Semen Characteristics and Testiculo-Epididymal Histology of Red Sokoto Bucks Fed Whole Cottonseed and Cottonseed Cake

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Abstract

This study evaluated the effect of cotton products (whole cottonseed and cottonseed cake) on semen characteristics and testiculo-epididymal histology of Red Sokoto bucks. A total of twenty-four (24) healthy red Sokoto bucks were used and randomly allocated to six dietary treatments of four animals (n=4) per group in a completely randomized design. The treatments contained 10, 20 and 30% inclusion levels of either Whole cottonseed (WCS) or Cottonseed cake (CSC) in the diets fed to the bucks over a period of 98 days. Semen obtained via electroejaculator was analyzed; semen parameters assessed include pH, volume, concentration, motility, live-dead ratio and sperm morphological abnormalities. Testes and epididymides were excised and processed routinely for histological evaluation. Spermatozoa motility, concentration and live to dead ratio decreased significantly with increasing WCS compared to the increasing trend observed in bucks fed CSC diets. Testicular histopathological lesions of moderate to severe seminiferous tubular depletion, reduced germ cell layers, emptied luminal contents and disrupted interstitial tissue in the bucks fed 20 and 30% of WCS compared to their others fed CSC diets. Also, epididymal parenchyma were remarkably distorted in bucks fed 30% WCS relative to CSC groups. In conclusion, this study has demonstrated that the higher inclusion levels of cotton products up to 30% had deleterious effect on semen characteristics and testiculo-epididymal histomorphology. Hence, farmers are encouraged to be cautious in the inclusion of cotton products in the buck's feed.

Keywords: whole cotton seed, cotton seed cake, testes, epididymides, histology, sperm quality

Caractéristiques du sperme et histologie Testiculo-Épididymaire des mâles Red Sokoto nourris de graines de coton entières et de tourteaux de graines de coton

Résumé

Cette étude a évalué l'effet des produits de coton (graines de coton entières et tourteaux de coton) sur les caractéristiques du sperme et l'histologie testiculo-épididymaire des boucs Red Sokoto. Un total de vingt-quatre (24) mâles Red Sokoto en bonne santé ont été utilisés et répartis au hasard dans six traitements alimentaires de quatre animaux (n = 4) par groupe dans une conception entièrement randomisée. Les traitements contenaient des niveaux d'inclusion de 10, 20 et 30 % de graines de coton entières (GCE) ou de tourteaux de graines de coton (TGC) dans les régimes alimentaires des boucs sur une période de 98 jours. Le sperme obtenu par électroéjaculateur a été analysé ; les paramètres du sperme évalués comprennent le pH, le volume, la concentration, la motilité, le rapport morts-vivants et les anomalies morphologiques du sperme. Les testicules et les épididymes ont été excisés et traités en routine pour une évaluation histologique. La motilité, la concentration et le rapport vivants/morts des spermatozoïdes ont diminué de manière significative avec l'augmentation du GCE par rapport à la tendance à la hausse observée chez les boucs nourris avec des régimes TGC. Lésions histopathologiques testiculaires de déplétion tubulaire séminifère modérée à sévère, couches de cellules germinales réduites, contenu luminal vidé et tissu interstitiel perturbé chez les boucs nourris avec 20 et 30% de GCE par rapport à leurs autres nourris avec

des régimes TGC. De plus, le parenchyme épididymaire était remarquablement déformé chez les boucs nourris avec 30 % de GCE par rapport aux groupes TGC. En conclusion, cette étude a démontré que les niveaux d'inclusion plus élevés des produits en coton jusqu'à 30% avaient un effet délétère sur les caractéristiques du sperme et l'histomorphologie testiculo-épididymaire. Par conséquent, les agriculteurs sont encouragés à être prudents dans l'inclusion de produits en coton dans l'alimentation du bouc.

Mots-clés : graine entière de coton, tourteau de coton, testicules, épididymes, histologie, qualité du sperme

Introduction

Whole Cotton seed (WCS) is a cheap by-product of cotton industry known for over a century as supplement in livestock feedstuff (Solaimon, 2007). Ruminant animals are believed to handle cotton seed diets very well because of the presence of the rumen which aids in detoxifying gossypol. The rumen environment promotes binding of free gossypol to proteins which render it physiologically inactive (Tiago do Prado *et al.,* 2016). It is a good source of protein for ruminants. cotton seed cake is another product derived by extracting oil from the cotton seed and has been recognized to be an excellent source of high amount of protein (40-46%) and energy required in the ration of domestic animals (Kenfack *et al*., 2015).

Despite the numerous nutritional benefits of cotton seed products use in domestic animal ration, its exclusive use is still being retarded by the toxicity level of the antinutritional substance, gossypol. The latter has been established to elicit severe reproductive pathologies in monogastric animals because of their inability to detoxify and impede the absorption of free gossypol by converting it into bound gossypol which is achievable only in the rumen of polygastric animals (Mena *et al*., 2004). In spite of this gossypol tolerance by ruminants, quite a number of studies with ruminants have revealed a range of variety of results, ranging from moderate (Chase *et al*., 1994) to severe pathological effects (Randel *et al*., 1992; Chenoweth *et al*., 1994). A good example of this effect is in the whole cottonseed fed to deer manifested as an effective male contraceptive as evidenced by low semen quality during both treatment and full recovery duration phases (Gizejewski *et al*., 2008).

The high cost of other protein sources used in livestock feed occasioned by man's competition in their consumption greatly favours the substitution of cotton seed cake in animal feed preparation (Kenfack *et al*., 2015). This study aimed at assessing the comparative effects of dietary levels of cotton products (whole cotton seed and cotton seed cake) inclusions on semen profiles and histo-architecture of reproductive organs (testes and epididymides) of red Sokoto bucks.

Materials and methods

Location of Experimental Site

The study was carried out at the Teaching and Research Farm, Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Kaduna state, Nigeria. Zaria is located on latitude $11^0$33'N and longitude $7^0$42'E. Annual rainfall in this area ranges from 1102 to 1904mm and lasts from late April or early May to mid-October. The site is situated within the Northern-Guinea Savannah zone of Nigeria at an altitude of 610 mm above sea level (Ovimaps, 2014).

Experimental Animals and their Management

Twenty-four (24) Red Sokoto goats bucks of about 6-8 months of age were sourced from Zaria metropolis. They were dewormed using albendazole oral tablets (Centre-Alben®- Aether Centre Biology Co., Ltd. Beijing) at the rate of 5 mg/kg body weight and also administered

tetracycline long acting-TLA (oxytetracycline dehydrate) 20% weight/volume injectable solution intramuscularly at the dosage of 0.1 mg/10 kg live weight against possible bacterial infection. Each buck was kept separately in individual crates. The animals were quarantined for a period of 3 weeks and then followed up with additional 2 weeks of stabilization period before the commencement of the study.

Experimental Diets and Design

The experimental diets containing 14% CP were fed to the bucks at 3% per kg body weight every morning with clean fresh water provided *ad libitum.* At the end of the adjustment period, the bucks were randomly allocated to six sub dietary groups of four animals (n=4) per group. Each group constituted a treatment and each buck within a group as a replicate in a completely randomized design. The treatments consist of either whole cotton seed (WCS) included at 10, 20 and 30% or cottonseed cake (CSC) included at 10, 20, and 30% levels (Table 1) in the diets fed to the bucks over a period of 98 days.

Table 1: Feed composition of Whole Cotton Seed and Cottonseed Cake diets fed to the bucks

Ingredients	Inclusion levels of whole cottonseed and cottonseed cake (%)					
	Whole Cotton Seeds			Cotton Seed Cake		
	10	20	30	10	20	30
Maize offal	20	15	10	23	21	19
WCS	10	20	30	-	-	-
CSC	-	-	-	10	20	30
BDG	26	21	16	23	15	7
Rice offal	40	40	40	40	40	40
Molasses	2	2	2	2	2	2
Bone meal	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100

WCS= Whole Cottonseed, CSC=Cottonseed cake, BDG= Brewers dried grain

Scrotal Circumference

The scrotal circumference was measured at the widest point, that is equidistant from the scrotal poles using a non-stretchable measuring tape.

Semen Collection and Evaluation

Semen samples was collected from bucks using an electro-ejaculator (Lane Manufacturing Inc. Kingstone, UK) every two weeks (fortnightly) between 9:00 am and 10:00 am during the study period into calibrated test-tubes. The ejaculates were examined for volume, colour, concentration, gross motility, live and dead sperm, pH, total sperm output and sperm morphological abnormalities. The volume of the ejaculates was measured by reading the semen level off the calibrated test-tubes. The colour of the ejaculates was observed visually immediately after collection as an indication of the semen density and the possibility of semen contamination.

Determination of pH

The pH of the semen samples was measured immediately after collection using pH paper (Bbr Chemocraft®) India. One inch of the paper was inserted into the semen for five seconds and removed to properly air dry. The colour change observed after drying was matched to the colour chart on the pH paper. The corresponding pH number for the colour on the chart was recorded as the pH of the sample.

Semen colour

Semen colour was determined at the point of collection. It was read of the collection test tube and graded as follows: 1-Watery, 2-Milky, and 3-Creamy.

Sperm motility

Sperm motility was determined immediately after semen collection from the bucks. This was done by placing a drop of semen on a warm glass slide maintained at 37°C with cover slide and observed under a microscope at x400 magnification. Gross motility was estimated as percentage score, using the 5-point numerical score (0 = very poor to 5 = excellent motility) according to the procedure of Rekwot *et al.* (1997).

Sperm Concentration

The concentration of the spermatozoa was determined using a haemocytometer according to the procedure outlined by Azawi and Ismaheel (2011). Diluted semen (dilution factor of 100) was placed on Neubauer haemocytometer (Rymet Scientific Industries®, India) and spermatozoa were subsequently counted in five squares of one chamber.

Live-Dead (Livability) Ratio

The percentage live and dead sperm cells were estimated by using Zemjanis (1977) methods. A drop of semen was placed on a well labelled warm glass slide, mixed with a drop of warm Eosin-Nigrosin stain and then observed under the microscope at x40 objective. Live sperm cells appeared as clear cells due to failure to absorb the stain; however, dead spermatozoa absorbed the stain and appeared purplish in colour. The live and dead sperm cells were separately counted from a total of 400 spermatozoa in smears stained with Eosin- Nigrosin and the ratio was determined according to the method of Zemjanis (1977).

Sperm morphology

On a clean, warm glass slide, a drop of semen was placed as well as two drops of Wells and Awa stain. The semen and stain were thoroughly mixed together with a smear made on another clean and warm slide. The smear was air-dried and observed using the light microscope starting with low power to high magnification. The presence of abnormal cells out of at least 400 sperm cells from several fields on the slide was counted and their total percentage was estimated.

Animal Sacrifice and Organ Excision

At the end of the experiment, the bucks were sacrificed. The scrotum and its adjoining structures were incised to access the testes and epididymis. The organs were then excised and placed in 10% formal saline for tissue processing.

Histopathological evaluation of Testicular and Epididymal Tissues

The tissue processing procedures described by Zahid *et al*. (2003) was adopted. The excised testicles and epididymides were trimmed and about 10×10×5 mm size was taken from the testicular centre or equator and from the caudal epididymis. The trimmed tissues were further placed in 10% formal saline for about seven days for proper fixation. The fixed tissues were subsequently dehydrated in ascending grades of alcohol (70%, 90%, 100%), cleared in xylene, embedded in paraffin blocks and sectioned at 5 microns thickness. Each section was stained with hematoxylin and eosin using standard staining procedures (Luna, 1968). Each slide was examined under light microscope (x250 for testis and x100 for epididymis).

Statistical Analysis

Data obtained on semen characteristics were analysed using GraphPad Prism Statistical Package, Version 4.00 for Window (GraphPad Software Incorporated, California, USA). The variations in the data were compared using analysis of variance (ANOVA) and level of significance was set at $p<0.05$. Pair-wise difference test was used to separate significant differences between the means of each group.

Results

Effect of dietary levels of WCS and CSC on Scrotal Circumference and Semen Quality of Bucks

The result of the effect of cotton seed products levels on scrotal circumference and semen quality of Red Sokoto bucks is presented in Table 2. Semen volume, pH, and sperm morphology

parameters were non-significantly (P>0.05) affected by cotton seed product levels.

Scrotal circumference

Scrotal circumference differed significantly (P<0.01) such that bucks fed WCS had decreasing scrotal circumference with increased levels of WCS while there was a decrease in scrotal circumference of bucks fed 20% CSC compared to those fed 10% CSC and 30% CSC.

Semen colour

Semen colour differed significantly (P<0.01) among the bucks fed WCS which had semen colour that ranged from milky on 10 and 20% inclusion levels to watery on 30% inclusion level. The bucks fed CSC on the other hand, had improved semen colour that ranged from milky to creamy as more cottonseed cake was added to their diets.

Spermatozoa motility

Sperm motility decreased with increase in level of whole cotton seed in the diet, being significantly (P<0.01) lower on 30% inclusion. However, sperm motility was significantly (P<0.01) lower on 20% inclusion of cottonseed cake than 10 and 30% levels of inclusion. Sperm motility ranged from 54.44% to 84.17% in this study.

Sperm concentration and live-dead ratio

Sperm concentration decreased as the level of WCS increased in the diet, being significantly (P<0.01) lower on 30% WCS than the control and 20%WCS, ranging from 289.11 to 143.39×10^6 spermatozoa. For the CSC group, sperm concentration was similar for all the levels of inclusion and the control. Sperm concentration ranged from 235.89 to 292.83×10^6 spermatozoa.

Table 2: Effect of cottonseed products on scrotal circumference, semen characteristics and sperm morphology of Red Sokoto bucks

	WCS (%)			CSC (%)				
Parameter	**10**	**20**	**30**	**10**	**20**	**30**	**SEM**	**P-value**
Scrotal circumference (cm)	15.41^{b}	14.76^{b}	14.32^{b}	16.12^{ab}	14.01^{b}	18.21^{a}	1.26	0.003
Semen colour	2.28^{ab}	2.17^{ab}	1.44^{b}	2.17^{ab}	1.89^{ab}	2.50^{a}	0.5	0.001
Semen Volume (ml)	0.20	0.21	0.17	0.23	0.22	0.25	0.13	0.83
Sperm Motility (%)	82.78^{ab}	80.00^{ab}	54.44^{c}	70.00^{abc}	67.78^{bc}	84.17^{a}	8.1	0.0003
Sperm Concentration (×10^6)	289.11^{a}	269.28^{a}	143.39^{b}	235.89^{a}	220.00^{ab}	292.83^{a}	43.17	0.004
pH	7.06	7.11	7.33	7.33	7.28	7.06	2.83	0.06
Live Spermatozoa (%)	80.56^{a}	78.61^{a}	61.39^{b}	73.33^{ab}	67.22^{ab}	79.17^{a}	7.43	0.001
Sperm. Morp. (%)								
Normal Cells	67.78	64.72	67.89	67.17	56.56	69.50	6.55	0.19
Detached Head	12.22	12.56	14.06	11.00	13.44	15.61	3.08	0.51
Coiled Tail	6.72	5.11	6.50	5.44	6.72	5.06	1.87	0.09
Bent Tail	5.17	5.72	6.06	4.78	5.67	5.06	1.79	0.79
Free Tail	5.39	7.06	6.11	5.78	6.22	5.89	1.38	0.55
Mid piece defect	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.37
Cyto. Droplet	1.11	1.39	1.00	0.56	0.72	0.89	0.50	0.34
Dag defect	2.44	1.94	1.22	2.06	2.00	2.39	0.89	0.09
Stunted tail	1.83	1.28	1.89	1.94	1.67	2.22	0.86	0.92

Means within rows with different superscripts are significantly different (P<0.05), SEM - Standard error of means, LOS - Level of significance, Semen colour: 1-Watery, 2-Milky, 3-Creamy, Scro. Circum – Scrotal circumference, Cyto. Droplet – Cytoplasmic droplet.

Testicular and Epididymal Histology

Testes

The testicular histological results of Red Sokoto bucks fed different inclusion levels of either Whole cottonseed (WCS) or Cottonseed cake (CSC) diets are presented in Plate 1 A-F. With respect to bucks fed with varying inclusion levels of WCS diet, testicular histoarchitecture of bucks

fed 10% WCS was devoid of histopathological lesions (Plate 1 A). However, in bucks fed 20 and 30% WCS, there was moderate to severe testicular histo architectural alterations such as seminiferous tubular depletion, reduced germ cell layers, empty luminal contents and disrupted interstitial tissue (Plate 1 C and E). Similarly, bucks fed the 10% cottonseed cake (CSC) diets, shared similar normal histological appearance observed for its counterpart 10% WCS (Plate 1 B). Regarding the testicular histology of bucks fed 20 and 30% of CSC diets, moderately visible lesions (germ cell depletion with clumps of luminal residual bodies, reduced germinal epithelial height and empty luminal content) were observed in their parenchyma (Plate 1 D and F)

Epididymides

The results of epididymal histological appearance of Red Sokoto bucks fed different inclusion levels of either Whole cottonseed (WCS) or Cottonseed cake (CSC) diets are contained in Plate 2 A-F. Concerning the epididymis of bucks fed with varied levels of WSC diet, both 10% and 20% WCS diets seemed not to precipitate alteration in the epididymal parenchyma (Plate 2 A and C). Though, the epididymal duct luminal spermatozoa were reduced in bucks fed with 20% WSC diet compared to those on 10% WSC. On the contrary, the epididymis of bucks fed 30% WSC diet was observed to have severe sloughing off of the ductal epithelial lining, moderate depletion of the epididymal interstitium and markedly emptied ductal lumen (Plate 2 E). On the epididymis of bucks fed CSC diets, 10% and 20% inclusion level diets shared similar histological appearance observed for those on WCS diets (Plate 2 B and D). However, bucks fed 30% CSC diet was observed to have empty ductal luminal content but morphological intact parenchyma (Plate 2 F).

Discussion

The scrotal circumference, an important gross testicular biometric parameter, has been established to provide an indirect measurement of testicular size, volume and even onset of active spermatogenesis (Bongso *et al.,* 1982). It is also known to have a high correlation with sperm production, service capacity and sexual development (Yarney *et al*., 1990). Therefore, the variation (decrease with percentage inclusions in WCS and increase with CSC inclusions) in scrotal circumference of bucks fed cotton seed products could have stemmed from the ongoing progressive testicular degeneration more particularly in the bucks fed with whole cotton seed. While for those with increased scrotal circumference,that is bucks fed with varying inclusions of cotton seed cake were adequately furnished with their morpho-functional needs. The general implication of this is that sperm production, service capacity and sexual development will be greatly impaired in bucks fed with whole cotton seed compared to those on CSC diets. This finding on cotton seed products influence on scrotal circumference wholly concurs with the reports of Andreazzi *et al*. (1995) on bucks and Babashani *et al.* (2014) on Yankasa rams.

Semen from males contains some abnormally formed spermatozoa. Usually, sperm abnormalities or morphological deviation from the normal structure in the sperm cells, indicate problem during spermatogenesis and this could be attributed to age, nutrition, gossypol content in feedstuff and pollution. It has been suggested that ejaculated semen containing some morphologically abnormal spermatozoa could not be associated with lowered fertility until the proportion of abnormal spermatozoa exceeds 20% (Hafez, 1993), although certain types of abnormalities may not be associated with infertility (Singh *et al*., 2012). In the light of these, the observed non-significant difference in the sperm abnormalities of the bucks fed different types of cotton seed products implies that the breeding soundness of all the category of bucks

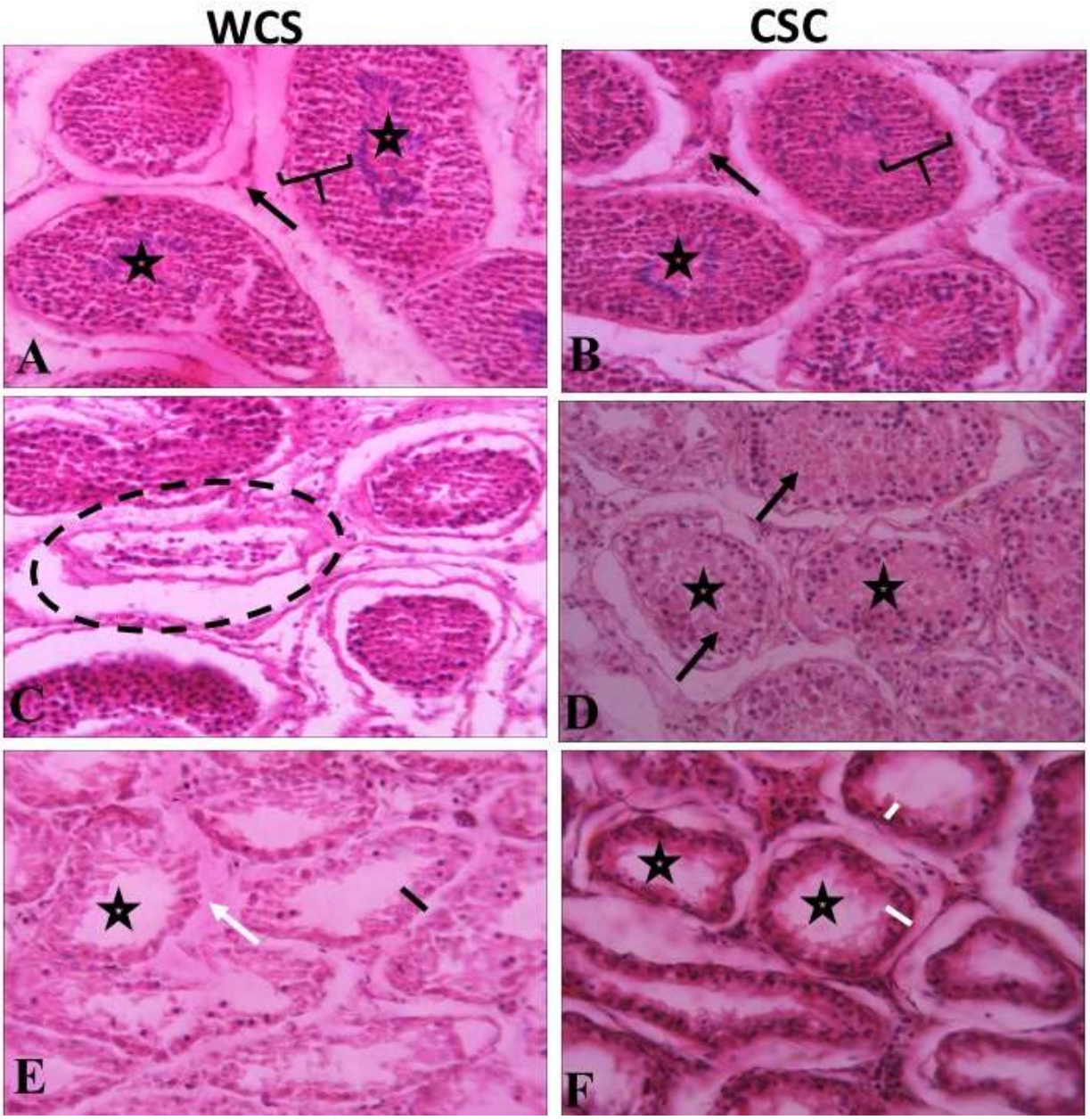

Plate 1 A-F. Light micrographs of the testes of Red Sokoto buck fed on different inclusion levels of either Whole cottonseed (WCS) or Cottonseed cake (CSC) diets. **A. 10% WCS, B. 10% CSC:** Testicular histoarchitectures were devoid of visible lesion and characterised by the presence of intact seminiferous tubular germinal epithelia containing spermatogonia, spermatocytes and spermatid (brace), lumen filled with spermatozoa (star) and distinct interstitial tissue (black arrow) **C. 20% WCS:** Moderate seminiferous tubular depletion (circle-like) **D. 20% CSC:** Moderate seminiferous tubular germ cell depletion (black arrow) with clumps of residual bodies filling the seminiferous tubular lumen (star) **E. 30% WCS:** Seminiferous tubules bear markedly reduced germinal epithelial height (black line), empty luminal contents (star) and severely depleted seminiferous tubular interstitial tissues (white arrow). **F. 30% CSC:** with the exception of markedly reduced germinal epithelial height and empty luminal content, testicular parenchyma appeared normal. **Magnification:** X250; **Stain:** Haematoxylin and Eosin

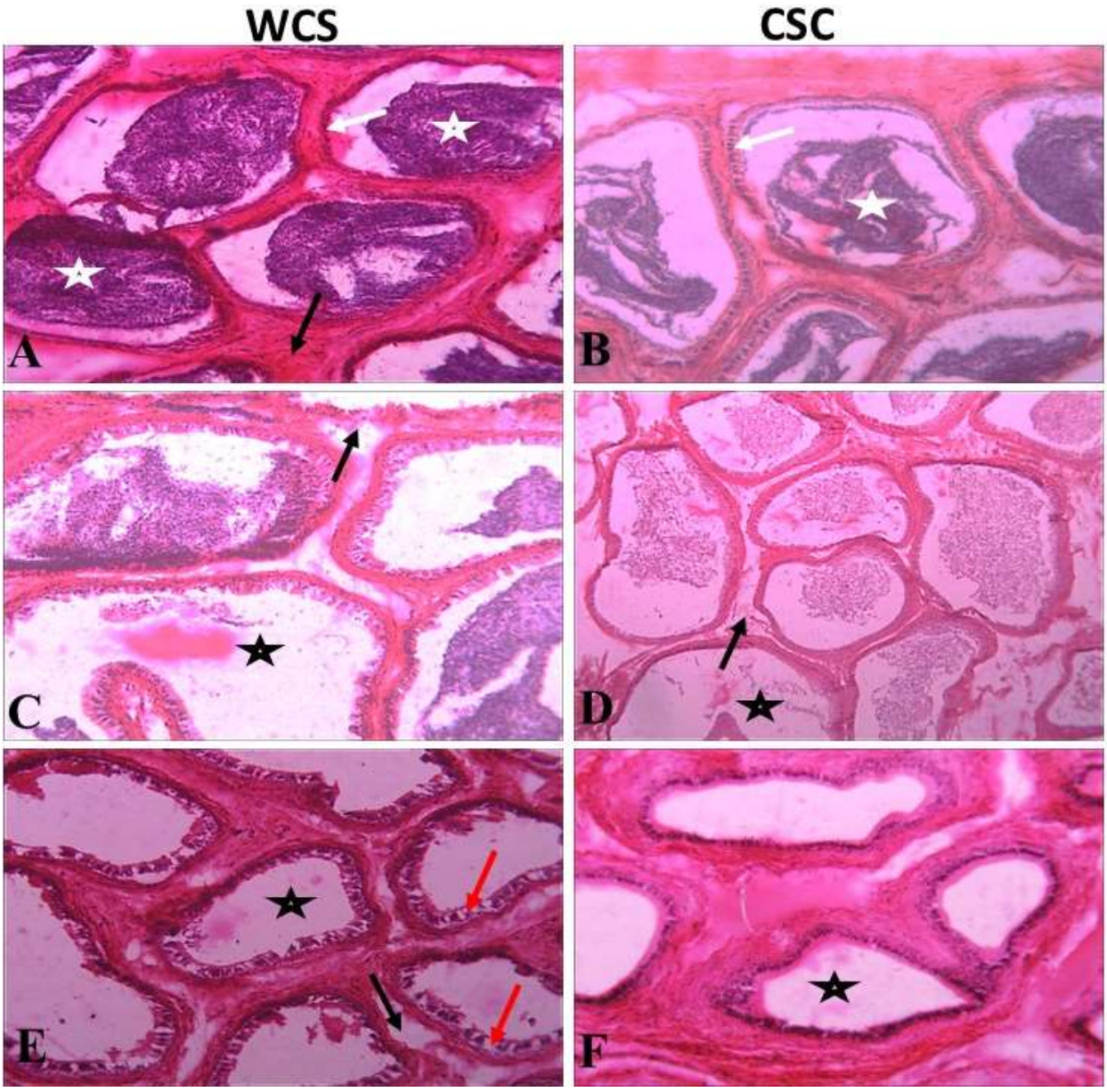

Plate 2 A-F. Plate 1 A-F. Light micrographs of the epididymides of Red Sokoto buck fed on different inclusion levels of either Whole cottonseed (WCS) or Cottonseed cake (CSC) diets. **A. 10% WCS, B. 10% CSC:** Epididymal parenchyma appeared normal without lesion and typified by the presence of intact pseudostratified columnar epithelium with stereocilia (white arrow), substantial presence of spermatozoa within the epididymal ductal lumen (star) and distinct epididymal interstitium (black arrow). **C. 20% WCS, D. 20% CSC:** There is moderate depletion of spermatozoa within epididymal ducts (star) **E. 30% WCS:** Severe sloughing off of the ductal epithelial lining (red arrow), moderate depletion of the epididymal interstitium (black arrow) and markedly empty ductal lumen (star). **F. 30% CSC:** Apart from the empty ductal luminal content (star), epididymal parenchyma appeared normal. **Magnification:** X100; **Stain:** Haematoxylin and Eosin.

might not be remarkably affected since the range of abnormalities recorded is fairly within normal range. Gossypol did not affect semen quality at puberty indicating that the relationship between high gossypol concentration and severe seminal pathologies in animals is not clear (Tiago do Prado *et al.,* 2016).

The observed non-significant difference in the ejaculate volume of bucks fed with different percentage inclusions of cotton seed products suggests that the diet does not elicit evident effect on the ejaculate volume. This observation agrees with numerous reports from other studies - Zahid *et al.* (2003) on Teddy male goats fed 1750 mg/kg and 2370 mg/kg of gossypol, Smith *et al.* (1991) on Holstein bulls fed 64 and 75 mg/kg body weight of free gossypol, Akhtar (1997) on Nili-Ravi buffalo bulls fed cottonseed meal with 1.68 g/kg of gossypol and Babashani *et al.* (2014) on Yankasa rams fed 48% of cotton seed products (whole cotton seed and cotton seed cake). These researchers also did not observe significant effect of cotton seed products or gossypol level on semen volume.

The live spermatozoa's percentage motility is positively correlate with the fertilizing capability of sperm cells (Oyeyemi and Ubiogoro, 2005). In this study, live and dead sperm were similar for the cotton products. However, the observed decreased sperm motility seen with 30% whole cotton seed diet indicates that the nutritional composition of the diets, more particularly the free gossypol contents, did not enhance sperm maturation process and in turn leads to poor spermatozoa fertilizing capacity. This coupled with the moderate seminiferous tubular depletion on 20% WCS and the markedly reduced germinal epithelial height, empty luminal contents and severely depleted seminiferous tubular interstitial tissues on 30% WCS, buttresses the deleterious effect of gossypol on spermatogenesis. This detrimental effect of gossypol on spermatozoa motility has earlier been linked to the induction of mitochondrial specific injury to the sperm tail through the inhibition of redox-cycling by electron transfer (Kovacic, 2003). On the other hand, the significant increased sperm motility displayed by bucks fed with increasing cotton seed cake diet reflects that the diet composition favours spermatozoa maturation process by maintaining the mitochondrial population within the sperm tail and hence, culminates in an excellent fertilizing ability. Effect of gossypol on the male reproductive system, results in impaired spermatogenesis and reduced sperm motility (Tiago do Prado *et al.,* 2016).

The sperm livability (live-dead) ratio and concentration profiles observed in this study follow similar trends stated above for motility. These two parameters are among the most important parameters to be considered when evaluating sperm qualities in a reproductively active mammalian male subject (Malmgren, 1997; Tabatabaei *et al.,* 2009). Thus, the presence of increased sperm livability ratio and concentration in bucks fed with increasing inclusion percentage of cotton seed cake still buttress the ability of the diets in the maintenance of normal semen characteristics. While the recorded reduced profiles of these two parameters in bucks fed on whole seed cake could again be associated with the anti-nutritive component within it, the gossypol. These findings on spermatozoa live-dead ratio and concentration corroborate the reports of El-Mokadem *et al.* (2012) in rams and Velasquez-Periera *et al.* (1998) in bulls treated with gossypol. It however contrasts the observation of non-significant effect observed by Zahid *et al.* (2003) in gossypol treated male goats.

The morphological integrity of the testes and epididymides were remarkably maintained in the bucks fed on lower levels (10 and 20%) of cotton seed products (whole cotton seed and cotton seed cake) inclusions. This could be suggestive of the reproductive safeness of the cotton seed products as viable alternative dietary supplements. These findings corroborate the report of absence of deleterious effects of cotton seed meal on the morphophysiology of the male reproductive organs of ram and buck (Nunes *et al.,* 2010; Guedes and Soto-Blanco, 2010; Kenfack *et al.,* 2015).

However, the moderate to severe testicular histo-architectural alterations (seminiferous tubular

germ cell depletion, reduced germ cell layers, empty luminal contents and disrupted interstitial tissue) observed in bucks fed with 20 and 30 percent inclusions of cotton seed products diets might be connected with the corresponding increase in free gossypol content within ruminal compartments with increased percentage inclusions in the diets used in this study. In spite of the recognized ruminant tolerability of gossypol laden diets, one could not have expected testicular lesions but Paim *et al.* (2016) had earlier postulated that the possible reproductive toxicity that could ensue from the permeability of reproductive organs to free gossypol varies with species, age and metabolic rate of the exposed animals. The histo-architectural changes observed in this study is consistent with changes reported in bulls by Arshami and Ruttle (1988), in rams by Babashani *et al.* (2014) and in bucks by Itodo *et al.* (2021). The morpho-functional implication of the testicular lesions stated above is in the potential arrest of spermatogenetic sequence and possibility of interference with the production of male gametes and sexual steroid (testosterone) by interstitial Leydig cells.

Feeding cotton seed products at 30% impaired the integrity of the epididymis in the bucks. This observed trend further validates the histo-architectural disruption pattern recorded for testes in this study. The presence of epididymal epithelia lining sloughed off cells, depletion of the epididymal interstitium and markedly emptied ductal lumen more remarkably, in the whole cotton seed groups, portends a serious disruption in the epididymal functional roles of sperm maturation and storage. The epididymal histological lesions seen in this study tallies with those reported by Nagalakshmi *et al*. (2000) in male lambs.

Conclusion

This study has demonstrated that feeding up 30% of cotton products to bucks particularly the whole cottonseed elicited deleterious histological alterations in the parenchyma of both the testes and epididymides as well as deranged sperm characteristics. The perturbed semen characteristics and morphological integrity of these organs could as well impair their functional roles via the arrest testicular spermatogenesis and epididymal sperm maturation, storage and transport. Hence, farmers are encouraged to be cautious in the inclusion of cotton products in buck's feed.

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References

Akhtar, N. 1997. Effect of cottonseed (gossypol) on the reproductive performance of male and female buffaloes.PhD. Thesis, University of Agriculture, Faisalbad, Pakistan.

Andreazzi, M. A., Moraes, G. V., Santos, G. T., Martins, E. N., Macedo, F. A. F., & Reis, A. C. F. 1995. Effect of cottonseed on the development and reproductive performance of male goats. *Arquivos de Biologia e Tecnologia*, 38(3):799-813

Arshami, J., and Ruttle, J. L. 1988. Effects of diets containing gossypol on spermatogenic tissue of young bulls. *Theriogenology,* 30:507-516.

Azawi, O. I., and Ismaeel, M. A. 2011. Effects of seasons on some semen parameters and bacterial contamination of Awassi ram semen. Reproduction in Domestic Animals, 47(3);403-406.

Babashani, M., Lawal, M., Njoku, C.O., Ate, I. U., and Rekwot, P.I 2014. Effects of dietary Gossypol on testicular histology and

ultrasonograms of Yankasa rams. *J. Vet. Adv.* 4 (7): 616-622.

Bongso, T.A., Jainudeen, M.R., and Zahrah, A. S. 1982. Relationship of scrotal circumference to age, body weight and onset of spermatogenesis in goats. *Theriogenology* 18 (5): 513-524

Chase, C. C. Jr., Bastidas, P., Ruttle, J.L., Long, C.R., and Randel, R.D. 1994. Growth and reproductive development in Brahman bulls fed diets containing gossypol. *Journal of Animal Science*, 72: 445-452.

Chenoweth, P.J., Risco, C.A., Larsen, R.E., Velez, J., Shaw, N., Chase, C.C., and Tran, T. 1994. Effects of dietary gossypol on aspects of semen quality, sperm morphology and sperm production in young Brahman bulls. *Theriogenology*, 42:1-13.

El-Mokadem, M.Y., Taha, T.A., Samak, M.A., and Yassen, M.A. 2012. Alleviation of reproductive toxicity of gossypol using selenium supplementation in rams. *Journal of Animal Science,* 90(9): 3274-3285.

Gizejewski, Z.; Szafranska, B.; Steplewski, Z.; Panasiewicz, G.; Ciereszko, A.; Koprowski, H. 2008. Cottonseed feeding delivers sufficient quantities of gossypol as a male deer contraceptive. *European Journal of Wildlife Research*, 54: 469-477.

Guedes F. C. B. and Soto-Blanco, B. 2010. Sperm quality of sheep fed cottonseed cake. *Acta Scientiae Veterinariae*, 38 (4): 415–418.

Hafez, E.S.E. 1993. Reproduction in Farm Animals. 6th ed., Lea and Febiger, Philadelphia, pp. 405-439.

Itodo, J.I., Ibrahim, R.P., Rwuaan, J.S., Aluwong, T., Shiradiyi, B.J., Owoicho, A.K., Azubuike, U.S., and Agbi, K.A. 2021. The effects of feeding graded levels of whole cottonseed on semen characteristics and testicular profiles of red Sokoto bucks. *Acta Scientiarum Animal Sciences,* 43, e50990.

Kenfack, A., Chombong, J.K., Ngoula, F., Vemo, N.B., Tsambou, A.M. M ., Zeukeng, G.M. Z., Mama, B., and Tchoumboué, J. 2015. Effect of feeding cottonseed cake on male fertility in rabbit. *Bang. J. Anim. Sci*., 44 (1): 16-20.

Kovacic, P. 2003. Mechanism of drug and toxic actions of gossypol: focus on reactive oxygen species and electron transfer. *Current Medicinal Chemistry,* 10: 2711-2718.

Luna, L.G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. pp 15-17**.** McGraw-Hill Book Co., New York.

Malmgren, L. 1997. Assessing the quality of raw semen: a review. *Theriogenology*, 48: 523 – 530.

Mena, H., Santos, J.E.P., Huber, J.T., Terazon, M., and Calhoun, M.C. 2004. The effects of varying gossypol intake from whole cottonseed and cottonseed meal on lactation and blood parameters in lactating dairy cows. *Journal of Dairy Science*, 87: 2506-2518.

Nagalakshmi, D., Sharma, A.K., and Sastry, V.R. 2000. Pathological lesions in lambs fed raw or processed lesions in lambs fed raw or processed cottonseed meal. *Vet. Res. Commun*., 24 (5): 349-59

Nunes, F. D. C. R. de Araujo, D. A. F. V. Bezerra, M. B., and Soto-Blanco, B. 2010. Effects of gossypol present in cottonseed cake on the spermatogenesis of goats. *Journal of Animal and Veterinary Advances*, 9 (1): 75–78.

Ovimaps 2014. Ovi Location Map; Ovi Earth Imagery, Dated April 3, 2014.

Oyeyemi, M. O., and Ubiogoro, O. 2005. Spermiogram and morphological characteristics in testicular and epididymal spermatozoa of large white boar in Nigeria. International Journal of Morphology, 23(3): 235 – 23.

Randel, R.D., Chase, C.C., and Wyse, S.J. 1992. Effects of gossypol and cottonseed products on reproduction of mammals. *Journal of Animal Science*, 70: 1628-1638.

Rekwot, P. I.,Oyedipe, E. O., Dawuda, P.M. and Sekoni, V. O. 1997. Age and Hourly related Changes of Serum Testosterone and Spermiogram of Pre- pubertal Bulls fed two Levels of Nutrition. *The Veterinary Journal*, 153: 341-347.

Singh, A., Veers, U., and Singh, S. 2012. Semen Evaluation in Farm Animals. *International Journal of Biomedical and Life Science*, 2(1): 35-42.

Smith, W.A., Vosloo, L.P., van Niekerk, C.H., and Theron, F.P. 1991. Effect of free gossypol in whole cottonseed on the semen quality of Holstein bulls. *South African Journal of Animal Science,* 21(1): 16-20.

Solaiman, S.G. 2007. Feeding value of whole cottonseed for goats.*Notes on Goats*. Tuskegee University, Technical Notes No. 07-08, Available at: http://www.boergoats.com/clean/articles/feeding/wholecottonseed

Tabatabaei, S., Batavani, R. A., and Talebi, A. R. 2009. Comparison of semen quality in indigenous and Ross broiler breeder roosters. Journal of Animal and Veterinary Advances, 8: 90 – 93.

Tiago do Prado P., Viana, P, Brandao, E, Amador, S., Barbosa, T., Cardoso, C., Lucci, C. M., de Souza, J. R., McManus, C., Abdalla, A. L., Louvandini, H. 2016. Impact of feeding cottonseed coproducts on reproductive system of male sheep during peripubertal period. Scientia Agricola. 73(6):489-497. http://dx.doi.org/10.1590/0103-9016-2015-0377

Velasquez-Pereira, J., Chenoweth, P.J., McDowell, L. R., Risco, C. A., Staples, C. A., Prichard, D., Martin, F. G., Calhoun, M. C., Williams, S.N., and Wilkinson, N.S. 1998. Reproductive effects of feeding gossypol and vitamin E to bulls. *Journal of Animal Science,* 76: 2894-2904.

Yarney, T.A.; Sanford, L.M., and Palmer, W.M. 1990. Pubertal development of ram lambs: body weight and testicular size measurements as indices of post-pubertal reproductive function. *Canadian Journal of Animal Science*, 70(1): 139-147.

Zahid, I. A., Lodhi, L.A., Qureshi, Z. I., Rehman, N. U., and Akhtar, M. S. 2003. Effects of gossypol on semen characteristics of Teddy male goats. *PakistanVeterinary Journal*, 23(4): 173-176.

Zemjanis, R. 1970. Collection and evaluation of semen. In: Diagnostic and Therapeutic techniques in Animal Reproduction 2nd Edition, Williams and Wilkins Co. Baltimore MD pp. 139-156.

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