

Gonadal and Epididymal Sperm Reserves of Yankasa Rams Treated with Cypermethrin

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Abstract: Cypermethrin 3% (30 gram/liter) at the dose rate of 3mg/kg (0.1ml/kg) was used as pour-on. Sixteen healthy Yankasa rams with clinically normal genitalia were used. The animals were divided into groups A and B i.e. experimental and control groups. The experimental group was given Cypermethrin treatment as pour-on fortnightly for a period of twelve weeks. The control group was given distilled water at the same rate (0.1ml/kg) and route of administration for the same length of time. Rams from both the experimental and control groups were sacrificed for estimation of gonadal and epididymal sperm/spermatids reserves at the end of the twelve week studies. Epididymal and gonadal sperm reserves were estimated using a haemocytometer. Results showed that gonadal sperm reserves was not significantly different between the two groups ($P>0.05$). Epididymal sperm reserves showed that there was a significant difference between the cauda epididymis of the experimental and the control rams with the experimental rams showing lower cauda epididymal reserves of $149.75\pm 13.13 \times 10^6/g$ while the control rams had a value of $244.56\pm 17.09 \times 10^6/g$ ($P<0.05$), the caput and corpus epididymis were not significantly different. It was concluded that 3% Cypermethrin at the dose rate of 3mg/kg given for a period of twelve weeks affects the epididymal sperm reserves at the cauda segment. It was therefore recommended that the use of Cypermethrin be applied with caution in rams as it may affect the fertility of Yankasa rams negatively.

Keywords: Cypermethrin, Sperm Reserves, Rams

1. Introduction

Protein is an essential food ingredient required for optimum growth of human beings in addition to carbohydrate, fats and other essential vitamins and minerals. It is required for growth and necessary repairs of the body cells [1]. To meet the nutritional requirement of people in developing countries of the world where protein and calorie malnutrition is a widespread phenomenon the quantity and quality of protein available to the people has to be increased.

Yankasa sheep have an important role in alleviating the problem of animal protein deficiency in Nigeria, because

they are the most numerous and most widely distributed among the 22.3 million indigenous sheep population [2] in Nigeria. Livestock have been reported to provide only 20% of human protein consumption in developing countries [3]. This low productivity is due among other things to animal diseases and infertility [4]. To improve the productivity of animals, strategies will have to focus on combating infertility, since this is a major problem in livestock production. The control of ectoparasites involves the use of chemicals in various forms. The frequent use of chemicals on the rams may be exposing them to some fertility problems. Cypermethrin dominates other acaricides in Nigerian

livestock markets. Cypermethrin is an insecticide in the synthetic pyrethroid family. It was first marketed in 1977. [5]. All of the insecticides in this family have chemical structures that are loosely based on pyrethrins which are insecticidal compounds found in chrysanthemum flowers. There are eight different ways that the atoms that make up the Cypermethrin molecules can arrange themselves in three dimensions. These are called isomers; Cypermethrin is a mixture of all eight isomers [5].

In animals, Cypermethrin has been used as a chemotherapeutic agent against ectoparasite infestations [6]. There have been reports of lowered fertility in experimental animals exposed to Cypermethrin [3, 7, 8, 21]. Scanty work has been documented on the fertility problems posed by this chemical on the rams. The knowledge about gonadal and extragonadal sperm reserves seems to be essential for a careful assessment of male fertility [9]. Extragonadal sperm reserves (ESR) represent sperm stored in the caput, corpus and cauda epididymis, and the number of spermatozoa stored in the epididymis has been said to be related to sperm production by the testes [10]. This study was designed to investigate gonadal and epididymal sperm reserves of Yankasa rams treated with Cypermethrin with the following objectives, (1) to determine the gonadal sperm/spermatids reserves of Yankasa rams treated with Cypermethrin. (2) To determine the epididymal sperm/spermatids reserves of Yankasa rams treated with Cypermethrin.

2. 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 between longitude 7° and 8°E at an elevation of 650m above sea level. The area has an annual rainfall of 1100mm. [11]. There are two seasons rainy season (May-October) and dry season (November-April).

2.2. Experimental Animals

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 Unit of NAPRI, after which they were judged to be in good health based on clinical findings, hematological and faecal examinations. The rams were housed at the Small Ruminant Research Unit of NAPRI. The house was made of brick concrete pens with concrete floors. The rams were divided into two groups of eight rams 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 *Digitaria simuthii* and water was given *ad-libitum*

2.3. Experimental Design and Treatment

The rams were divided into groups A and B, where A was the treated group and B the control group of eight rams each for the study. The animals were acclimatized for two weeks during which blood and faecal samples were collected and analyzed for haemoparasites and helminthes and treatments were given where necessary.

Administration of 3% Cypermethrin

The rams in group (A) were administered Cypermethrin 3% at the dose rate of 3mg (0.1ml)/kg body weight, topically as pour-on. The control group (B) were administered distilled water at the same rate of 0.1ml/kg body weight topically as pour-on. These treatments were repeated every two weeks for a period of 12 weeks.

2.4. Sample Collection and Analysis

The rams were sacrificed at the end of 12 weeks and the testes were collected for estimation of gonadal and epididymal sperm reserves.

Determination of Gonadal and Epididymal Sperm Reserves

Four rams each from groups A and B were sacrificed and the testes removed intact then dissected free from any extraneous tissue. Tunica albuginea was removed using a scapel blade and the testicular parenchyma was weighed, the left and right epididymis were separated into caput, corpus and cauda based on gross anatomy and were weighed, then were placed in normal saline for onward estimation of epididymal sperm reserves. Gonadal and epididymal sperm reserves were determined as described by [12; 13; 14]. The testicular parenchyma was weighed, sliced and homogenized for two minutes with 50ml of 0.9% NaCl containing antibiotics (sodium penicillin G, 100 iu/ml and streptomycin sulphate, 1mg/ml) to prevent bacterial growth. In determining the epididymal sperm reserves, caput, corpus and cauda epididymis were isolated, weighed and minced with a pair of scissors separately in 20ml of 0.9% NaCl solution. All tissues were homogenized 2 - 6 hours after collection, testicular homogenates and epididymal samples were refrigerated overnight. After 24 hours the samples were filtered through gauze and the filtrate volume measured. 1 ml of epididymal filtrate was diluted with 2 ml of saline solution. Sperm reserves of the gonads as well as epididymal sperm reserves were determined using a haemocytometer and light microscope, by charging the haemocytometer with a drop of the filtration from each sample. Sperm cells and spermatids were counted diagonally from top left to bottom right in five large squares according to the method of [15].

2.5. Statistical Analysis of Data

Results were expressed as means and Standard Error of Mean (SEM). Data was analyzed using 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 [16].

3. Sperm Reserves

3.1. Gonadal Sperm Reserves

The mean parameters for the gonadal sperm reserves of the experimental and control groups are presented (Table 1). The mean testicular weight for the experimental and control groups after sacrificing the animals were 143.81±7.71g and 130.43±0.63g respectively (Table 1) there was a statistically significant difference in the testicular weight of the rams (P<0.05). The difference between the live weight of the experimental and control rams was not statistically significant during sacrifice (P>0.05).

The mean testicular volume for the experimental and control groups were 53.25±0.63ml and 56.25±1.65ml respectively (Table 1). The difference was statistically not significant (P>0.05). The testicular sperm/spermatids reserves were 77.50±12.94 x 10⁶/g and 86.75±12.98 x 10⁶/g respectively (Table 1), the difference was statistically not significant (P>0.05).

Table 1. Mean live weight, testicular volume, weight and gonadal sperm reserves of the experimental and control rams at the end of the experiment (± SEM).

	Experimental n=4	Control n=4
Live weight (kg)	29.80 ± 1.40	26.30 ± 1.30
Paired Testicular weight	143.80 ± 7.71 ^a	130.13 ± 15.03 ^b
Paired testicular volume (ml)	53.25 ± 0.63	56.25 ± 1.65
Paired testicular sperm reserves (x10 ⁶ /g of testis)	77.50 ± 12.94	86.75 ± 12.98

(a, b) means in the same row with different superscript alphabet are statistically significant (P<0.05).

3.2. Epididymal Sperm/Spermatids Reserves

The mean parameters measured for the epididymal sperm reserves of the experimental and the control groups are presented (Table 2). The mean epididymal sperm/spermatids reserves of the experimental and control groups were (1) caput: 96.00±6.12 x 10⁶/g and 83.50±20.84 x 10⁶/g respectively, (2) corpus: 94.50±18.68 x 10⁶/g and 77.50±43.70 x 10⁶/g respectively and (3) cauda: 149.75±13.13 x 10⁶/g and 244.25±17.09 x 10⁶/g respectively (Table 2). There was a statistically significant difference between epididymal sperm/spermatids reserves in the cauda epididymis of the experimental and control groups (P<0.05).

The mean filtrate volume (ml) of the experimental and control groups were (1) caput: 19.50±0.29ml and 18.75±0.25ml respectively, (2) corpus: 17.25±2.43ml and 18.75±0.25ml respectively and (3) cauda: 19.25±0.48ml and 19.50±0.29ml respectively (Table 2). There was no statistically significant difference in filtrate volume between the two groups in any part of the epididymis (P>0.05). The mean epididymal length (cm) of the experimental and control groups were (1) caput: 4.93±0.29cm and 5.48±0.31cm respectively, (2) corpus: 8.70±0.29cm and 8.15±0.90cm respectively and (3) cauda: 3.83±0.23cm and 4.35±0.27cm respectively. There was no statistically significant difference

in epididymal length between the two groups in any part of the epididymis (P>0.05). The mean epididymal weight (g) of the experimental and control groups were (1) caput: 5.70±0.47g and 6.25±1.08g respectively, (2) corpus: 2.53±0.66g and 2.45±0.68g respectively and (3) cauda: 7.83±36g and 8.15±0.23g respectively. There was no statistically significant difference in epididymal weight between the two groups in any part of the epididymis (P>0.05).

Table 2. Mean epididymal length, weight, epididymal sperm/spermatids reserves and filtrate volume of experimental and control rams after sacrifice (± SEM)

Parameters	Experimental n=4	Control n=4
Paired epididymal length (cm)		
Caput	4.93 ± 0.29	5.48 ± 0.31
Corpus	8.70 ± 0.29	8.15 ± 0.90
Cauda	3.83 ± 0.23	4.35 ± 0.27
Paired epididymal weight (g)		
Caput	5.70 ± 0.47	6.25 ± 1.08
Corpus	2.53 ± 0.66	2.45 ± 0.68
Cauda	7.83 ± 36	8.15 ± 0.23
Paired epididymal sperm/spermatids reserves (x 10 ⁶ /g testis)		
Caput	96.00 ± 6.12	83.50 ± 20.84
Corpus	94.50 ± 18.68	77.50 ± 43.70
Cauda	149.75 ± 13.13 ^a	244.25 ± 17.09 ^b
Paired filtrate volume (ml)		
Caput	19.50 ± 0.29	18.75 ± 0.25
Corpus	17.25 ± 2.43	18.75 ± 0.25
Cauda	19.25 ± 0.48	19.50 ± 0.29

(ab) means in the same row with different superscript alphabets are statistically significant (P<0.05).

The mean epididymal weight (g) of the experimental and the control groups were (1)

4. Discussion

The result of this experiment on gonadal sperm reserves showed that gonadal sperm/spermatids reserves was not affected. Testicular volume was not affected. Weight measurement of the testes revealed that testicular weight was statistically significantly higher in experimental rams than in the control rams. Live weight also had higher values in the experimental rams the differences were not statistically significant. However, the epididymal component showed that there was a statistically significant difference between the epididymal sperm/spermatids reserves (cauda) of the experimental and control rams. Although, in all the parts of the epididymis (Caput, Corpus and Cauda) there was no significant difference in epididymal length, weight and filtrate volume between the experimental and control rams. Oral administration of Cypermethrin at a dose of 55.1mg/kg body weight (1/5LD₅₀) for 60 successive days to male white rats induced deleterious effects on male fertility as elucidated by the significant decrease in testicular index, sperm mass motility and spermatozoa concentration in the epididymis [7]. This report supports the result of the present work in rams. Even at a low dose of 3mg/kg body weight, if

treatment is prolonged as long as three months as in our studies, concentration of Cypermethrin in the body is likely to increase and constitute toxic dose to sperm cells.

The interesting finding here is decreased epididymal sperm reserves as reflected in the cauda epididymis and testicular weight which was statistically significantly higher in experimental rams which corroborates the following reports, ingestion of Cypermethrin at a concentration of 18.93 or 39.66mg/day by Sprague Dawley rats resulted in significant increase in the weight of testes and seminal vesicles and significant decrease in epididymal and testicular sperm counts. [17]. The restricted finding in the cauda epididymis may be because that is the major reservoir that is very sensitive to reflect mild reduction or early reduction in sperm reserves. The result of the gonadal sperm reserves which is not significant may be due to the low dose of 3mg/kg. The higher values observed in the weights of the testes in the experimental rams may be relative to the live weight of the rams which was higher in experimental than in control rams and the sizes of these organs are related to the body weight of rams. This finding is in agreement with reports of Jalal [18]. In contrast three doses of beta - Cypermethrin decreased body weight gain and weight of testosterone-sensitive organs such as testes, epididymis, seminal vesicles and prostate. [6, 19]. Cytotoxic effects of aerosols of the pyrethroid insecticide Matox[®] on male rats exposed to Matox[®] daily up to 2, 4 and 8 weeks, which exhibited a significant decrease in weights of testes, epididymis, seminal vesicle and prostate glands have been reported [20]. Slight to severe skin irritation, decreased food consumption, body weight and absolute and relative gonad weights have been observed in rabbits treated with Cypermethrin [21].

These conflicting reports on the effect of Cypermethrin on testicular weight and the accessory glands may be attributed to inaccurate interpretation since the weight of these organs are related to the live weight of the animals. Secondly, dose rate may be an important factor determining whether the effect on the weight of the testes will be positive or negative and finally, the species of animals involved in the study may be important.

In our studies 3mg/kg body weight over three month period could have built up a concentration that started affecting the sperm cells within the epididymis and this reflected in the decrease in the epididymal sperm reserves (cauda) of the experimental rams. Concentration of Cypermethrin in the body may not just depend on the dose but also on the duration of exposure.

5. Conclusion

Based on the findings of this research it was concluded that treatment of rams with 3mg/kg body weight of Cypermethrin (pour-on) led to a significant reduction in the cauda epididymal sperm reserves but did not significantly affect gonadal sperm reserves and sperm reserves in the caput and corpus epididymis. It was recommended that the

use of 3% Cypermethrin at the dose of 3mg/kg body weight be applied with caution in rams as it may affect the fertility of the rams. The use of this formulation in rams should not be prolonged up to twelve weeks.

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