

Effects of Experimental *Fasciola gigantica* Infection on Serum Testosterone Profiles in Relationship to Semen Characteristics of Yankasa Ram

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Abstract: This study was designed to evaluate the effects of Fasciolosis on serum testosterone profiles and semen characteristics of Yankasa rams. Twelve, apparently healthy, Yankasa rams aged 18-24 months were randomly divided into two groups (infected group A and control group B) with seven and five rams, respectively. The rams were kept under intensive management in different pens throughout the study period. Group A rams were inoculated with 800 metacercariae orally and monitored for 12 weeks Post Infection (PI). Clinical signs were manifested 2 weeks PI. Faecal examination revealed *Fasciola* eggs at week 7 PI in group A. Three mL of blood were collected aseptically via the jugular vein at 30 min interval, weekly between 08:00-9:00am for serum testosterone assay. Testosterone assay was done using ELISA technique. There was significant decrease in testosterone concentration in group A at different time intervals of the weeks of post-infection. The semen analysis showed the lowest mean (\pm SEM) of semen volume, semen motility, semen concentration, of group A, as (0.01 \pm 0.0 mL), (3.6 \pm 2.4%), (16.1 \pm 4.2%) compared to group B with mean (\pm SEM) (1.6 \pm 0.1 mL), (93.0 \pm 1.2%), (440.0 \pm 13.0%) at week 7 post-infection, respectively. This study revealed that Fasciolosis has a great impact on sheep mainly by diminishing their reproductive efficiency and rendering infected rams infertile. It is concluded that *F. gigantica* infection had advanced effects on testosterone production and semen quality of Yankasa rams.

Keywords: Electro ejaculator, semen characteristics and Yankasa ram, testosterone profiles

INTRODUCTION

The Yankasa breed of sheep has been the most extensively studied in Nigeria (Blench *et al.*, 2006). Within the indigenous breeds of sheep about 22.3 million (FDLPCS (Federal Department of Livestock and Pest Control Services), 1991) are kept mainly for meat in Nigeria, the Yankasa sheep is the most numerous and most widely distributed breed throughout the various ecological zones, particularly the Guinea and Sudan Savannah zone of Nigeria. It constitutes about 60% of the total national flock (Afolayan, 1996) and it plays an important role in Nigerian livestock economy (Lakpini *et al.*, 2002). The use of sheep and goats for religious and social rites has also increased their economic importance in the society. The animals are valuable source of protein to human and their products such as meat, milk, skin, hair and other byproducts serve as major sources of economic income (Adeloye, 1998). Fasciolosis is a common form of liver infection, which has a cosmopolitan distribution and is pathogenic in all classes of livestock (Roseby, 1970; Dargie, 1987). The two most important species, are

Fasciola hepatica, found in temperate and in cooler areas of high altitude in the tropics and subtropics and *Fasciola gigantica*, which predominates in tropical areas. Both species co-exist where ecology is conducive for the snail hosts (Yilma and Mesfin, 2000). The life cycle of these trematodes involves snail species of *Lymnaea natalensis* and *Lymnaea auricularia* as intermediate host (Max *et al.*, 2006; Walker *et al.*, 2008). Chemotherapy is the only treatment available (Mas-Coma *et al.*, 1999). Parasitic infection is known to compromise the immune status and give chance to mix infections that can affect productivity and reduce reproduction performance of the animals. Fasciolosis in particular is known to impair liver function which has direct effect on metabolic activities and synthesis of cholesterol, the primary precursor for testosterone. The results of research into testosterone show that the hormone participates in sexual (O'Connor *et al.*, 2011), social (Gabor *et al.*, 2011) and spatial (Spritzer *et al.*, 2011) behaviours. Testosterone also played a vital role in exhibition of secondary sexual characteristics.

There is paucity of information on the effect of fasciolosis on spermatogenesis in Yankasa rams. Out of

this was to investigate the effect of fasciolosis on testosterone profiles and semen characteristics in Yankasa rams.

MATERIALS AND METHODS

Experimental animals: Twelve apparently healthy Yankasa rams, aged 18-24 months were purchased from Makarfi market in Makarfi Local Government area of Kaduna State, Nigeria. The rams were housed at small ruminant experimental pens of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University Zaria, Nigeria. Two weeks prior to the arrival of the rams the pens were fumigated, washed and disinfected. The rams were kept for two weeks to acclimatise. Thereafter, they were screened for parasitic infections and other diseases. Subsequently, the experimental rams were routinely dewormed with Albendazole bolus (Shanuzole®) at a dose rate of 7.5 mg/kg, administered per os. The rams were fed on basal diet of *Digiteriasmutssi* and concentrate supplement of 0.5 kg/ram/day. Water and salt lick were provided *ad libitum* throughout the experimental period.

Isolation and preservation of materials: *Fasciolagigantica* metaceariae are isolated from naturally infected snails (*Lymnaea natalensis*), obtained along stream in area BZ staff quarters and Ahmadu Bello University Zaria dam. Collected *Lymnaeanatalensis* were transported to helminthology laboratory, Faculty of Veterinary Medicine Ahmadu Bello University, Zaria, where they were crushed in water using Petridish snails tissues removed. The swimming cercariae were viewed under the microscope and left to encyst and attach to the Petridish. Thereafter, they were left in water in Petridish for 3 days and uncovered at 24°C room temperature to become infective (Ajanusi, 1987). Before infections, metaceariae were examined under stereomicroscope to confirm their viability.

Animals infection: The 12 rams were randomly assigned into two groups, comprising infected group A (n = 7) and control group B (n = 5). Each ram in infected group A was orally inoculated with 800 *Fasciolagigantica* metaceariae as described by Ahmed *et al.* (2007).

Post infection samples collection: Three mL of blood was collected aseptically via the jugular vein at 30 min interval between 08:00-9:00 am. After clot formation, sample were centrifuged at 1200×g for 15 min and the recovered serum was stored at -20°C until analyzed. The serum was randomly selected from weeks 3, 5, 7 and 9 from group A and B for testosterone assay.

Semen collection: Semen was collected from the rams once a week for 10 weeks. After restraining semen was collected from each ram using electro ejaculator Bailey ejaculator (MOD2, Western Instrument Company,

Denver, Colorado USA). The Bailey ejaculator is powered by a 6-volt battery. The probe of the ejaculator was lubricated using petroleum jelly for easy insertion into the rectum. Collection of semen involved three persons, one restraint the ram, the second person operated the ejaculator, while the third person collected the semen into a calibrated test tube. The tail of the ram was grasped firmly and the probe inserted gently into the rectum, pushed forward slowly and stimulation was done intermittently for 2-3 sec. Within few seconds the penis became erected and ejaculation was achieved (Fadason, 2008). Immediately after collection, the ejaculates were examined grossly for colour and volume. Thereafter ejaculates were placed in water bath at 37°C for microscopic examination.

Semen evaluation: Semen samples were evaluated as described by Zemjanis (1970). The volume of semen was measured directly from the calibrated tube used for collection. The gross semen characteristics examined included volume, colour, pH and presence or absence of foreign bodies as described by Maina *et al.* (2006). The volume was read from the graduated collecting tubes. The colour was coded as 1, 2 and 3 corresponding to creamy, milky and watery colours, respectively. The pH colour changed was matched with standard chart, reflecting the pH value. A drop of semen was placed on pH paper (Universal pH 1-14®MOD2, Western Instrument Company, Denver, Colorado USA) and immediate colour change or pH reading was recorded.

Microscopic examination for gross sperm motility was determined as described by Oyeyemi *et al.* (2009). A drop of raw undiluted semen on a pre-warmed slide was covered by a slip and viewed under a field microscope at x40 magnification.

Sperm concentration was determined by using the haemocytometer method (Bearden and Fuquay, 1992). Semen sample was sucked into the red blood-cell diluting pipette up to 0.1 mark and the volume made up to 101 mark with 10% formal saline and mixed thoroughly. The mixture was dropped and allowed to spread under the cover slip, placed tightly on the haemocytometer after discarding few drops. The cells were allowed to settle before counting under x40 magnification. Sperm cells were counted diagonally from top left to right bottom in 5 small squares of the improved Neubauer haemocytometer. Sperm output of individual ram was calculated as described by Verstegen *et al.* (2002).

Live-dead ratio of the sperm cells was determined as described by Estes *et al.* (2006). A thin smear of the semen sample was made on clean grease-free glass slides and stained with Eosin-Nigrosin stain. At least 400 sperm cells were counted using light microscope at x40 magnification.

Sperm abnormalities were determined as described by Estes *et al.* (2006). A thin smear of the semen

sample on clean grease-free glass slides and fixed with buffered-formal saline and at least 400 sperm cells were counted per slide using light microscope at x40 magnification. Sperm abnormalities were determined as described by Estes *et al.* (2006). A thin smear of the semen sample was made on a clean grease-free glass slide and fixed with buffered-formal saline. The preparation was then examined and abnormal sperm cells were counted in a regular sequence using light microscope at x100 magnification with oil immersion. A total of 400 well-spaced spermatozoa were carefully examined in each preparation and the percentage of head, mid-piece and tail sperm abnormalities were determined as described by Melrose and Laing (1970).

Testosterone assay: Testosterone assay was done using testosterone kits (Accu-bind®) by ELISA technique. Reagent was constituted as described by the manual. The assay procedure was as follows:

Microplate well for each serum reference was assayed in duplicate. Serum sample (10 µL) was pipetted into the assigned wells. The working testosterone enzyme reagent (50 µL) was added to all wells and the microplate were swirled gently for 20-30s. Testosterone biotin reagent (50 µL) was added to all wells and the microplates was swirled gently for 20-30s. The microplates were covered with parafilm paper and incubated for 60 min at room temperature 24°C. The contents of the microplates were discarded by decantation and the microplates were dried using absorbent paper. Wash buffer (350 µL) was aspirated into the wells of the microplate trice and discarded. Working substrate solution (100 µL) was added to all wells. The microplate was incubated for 15 min and 50 µL of stop solution was added to each well and was gently mixed for 15-20 s. The absorbance was read in each well at 450 nm using a reference wavelength of

620-630 nm to minimize well imperfections in the microplate reader (SpectraMax plus 384®).

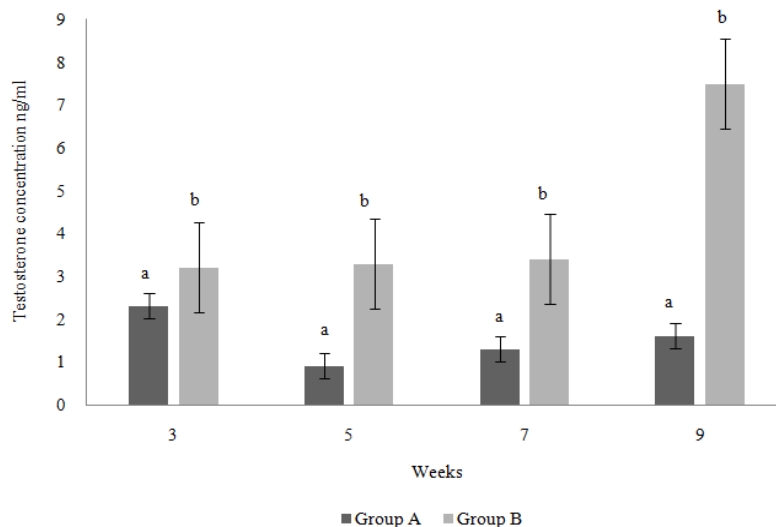
Data analysis: The data generated are presented in tables and graphs. Data on testosterone profiles, semen characteristics and EPG of *Fasciolagigantica* were expressed as mean (±SEM). Student t-test was used for the statistical analysis and to determine the difference between the two groups mean values of p<0.05 were considered significant.

RESULTS

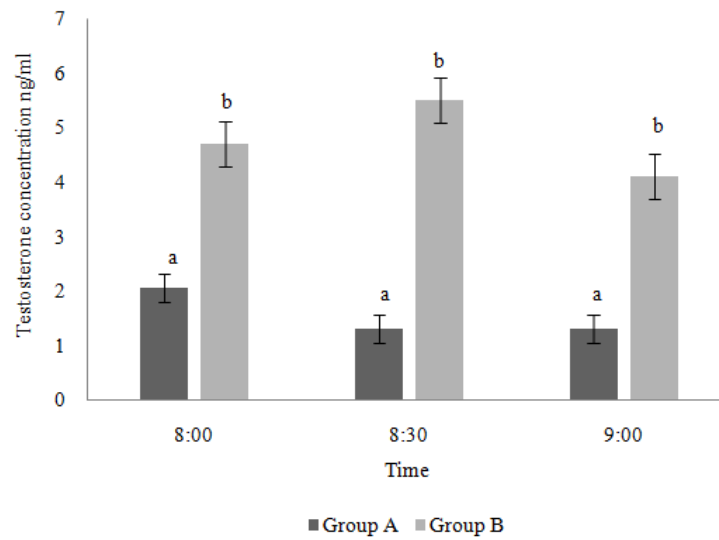
Two weeks Post-Infection (PI). The infected rams (group A) showed clinical signs, which included distended abdomen, rough hair coat, emaciation, anorexia, diarrhoea, weakness, muco-purulent nasal and ocular discharges, while no clinical sign were observed group B rams.

There were decreases in serum testosterone concentration of group A rams at different time intervals of the study period 12 weeks. Group A had the highest serum testosterone concentration of (2.7±0.7 ng/mL) at week 3 PI by 8:30 am compared to group B with (6.4±3.1 ng/mL) at week 9 PI by 8:00am. The concentration consistently decreased in group A with respect to week and time as presented in Fig. 1. Coprological examination revealed *Fasciolagigantica* eggs in group A rams at week 7 Post-Infection (PI) but no *Fasciola* eggs were found in group B rams (Fig. 2).

The effects of *Fasciolagigantica* infection on semen volume are presented in Table 1. There were significant (p<0.05) differences in the semen volume of group A rams at week 3 Post-Infection (PI), with a mean value of 0.5±0.2 mL compared to group B rams with mean value of 1.1±0.3 mL at the same week. The semen volume fluctuated first and progressively thereafter continued to decrease in group A.



(a): Testosterone concentration (ng/mL) with respect to week



(b): Testosterone concentration (ng/mL) with respect to time

Fig. 1: Changes in Testosterone concentration of Yankasa rams (n = 12) experimentally infected with *Fasciolagigantica*: ^{ab}Mean (\pm SEM) figure with different superscript alphabets between group A and B are significantly ($p < 0.05$) different; X = Testosterone concentration with respect to weeks; Y = Testosterone concentration with respect to time

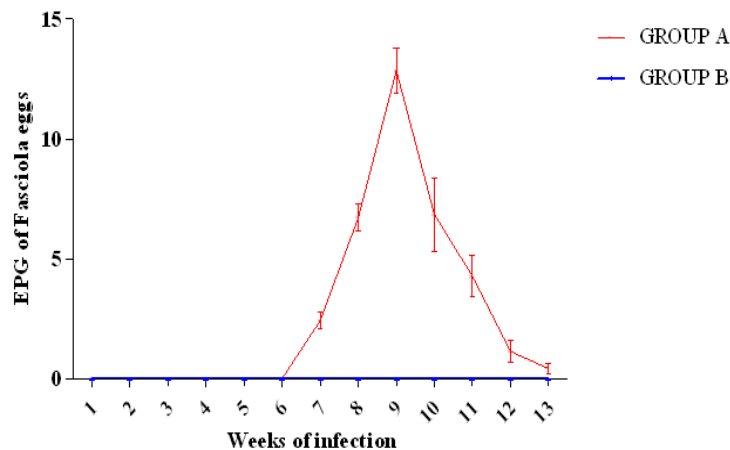


Fig. 2: Egg Per Grams (EPG) of faeces of Yankasa rams following experimental infection with *F. gigantica*

Rams in group A showed a consistent decrease in sperm motility from week 3 PI, with significant ($p < 0.05$) difference between the highest and lowest sperm motility of 51 and 0.7%, obtained at weeks 3 and 12 PI, respectively. Group B rams showed consistent increase in sperm motility at PI week 3 with the highest and lowest semen motility of 94 and 64% at weeks 12 and 3, respectively, Table 1.

The sperm concentration of rams in group A fluctuated between week 3-7 PI and the values obtained were significantly ($p < 0.05$). There was a progressive decrease in sperm concentration between weeks 8 and 12 PI. The highest sperm concentration of $222 \times 10^6/\text{mL}$ was recorded at week 4 PI and the lowest sperm concentration of $120 \times 10^6/\text{mL}$ was obtained at week 12

PI. There was significant increase in sperm concentration in group B rams.

Fasciolagigantica infection had significantly ($p < 0.05$) impacted on percentage of sperm morphology in group A, with consistent fluctuation occurring between weeks 3 and 12 PI. The highest morphological defect of 50.9% and the lowest value of 29% were recorded at week 9 and 4 PI, respectively. In group B the highest morphological defect of 10.2% and the lowest of 7.5% were obtained at week 4 and 6 PI, respectively (Table 2).

There were significant difference ($p < 0.05$) in the percentage of live sperm cells of group A rams with the highest percentage of 51.0% at week 4 PI, was higher than that of the group B rams that had 96.0% at week 9 PI (Table 1).

Table 1: Mean (\pm SEM) Semen characteristics of Yankasa rams experimentally infected with *Fasciolagigantica*

Weeks	Groups		Semen pH	Semen colour	Semen volume (mL)	Semen motility (%)	Sperm concentration (x106/mL)	% sperm viability
	A (n = 7), B (n = 5)							
0-2	ND		ND	ND	ND	ND	ND	ND
3	A		7.0 \pm 0.0	1.0 \pm 0.0	0.5 \pm 0.2 ^a	51.4 \pm 10.4 ^a	218.7 \pm 12.4 ^a	36.0 \pm 12.0 ^a
	B		7.0 \pm 0.0	1.0 \pm 0.0	1.1 \pm 0.3 ^b	64.0 \pm 7.5 ^b	402.0 \pm 8.6 ^b	66.0 \pm 14.0 ^b
4	A		7.0 \pm 0.0	1.0 \pm 0.0	0.4 \pm 0.1 ^a	35.7 \pm 5.6 ^a	222.1 \pm 11.7 ^a	51.0 \pm 6.0 ^a
	B		7.0 \pm 0.0	1.0 \pm 0.0	1.6 \pm 0.3 ^b	74.0 \pm 5.1 ^b	497.0 \pm 8.6 ^b	86.0 \pm 2.0 ^b
5	A		7.0 \pm 0.0	1.0 \pm 0.0	0.3 \pm 0.1 ^a	37.1 \pm 9.4 ^a	208.0 \pm 7.2 ^a	14.0 \pm 4.0 ^a
	B		7.0 \pm 0.0	1.0 \pm 0.0	1.6 \pm 0.4 ^b	81.0 \pm 3.7 ^b	456.8 \pm 11.6 ^b	88.0 \pm 4.0 ^b
6	A		7.0 \pm 0.0	1.0 \pm 0.0	0.2 \pm 0.0 ^a	37.9 \pm 2.9 ^a	207.4 \pm 5.3 ^a	9.0 \pm 3.0 ^a
	B		7.0 \pm 0.0	1.0 \pm 0.0	1.0 \pm 0.2 ^b	83.0 \pm 3.7 ^b	446.0 \pm 6.1 ^b	90.0 \pm 3.0 ^b
7	A		7.0 \pm 0.0	1.0 \pm 0.0	0.5 \pm 0.1 ^a	13.6 \pm 3.7 ^a	191.0 \pm 2.3 ^a	11.0 \pm 3.0 ^a
	B		7.0 \pm 0.0	1.0 \pm 0.0	1.4 \pm 0.1 ^b	91.0 \pm 1.9 ^b	444.0 \pm 13.4 ^b	90.0 \pm 3.0 ^b
8	A		7.0 \pm 0.0	1.0 \pm 0.0	0.1 \pm 0.0 ^a	7.1 \pm 4.2 ^a	179.0 \pm 3.5 ^a	11.0 \pm 3.0 ^a
	B		7.0 \pm 0.0	1.0 \pm 0.0	1.6 \pm 0.1 ^b	86.0 \pm 2.9 ^b	444.0 \pm 10.8 ^b	86.0 \pm 4.0 ^b
9	A		7.0 \pm 0.0	1.0 \pm 0.0	0.06 \pm 0.01 ^a	3.6 \pm 2.4 ^a	157.1 \pm 4.2 ^a	6.0 \pm 3.0 ^a
	B		7.0 \pm 0.0	1.0 \pm 0.0	1.6 \pm 0.1 ^b	93.0 \pm 1.2 ^b	440.0 \pm 13.0 ^b	94.0 \pm 2.0 ^b
10	A		7.0 \pm 0.0	1.0 \pm 0.0	0.04 \pm 0.00 ^a	0.7 \pm 0.7 ^a	143.6 \pm 6.4 ^a	4.0 \pm 2.0 ^a
	B		7.0 \pm 0.0	1.0 \pm 0.0	1.6 \pm 0.1 ^b	93.0 \pm 1.2 ^b	436.0 \pm 9.3 ^b	96.0 \pm 2.0 ^b
11	A		7.3 \pm 0.0	1.0 \pm 0.0	0.03 \pm 0.00 ^a	10.0 \pm 3.9 ^a	130.0 \pm 7.6 ^a	6.0 \pm 2.0 ^a
	B		7.0 \pm 0.0	1.0 \pm 0.0	1.6 \pm 0.1 ^b	94.0 \pm 1.0 ^b	432.0 \pm 9.7 ^b	90.0 \pm 3.0 ^b
12	A		7.3 \pm 0.0	1.0 \pm 0.0	0.01 \pm 0.00 ^a	6.4 \pm 3.2 ^a	120.0 \pm 7.6 ^a	3.0 \pm 2.0 ^a
	B		7.0 \pm 0.0	1.0 \pm 0.0	1.5 \pm 0.12 ^b	94.0 \pm 1.0 ^b	432.0 \pm 9.7 ^b	94.0 \pm 2.0 ^b

^{ab}Mean in the same rows for each parameter with different superscript alphabets between group A and B are statistically ($p < 0.05$) different; ND = Not determined.

Table 2: Mean (\pm SEM) Semen morphological abnormalities of Yankasa rams experimentally infected with *Fasciolagigantica*

Weeks	Groups	DMP (%)	CT (%)	BT (%)	AC (%)	FT (%)	DH (%)	Abnormal sperm (%)
0-2	ND		ND	ND	ND	ND	ND	ND
3	A	3.1 \pm 0.7 ^a	3.1 \pm 0.3 ^a	4.1 \pm 0.6	8.9 \pm 1.0 ^a	5.0 \pm 0.4 ^a	5.0 \pm 0.7 ^a	29.2 \pm 3.7 ^a
	B	1.0 \pm 0.5 ^b	1.1 \pm 0.4 ^b	1.4 \pm 0.5	1.4 \pm 1.4 ^b	1.4 \pm 0.4 ^b	2.0 \pm 0.5 ^b	9.3 \pm 3.7 ^b
4	A	1.1 \pm 0.3	3.3 \pm 0.7	7.1 \pm 0.4 ^a	19.4 \pm 1.1 ^a	4.0 \pm 0.4 ^a	3.8 \pm 0.5 ^a	35.2 \pm 2.5 ^a
	B	0.6 \pm 0.4	3.2 \pm 0.3	3.4 \pm 1.1 ^b	0.6 \pm 1.5 ^b	1.2 \pm 0.5 ^b	1.2 \pm 0.3 ^b	10.2 \pm 4.1 ^b
5	A	0.7 \pm 0.3	1.6 \pm 0.5 ^a	4.4 \pm 0.7 ^a	13.6 \pm 1.1 ^a	1.8 \pm 0.4 ^a	5.3 \pm 0.7 ^a	27.4 \pm 3.7 ^a
	B	0.6 \pm 0.4	1.2 \pm 0.5 ^b	2.0 \pm 0.4 ^b	2.1 \pm 0.7 ^b	1.3 \pm 0.9 ^b	1.9 \pm 0.4 ^b	10.0 \pm 0.7 ^b
6	A	1.3 \pm 0.4	1.9 \pm 0.5 ^a	2.4 \pm 0.4 ^a	4.3 \pm 1.8 ^a	4.0 \pm 0.8 ^a	10.1 \pm 0.5 ^a	24.1 \pm 2.2 ^a
	B	1.3 \pm 0.1	0.8 \pm 0.3 ^b	1.0 \pm 0.6 ^b	1.0 \pm 0.3 ^b	0.8 \pm 0.4 ^b	2.3 \pm 1.4 ^b	7.5 \pm 1.3 ^b
7	A	0.7 \pm 0.2	3.4 \pm 0.5 ^a	5.1 \pm 0.6 ^a	18.1 \pm 0.6 ^a	3.4 \pm 0.7 ^a	2.5 \pm 0.4 ^a	36.9 \pm 1.6 ^a
	B	0.6 \pm 0.2	1.6 \pm 0.2 ^b	1.2 \pm 0.2 ^b	8.2 \pm 0.7 ^b	3.2 \pm 0.3 ^b	1.7 \pm 0.2 ^b	8.9 \pm 0.7 ^b
8	A	2.9 \pm 0.5 ^a	3.9 \pm 0.6 ^a	6.7 \pm 0.7 ^a	25.1 \pm 2.3 ^a	6.4 \pm 0.7 ^a	5.6 \pm 0.8 ^a	50.9 \pm 2.3 ^a
	B	0.6 \pm 0.2 ^b	1.6 \pm 0.2 ^b	1.3 \pm 0.2 ^b	1.2 \pm 0.7 ^b	2.3 \pm 0.4 ^b	1.6 \pm 0.2 ^b	8.8 \pm 0.7 ^b
9	A	3.5 \pm 0.7 ^a	3.1 \pm 0.3 ^a	3.1 \pm 0.6	18.9 \pm 1.0 ^a	5.0 \pm 0.4 ^a	5.8 \pm 0.7 ^a	39.1 \pm 1.0 ^a
	B	1.0 \pm 0.5 ^b	1.1 \pm 0.3 ^b	1.4 \pm 0.5	2.4 \pm 1.4 ^b	1.4 \pm 0.4 ^b	2.0 \pm 0.5 ^b	9.6 \pm 1.4 ^b
10	A	3.7 \pm 0.3 ^a	1.6 \pm 0.4	4.7 \pm 0.7 ^a	15.6 \pm 1.1 ^a	1.6 \pm 0.4 ^a	5.2 \pm 0.7 ^a	32.4 \pm 1.1 ^a
	B	0.6 \pm 0.4 ^b	1.2 \pm 0.5	1.0 \pm 0.4 ^b	3.0 \pm 0.7 ^b	1.2 \pm 0.9 ^b	1.8 \pm 0.4 ^b	8.0 \pm 0.7 ^b
11	A	2.7 \pm 0.5 ^a	3.2 \pm 0.6 ^a	6.7 \pm 0.6 ^a	25.1 \pm 2.3 ^a	6.5 \pm 0.8 ^a	5.3 \pm 0.8 ^a	49.9 \pm 2.3 ^a
	B	0.5 \pm 0.2 ^b	1.7 \pm 0.2 ^b	1.2 \pm 0.2 ^b	5.2 \pm 0.7 ^b	1.2 \pm 0.4 ^b	1.6 \pm 0.2 ^b	8.8 \pm 0.7 ^b
12	A	0.7 \pm 0.2 ^a	1.6 \pm 0.5 ^a	7.7 \pm 0.7 ^a	23.5 \pm 1.0 ^a	1.7 \pm 0.4 ^a	5.4 \pm 0.7 ^a	40.4 \pm 1.0 ^a
	B	0.4 \pm 0.2 ^b	0.7 \pm 0.2 ^b	0.6 \pm 0.4 ^b	1.0 \pm 0.3 ^b	0.6 \pm 0.4 ^b	1.0 \pm 0.4 ^b	7.8 \pm 0.6 ^b

^{ab}Means in the same rows for each parameter with different superscript alphabets between group A and B are statistically ($p < 0.05$) different; ND = Not Determined; MPD = Mid Piece Detached; CT = Coil Tail; BT = Bent Tail; AC = Abnormal Acrosome; FT = Free Tail; DH = Detached Head

DICUSSION

This study revealed the adverse effects of *Fasciolagigantica* infection on Testosterone profiles at different time intervals of the weeks. Testosterone concentration remarkably decreased in infected rams in group A and this result agreed with the findings of Wiedosari and Copeman, (1990). The testosterone profiles were episodic and the peak of the concentration, occurring in the morning which agreed with the result obtained by Rekwot *et al.* (1987a). The

results of semen volume, sperm motility and sperm concentration obtained in the present study agreed with the finding of Kishk (2008), who showed that testosterone levels are good markers of semen quality and sperm production.

There was a significant decline in semen volume in group A, this agreed with the findings of Ahmed *et al.* (2007). The reason for this progressive drop in ejaculate volume may be due to anorexia and pyrexia associated with fasciolosis. This progressive decrease in semen volume might attributed to the deleterious effect of

fasciolosis on the reproductive organs, particularly the accessory sex glands responsible for the bulk of the semen. The progressive decrease in semen volume agrees with the findings of Sekoni and Rekwot (2003) and Ahmed *et al.* (2007). The continued increase in semen volume of the control group was probably due to improved nutrition (Rekwot *et al.*, 1987a).

The sperm motility was adversely affected by *Fasciolagigantica* infection. This finding may be attributed to the total disruption of energy supply required for semen motility due to the destruction of the hepatocytes, as this may affect the secretion of accessory sex glands that is rich in carbohydrate and amino acids. The decrease sperm motility agrees with findings of others Sekoni *et al.* (1990), Ahmed *et al.* (2007), Marai *et al.* (2007) and Raadsma *et al.* (2007) attributed faulty spermatogenesis to anorexia, pyrexia and inability of the liver to synthesis cholesterol, which is attributable to Fasciolosis.

Semen concentration was also adversely affected in the present study and this result agreed with the findings of Ahmed *et al.* (2007). The progressive decrease in sperm concentration in the infected rams in group A may be attributed to faulty spermatogenesis due to anorexia and low protein level, associated with the disease. Sperm concentration was also apparently, affected by immunological disorders induced by the parasite infection immune system perceives sperm cells as non-self, sperm cells may come under attack inside the testes (Raadsma *et al.*, 2007; Rodriguez-Martinez, 2003).

The semen morphology and viability were affected in group A rams. This agreed with findings of Ahmed *et al.* (2007). This finding may be attributed to decreased appetite and interference with post-absorptive metabolism of protein, carbohydrates and minerals, which had significant negative effect on sperm production as reported by Sekoni *et al.* (1990) and Kumar *et al.* (2008).

CONCLUSION

This study shows that experimental *F. gigantica* infection in Yankasa rams, had advanced effects on sperm production. Fasciolosis compromised the metabolic activities of the liver and synthesis of cholesterol that play a vital role in realisation of testosterone. Infected rams continued to lose weight, became weak and anaemic.

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