

# Gonadal and Extragonadal Sperm Reserves of Yankasa Rams Fed Graded Levels of Dietary Protein Using Cotton Seed and Palm Kernel Cakes

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**Abstract:** The aim of this study was to evaluate the effects of graded levels of dietary protein using cotton seed and palm kernel cakes on gonadal and extragonadal sperm reserves of Yankasa rams. A total of 15 rams aged  $19.06 \pm 2.4$  months and weighing  $19.4 \pm 1.6$  kg with good body condition scores of 3.5 were divided into three treatment groups (A, B and C) according to the dietary protein level. Group A (n=5) received 10% crude protein level feed, group B (n=5) received 15% crude protein level feed, while group C (n=5) received 20% crude protein level feed. The rams were fed for a period of 12 weeks before the testicles were harvested. Gonadal reserves of rams fed 20%, was highest with values of  $43.3 \pm 3.9$  ( $\times 10^6$ /g) and  $47.0 \pm 2.5$  ( $\times 10^6$ /g). This was followed by rams fed 15% C. P. and 10% in decreasing order ( $28.0 \pm 3.9 \times 10^6$ /g,  $37.7 \pm 2.5 \times 10^6$ /g and  $20.7 \pm 10.1 \times 10^6$ /g and  $36.0 \pm 14.6 \times 10^6$ /g). These differences were not statistically ( $P > 0.05$ ) different. Rams fed 15% had the highest *caput* epididymal reserves with values of  $15.3 \pm 0.7$  ( $\times 10^6$ /g) for right epididymis and  $16.7 \pm 11.6$  ( $\times 10^6$ /g) for left epididymis. It was observed from this study that varying levels of protein diets using cotton seed and palm kernel cakes had no significant difference in gonadal and extragonadal sperm reserves of Yankas rams.

**Keywords:** Protein, Gonadal and Extra-gonadal Reserves, Yanksa Rams

## 1. Introduction

Chronic low animal protein intake in developing countries is a basic problem that needs an urgent solution [1]. The low animal protein intake may be attributed to low livestock productivity and therefore available animal protein are very expensive for the growing population with a very low per capita income. Successful reproduction as an important factor in livestock production economy depends on genetic merit, physical environment, nutrition and management [2].

Nutrition is a major factor in the effectiveness of reproductive function. It may affect the efficiency of related hormone production and the growth of reproductive organs.

Studies have demonstrated that luteinizing hormone (LH) secretion during early gonadotropin rise is elevated and can be sustained for a longer period when calves are fed with improved nutrition during calthood [3]. There is a serious shortage in the supply of convectional feed ingredients such as soybean meal, maize and groundnut cake for concentrate diet for ruminants. In addition, production of grains in developing countries is mostly for human consumption, leading to very high prices of the ingredients [4]. The utilization of unconventional feed resources holds great relevance to developing countries, where the main constraint

to livestock production is the scarcity and fluctuation of the quality and quantity of all-year-round animal feed supply.

Over the years, groundnut cake (GNC) and soybean (SBM) meal have remained the major protein sources in the diets of non-ruminant animals. These ingredients are also highly consumed by man, and as such, there is stiff competition between man and livestock for their consumption. Hence their prices are becoming more and more prohibitive. A possible alternative for feeding ruminants is cottonseed cake (CSC) and Palm kernel cake.

The knowledge about gonadal and extragonadal sperm reserves seems to be essential for a careful assessment of male fertility [5]. 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 [6]. This study was designed to investigate gonadal and epididymal sperm reserves of Yankasa rams that were fed graded levels of dietary protein using cotton seed and palm kernel cakes.

## 2. Materials and Method

### 2.1. Experimental Animals

A total of 15 rams aged  $19.06 \pm 2.4$  months and weighing  $19.4 \pm 1.6$  kg with good body condition scores of 3.5 were divided into three treatment groups (A, B and C) according to the dietary protein level. Group A (n=5) received 10% crude protein level feed, group B (n=5) received 15% crude protein level feed, while group C (n=5) received 20% crude protein level feed. The rams were screened for blood and helminth parasites, and appropriate treatment carried out before the commencement of the research.

### 2.2. Experimental Diets

The three levels of protein were formulated to contain 10% crude protein, 15% crude protein and 20% crude protein.

### 2.3. Gonadal Sperm Reserves

Gonadal and epididymal spermatozoa reserves were determined as described by [7]. Three rams from each treatment group were sacrificed, the length and weight of each testis were determined using a measuring tape and the digital weighing balance respectively. The *tunica albuginea* was removed from each testis. The epididymis were separated from each testis and divided into caput, corpus and cauda which was measured and weighed using a measuring tape and a digital weighing balance. Thereafter, testicular and epididymal spermatozoa numbers were determined by homogenization [8]. Each fraction was homogenised in 25ml physiological saline solution using mortar and pestle. Antibiotics (Streptomycin sulphate 1mg/ml and Sodium G 100 IU/ml) were added to saline. The homogenate volume was measured after rinsing the mortar with 10ml of physiological saline solution and adding the effluent. Exactly 2.5 ml of the homogenate was transferred into a conical flask

and further diluted with 40ml of saline. The diluted testicular homogenate sample were stored overnight at 5°C and filtered through gauze, and then the filtrate volume will be measured. Spermatozoa/spermatids concentrations were determined using a haemocytometer according to the method of [9]. The determination of spermatozoa and spermatid reserves was done according to the method of [10].

### 2.4. Gonadal Sperm Reserves

Gonadal and epididymal spermatozoa reserves were determined as described by [7]. Three rams from each treatment group were sacrificed, the length and weight of each testis were determined using a measuring tape and the digital weighing balance respectively. The *tunica albuginea* was removed from each testis. The epididymis were separated from each testis and divided into caput, corpus and cauda which was measured and weighed using a measuring tape and a digital weighing balance. Thereafter, testicular and epididymal spermatozoa numbers were determined by homogenization [8]. Each fraction was homogenised in 25ml physiological saline solution using mortar and pestle. Antibiotics (Streptomycin sulphate 1mg/ml and Sodium G 100 IU/ml) were added to saline. The homogenate volume was measured after rinsing the mortar with 10ml of physiological saline solution and adding the effluent. Exactly 2.5 ml of the homogenate was transferred into a conical flask and further diluted with 40ml of saline. The diluted testicular homogenate sample were stored overnight at 5°C and filtered through gauze, and then the filtrate volume will be measured. Spermatozoa/spermatids concentrations were determined using a haemocytometer according to the method of [9]. The determination of spermatozoa and spermatid reserves was done according to the method of [10].

### 2.5. Epididymal (Extragonadal) Sperm Reserves

The epididymis was carefully separated from the testis with a scalpel and the lengths and weights of the *caput*, *corpus* and *cauda* portions were determined using a measuring tape and a digital weighing balance. The *caput*, *corpus* and *cauda* epididymis were separated using sharp scissors, minced separately in 20 ml of saline and stored overnight at 5°C and filtered through gauze, then the filtrate volume measured. Spermatozoa/spermatid concentration was determined using a haemocytometer according to the method of [9].

### 2.6. Statistical Analysis

Data collected were expressed as means and standard error of the mean ( $\pm$  SEM). Significance of differences between treatments means were estimated at  $P \leq 0.05$  with Tukey-Kramer multiple comparison test of repeated measure analysis of variance (ANOVA). Statistical analysis was conducted using the Graphpad Instat computer programme (GRAPHPAD for Windows, Inc., version 3.05 of 2000).

**Table 1.** Ingredients and nutrient composition of experimental diets.

Groups/Ingredients (%)	A (10% C.P)	B (15% C.P)	C (20% C.P)
Maize Bran	23.5	10	2
Bagasses	20	8.5	2.5
Palm Kernel Cake	10	30	45
Cotton Seed Cake	15	30	48
Rice Bran	30	20	1
Bone meal	1	1	1
Common Salt	0.5	0.5	0.5
Total	100.00	100.00	100.00

**Table 2.** Proximate analysis of feeds (10%, 15% and 20% CP levels respectively).

Group and %CP	% DM	% Ash	% CF	% N	% CP	Energy (MJ/Kg DM ME)
A (10%)	96.21	11.94	35.69	1.67	10.44	2.060
B (15%)	95.57	9.26	31.15	2.43	15.19	2.120
C (20%)	96.05	6.70	30.50	3.30	20.63	2.210

DM= Dry matter, CF= Crude fibre, N= Nitrogen, CP= Crude protein

### 3. Results

Rams fed 10% crude protein had the lowest testicular weights for both left and right testes with values of  $98 \pm 23.0$ g and  $98.8 \pm 26.3$ g, respectively. Rams fed 20% crude protein had the highest testicular weights with values of  $149.4 \pm 19.9$  and  $158.6 \pm 15.7$  (Table 3). Rams fed 15% had values of  $131.6 \pm 8.2$ g and  $137.3 \pm 9.7$ g (Table 3). Left testes of groups fed 10% and 15% C.P and 20% had higher weights than right testes. All the differences were not statistically ( $P > 0.05$ ) different. Gonadal reserves of rams fed 20% was highest with values of  $43.3 \pm 3.9$  ( $\times 10^6$ /g) and  $47.0 \pm 2.5$  ( $\times 10^6$ /g). This was followed by rams fed 15% C.P. and 10%

in decreasing order ( $28.0 \pm 3.9 \times 10^6$ /g,  $37.7 \pm 2.5 \times 10^6$ /g and  $20.7 \pm 10.1 \times 10^6$ /g and  $36.0 \pm 14.6 \times 10^6$ /g). These differences were not statistically ( $P > 0.05$ ) different. Rams fed 15% had the highest *caput* epididymal reserves with values of  $15.3 \pm 0.7$  ( $\times 10^6$ /g) for right epididymis and  $16.7 \pm 11.6$  ( $\times 10^6$ /g) for left epididymis. This is followed by rams fed 20% C.P and 15% C.P. in decreasing order. *Corpus* epididymal reserves was highest in rams fed 20% C.P. followed by rams fed 15% and 10% in decreasing order. *Cauda* epididymal reserves was highest in rams fed 15% C.P., this was followed by rams fed 20% and 10% in decreasing order. These differences however were not statistically different.

**Table 3.** Mean Gonadal and Epididymal Sperm/Spermatid Reserves of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cakes.

Parameters	Group A (10%)			Group B (15%)		Group C (20%)	
LW (kg)	19.4±1.0 <sup>a</sup>			24.8±3.0 <sup>b</sup>		27.8±1.4 <sup>b</sup>	
SC (cm)	24.7 ±1.7 <sup>a</sup>			28.4±1.6 <sup>b</sup>		30.0±1.0 <sup>b</sup>	
Testes		Right	Left	Right	Left	Right	Left
	Length (cm)	8.2 ± 0.6	8.7±0.2	8.3 ± 0.3	8.5 ± 0.3	9.3 ± 0.3	9.3 ± 0.3
	Weights (g)	98.7±23	99.8±26.3	131.6 ± 8.2	137.3 ± 9.7	149.4±19.9	158.6±15.7
	GR (x10 <sup>6</sup> /g)	20.7±10.1	36±14.6	28.0 ± 3.9	37.7 ± 2.9	43.3 ± 3.9	47.0 ± 2.5
Epididymis		Right	Left	Right	Left	Right	Left
<i>Caput</i>							
	Length (cm)	4.9 ± 0.6	4.8 ± 0.3	5.4 ± 0.4	5.3 ± 0.3	5.9 ± 0.7	5.4 ± 0.2
	Weight (g)	6.1 ± 1.8	6.3 ± 1.7	8.2 ± 0.4	8.0 ± 0.1	7.7 ± 0.5	7.83 ± 0.3
<i>Corpus</i>	Epididymal reserves (x10 <sup>6</sup> /g)	11.0± 9.2	12.3 ± 3.7	15.3 ± 0.9	16.7 ± 11.6	14.7 ± 2.9	10.3 ± 5.6
	Length (cm)	4.6 ± 0.7	4.0 ± 0.0	3.8 ± 0.6	4.2 ± 0.2	5.5 ± 1.2	4.8 ± 0.7
	Weight (g)	0.7 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.2	0.7 ± 0.0
<i>Cauda</i>	Epididymal reserves (x10 <sup>6</sup> /g)	7.3 ± 6.4	2.0 ± 1.1	2.7 ± 1.7	13.3 ± 9.6	6.7 ± 2.7	13.0 ± 7.5
	Length (cm)	4.4 ± 0.7	4.6 ± 0.9	5.9 ±0.1	5.1 ± 0.3	5.4 ± 0.0	5.6 ± 0.7
	Weight (g)	7.9 ± 2.0	8.1 ± 2.2	11.3 ± 0.5	11.5 ± 0.5	10.1 ± 0.6	10.6 ± 0.8
	Epididymal reserves (x10 <sup>6</sup> /g)	103.1±59	61.6±38.1	141.2 ± 26.6	168.8±24.1	142.3± 16.4	133.0 ± 14.0

## 4. Discussion

Testicular weights observed in this study had a similar trend with gonadal reserves. This is similar to findings of [11] and [12]. Also, [13] stated that rams with larger testes tend to produce more spermatozoa, a finding which was similar to what was observed in this study. [14] also reported a positive correlation between testicular weights and gonadal reserves in West African Dwarf bucks. It was observed from this study that the left testes were heavier than the right testes in all groups, regardless of level of crude protein fed. This finding is similar to the observations of [15] in Red Sokoto bucks fed with *Moringa oliefera*-supplemented diets. Also similar finding regarding the left testes having higher sperm reserves was observed in camel by [16]. [17] reported similar findings in Yankasa rams that were fed non gossypol containing diets. This increase in weight of the left testes might be due to higher proportion of Sertoli cells and seminiferous tubules in the left testes, which have been reported to be responsible for sustenance of spermatogenesis [18].

The corpus of the epididymis had the lowest sperm reserves across all groups, compared with the *caput* and *cauda epididymis*. This result is similar to reports by [19], regarding gonadal reserves in Red Sokoto bucks fed cotton seed cake. This could be explained by the fact that the major storage point of spermatozoa is the *cauda epididymis*, and therefore the *corpus* might be serving as a transit point only. Similar findings were made by [20], that the *corpus epididymis* had the lowest sperm reserves in goats.

From this study, rams fed 15% C.P. had higher extragonadal sperm reserves than rams fed 10% and 20% CP. This could be because at 15% CP, there is optimum utilization of protein to facilitate spermatogenesis. Rams fed 20% had higher gonadal sperm reserves compared to other groups. This is positively correlated with testicular weights recorded, which could be as a result of presence of more sertoli cells and seminiferous tubules, leading to increased production of sperm cells.

Regardless of crude protein levels, left testes weighed more than the right testes. Testicular weights and gonadal reserves were highest in rams fed 20%, but the difference with other groups were not statistically ( $P > 0.05$ ) different. Regardless of crude protein intake, the *corpus epididymis* had the lowest reserves, while the *cauda* had the highest reserves.

## 5. Conclusion

It was observed from this study that varying levels of protein diets using cotton seed and palm kernel cakes had no significant difference in sperm reserves in the gonads and sperm reserves in the caput, corpus and cauda epididymis of Yankas rams. Cauda epididymis had the highest reserves across all treatment groups.

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