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The effects of feeding graded levels of whole cottonseed on semen characteristics and testicular profiles of Red Sokoto Bucks

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ABSTRACT. The present work evaluated the effects of feeding graded levels of whole cottonseed on reproductive parameters of Red Sokoto bucks. Twenty Red Sokoto bucks were used for the experiment. After a 14-day pre-treatment period, bucks were assigned for 90 days to one of four isonitrogeneous treatments: control (diet A); 0 mg kg^{-1} of total gossypol, (diet B); 15% mg kg^{-1} of total gossypol, (diet C); 30% mg kg^{-1} of total gossypol and (diet D); 45% mg kg^{-1} of total gossypol. The mean percentage sperm gross motility was significantly ($p < 0.05$) lower in group D (45% WCS) compared to groups C (30% WCS) and A (control) at days 60, 75 and 90. The mean semen pH and reaction time were not significantly ($p > 0.05$) different among treatment groups. Semen colour of the bucks in the control group was majorly creamy, in group C (30% WCS) and B (15% WCS) creamy to milky and group D (45% WCS) colourless. The mean semen volume was significantly ($p < 0.05$) higher in groups C and A compared to group D (45% WCS) at days 60, 75 and 90. The mean semen concentration was significantly ($p < 0.05$) lower in group D (45% WCS) when compared to group A (control) at days 30, 45, 60, 75 and 90. Group A (control) bucks had significantly ($p < 0.05$) higher percentage live sperm compared to those in group D (45% WCS) at days 45, 60, 75 and 90. Mean Sperm morphological abnormalities including detached head, free tail curved tail and midpiece droplets were significantly ($p < 0.05$) higher in group D (45%) than in group A (Control) at day 15 (for free tails) and day 90 (for all). Testicular and epididymal sperm reserves were higher in animals supplemented with up to 30% whole cottonseed. In conclusion, feeding bucks above 30% WCS resulted in more deleterious effects on the semen characteristics and testicular profile.

Keywords: bucks; gossypol; histology; semen; sperm reserves; testosterone concentration.

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Introduction

Whole cottonseed is used as source of proteins for animal feeding. However, cottonseeds present a substance with toxic potential in their composition called gossypol. Gossypol is a phenolic compound produced by the cotton plant and it is highly reactive, binding rapidly to different substances, including minerals and amino acids. Gossypol appears to exert unique and selective effects upon the male reproductive system (Gadelha, Fonseca, Silvia Oloris, Marília, & Soto-Blanco, 2014). Earlier work showed that chronic administration of gossypol acetic acid (GAA) caused a consistent and marked reduction in the number of ejaculated spermatozoa, usually preceded by lowered sperm motility (Guedes & Soto-Blanco, 2010). In non ruminants, this reduction in spermatozoa was associated with variable damage to the spermatogenic epithelium, leading to reduced germinal cell layers (Nunes, Araújo, Bezerra, & Soto-Blanco, 2010; El-Mokadem, Taha, Samak, & Yassen, 2012). In bulls, reported gossypol effects have included degeneration and reduction of spermatogenesis (Tanyildizi & Boskurt, 2004) as well as damage to the basement membrane of spermatogenic tubules. In all species studied, the spermatoxic effects of gossypol appear to be both time and dose dependent.

Material and methods

Study location, diet preparation, bucks and experimental design

The study was carried out at the Small Ruminant unit, Faculty of Veterinary Medicine, Ahmadu Bello University, Samaru – Zaria. Samaru is located in the Northern Guinea Savannah zone of Nigeria, between

latitude 11 °N and 12 °N and between longitude 7 °E and 8 °N and at an elevation of 650 m above sea level. It has an average annual maximum and minimum temperatures of 31.0 ± 3.2°C and 18.0 ± 3.7°C, respectively. Samaru has an average annual rainfall of 1100 mm, usually lasting from May to October, with a mean relative humidity of 72%. The dry season lasts from November to April, with mean daily temperatures ranging from 15 – 36°C, and mean relative humidity of between 20 – 37% (Wikipedia, 2014).

Diet preparation

Whole cottonseed was purchased by from sunseed oil and mills, Sabo-gari Zaria, metropolis. The WCS was crushed into a meal using cereal grinding machine of 5-mm diameter sieve. Four (4) experimental diets were formulated and compounded to contain Whole cottonseed replacing maize offal at the rates 0, 15, 30, 45.

Experimental animals and design

Twenty (20) apparently, healthy Red Sokoto bucks with clinically- normal genitalia, aged 12-18 months and weighing 6-10 kg were randomly used for the experiment. The animals were purchased from goat sellers within Zaria metropolis. There were randomly distributed into four (4) treatment groups of five (5) animals each in a completely randomized design and each animal was a replicate.

Management

The bucks were screened and treated with albendazole at 10mg kg^{-1} for haemoparasites and helminthes before the commencement of the experiment. The bucks were managed under intensive system and kept in separate pens. They were given access to a balanced ration, comprising maize offal, wheat bran, bone meal, common salt, groundnut haulms and water *ad libitum*. No vaccine was administered within the two-week pre–conditioned period. Ingredients and samples of the diet were analysed for dry matter, crude protein, ether extract and ash content in animal nutrition laboratory, Faculty of Agriculture, Ahmadu Bello University, Zaria using the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1990). Determinations of the crude fibre and nitrogen-free extract fraction as carried out as described by Goering and Van Soest (1970). Each analysis was done in triplicate.

Semen collection

Before the commencement of the experiment, semen samples were collected once from bucks for two weeks in each group. After the pre-conditioned period, semen was collected fortnightly from each buck in the morning between 09 and 11h on collection days, using a portable battery-powered electro-ejaculator (Lane Manufacturing Inc, Kingston, U.K. No. 72707C) for small ruminants. Each buck was adequately restrained and its prepuce was washed and dried with warm water and a clean towel. The bipolar rectal probe of the electro-ejaculator was lubricated using petroleum jelly, inserted gently into the rectum and switched on, and stimulation was done intermittently for 2-3 seconds. This process resulted in erection and subsequently, ejaculation. The reaction time, semen volume, color and consistency were recorded. This procedure was repeated for each buck in each group.

Reaction Time: The time of erection to the first ejaculation and was measured using a digital clock. Semen Evaluation Semen samples collected were evaluated as described by Zemjanis (1970) as follows: Semen volume: The volume of semen was measured directly from the calibrated tube used for collection. Sperm motility: Microscopic examination for wave pattern (gross sperm motility) was carried out. The sample was examined as quickly as possible after collection. The slide was examined without a cover slip and the cells were examined under x 10 magnification. Sperm concentration: Briefly, sperm concentration was determined after semen was diluted with 3% NaCl. The diluted semen was placed on a Neubauer haemocytometer and the spermatozoa were counted in five squares of one chamber. Spermatozoa concentration was determined using a Neubauer haemocytometer as described by Azawi and Ismaeel (2011). Live-dead ratio: Live-dead ratio of the sperm cells was determined as described by Esteso, Soler, Fernandez-Santos, Quinteromoreno and Garde (2006). A thin smear of the semen sample was made on a clean grease-free glass slide and stained with eosin-nigrosin stain. The staining mixture contained 1 g of eosin-B and 5 g of nigrosin in 3 g of sodium citrate dehydrate solution. At least 100 sperm cells were counted using light microscopy at x 40 magnification. Dead spermatozoa stained pink or reddish, while live spermatozoa remained colourless. Morphological abnormalities: Sperm abnormalities were determined by making a thin smear of the semen sample on clean grease-free glass slide and fixed with buffered-formol saline. One hundred sperm cells counted per slide using light microscope at X 40 magnification.

Blood sampling and evaluation

Testosterone assay

Blood for testosterone assay was collected from the jugular vein from two bucks in each group, hourly for six hours between 8 - 14h. Sampling was done every forty five days (twice) during the 90-days feeding period. The collected blood was put into clean vials and left to clot at room temperature (37^0C) for 1 hour. The blood was centrifuged at 3,000 *g*, and the serum was harvested and stored at – 20^oC until used for testosterone assay.

Testosterone assay was carried out using the Accubind testosterone kit according to the manufacturer's recommendation (Monobind Inc. 100, North Pointe Drive, Lake Forest, California USA). All reagents were brought to room temperature (37ºC) before use. Briefly, 50 µL of standard, sample and control solutions was pipetted into appropriate wells. Testosterone enzyme conjugate solution 100 µL was added to each well (except those set for blanks). Fifty (50) µL of rabbit anti-testosterone antibodies was used. The content of each well was mixed for 30 seconds and incubated for 1 hour at 37ºC. A zip-lock bag was used to store the plate during incubation. The content of the well was discarded and the plate was washed five times with Wash (cleaning) solution (250-300 µL) per well. The plate was inverted and tapped firmly against absorbent paper to remove any residual moisture. Tetra-methyl benzidine (TMB) colour (100 µL) was added into each well, including the blanks. The reaction was stopped by adding 50 µL of stopping solution to wells in the same sequence that the substrate solution was added and gently mixed. The absorbance was read at the wavelength of 450 nm with a microwell reader. The mean absorbance values (A) for each set of reference standards, controls, samples and blanks were calculated. The value for blank was subtracted from those for standard, control and unknown samples. The B Bo^{-1}% (standard absorbance plotted against concentration absorbance) values were calculated by dividing each value by the zero-standard. A graph was plotted on the semi-log graph paper with B BO^{-1}% values for the standards on the ordinate and the testosterone concentration (ng mL^{-1}) on the abscissa. Using the graph, the testosterone concentrations were read for the unknown samples and sensitivity of this assay was 0.1 ng mL^{-1}.

Statistical analysis

Data obtained were expressed as mean ± standard error of the mean (Mean ± SEM). Semen characteristics data were compared using unpaired *t* test (GraphPad Prism v.5.0 for Mac). The level of significance was set at $p < 0.05$.

Ethical considerations

The handling, management and semen collection of the bucks were carried out humanely in accordance with the guidelines, governing the welfare of research animals by the Ahmadu Bello University, and as approved by Ethics Research Committee of the Department of Theriogenology and Production, Ahmadu Bello University, Zaria.

Result and discussion

The present study revealed that bucks fed 45% WCS had a significant effect on semen volume, as feeding progressed (Table 1). The decrease in semen volume observed in the groups fed 30 and 45% WCS agrees with the findings of Babashani et al. (2015) in Yankasa Rams and El-Mokadem et al. (2012) who reported a decrease in semen ejaculate in rams fed low and high levels of gossypol. But it is in contrast to the findings of Jimenez et al. (1989) who reported that feeding post-pubertal bulls with 30% WCS for a period of 60 days did not have any effect on the semen volume. This finding may be attributed to the fact that WCS contains gossypol, which induces infertility and causes spermatogenesis arrest when the gossypol was fed over a long period of time (Coutinho et al., 2000). This also led to decrease in scrotal circumference and weight which may be responsible for the decreased semen volume observed in bucks fed 45% WCS in this study (Table 1). This is also in agreement with the works of Al-Kawmani, Alfuraiji, Abou-Tarboush, Alodan, & Farah (2014), and Almaguer, Font, Cabrera and Arias (2017) in rams. The presence of free gossypol in WCS adversely affected sperm production, associated with damage to the spermatogenic epithelium and leading to reduced germinal cell layers. The anti-fertility effect of gossypol is related to the ability of gossypol in WCS to efficiently cross the general circulation – gonadal barrier (El-Mokadem et al., 2012; Al-Kawmani, Alfuraiji, Kandeal, Farah, & Alanazi, 2017).

Table 1. Semen characteristics of bucks fed whole cottonseed from day 0 to 90 (Mean ± S.E.M).

Parameter	Day 0				Day 15			
	A	B	C	D	A	B	C	D
Motility	82. 00± 4.64	76. 00±9.93	82.50±3.23	67. 00±3.74	91.00±1.81	81.0±6.2	78.0±1.23	66.0±10.89
Ph	6.4±0.24	6.8±0.37	7.8±0.37	7.2±0.37	6.8±0.20	6.8±0.20	6.60±0.24	7.0±0.45
Semen colour	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Milky	Milky
Semen Volume	0.58±0.06	0.50±0.07	0.46±0.05	0.56±0.07	0.54±0.02	0.50±0.03	0.52±0.07	0.57±0.03
Concentration	437.5±65.24	403.8±117.0	505.0±90.0	363.3±108.5	525.8±66.49	544.7±171.3	510.8±32.78	657.3±17.6
Reaction Time	51.80± 16.91	58.20 ±12.14	51.00±8.58	61.60±10.11	32.25±6.09	39.60±11.10	26.50±5.95	36.40±2.1
Live sperm %	81.00±4.00	88.00±2.00	85.00±4.47	84.00±4.0	82.00±3.74	84.0±4.30	83.0±4.36	80.0±3.16
Dead sperm %	16.25±3.75	12.00±2.00	15.00±4.47	16.10±4.0	18.00±2.89	16.0±4.30	17.0±4.36	20.0±3.16
Detached head	15.67±3.38	12.80±2.48	16.00±3.11	17.00±1.87	13.60±2.11	14.50±3.75	15.20±4.07	16.40±1.97
Free Tail	10.60±2.04	9.60±2.36	7.4±1.17	13.0±0.84	$3.25±1.11^{a}$	8.0±2.03	4.80±0.9	$10.00±1.82^{a}$
Curve Tail	2.60±0.60	5.60±0.75	4.40±1.44	4.40±1.33	1.75±0.75	4.25±1.11	1.67±0.33	4.60±0.87
Bent Tail	3.75±0.48	8.00±1.08	6.00±1.73	6.80±1.32	4.40±1.47	5.80±2.04	6.25±1.70	7.50±2.63
Mid –piece	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.40±0.24	0.60±0.40	0.40±0.24	0.0±0.0
	Day 30				Day 45			
Motility	65.50±16.01	58.75±13.90	78.33±6.0	42.50±2.50	82.50±7.50	60.00±11.73	68.33±4.41	41.67±13.02
pH	8.0±0.58	8.20±0.80	7.67±0.33	8.40±0.40	6.5±0.64	7.6±0.40	7.0±0.58	7.0±0.32
Semen Colour	Creamy	Creamy	Milky	Milky	Creamy	Milky	Milky	Watery
Semen Volume	0.50±0.040	0.46±0.02	0.17±0.033	0.30±0.0	0.85±0.41	0.35±0.15	0.33±0.12	0.33±0.10
Concentration	$373±9.0^{a}$	$321.5±87.5^{b}$	192.3±29.42	$31.0±9.00^{ab}$	$373.0±9.0^{a}$	$321.5±87.50^{b}$	192.3±29.42	$31.0±9.00^{ab}$
Reaction Time	78.25±17.57	83.20±28.02	83.33±6.67	142.5±23.27	44.50±13.77	20.60±7.42	45.00±14.00	36.40±5.49
Live sperm %	85.00±7.64	77.50±12.50	71.67±6.0	42.5±22.5	$90.0±2.04^{a}$	$85.00±4.74^{b}$	$93.33±1.67^{c}$	$30.00±7.15^{abc}$
Dead sperm %	15.00±10.93	22.50±12.50	30.00±4.56	57.5±22.50	$10.00±2.04^{a}$	$15.00±4.74^{b}$	$16.67±4.41^{c}$	$70.00±7.15^{abc}$
Detached head	14.75±9.67	15.67±8.0	10.50±5.24	15.00±2.89	10.33±4.41	19.20±7.07	7.33±3.71	19.20±7.20
Free Tail	3.75±2.50	3.67±1.76	5.25±2. 06	8.50±1.50	3.75±0.95	2.40±0.75	2.67±0.88	4.75±1.65
Curve Tail	2.0±0.82	2.0±0.82	3.40±1.40	7.50±2.50	3.50±0.65	3.20±1.28	2.67±0.67	5.80±1.59
Bent Tail	2.50±1.66	4.25±0.25	4.00±1.70	4.33±0.88	2.25±0.85	2.40±0.75	2.0±1.15	3.60±0.81
Mid-piece	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.58
	Day 60				Day 75			
Motility	$78.75±2.39^{a}$	$88.00±2.55^{b}$	$73.33±6.0^{c}$	$37.00±6.63^{abc}$	$72.75±1.09^{a}$	$69.00±2.56^{b}$	$65.33±6.1^{c}$	$32.00±7.12^{abc}$
Ph	6.50±0.65	7.60±0.40	7.0±0.58	7.0±0.32	6.80±0.65	7.60±0.40	7.2±0.58	7.2±0.32
Semen Colour	Creamy	Creamy	Creamy	Watery	Creamy	Creamy	Milky	Watery
Semen Volume	$0.40±0.04^{a}$	$0.66±0.06^{b}$	$0.50±0.15^{c}$	$0.12±0.04^{abc}$	$0.80±0.06^{a}$	$0.56±0.01^{b}$	$0.60±0.16^{c}$	$0.14±0.05^{abc}$
Concentration	219.7±49.36	$380.0±77.8^{a}$	$245.3±52.26^{b}$	$15.00±4.12^{ab}$	$380.7±76.04^{a}$	$245.0±82.10^{b}$	$291.3±42.56^{c}$	$18.30±4.32^{abc}$
Reaction Time	59.0±12.20	76.80±15.39	84.67±35.88	74.80±11.51	59.0±12.20	76.80±15.39	74.67±35.88	84.80±11.51
Live sperm %	$81.67±4.41^{a}$	$91.60±2.25^{b}$	84.33±4.70	$36.40±4.74^{abc}$	$90.67±4.41^{a}$	$82.60±2.25^{b}$	$70.33±4.70^{c}$	$36.40±4.74^{abc}$
Dead sperm %	$18.33±4.41^{a}$	$8.40±2.25^{b}$	$15.67±4.70^{c}$	$63.60±4.74^{abc}$	$10.33±4.41^{a}$	$18.40±2.25^{b}$	$30.67±4.70^{c}$	$64.60±4.74^{abc}$
Detached head	7.75±1.55	8.20±3.31	6.33±1.45	12.80±2.85	7.75±1.55	8.20±3.31	6.33±1.45	12.80±2.85
Free Tail	3.50±0.96	5.60±2.11	4.0±1.15	5.7±0.88	3.50±0.96	5.60±2.11	4.0±1.15	6.7±0.88
Curve Tail	5.67±2.03	3.75±1.70	8.0 ± 0.0	7.0±1.52	3.67±2.03	3.75±1.70	7.0±0.0	7.0±1.52
Bent Tail	$0.75±0.25^{a}$	$0.60±0.40^{b}$	0.50±0.50	$5.80±1.83^{ab}$	$0.45±0.15^{a}$	$0.50±0.80^{b}$	0.55±0.50	$6.20±1.^{53ab}$
Mid-piece	0.0±0.0	0.20 ± 0.20	0.67 ± 0.67	2.0 ± 0.71	0.0±0.0	0.20 ± 0.20	0.47 ± 0.67	2.0 ± 0.71
Day 45	Day 90							
Motility	$80.00±2.04^{a}$	$65.00±2.24^{b}$	$55.02.±2.89^{c}$	$37.00±6.63^{a}$				
Ph	6.75±0.75	7.60±0.40	7.0±0.58	7.00±0.32				
Semen Colour	Milky	Milky	Milky	Watery				
Semen Volume	$0.70±0.04^{ab}$	$0.66±0.06^{cd}$	$0.33±0.033^{ac}$	$0.14±0.02^{bd}$				
Concentration	$125.6±9.8^{ade}$	$219.7±30.61^{abc}$	$54.67±16.37^{bd}$	$14.40±4.62^{ce}$				
Reaction Time	59.0±12.20	76.80±15.39	84.67±35.88	78.80±11.51				
Live %	$76.25±6.25^{a}$	$91.60±2.25^{bc}$	$65.00±2.89^{bd}$	$36.40±4.74^{acd}$				
Dead %	$23.75±6.25^{a}$	$8.40±2.25^{bc}$	$35.00±2.89^{bd}$	$63.60±4.74^{ac}$				
Detached head	$7.75±1.55^{a}$	$6.40±1.63^{b}$	18.0±3.06	$30.80±4.68^{ab}$				
Free Tail	$3.50±0.96^{a}$	$5.60±2.11^{b}$	12.67±4.41	$17.50±1.04^{ab}$				
Curve Tail	$3.25±0.75^{ab}$	8.40±1.03	$14.33±1.86^{a}$	$15.00±2.88^{b}$				
Bent Tail	$0.75±0.25^{a}$	$0.60±0.40^{b}$	$0.50±0.50^{c}$	$7.80±1.59^{abc}$				
Mid-piece	$0.0±0.0^{a}$	$0.20 ± 0.20^{b}$	1.00 ± 0.58	$2.20 ± 0.58^{ab}$				

[abc]Means on the same row with same superscript letters are significantly different from one another.

The change in semen colour associated with increased WCS levels and duration observed in this study indicates feeding bucks with WCS above 30% had adverse effect on semen colour (Table 1). This is in agreement with Gomes, Gonzales, Carvalho and Soares (2012) who stated that semen colour was watery to colorless in sheep fed 40% WCS. The semen colour in this study ranged from creamy to colourless. Bucks fed on 0% (control) had mostly creamy coloured semen, while those fed 45% had pale milky to colourless semen colour this could be attributed to the increased levels of WCS, exerting adverse effects on spermatogenesis.

There were no significant differences among the treatment groups in semen pH all through the 90 days of the conducted feeding trials in this present study(Table 1). This result is in agreement with the works of Nunes et al. (2010), who showed that semen pH was not altered when bucks were fed 50% cottonseed cake of their daily ration for a period of 120 days.

The results of the present study showed that as the level of WCS increased in diet and the days of administration progressed, abnormalities such as dead sperm percentage, detached head, curved tail, bent tail and mid–piece droplet increased in values and the abnormalities were more pronounced in bucks fed 45% WCS especially from days 60,75 and 90 (Table 1). These observations are in agreement with the earlier reports by El-Mokadem et al. (2012) who reported a significant increase in percentage of total abnormal sperm in rams fed 30% WCS. Also, Tanyildizi and Boskurt (2004) reported that gossypol is both time and dose–dependent and that sperm abnormalities became more pronounced by increasing the quantity of WCS in the diets fed to bulls. Tanyildizi and Boskurt (2004) found that bulls fed cottonseed meal had significant increase in mid–piece sperm abnormalities appearing three weeks after the onset of the feeding trial. Ming et al. (2011) also reported that gossypol treatment in piglets increased the percentage of dead spermatozoa. In contrast with the findings of Solaiman (2007) who reported that WCS as high as 30% in a buck diet may not cause any deleterious effect on sperm normality and progressive motility. Also Nunes et al. (2010) observed no morphological abnormalities in bucks fed cottonseed cake at 40%. As sperm morphology is related to sperm hydrodynamics, even small differences in geometrical variables of morphology may impair sperm hydrodynamics (Ivana et al., 2014). Sperm with certain type of sperm tail defects failed to bind to zona pellucida because of impaired motility. It has also been reported that sperm with abnormal head shape or tail defects had reduced capability to bind and penetrate the zona pellucida and the resulting zygotes had a reduced ability for cleavage and a high rate of embryonic loss (Braga et al., 2012). Feeding WCS above 30% over a period of 60 days above may lead to these sperm abnormalities.

Semen concentration in the present study was significantly reduced by increases in WCS supplementation and the duration of the feeding trial especially in bucks fed 45% WCS (Table 1). The observation confirmed earlier reports by Ranga, Kalla and Kanwar (1990) who observed that gossypol at low doses of 15% WCS supplementation produced significant decreases in sperm concentrations. A reduction in sperm concentration has also been observed previously in gossypol treated– ruminants. However, El-Mokadem et al. (2012) reported an increase in semen concentration in ruminants fed diet containing free gossypol for 8 weeks at 30% supplementation. The decrease in semen concentration observed especially in bucks fed 30 and 45% WCS as the feeding progressed to 45 to 90 days, may be due to increase in sperm cell abnormalities.

Whole cottonseed supplementation in this present study did not significantly affect sperm motility of the treatment groups at days 0, 15, 30 and 45. However, on days 60, 75 and 90, the bucks fed on 45% supplementation level had significantly decreased sperm motility, compared to other groups (Table 1). This is in agreement with works of Ake-Lopez, Ake-Villanueva, Segura-Correa, Ake-Villanueva and Montes Perez (2016) where they observed an immobilizing effect of gossypol on ram sperm fed above 30% WCS. Nunes et al. (2010) also reported reduced sperm motility in bulls fed 40% cottonseed cake. Timurkaan, Yilmaz and Timurkaan (2011) also found out that diets containing 15% WCS produced significant decrease in sperm motility in rats. Significant decrease in sperm motility through an inhibitory effect of gossypol in WCS has been attributed to its effects on mitochondrial ATP production has been reported. (Ming et al., 2011; Timurkaan et al., 2011). It has also been reported that gossypol contained in WCS promotes formation of oxygen radicals, which may be the underlying basis of its biological activity (Braga et al., 2012). However, the result obtained in this study is in contrast with the works of Nunes et al. (2010) who observed no mobility abnormalities in bucks fed 30% cottonseed cake. In this study, changes in testicular size were associated with changes in the spermatogenic capability of the bucks, thus implying a clear association between the effects of diet on the testosterone-secreting and the spermatogenic functions of the testes of the experimental bucks (Gadelha, Rangel, Silva, & Soto-Blanco, 2011). Bucks on 30% WCS supplementation

had a significantly higher scrotal circumference than those on 0 and 15%, while bucks fed 45% had their scrotal sizes significantly reduced (Figure 1). This result may be attributed to the deleterious effect of very high gossypol level on the architecture of the seminiferous tubules. El-Mokadem et al. (2012) reported significant reduction in scrotal sizes of rams fed 40% WCS of thier diet for a period of 120 days.

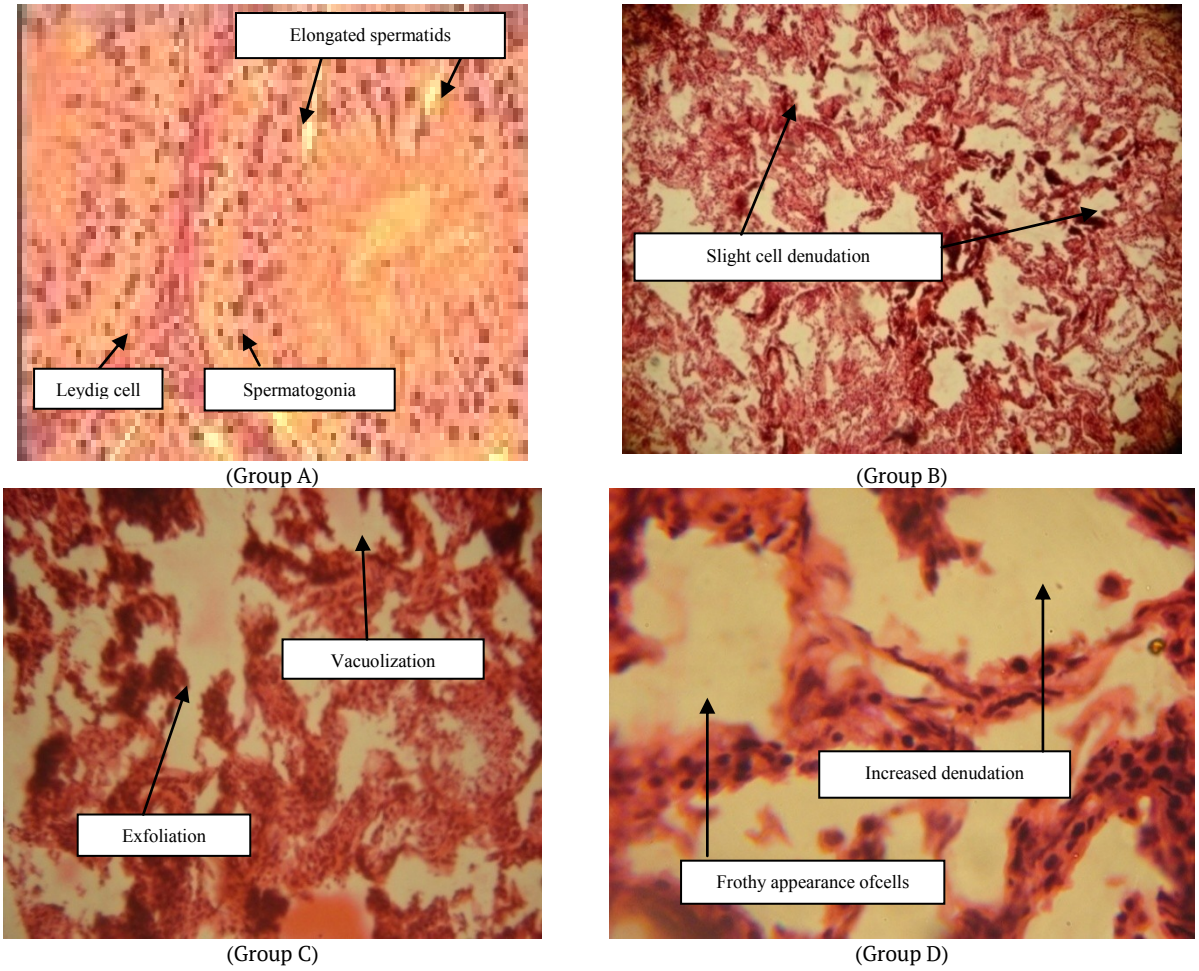

Figure 1. Control Group A (0% Whole cottonseed inclusion) - Seminiferous tubules showing normal Cells and architecture X 40 H&E; Group B (15% Whole cottonseed inclusion) - Slight cell denudation (X40) H&E; Group C (30% Whole cottonseed inclusion) - vacuolization and exfoilation (X40) H&E; Group D (45% Whole cottonseed inclusion) - Enlarged frothy appearance of cells and increased denudation (X40) H&E.

Testicular histology of Red Sokoto Bucks fed whole cottonseed

The histhopathological leisions showed cellular denudation of the tubules, as similarly reported by Babashani et al. (2015), who observed smaller scrotal circumference in goats receiving 45% of whole cottonseed in relation to the control diet.

Several studies conducted on animals showed that gossypol reduced fertility without alteration in testosterone, other androgens, or luteinizing hormone (Timurkaan et al., 2011). However, some other studies indicated that gossypol has an inhibitory effect on testosterone production by the Leydig cells via a lesion subsequent to pregnenolone formation. El-Mokadem et al. (2012) reported that WCS caused a reduction in serum testosterone concentration in rams. In this study, the antifertility effect of gossypol appears secondary to the decrease of testosterone synthesis (Gadelha et al., 2014) (Figure 2). It is very likely that the great contradiction of the endocrinal effects of gossypol can be due to the use of different animal species, different doses and times of treatment, or on account of different administration routes However, it is clear that infertility can be induced by gossypol with altered androgen status (Braga et al., 2012).

The significantly decreased sperm reserves of bucks fed 45% WCS over 90 day period could be attributed to the deleterious effects of increased WCS on spermatogenesis in the seminiferous tubules of the testes and massive destruction or cellular denudation as observed in this study (Figure 2A and B). The result is in agreement with the works of Timurkaan et al. (2011) who observed a decrease in the testicular and

epididymal sperm reserves in rats fed whole cottonseed over a period of 3 months, but in contrast with the findings of Nunes et al. (2010), who did not observe depressed fertility and impaired spermatogenesis in young bulls fed above 45% cottonseed meal.

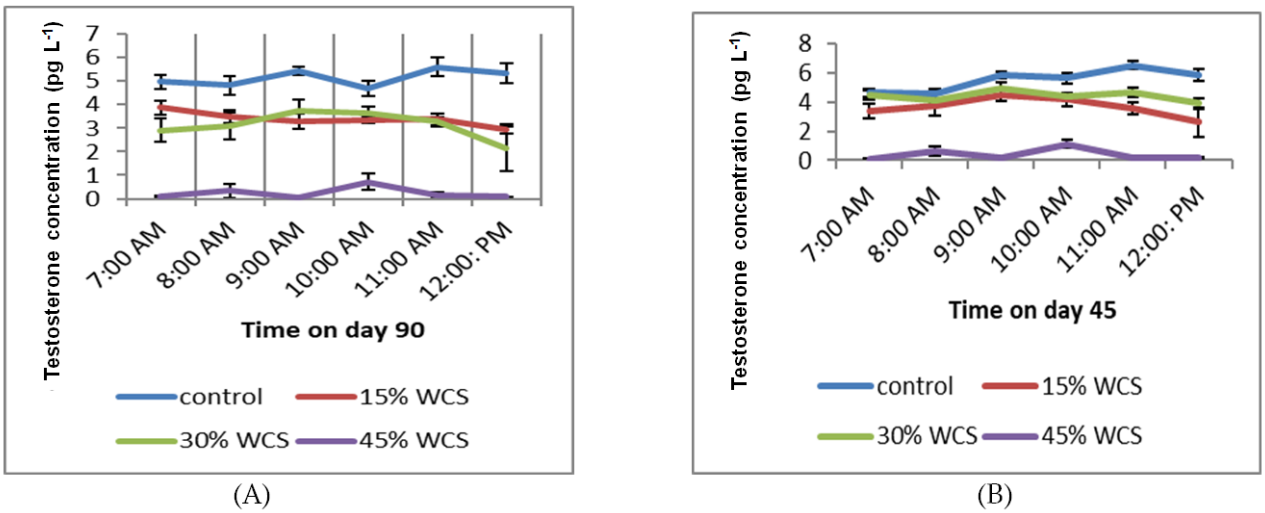

Figure 2. (A) Testosterone Concentrations of Bucks fed graded levels of WCS between 7am – 12pm on day 90; (B) Testosterone Concentrations of Bucks fed graded levels of WCS between 7am – 12pm on day 45.

Conclusion

Sperm morphological abnormalities (detached head, mid piece droplets, curved, coiled bent and free tails) were also increased by feeding increasing levels of whole cottonseed and duration of feeding with those on 45% having the highest abnormalities. Bucks fed above 30% of whole cottonseed had deleterious and adverse effect on the epididymal and gonadal sperm reserves. The testes of bucks fed WCS 30% and above showed severe loss of architecture and cellular denudation.

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