Testosterone Profiles and Haematological Parameters of Yankasa Rams following Cypermethrin Treatment

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ABSTRACT

This study was designed to investigate the testosterone profiles and haematology of Yankasa rams treated with Cypermethrin. Sixteen rams aged 18 - 30 months and weighing between 21.5 - 46.5kg were used. The rams were divided equally into two groups (A and B) A served as the treatment group while group B was the control. The rams in (A) were given Cypermethrin (3%) at the dose rate of 3mg/kg (0.1ml/kg) b.w. (B) rams were given distilled water. These treatments were repeated forthrightly for a period of 12 weeks. 5ml of blood was aseptically collected weekly by jugular venipuncture using a 5 ml syringe and 18 gauge sterile needle from each of the animals between 8.00 and 10.00am. The collected blood samples were divided into two for haematology and testosterone assay. Packed cell volume, total white blood cell count and differential white blood cell count were done using the blood in anticoagulant bottle, while the serum samples were used for testosterone assay. Bovine testosterone (T) ELISA KITS (Wkea®) were used. Results showed no statistically significant difference between the two groups during the treatment period ($P>0.05$) across the parameters measured. It was concluded that topical application of Cypermethrin at the dose rate of 3mg/kg body weight for twelve weeks to Yankasa rams did not affect the serum testosterone profiles and haematological parameters measured. It was recommended that further studies be extended to the bovine species to establish more data about testosterone profiles and haematology in ruminants treated with Cypermethrin.

Keywords :

Testosterone,
Haematology,
Cypermethrin,
Yankasa,
rams.

I. INTRODUCTION

Testosterone is required for the production of sperm and their subsequent maturation in the epididymis, for the function of accessory sex glands and for the development of masculine secondary sexual characteristics [1]. Within the testis, androgen receptors are present in Leydig, Sertoli and myoid cells [2] but not in germ cells. Testosterone is converted to 5α – dihydrotestosterone (DHT) by 5α – reduction in the Sertoli cells and in accessory sex glands [3]. DHT which is not susceptible to aromatization and is a more potent androgen than testosterone itself, appears to be the primary androgen controlling accessory sex gland activity, whereas testosterone is the primary androgen involved in spermatogenesis [4]. Both testosterone and DHT are bound within the tubule lumen by the secretary product of the Sertoli cell, androgen-binding protein (ABP). The role of ABP therefore appears to be to maintain high androgen concentrations in the lumina of the seminiferous tubule and epididymis.

The main target of follicle stimulating hormone (FSH) is the Sertoli cell, where it also acts through adenylate-cyclase-linked enzyme systems. Under the influence of FSH, Sertoli cells secrete ABP [5] and aromatize testosterone into oestrogens [6], while FSH is also responsible for switching on a great many genes in the Sertoli cell that regulate or support spermatogenesis [4]. Most aspects of spermatogenesis require support by FSH and/or testosterone.Cypermethrin has a molecular formula: $C_{22}H_{19}O_3NCL_2$, with a relative molecular mass of 416.3. Cypermethrin is the ISO name for the pure racemic compound, consisting of 8 stereo isomers. The technical products commonly available contain more than 90% Cypermethrin and the ratio of cis to trans isomer varies from 50:50 to 40:60 [7].Technical Cypermethrin varies from a viscous, yellow liquid to a semi solid crystalline mass at ambient temperatures [7]. Cypermethrin can be found in trace amounts or at higher concentrations in soil and air [8]. In mammals, Cypermethrin can accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, lung, blood and heart. However, the main target for Cypermethrin is the central nervous system [8].The absorption and elimination of Cypermethrin is reported as rapid in the different mammalian species tested [9]. The major metabolic reaction is cleavage of the ester bond followed by hydroxylation and conjugation of cyclopropane and phenoxybenzyl moieties. The half-life in the fat of rats is about 9 - 12 days for the cis-isomer and 3-4 days for the trans-isomer, the acute toxicity of Cypermethrin for mammals is of moderate order. The oral LD_{50} for the rat ranged from 200 - 400 mg/kg body weight, short term and long-term toxicity studies on rats, mice, and dogs have shown effects on growth, the liver and kidneys, the nervous system and the blood. [9].Cypermethrin is also a skin and eye irritant, slight to severe skin irritation, decreased food consumption, body weight and absolute and relative gonad weights have been observed in rabbits treated with Cypermethrin [10]. Dosing of adult male NMRI mice for 35 consecutive days with Cypermethrin decreased sexual behaviours (sniffing, following, mounting and coupling). However, continued treatment with Cypermethrin lowered circulating testosterone levels significantly and increased the serum FSH and LH levels [11].

It has been determined that pyrethroid type II insecticides are involved in interplay with the picrotoxin site of the gama aminobutyric acid (GABA) receptor complex. Thus sexual behaviour is encompassed by the many behaviours affected by the GABA neutrotransmitter. This neutrotransmitter is deemed to suppress male copulatory behaviour and the thorough erectile response in particular [11]. Stimulation of GABA receptors in the middle preoptic area (MPOA) has been shown to diminish the number of animals that demonstrated mounts and ejaculations. Treatment of rats with Cypermethrin at doses of 18.93 or 39.66mg/kg per day decreased FSH and LH levels as well as testosterone levels. However treatment with lower doses of Cypermethrin decreased serum testosterone levels while increasing serum LH and FSH levels [11].

The key role of testosterone in health (enhanced production of red blood cells, increased energy and protection against osteoporosis) as well as sexual functioning (increased libido) is noticeable and disruption of its production may impair male reproductive health (Jalal *et al*., 2010). Some pyrethroid exposures in rats have been reported to cause significant decreases in testicular enzymes involved in testosterone biosynthesis, such as 17β-hydroxysteroid dehydrogenase (17β-HSD) and glucose-6- phosphate dehydrogenase, which might be due to interference with testicular testosterone synthesis. Insufficiency of the above-mentioned proteins significantly decreases testosterone biosynthesis [11]. Elevated levels of FSH and LH can result from the direct effects of pyrethroid on the CNS or they can be in response to decreased testosterone by negative feedback of the anterior pituitary. It has been suggested that the insecticide Cypermethrin modifies normal sexual behavior and testosterone levels and might have exert certain toxic effects on humans [11].A research has shown that a receptor protein found in high concentration in the testes is inhibited by Cypermethrin and could disrupt the normal functioning of sex hormones [12].In addition to neurons, reproductive organs are other targets of Cypermethrin. Cypermethrin decreased the weight of testosterone-sensitive organs, increase the height of seminal gland epithelium and reduced sperm count and maturity in male mice. Moreover, Cypermethrin significantly reduced serum concentrations of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [8]. The mechanism by which Cypermethrin affects male reproduction is unclear [8]. Pyrethroids are rapidly metabolized in mammals and several studies have shown that Cypermethrin damages the brain, liver and erythrocytes by causing oxidative stress. [8]. Three doses of β-Cypermethrin decreased body weight gain and weight of testosterone-sensitive organs such as testes, epididymis, seminal vesicles, and prostate glands. Sperm count, viability and intact acrosome population [8].Qualitative analyses revealed that low dose (1mg/kg) of beta-Cypermethrin decreased the number of interstitial Leydig cells but did not affect the intratubular compartment of seminiferous tubules [8]. Low dose of beta- Cypermethrin did not significantly affect sperm concentration, while a high dose of (20mg/kg) significantly reduced the number of sperm cells in the seminiferous tubules, serum testosterone and steroidogenesis acute regulatory protein (sTAR) [8]. A dose of 10mg/kg and 20mg/kg, reduced serum testosterone to approximately 65% and 30% of control, respectively and sTAR was reduced to approximately 5.3% of control by (20mg/kg) [8]. β- Cypermethrin affected the ultrastructure of Leydig cells as evidenced in an experiment where vehicle-treated controls had Leydig cells with normal smooth endoplasmic reticulum (SER) and mitochondria profiles [8]. Most of the reports of the effects of Cypermethrin on testosterone profiles and haematology were in species other than the ruminants. This study was designed to investigate the testosterone profiles and haematological parameters of Yankasa rams treated with Cypermethrin topically.

II. MATERIALS AND METHODS

2.1. Study location

The research was carried out at the National Animal Production Research Institute (NAPRI) Shika, Ahmadu Bello University Zaria, which is situated in the Northern Guinea Savannah and lying between latitudes 11° and 12°N and longitude 7° and 8°E, at an elevation of 650m above sea level. The area has an annual rainfall of 1100mm. [13]. There are two seasons {rainy season (May-October) and dry season (November-April)}.

2.2. Experimental animals

The animal experiments followed the principles of the Laboratory animal care [14].Sixteen sexually-mature, healthy Yankasa rams aged 18 - 30 months and weighing between 21.5 - 46.5kg with clinically normal genitalia were used. The rams were purchased from the open market in Sabua Local Government Area of Katsina State. They were acclimatized for two weeks at the Small Ruminant Research Programme Experimental Unit of NAPRI, after which they were judged to be in good health based on clinical findings, haematological and faecal examinations. They were housed at the Small Ruminant Research Programme Experimental Unit of NAPRI. The house was made of brick concrete pens with concrete floors. The rams were divided into two groups of eight each. They were given concentrate feed *ad- libitum* (cotton seed, maize offal, maize, wheat offal, bone meal and salt) in the morning and later in the evening; hay was made available during the day at intervals. The hay used was *Digitariasimuthii*, and water was given *ad- libitum*.

2.3. Experimental design and treatment

The 16 rams were divided equally into two groups (A and B) Group A served as the treatment group while group B served as the control. The animals were acclimatized for two weeks during which time blood and faecal samples were collected and analyzed for haemoparasites and helminths and treatments given where necessary. Blood and serum samples were taken to establish base line data. This was done once before the experiment started.

2.3.1. Administration of 3% cypermethrin

The rams in group (A) were given Cypermethrin (3%) at the dose rate of 3mg/kg (0.1ml/kg) body weight, topically as pour-on. The control group (B) rams were given distilled water at the same dose rate of 0.1ml/kg body weight topically as pour-on. These treatments were repeated every two weeks for a period of 12 weeks. Blood and serumsamples were collected before the administration of 3% Cypermethrin to establish base line data. This was done by getting the average values for the eight animals in each group before the experiment started.

2.4. Sample collection and analysis

2.4.1. Haematological parameters

5ml of blood was aseptically collected weekly by jugular venipuncture using a 5 ml syringe and 18 gauge sterile needle from each of all the animals between 8.00 and 10.00am. The collected blood samples were divided into two: 2ml in a sample bottle containing ethylene diamine tetra acetate (EDTA) anticoagulant at the rate of 2mg/ml of blood for haematological analysis, and the other 3ml of blood without anticoagulant was analyzed for serum sample. Packed cell volume (PCV), total white blood cell count (TWBC) and differential white blood cell count (DWBC) were done using the blood in anticoagulant bottle, while the serum samples were used for testosterone assay.

Blood samples for testosterone assay were allowed to clot at room temperature for 30 minutes before being centrifuged at 1000g for 15 minutes. The supernatant (sera) were collected in serum vials and stored in the deep freezer at -20^{o}C at the Theriogenology Laboratory of the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, until assay.

2.4.2. Haematological analyses

The white blood cell (WBC) counts were done using a haemocytometer [15]. The packed cell volume (PCV) was estimated by the microhaematocrit method [16].Non-heparinized capillary tubes (John Poulten Limited, England) were filled with the blood collected from the animals in bottles containing EDTA. The tubes were loaded into a microhaematocrit centrifuge machine (Hawksley, England) and centrifuged at 1000g for ten minutes. A haematocrit reader (Hawksley, England) was used to read the PCV value for each of the tubes.The method described by Dacie and Lewis (1991) [17] was used. Total leucocyte counts were determined using a haemocytometer. For the differential leucocyte count. A drop of blood was placed on one end of a glass slide (Unescope, England) and a (blood) film was made using a spreader. It was then allowed to air-dry. Leishman stain was poured over the film to cover it for two minutes. The film afterwards was rinsed with phosphate buffered saline and allowed to stand for ten minutes. The film was then viewed under a microscope using oil immersion at x 100 magnification.

2.4.3. Testosterone assay using ELISA

The assay was done in the Endocrinology laboratory of NAPRI. Bovine testosterone (T) ELISA KITS (Wkea®) were used. The microtiter wells were coated with antibody directed towards a unique antigen site on a testosterone molecule. An aliquot of the serum samples containing endogenous free testosterone were incubated in the coated wells with enzyme conjugates, which were antifree testosterone antiserum conjugated with horseradish peroxide, for 30 minutes at 37^{o}C after closing plates with closure plate membranes.

After the incubation, the unbound conjugates were washed off with distilled water. The amount of bound peroxide was proportional to the concentration of free testosterone in the sample. Substrates A and B were added to each well, covered and incubated again for 15 minutes at 37^{o}C. After adding substrate solutions, the intensity of colour developed was proportional to the concentration of free testosterone in the serum samples. The reactions were stopped with stop solution and the optical density of each well was determined within 15 minutes using a micro plate reader/ EL-800, the results obtained were multiplied by the sample dilution. Details of the assay procedure was as described by the manufacturer. [18].

2.5. Statistical analysis of data

Data were expressed as means and Standard Error of Mean (SEM). Data were analyzed using descriptive statistics and paired student's t-test with SPSS/PC computer program (Version 20.0, SPSS®, Chicago IL, USA). Differences with confidence values of $p < 0.05$ were considered statistically significant [19].

III. RESULTS

3.1.Serum testosterone

The mean weekly serum testosterone of the experimental and control groups are presented (Fig. 1). The values fluctuated throughout the study however there were no statistically significant difference between the two groups during the treatment period ($P>0.05$). The base line data for testosterone profiles and haematological parameters in both the treated and control rams is presented (Table 1).

Table 1. (Mean ± SEM)Base line data for testosterone profiles and haematological parameters in both the treated and control rams.

Parameters	Treated n = 8	Control n = 8
Testosterone (ng/ml)	7.50 ± 2.02	6.19 ± 1.73
Pack Cell Volume (%)	33.25 ± 2.30	35.87 ± 1.44
TWBC (x103/ml)	7.93 ± 0.99	8.20 ± 0.68
Neutrophils (%)	38.87 ± 1.75	34.13 ± 2.22
Lymphocytes (%)	60.00 ± 2.11	64.13 ± 1.92
Monocytes (%)	0.00 ± 0.00	1.00 ± 0.68
Eosinophils (%)	0.25 ± 0.25	0.50 ± 0.38
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00
Bands (%)	0.88 ± 0.64	0.25 ± 0.25

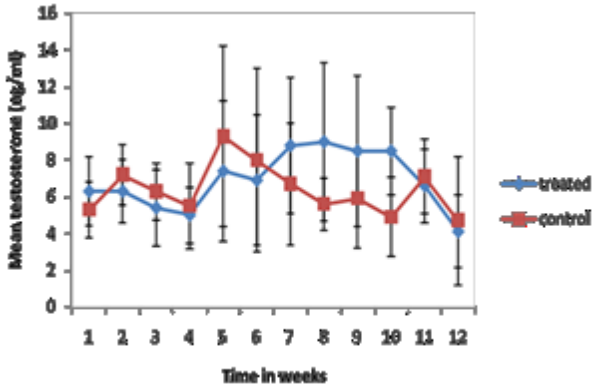

Fig : 1 : Mean weekly testosterone (ng/ml) of Yankasa rams during the treatment period.

3.2.Haematological parameters

There was a variation in the mean weekly Packed Cell Volume of the treated and control groups (Fig. 2). The difference was not statistically significant (P> 0.05). The mean weekly Total White Blood Cell Count (TWBC) of the treated and the control group are presented (Fig. 3). There was no statistically significant difference between the two groups during the treatment period (P>0.05).The mean weekly lymphocyte count of both the treated and control groups are presented (Fig. 4). There was no statistically significant difference between the two groups during the treatment period (P>0.05). The mean weekly neutrophil count of the treated and control groups are presented (Fig. 5). There was no statistically significant difference between the two groups during the treatment period (P>0.05).

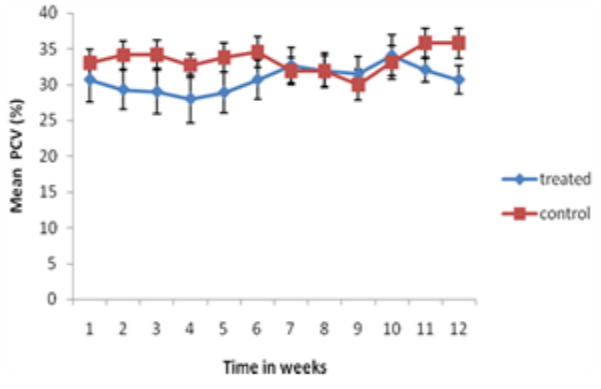

Fig : 2 Mean weekly packed cell volume (%) of Yankasa rams during the treatment period

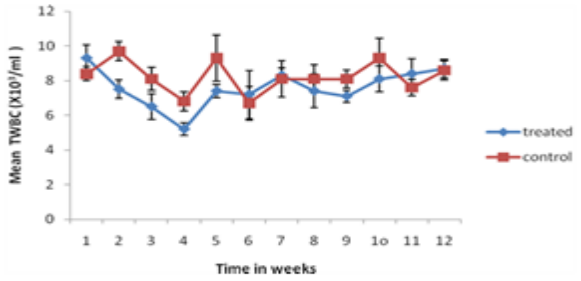

Fig : 3 Mean weekly total white blood cell count (x103 /ml) of Yankasa rams during the treatment period

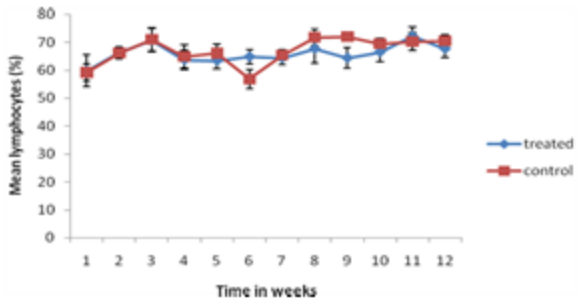

Fig : 4 Mean weekly lymphocytes (%) of Yankasa rams during the treatment period

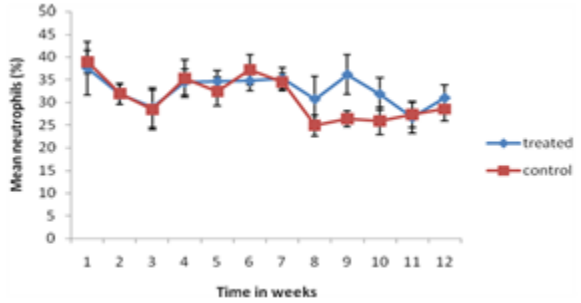

Fig : 5 Mean weekly neutrophils (%) of Yankasa rams during the treatment period

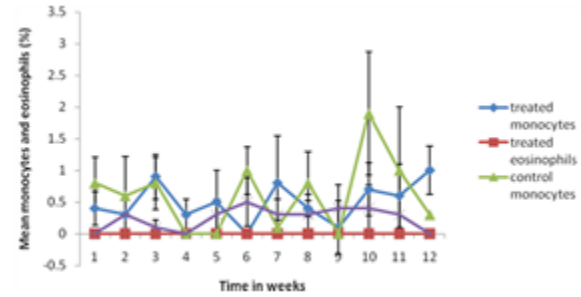

Fig : 6 Mean weekly monocytes and eosinophils (%) of Yankasa during the treatment period

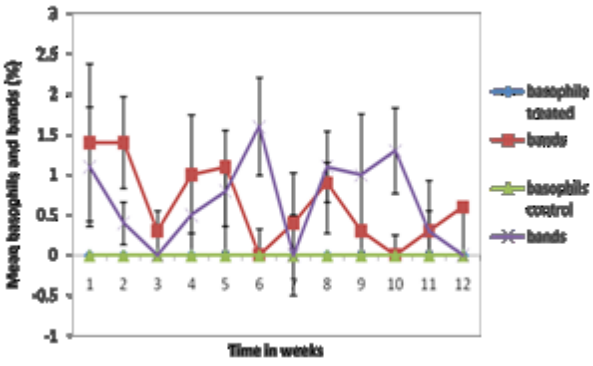

Fig : 7 Mean weekly basophils and bands (%) of Yankasa rams during the treatment period

Other cells of differential white blood cell count which include the monocytes, eosinophils, basophils and bands are presented (Fig. 6 and Fig. 7). Basophils were not seen in both the treated and control (Fig. 7), eosinophils were seen in the treated group. They were more frequent in the control group. The bands were present in the two groups (Fig. 6). The monocytes were also present in the two groups (Fig. 7). There was no statistically significant difference in the differential leucocyte count between the two groups during the treatment period (P>0.05).

IV. DISCUSSION

The results of this work revealed that Cypermethrin did not affect the packed cell volume (PCV) of the treated rams. There was no statistically significant difference between the PCV of the treated and the control rams during the treatment period (p>0.05). This finding is not in agreement with reports on chronic effects of Cypermethrin on the blood. Long-term feeding studies with laboratory animals have shown it caused reduced growth rate increased liver weight. In mice, it caused reduced weight gain, mild anemia and increased liver weight, [12]. Our result did not concur with this report in terms of mild anemia. This may be due to the route of administration.

Cypermethrin had no adverse effect on the total white blood cell count (DWBC) and the differential white blood cell count (DWBC) of Yankasa rams at the dose rate of 3mg/kg body weight topically. There was no significant difference between the (TWBC) and (DWBC) of the treated and control rams (p>0.05). This finding however, did not support the report that Cypermethrin has effect on the immune system [12]. It has been reported that in both rabbits and rats, Cypermethrin has been shown to suppress the immune system function. [12]. The variation in this report may be due to the dose, route of administration and species of animals involved.

Cypermethrin did not affect the serum testosterone of treated Yankasa rams. There was no statistically significant difference between the serum testosterone of the treated and control rams during the treatment period (p>0.05). Reduction in serum testosterone of exposed animals have been reported, dosing of adult male NMRI mice for 35 consecutive days with Cypermethrin decreased sexual behaviours (sniffing, following, mounting and coupling) [11]. However, continued treatment with Cypermethrin lowered circulating testosterone levels significantly and increased the serum FSH and LH levels [11]. Treatment of rats with Cypermethrin at doses of 19.93 or 39.66mg per day decreased FSH and LH levels as well as testosterone levels, however, treatment with lower doses of Cypermethrin decreased serum testosterone levels while increasing serum LH and FSH levels [11].

Serum testosterone was not affected by low dose (1mg/kg) of beta- Cypermethrin, but was reduced to approximately 65% and 30% of control with the moderate (10mg/kg) and high doses (20mg/kg) respectively (Wang *et al*., 2009).It has also been reported that ingestion of Cypermethrin at a concentration of 18.93 or 39.66mg/day by Sprague Dawley rats resulted in significant increase in weight of testes as well as significant reduction in serum levels of testosterone, follicle-stimulating hormone and luteinizing hormone [20]. The adverse effect of Cypermethrin on the blood circulating testosterone have also been emphasized by Abd-Allah, (1995) [21]. Some pyrethroid exposures in rats have been reported to cause significant decreases in testicular enzymes for testosterone biosynthesis [11].

The implication of our finding and other reports is that in general, route of administration, dose and duration of treatment are most likely factors influencing the effect of Cypermethrin on the serum testosterone. The report that low dose (1mg/kg) of Cypermethrin did not affect the serum testosterone may be because Cypermethrin is rapidly metabolized by the body and if a single low dose is given, it may not have an effect. This is in agreement with our finding. The administration of Cypermethrin at a low dose of 3mg/kg via the skin as in our study is not likely to affect the serum testosteronebecause it is rapidly metabolized in the body starting from the skin before systemic circulation occurs. Synthetic pyrethroids are generally metabolized in mammals through ester hydrolysis, oxidation and conjugation (WHO, 1989b). The major urinary metabolites of Cypermethrin are a variety of conjugates of cis and trans (DCVA) 3 – phenoxybenzoic acid (3 PBA) and 3 – (4 – dydroxyphenoxy) benzoic acid (40H3 PBA). Marked differences in the urinary metabolite profile by oral and dermal routes in human volunteer studies suggest that Cypermethrin could be significantly metabolized in the skin before systemic circulation occurs. [22]. Therefore, several other factors may contribute to the concentration of Cypermethrin in the blood and thus the effect of the chemical on serum testosterone and haematological parameters. Testosterone enhances production of red blood cells, therefore, looking at the result in general, testosterone, PCV and other blood parameters measured followed the same trend. This shows that other health functions of testosterone were not likely to be affected in the study.

V. CONCLUSION

It was concluded that topical application of Cypermethrin at the dose rate of 3mg/kg body weight for twelve weeks to Yankasa rams did not affect the serum testosterone level, packed cell volume, total white blood cell count and differential white blood cell count. The implication of our finding and other reports is that in general, route of administration, dose and duration of treatment are most likely factors influencing the effect of Cypermethrin on the serum testosterone and blood picture. The report that low dose (1mg/kg) of Cypermethrin did not affect the serum testosterone may be because Cypermethrin is rapidly metabolized by the body and if a single low dose is given, it may not have an effect. The administration of Cypermethrin at a low dose of 3mg/kg body weight topically, like our study is not likely to affect the serum testosterone, PCV and other haematological parameters because it is rapidly metabolized in the body starting from the skin before systemic circulation occurs. It was recommended that further studies be extended to the bovine species to establish more data concerning Cypermethrin and testosterone cum haematology in ruminants.

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