sperm reserves of Yankasa rams is shown in Table 5. A significant difference ($p < 0.05$) was observed for both left and right epididymides volume. For caput, T_1 had the highest value for both left (18 x10^6 /ml) and right (21 x10^6 /ml) while the lowest was observed in T_3 (7 x10^6 /ml and 9 x10^6 /ml) for left and right epididymides respectively. For corpus, the value for the left and right epididymides for T_1 was highest (7 x10^6/ml and 6 x10^6/ml), and lowest in T_3 (0 x10^6 /ml and 2 x10^6 /ml). For cauda, T_1 had higher values for both the left (98 x10^6 /ml) and right (103 x10^6 /ml) epididymides, and lowest in T_3 (18 x10^6 /ml and 21 x10^6 /ml) for the left and right epididymides respectively.

Table 4: Effect of Tiger nuts chaff and Cowpea husk in a mixed ration on the volume of Testicular homogenate and filtrate of Yankasa rams (mls)

	Treatments						P-value		
Parameter	T_1	T_2	T_3	T_4	T_5	SEM	Linear	Quadratic	Cubic
Right testis homogenate	100.00^a	80.00^b	35.00^c	30.00^c	40.00^c	13.93	0.117	0.381	0.041
Left testis homogenate	95.00^a	70.00^a	30.00^b	31.00^b	40.00^b	12.72	0.104	0.047	0.225
Right testis filtrate	185.00^a	158.00^b	70.00^c	60.00^c	78.00^c	11.96	0.241	0.052	0.623
Left testis filtrate	170.00^a	130.00^b	65.00^c	60.00^c	70.00^c	10.72	0.154	0.413	0.025

a,b,c means in the same row with different superscripts are significantly ($p<0.05$) different, SEM=Standard error of mean; T_1 = 40% Cowpea husk, 0% Tiger nut residue; T_2 = 30% Cowpea husk, 10% Tiger nut residue; T_3 = 20% Cowpea hus k, 20% Tiger nut residue; T_4 = 10% Cowpea husk, 30% Tiger nut residue; T_5 = 0% Cowpea husk, 40% Tiger nut residue

Table 5: Effect of Tiger nuts chaff and Cowpea husk in a mixed ration on Gonadal sperm reserves ($x10^9$)/testes of Yankasa rams

	Treatments						P-value		
Parameter	T_1	T_2	T_3	T_4	T_5	SEM	Linear	Quadratic	Cubic
Right testes	2.84^a	1.88^b	1.24^c	2.47^c	1.41^c	0.35	0.151	0.064	0.009
Left testes	2.83^a	1.87^a	1.03^b	2.03^b	1.24^b	0.32.	0.601	0.126	0.044

^{a,b,c} means in the same row with different superscripts are significantly ($p<0.05$) different, SEM=Standard error of mean; T_1 = 40% Cowpea husk, 0% Tiger nut residue; T_2 = 30% Cowpea husk, 10% Tiger nut residue; T_3 = 20% Cowpea husk, 20% Tiger nut residue; T_4 = 10% Cowpea husk, 30% Tiger nut residue; T_5 = 0% Cowpea husk, 40% Tiger nut residue

Effect of Tiger nuts chaff and Cowpea husk in a mixed ration on the volumes of Testicular homogenate and filtrate of Yankasa rams

Table 6 presents the results of Tiger nuts chaff and cowpea husk in a mixed ration of diets offered to Yankasa rams. The result indicates quadratic and cubic differences ($p<0.04$) among the homogenate volumes for both the left and right testes across treatments. For the left testes, T_1 had the highest (95 mls), and lowest in T_3 (30 mls). On the other hand, the homogenate volumes for the right testes were highest for T_1 (100 mls) and lowest observed in T_4 (30 mls). There were quadratic ($p<0.05$) and cubic ($p<0.03$) differences observed among the filtrate volumes for the right and left testis respectively. For both the right and left testicular filtrates, the highest values were observed in T_1 with 185 mls and 170 mls and the lowest values were observed in T_4 with 60 mls for both respectively.

The Photomicrograph of a section of the testis

The photomicrograph of the testes reveals degenerated seminiferous tubules for T_1, T_2, T_3, and T_4 but T_5 as presented in Plates 1 to 10, showed a normal histological appearance with aggregated luminal spermatids, well-outlined seminiferous tubule with remains of Leydig cells in between the interstitial spaces.

Discussion

Chemical composition of the diet

The crude protein content was observed to decrease as the level of cowpea husks

Table 6: Effect of Tiger nuts chaff and Cowpea husk in a mixed ration on Extra Gonadal sperm reserves of Yankasa rams (x10^6/mls)

	Treatments						P-value		
Parameter	T_1	T_2	T_3	T_4	T_5	SEM	Linear	Quadratic	Cubic
Right caput epididymides	21.00^a	19.00^a	9.00^b	15.00^{ab}	12.00^b	2.20	0.205	0.496	0.583
Left caput epididymides	18.00^a	17.00^a	7.00^b	14.00^{ab}	11.00^b	2.01	0.678	0.344	0.231
Right carpus epididymides	6.00^a	5.00^{ab}	2.00^c	4.00^b	2.00^c	0.15	0.905	0.451	0.152
Left carpus epididymides	7.00^a	4.00^{ab}	0.00	3.00^b	2.00^b	0.03	0.292	0.705	0.284
Right cauda epididymides	103.00^a	80.00^b	21.00^c	60.00^b	71.00^b	7.80	0.413	0.510	0.702
Left cauda epididymides	98.00^a	73.00^{ab}	18.00^c	62.00^b	57.00^b	4.52	0.252	0.946	0.853

^{a,b,c} means in the same row with different superscripts are significantly ($p<0.05$) different, SEM=Standard error of the mean; T_1 = 40% Cowpea husk, 0% Tiger nut residue; T_2 = 30% Cowpea husk, 10% Tiger nut residue; T_3 = 20% Cowpea husk, 20% Tiger nut residue; T_4 = 10% Cowpea husk, 30% Tiger nut residue; T_5 = 0% Cowpea husk, 40% Tiger nut residue.

decreased and the level of Tiger nuts chaff increased. The crude protein level in this study fell within the range of 139 g kg^{-1} DM to 220 g kg^{-1} DM reported by (24) who used cowpea husks to feed goats but lower than the range of 162.1 – 203.3 g kg^{-1} DM (25) and 354 g kg^{-1} DM to 383 g kg^{-1} DM (26) reported. (27) and (28) reported 102.5 and 137.3 g kg^{-1} DM which is lower than that obtained from this study. The variation observed between previous reports and results from this study could be attributed to the difference in the composition of diets offered to the experimental animals. The crude protein content in this study is above 7% CP recommended for rumen microbes of tropical livestock by (29) below which performance will be deficient. The NDF and ADF were highest at T_1 (453.7 g kg^{-1} and 265.1 g kg^{-1} DM respectively) and lowest at T_5 (420.6 g kg^{-1} and 232.0 g kg^{-1} DM) which implies that increasing the inclusion of Tiger nuts chaff decreases and the values reported from this experiment is lower than the range of 527 g kg^{-1} DM – 540.31 g kg^{-1} DM for NDF and 321.31 g kg^{-1} DM – 325 g kg^{-1} DM for ADF as reported by (25). (30) posited that NDF level of forages beyond 65% can limit feed intake. The EE obtained from this study fell within the range of 22.10 g kg^{-1} DM - 42.21g kg^{-1} DM reported by (25). The Lignin content reported from this study is higher than that reported by (25) and (26). Differences observed in the composition of the feed are attributed to the ration used as (25) used a mix of soya bean meal and *Gmelina arborea* leaves, (26) used cotton seed cake while a mixed ration of cowpea husk and tiger nuts chaff was used for this study.

Testicular histometry

According to (31) and (32), any physical testicular variables that can be measured are closely associated with the ability of the sperm to fertilize, which can be used as an assessment of sperm quality. The size of the reproductive organ and the animal's physiological health might affect the production of semen. The difference observed in the testicular weight and length among treatments may be because the varying levels of cowpea husk and tiger nut chaff had a significant effect on the testicular morphometry as observed in this study, while the testicular weight range from 38 g to 150 g.

Testicular length in this study ranged from 6 cm to 13cm. This report falls within the range reported by (33) for the testicular weight (98.7 g - 158.6 g) and the testicular length (8.2 cm - 9.3 cm) in Yankasa rams. (26) reported lower values for testicular weights but higher values for testicular lengths. The variation observed could be because different animal species and feed rations were used for the studies. (26) used Red Sokoto bucks and fed cotton seed cake while growing Yankasa rams fed a ration mix of cowpea husks and tiger nuts chaff was used for this study. The most suitable testicular parameter that indirectly leads to improving the reproductive performance of the herd, status of the reproductive organ growth, spermatogenesis, and seminal characteristics is the testicular size (34) and it has a positive and strong correlation with sperm production capacity as reported by (35) and (36). (37) reported that heavy-weight testicles generate more spermatozoa than light-weight testicles, as they possess a higher number of Sertoli cells and an excessive number of sperm. Where rams are raised for meat or other purposes aside from reproduction, varied levels of tiger nut chaff could be fed, however, for reproductive purposes, rams fed 40% cowpea husks, and 0% tiger nuts will have optimum performance as they will be sexually more viable.

Testicular homogenate and filtrate

There were significant variations observed in this study on the testicular homogenate and filtrate which does not agree with the findings of (26) and (38) who reported little variation and differences between homogenate and filtrate volumes? respectively in Red Sokoto bucks fed cotton seed cake. Yankasa rams were used for this study which could have resulted in the observed differences from previous findings because of the variation in the testicular size, shape, and mass. Additionally, variation in testicular homogenate and filtrate volume could also arise from the tissue and its water absorption rate. There is scanty information about the effect of cowpea husk and tiger nut chaff on the testicular homogenate and filtrate of ruminants. Homogenate and filtrate volumes are important indicators of testicular and epididymal sperm reserves in rams, reflecting sperm production, storage, and maturation. Changes in these volumes can impact sperm quality, concentration, and output, ultimately influencing fertility.

Gonadal sperm reserves

(26) and (39) reported lower values for left and right testes (2.76) compared to the values obtained from this study, which could be because Yankasa rams fed cowpea husk, and tiger nuts chaff was used in this study while (26) and (39) used Red Sokoto and Canarian bucks respectively. The results from this study suggest that rams fed 40% cowpea husks had higher spermatogenic action compared to the rams from other treatments. There is a paucity of information on the effect of varying levels of cowpea husks and Tiger nut chaff on gonadal sperm reserves for ruminants.

Extra gonadal sperm reserves

The results from this study do not agree with the report from (26) in Red Sokoto bucks but concur with the findings of (40) who reported that the epididymides homogenate and filtrate volumes were statistically different ($p<0.05$) in Akkararnan and Awassi rams. The 'sperm', which is the male reproductive cell, typically takes 49 days (7 weeks) to be generated. Rams' testicular structure is regarded to be a highly accurate predictor of their ability to produce sperm. The relationship between epididymides homogenate and filtrate volumes and spermatogenesis is complex and bidirectional. Changes in volume or composition can impact sperm maturation, concentration, quality, and spermatogenesis regulation, ultimately influencing fertility. The epididymis tail can be palpated to determine sperm reserves. Furthermore, a large, firm tail strongly suggests good storage, while a little, soft tail would suggest the opposite (41). It, therefore, implies that feeding up to 40% of cowpea husk supports increased production of sperm reserves which indicates better reproductive performance.

Histological study

In Plates (1 - 8) there are distorted and degenerated seminiferous tubules in rams fed varying levels of cowpea husk and tiger nuts chaff. The distorted and degenerated seminiferous tubules indicate that there is a limited production of viable sperm cells capable of fertilization which could lead to infertility in extreme cases. Where interstitial edema was observed from the histology of the testes, that could be because of a blockade in spermatogonia differentiation by testosterone thereby indirectly producing edema as also reported in rats by (42). Inflammatory responses in the testis are very uncommon and are usually seen only in response to seminiferous tubular necrosis or tissue damage sufficient to break down the Sertoli cell tight junctions that make up the blood testis barrier. There are five standard categories of inflammation: acute, suppurative, chronic, chronic-active, and granulomatous (43). Seminiferous tubule vacuoles are in most cases within or between Sertoli cells which is a common early feature of morphologic injury to Sertoli cells that may be seen before any germ cell degeneration (44). Generally, vacuoles often accompany generalized germinal cell degeneration/atrophy (43). Testicular histographs from rams fed a mixed ration of 0% cowpea husk and 40% inclusion of tiger nuts chaff, indicated the existence of well-outlined seminiferous tubules with normal histological appearance of luminal spermatids. The testicular histological changes seen in the present study may be attributed to the low levels of testosterone hormone either directly through the limited number of Leydig cells or indirectly by affecting the central nervous system and release of gonadotropin-releasing hormones (GnRH) from the pituitary as documented by (45). The male gonads' testes contain Leydig cells, which are vital. Together, these cell types support male pubertal sexual traits, oversee hormone balance, and uphold spermatogenesis (46).

Conclusion and Applications

1. The testicular morphometry, gonadal, and extragonadal sperm

reserves gave a better output with a ration mix of 40% cowpea husk and 0% tiger nuts chaff.

2. Semen from animals fed a mixed ration of cowpea husk and tiger nuts chaff should be further investigated to determine the impact of the mixed ration on the fertility of breeder rams.

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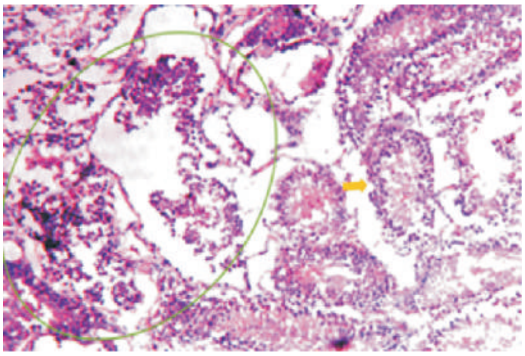

Plate 1: Testicular section (Right) of Yankasa Rams in group T1. The section is showing numerous inflammatory cells (circle) with degenerated and distorted Seminiferous Tubules with what appears to be the remains of spermatids (arrowhead). H&E x250

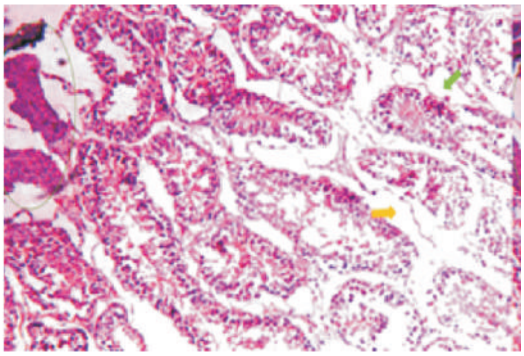

Plate 2: Testicular section (Left) of Yankasa Rams in group T1. The section shows mostly degenerated Seminiferous Tubules (arrowheads) with the complete destruction of some of the spermatozoa (circle). H&E x250

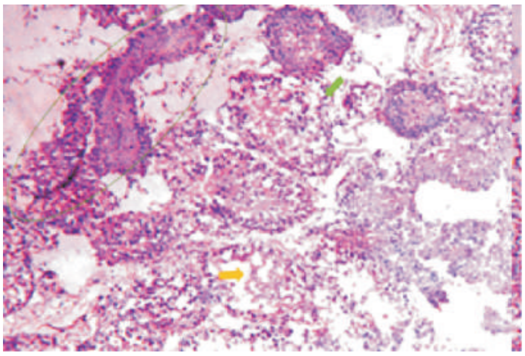

Plate 3: Testicular section (right) of Yankasa Rams in group T2. The section shows mostly degenerated and vacuolated Seminiferous Tubules (arrowheads) with the complete destruction of some of the spermatozoa (circle). H&E x250

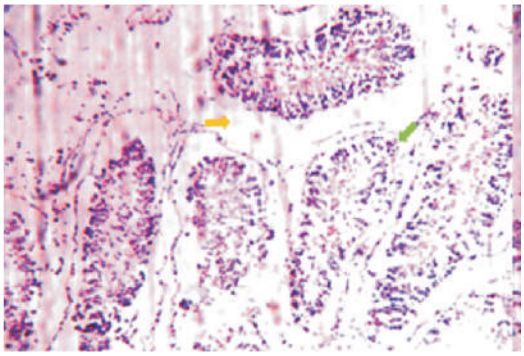

Plate 4: Testicular section (left) of Yankasa Rams in group T2. The section presented ghost Seminiferous Tubules (arrowheads) with the complete destruction of most of the spermatozoa (green arrow) and interstitial

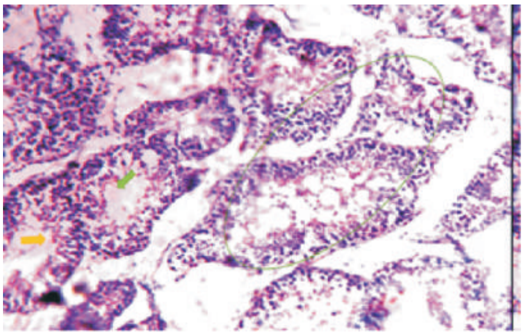

Plate 5: Testicular section (right) of Yankasa Rams in group T3. The section is presenting degenerated (arrowheads) and vacuolated Seminiferous Tubules (circle). H&E x250

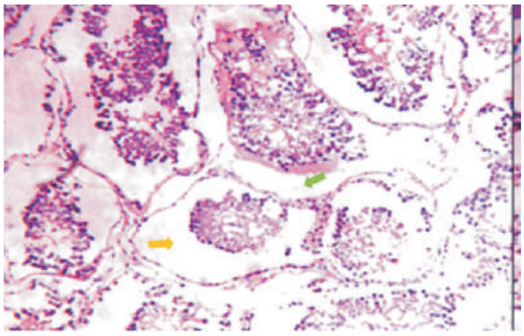

Plate 6: Testicular section (left) of Yankasa Rams in group T3. The section is marked with extensive degenerated Seminiferous Tubules with an appearance of interstitial edema (arrowhead). H&E x250

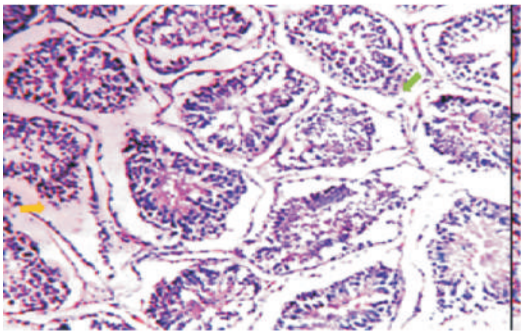

Plate 7: Testicular section (right) of Yankasa Rams in group T4. Most of the Seminiferous Tubules and the spermatozoa appeared degenerated. Note the appearance of interstitial edema (arrowhead). H&E x250

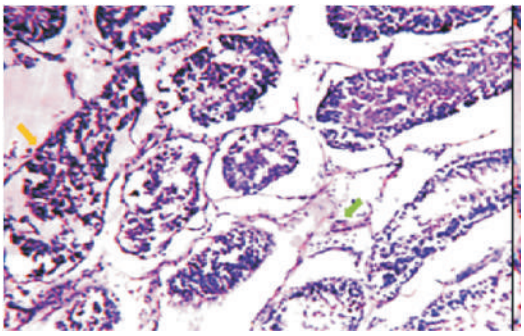

Plate 8: Testicular section (left) of Yankasa Rams in group T4. The section is characterized by degenerated and disoriented spermatozoa and Seminiferous Tubules (arrowheads). The Leydig cells (green arrow) appear preserved. H&E x250

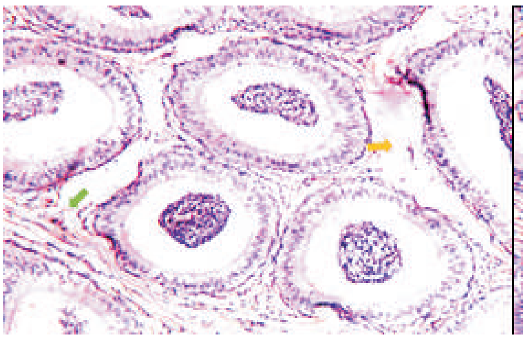

Plate 9: Testicular section (right) of Yankasa Rams in group T5, showing normal histological appearance with aggregated luminal spermatids. The seminiferous tubule is well outlined with remains of Leydig cells (arrow) in between the interstitial spaces. H&E x250

Plate 10: Testicular section (left) of Yankasa Rams in group T5, showing normal histological appearance with aggregated luminal spermatids. The seminiferous tubule is well outlined with remains of Leydig cells (arrow) in between the interstitial spaces. H&E x250

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