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ORIGINAL RESEARCH ARTICLE

Semen and testicular characteristics in Red Sokoto and Sahel bucks fed different protein sources

***[1]Daudu, O. M.,[2] Makun, H., [1]Zechariah, A. J., [1]Titus, G., [1]Oluwa, D. E., [2]Olutunmogun, A. K., [2]Barje, B. P. and [2]Otaru, S. M.**

[*1]Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
[2]National Animal Production Research Institute Zaria, Kaduna State, Nigeria
*Corresponding author: remidaudu@yahoo.com

ABSTRACT

The experiment was conducted to determine the effect of dietary intake of groundnut cake, cotton seed meal and soybean meal (20.97, 46.43 and 18.57%) respectively) on semen quality of Red Sokoto and Sahelian bucks. Twenty seven, 7 months old bucks (12 Red Sokoto and 15 Sahelian) were distributed into three treatments and fed three different protein sources for a period of ninety days. The experimental design was a 2×3 factorial arrangement in a completely randomized design with two breeds of bucks and three protein sources. Semen was collected at the end of the feeding trial by electrical stimulation method to analyze the semen and determine the sperm morphology. Data collected were subjected to GLM procedure of SAS (2002) while the Tukey post hoc test was used to separate significant means and presented in standard error (±SE) while probabilities of p<0.05 were considered significant. The principal component analysis (PCA) was carried out using SPSS (PASW Statistics 18). Data analyzed revealed that breed had significant effect on semen volume with the Red Sokoto bucks having higher volume (0.61ml) per ejaculate than the Sahelian bucks (0.44ml). The physical testicular characteristics of the two breeds were not significantly different. The result of the morphological defects of the sperm showed that Red Sokoto bucks had significantly higher values for detached head and curled tail. Dietary protein source had no significant effect on body weight and the semen quality parameters of the bucks. The circumference and length of testis was significantly higher in bucks fed soybean meal and cotton seed meal respectively. There was no significant effect of the different protein sources on the sperm morphological abnormalities. The results obtained suggest that the levels of the protein sources utilized in the rations had no detrimental effect on semen quality, physical testicular characteristics and sperm morphology of Red Sokoto and Sahelian bucks.

Keywords: Red Sokoto, Sahel bucks, Oil seed meal semen quality, sperm morphology.

INTRODUCTION

Goats, over the years have provided mankind with useful products like meat, milk and skin and they are the widest spread breed of domestic animals in terms of adaptability to all climatic zones from the mountains of Siberia to the desert and to the tropical parts of Africa (Luikart *et al.* 2001). Goats are unique in subsistence animal husbandry because of their adaptability to harsh environmental conditions and catholic taste for common and local feedstuffs, mostly roughages and plant by-products (Ebegbulem *et al.,* 2011).

In Nigeria, goats are the most numerous of all the types of livestock numbering above 53 million (FOA, 2010). The average numbers of goats and sheep per owner have been estimated to be between three to four animals with goat predominating (Abu *et al.,* 2011). The Red Sokoto and Sahel breeds are predominantly found in Northern Savanna and Sahel areas (Semi arid zone) respectively Fayeye and Omolosho (2015) while the West African dwarf breed is common in the humid forest zone of Southern Nigeria.

Semen quality, like other phenotypic expressions, no doubt consists of a genetic component, an environmental component and a variety of interactions between the two traits. The genetic component is generally thought to be small because the heritability of fertility usually is low. However, sperm output has been shown to be positively associated with body weight (Mekasha *et al*., 2007). With low heritability, any genetic variability is dwarfed by the numerous environmental factors involved. Semen quality parameters such as motility, sperm number and sperm morphology are of value in identifying animals of low fertility in pastoral herds (Parkinson, 2004). Of the components of semen quality, spermatozoa morphology of abnormal sperm which is controlled by single gene pair is of utmost importance in animals extensively reared in the tropics (Chacon, 2001).

Protein is an expensive component in animal rations and one that may be in short supply especially in developing countries. One of the critical pressing problems today is how to augment the shortage of protein in diets, curtail the presence of anti-nutrients which could hinder their utilization (Amani and Salih, 2009) and possibly improve the reproductive status and ability of animals. Balanced nutrition has profound effects on the evolution of the reproductive and puberty traits in all animal species (Makawi, 1994) however, the male fertility is not affected by the level of protein although high and varying protein levels in the ration is known to increase the sperm cell concentration and reduces the semen volume and sperm motility (Zayed and Salhad, 1994). Different sources of protein like soy beans meal, sun flower meal, ground nut meal and sesame meals can be used to supplement the rations of bucks.

Groundnut meal contains mycotoxins an antinutritional factor, when consumed has often resulted in incoordination, changes in reproductive cycles, fertility in male animals, low sperm motility and reproduction defects (Annor *et al*., 2004). Cotton seeds present a substance with toxic potential in their composition, the gossypol, a compound that binds rapidly to different substances, including minerals and amino acids. Even though mature ruminants seem to have a large capacity to detoxify gossypol, its intake may overwhelm ruminal detoxification and can be absorbed at potentially toxic concentration (Guedes and Soto-Blanco, 2010). Santa Inês sheep were fed 0.5 kg/animal/day cottonseed cake and no significant differences were found in semen volume, spermatozoa concentration, total spermatozoa, motility and percentage of abnormal spermatozoa (Guedes and Soto-Blanco, 2010). In males, gossypol promotes reduction of motility and spermatozoa concentration (Guedes and Soto-Blanco, 2010).

Soybean contains chemicals that mimic estrogen and lower testosterone levels (Daniel, 2005). The adverse estrogenic effects of phytoestrogens on reproductive development have been observed in domestic and experimental animals (Rickard and Thompson, 1997). Severe reproductive defects and infertility have also been reported (Hess, 2003). Carlsen *et al*. (1992) have shown evidence to confirm that there has been significant decline in sperm quality and quantity with increased phyto-estrogens in soybean. Processed soybean meal was fed at 0, 100, 200 and 300 mg/kg body to rats and it affected spermatogenic activities and sperm quality that could result in reproductive toxicity, reproductive dysfunctions and infertility in male animals (Ekaluo *et al*., 2013). This study was therefore undertaken to evaluate the effect of three different dietary protein sources on the reproductive performance of two breeds of goats in Nigeria.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the National Animal Production Research Institute, Shika, Nigeria. Shika is geographically situated in the Northern Guinea Savannah zone between latitude 11º 12’ N and longitude 7º 33’ E at an altitude of 640m above sea level. The climate is characterized by a well defined dry and wet season. The total annual rainfall ranges from 617 to 1365mm with an average of 1041 mm.

A total of twenty seven bucks aged seven months were used in this experiment which comprised two breeds, 4 Red Sokoto and 5 Sahelian bucks in each treatment. The animals were fed 4% of body weight, with different rations (Table 1) containing three protein sources, groundnut cake (GNC), cotton seed cake (CSC) or soybean cake (SBC) as supplement and *Digitaria smutsii* serving as basal diet. Feed and water was provided *ad libitum*. All routine managerial practices were observed including deworming and treatments against endemic diseases.

Table 1: Composition of experimental diets

Ingredients (%)	GNC	CSC	SBC
Maize	30.00	30.00	30.00
Maize offal	46.03	20.57	48.43
Groundnut cake	20.97	0	0
Cottonseed cake	0	46.43	0
Soy Cake	0	0	18.57
Salt	1.00	1.00	1.00
Bone	2.00	2.00	2.00
Total	100	100	100
Crude Protein (%)	15.97	15.97	15.93
Energy (MJ/Kg)	8.93	8.88	8.94

GNC-Groundnut cake; CSC-Cotton seed cake; SBC-Soybean cake

The experimental design was a 2×3 factorial arrangement in a completely randomized design with two breeds of bucks and three protein sources. The parameters measured were body weight, semen characteristics and physical testicular parameters.

Data collection and traits measured

At the end of the 90 days feeding trial, the testicular length (proximal to distal) was measured in centimeters with a flexible measuring tape as the distance along the caudal surface of the scrotum, from its point of attachment to the tip of the scrotum with the help of calipers, taking care not to modify the normal shape of the organ (Shrestha *et al.,* 1983; Akpa *et al*., 2012). Testicular circumference is the maximum dimension around the pendulous scrotum after pushing the testes firmly into the scrotum and then measuring the greatest circumference with a flexible tape in centimeters, Ahmad *et al*. (2006); Akpa *et al*. (2006) and the testicular width (medial-lateral) is calculated by dividing the value of testicular circumference by two measured in centimeters.

Semen samples were collected into labeled tubes from each animal at the end of the 90 days feeding trial using an electro-ejaculator at about 8-10am. The semen samples were evaluated immediately for colour, volume, motility and pH (Zemjanis, 1970). Smear of each semen sample was prepared; air dried, labeled and kept for further examination, vis determination of sperm concentration using formaldehyde and determination of live and dead ratio using eosin-nigrosin stain. The concentration of the spermatozoa was determined using the haemocytometer crossed with microscopic grids containing 25 large squares with each containing sixteen smaller squares. Sperm cells were counted diagonally from top left to the bottom right and from top right to the bottom left in five large squares or a total of 80 smaller squares (Rekwot *et al*., 1997). Prior to counting, formaldehyde was used as a dilution reagent.

A drop of semen was taken from each sample using automatic pipette and diluted with formaldehyde at 1:100. The haemocytometer was mounted on the microscope and an absorbable tube and O-no pette was used to pipette a drop of the solution into the haemocytometer chamber. After counting in the 5 large squares, the number obtained was multiplied with 100 (dilution factor), 16 (the number of smaller squares in a larger square and the volume of the semen sample collected, multiplied by 10^9). The result obtained was recorded as the sperm cell concentration for each sample.

The live and dead ratio was estimated by the preparation of a smear of individual semen sample using Eosin-Nigrosin stain immediately after collection. A drop of semen was diluted and placed on a clean glass slide using automatic pipette. A drop of the Eosin-Nigrosin stain was placed alongside the semen on the slide and a gentle circular turning of the slide was done to allow a uniform mixture of the two samples. A quarter of another clean slide was placed on top of the first sample and the two slides were gradually and carefully drawn apart to prepare a thin smear on the first slide. This was allowed to dry and thereafter labeled. This was done for each sample and they were later mounted on an electric microscope for counting the live and dead sperm cells**.** The dead sperm cells accept the stain and appear purple while the live sperm cells rejected the stain and remain unstained.

The slides prepared for live and dead ratio were used for the morphology studies. Live and abnormal spermatozoa percentages were counted using hand counter. Fifty spermatozoa were examined for each sample. The total number of abnormal cells were counted and recorded. The following were the abnormalities observed: detached tail, detached head, coiled and bent tail, and acrosomal abnormality of head. The acrosomal abnormality was determined by using smears made from the fresh semen and stained by Giemsa stain (Watson 1975).

The principal component analysis (PCA) was carried out using SPSS (PASW Statistics 18). It allows identification of clusters of parameters that are interrelated by identifying latent principal components. The eigenvalues of the principal component were used to explain the extent of multiple correlations of the analyzed parameters (Burstyn, 2004). The data were subjected to GLM procedure of SAS (2002) while the Tukey post hoc test was used to separate significant means and presented in standard error (±SE) while probabilities of $p<0.05$ were considered significant.

Results and discussion

Tables 2 and 3 show the interaction of breed and protein source for semen quality characteristics and sperm morphological defects. The result showed there was no interaction among the variables.

Table 4 shows effect of breed on body weight and semen quality traits. There was no significant ($P>0.05$) difference in body weight, concentration, motility, semen pH, concentration and live cells but there was a significant ($P<0.05$) difference in semen volume (0.61ml and 0.44ml) for Red Sokoto and Sahel bucks respectively. The report of Ibrahim (1997) on semen traits agrees with this work that breed has no significant effect on semen characteristics but differs with respect to the semen volume. Kridli *et al*. (2007) also reported significant differences in semen volume for Black Bedouin and Black Bedouin-Damascus crossbred bucks. The difference in semen volume could be due to individual differences in the animals or difference in breed. Table 5 shows that all the physical testicular characteristics, circumference of scrotum, width of scrotum and length of scrotum) of the two breeds were not significantly ($P>0.05$) different. This shows that the two breeds studied had similar testicular biometrics.

Table 2: Effect of different protein sources and breed on semen quality characteristics of Red Sokoto and Sahelian bucks

Parameters	Breed	GNC	CSC	SBC	SEM	P value
Volume (%)	RS	0.53	0.53	0.65	0.06	0.30
	SH	0.38	0.50	0.44		
Colour	RS	2.00	1.75	1.75	0.26	0.95
	SH	2.20	2.00	2.00		
Motility (%)	RS	77.50	68.75	66.25	11.16	0.81
	SH	44.00	56.00	49.00		
Conc. ($x10^6$)	RS	1.57	1.99	2.35	4.48	0.76
	SH	127.20	151.80	132.00		
Semen pH	RS	7.25	7.25	7.50	0.20	0.45
	SH	7.40	7.40	7.20		
Live cells	RS	67.50	70.00	71.25	11.00	0.99
	SH	44.00	50.00	46.67		

RS- Red Sokoto; SH-Sahel; GNC-Groundnut cake; CSC-Cotton seed cake; SBC-Soybean cake; conc.-concentration

Table 3: Effect of different protein sources and breed on semen morphological abnormalities of Red Sokoto and Sahelian bucks

Parameters	Breed	GNC	CSC	SBC	SEM	P value
NSC	RS	77.50	67.75	63.25	10.79	0.71
	SH	46.00	60.00	46.40		
DH (%)	RS	9.00	13.75	9.50	1.80	0.91
	SH	5.20	8.00	4.50		
DT (%)	RS	6.25	4.25	9.75	1.98	0.62
	SH	4.80	3.60	3.80		
AH	RS	0.75	0.00	0.75	0.33	0.32
	SH	1.00	0.80	0.20		
BT (%)	RS	3.50	8.25	11.00	2.16	0.63
	SH	2.40	5.20	3.80		
CT (%)	RS	3.00	5.25	5.25	0.89	0.95
	SH	0.60	2.40	2.00		

RS- Red Sokoto; SH-Sahelian; GNC-Groundnut cake; CSC-Cotton seed cake; SBC-Soybean cake; NSC-number of sperm cells; DH- detached head; DT-detached tail; AH-acrosomal head; BT-bent tail; CT-coiled tail

Table 4: Body weight and semen quality characteristics of Red Sokoto and Sahel bucks

	Breeds		
Parameters	**Red Sokoto**	**Sahel**	**P values**
Initial weight (kg)	9.35±0.55	10.24±0.55	0.16
Final weight (kg)	13.18±0.77	13.21± 0.77	0.97
Weight gain (kg)	3.21±0.47	3.41±0.47	0.86
Volume (ml)	$0.61±0.05^a$	$0.44±0.05^b$	0.04
Colour	Milky white	Milky white	
Motility (%)	70.83±9.67	48.61±8.40	0.09
Ph	7.33±0.17	7.38±0.14	0.86
Concentration ($x10^6$)	1.97±38.42	1.25±33.39	0.16
Live cell (%)	69.58±9.52	46.88±8.28	0.08

^{ab}-Means with different superscripts in the same row differ (P<0.05)

Table 5: Testicular characteristics of Red Sokoto and Sahel bucks

Parameters	Breeds		P values
	Red Sokoto	Sahel	
Scrotal Circum. (cm)	15.16±1.56	17.75±1.36	0.22
Scrotal width (cm)	7.58±0.78	8.87±0.68	0.22
LS (cm)	8.04±0.85	9.84±0.74	0.12

Circum-circumference; LS- Length of scrotum

Table 6: Effect of breed on sperm morphological defects of bucks (±SE) fed different protein sources

	Breeds		
Parameters	Red Sokoto	Sahel	P values
Detached Head (%)	10.75±1.55[b]	5.90±1.35[a]	0.02
Detached Tail (%)	6.75±1.72	4.13±1.49	0.26
Acrosome (%)	0.50±0.28	0.65±0.24	0.68
Bent Tail (%)	7.83±1.86	3.81±1.62	0.12
Curled Tail (%)	4.5±0.77[b]	1.72±0.67[a]	0.01

[ab]- Means with different superscripts in the same row differ ($p<0.05$); n=27

Table 6 shows sperm morphological defects in the semen of the two breeds with significant ($P<0.05$) differences in only detached head and curled tail with the higher values in the Red Sokoto bucks. Saacke (1968) reported that most males contain some abnormal form of spermatozoa which is not associated with low fertility rate until the proportion of abnormalities exceed 20% even then certain types of abnormalities may not be associated with infertility. Table 7 shows that the different sources of dietary protein had significant ($p>0.05$) effect on final weight and weight gain but no significant ($P>0.05$) effect on semen quality parameters of the bucks. Bucks fed SBC and CSC had better final weight and weight gain than bucks fed GNC). SBC is the best protein source for animal feeding hence it is expected that animals will grow optimally when fed SBC. The comparable performance of bucks fed CSC is an indication that animals can also grow optimally when fed CSC. The comparable performance is likely due to proper processing of the cotton seed to remove gossypol.

Table 7: Effect of protein source on body weight and semen characteristics of Red Sokoto and Sahel bucks

Parameters	Protein sources			±SE	P values
	GNC	CSC	SBC		
IW(kg)	9.74	9.98	9.88	1.53	0.9473
FW(kg)	11.75[b]	12.54[ab]	15.29[a]	1.91	0.0570
WTG (kg)	2.11[b]	3.44[ab]	4.63[a]	0.47	0.0006
Volume (ml)	0.52	0.51	0.55	0.06	0.92
Colour	Milky white	Milky white	Milky white	0.25	0.83
Motility (%)	60.75	62.38	56.04	11.23	0.91
pH	7.33	7.43	7.33	0.19	0.86
Conc. ($x10^6$)	1.42	1.66	1.74	4.64	0.91
Live (%)	55.75	60.00	58.95	11.06	0.95

GNC- Ground nut cake, CSC- Cotton seed cake, SBC- Soy bean cake; IW-initial weight; FW- final weight; WTG-weight gain; conc.-concentration

Table 8 shows the effect of different dietary protein supplements on scrotal circumference, number of testes and scrotal length. Protein supplement had significant ($P<0.05$) effect on scrotal circumference and length of scrotum.

Animals fed SBC and CSC had significantly higher (P<0.05) scrotal circumference (19.32 and 19.17cm) and length of scrotum (11.17 and 10.56cm) respectively. The higher values of scrotal circumference and length of scrotum observed in bucks is an indication of better utilization of dietary SBC and CSC than GNC by the bucks. SBC is one of the best protein sources. Its feeding value is unsurpassed by any other plant protein source (Romwell *et al*., 1999). Bucks fed CSC compared favourably to bucks fed SBC. The positive effect of CSC could likely be attributed to proper processing thus minimizing the toxic effect of gossypol found in cotton seed. This result also shows that with proper processing, testicular biometrics of animals can be improved using other cheaper sources of protein. The findings of Nasir and Adil, (2014) showed that feeding pre-puberty male goat kids on a ration supplemented with CSC reduced their scrotal circumference is not in consonance with the findings of this study in which the dietary inclusion of CSC in the diet improved the scrotal circumference of the goats. Nasir and Adil, (2014) also reported a scrotal circumference of 22.2cm was reported for bucks fed GNC as against the 15.28cm reported in this study. The differences observed could be due to difference in age or individual differences in the bucks.

Table 9 shows the effect of different protein supplements on semen morphological abnormalities of Red Sokoto and Sahelian bucks. There was no significant (P>0.05) effect of the different protein sources on the semen morphological abnormalities. This is an indication that different protein sources had similar effect on semen morphological abnormalities of the bucks.

Table 8: Effect of protein source on physical testicular characteristics of Red Sokoto and Sahelian bucks

	Protein Sources				
Parameters	**GNC**	**CSC**	**SBC**	**SEM**	**P value**
Scrotal circum (cm)	15.28[b]	19.17[a]	19.32[a]	0.07	0.001
Length of scrotum (cm)	8.28[b]	10.56[a]	11.17[a]	0.05	0.001

n=27; GNC= Ground nut cake, CSC= Cotton seed cake, SBC= Soybean cake; circum-circumference

Table 9: Effect of different protein supplements on sperm morphological defects of Red Sokoto and Sahelian bucks

	Protein Sources				
Parameters	**GNC**	**CSC**	**SBC**	**SEM**	**P value**
Number of sperm cells	60.00	63.44	53.89	1.20	0.67
Detached head (%)	6.89	10.56	6.33	0.20	0.15
Detached tail (%)	5.44	3.89	6.44	0.23	0.69
Acrosomal head (%)	0.89	0.44	0.44	0.04	0.64
Bent tail (%)	2.89	6.56	7.00	1.25	0.43
Coiled tail (%)	1.67	3.67	3.44	0.10	0.11

GNC-Groundnut cake; CSC-Cotton seed cake; SBC-Soybean cake

The PCA was aimed at identifying clusters of analyzed semen quality characteristics that would explain significant proportion of total variation (Table 10). Five PCs are shown with significant loadings that collectively explained 75.67% of the total variance. PC 1 is strongly correlated with six of the variables hence it increased with motility, semen concentration,

live cells, number of cells counted, detached head and curled tail and explained 29.70%. This suggests that these variables vary together. The values obtained for each variable is a measure of the quality of the variables. High values for motility, semen concentration, live cells, number of cells counted reflect high quality while high values for detached head and curled tail indicates lack of quality. The negative value of colour means that colour is lacking in a latent variable associated with PC 1. Only number of testes, circumference and length clustered in PC 2 which accounted for 17.90% of the total variance. PC 2 increases with these parameters suggesting that the variables are related. In PC 3 only acrosomal head formed a cluster. Live cells had the highest loading in PC 1 indicating strong correlation between the two hence PC 1 is primarily a measure of life cells. Therefore, live cells seem to be the principal controlling factor in the trial. Communality values are indicative of the proportion of each parameter explained by PCA. In this analysis, communality values generally ranged from above average (0.641for HOS) to high (0.965 for scrotal circumference) (Table 10). The first factor was the semen quality characteristics and the second was physical testicular characteristics, dominated by scrotal circumference, and the third cluster, which described less variability was sperm morphology (acrosomal head). A high proportion of most of the analyzed parameters were effectively explained by the PCA and this can be confirmed from their communality values.

Table 10: Principal component loadings and estimated communalities of the parameters along a transect

Parameters	Unit	PC1	PC2	PC3	PC4	PC5	Communalities
Weight	Kg	-	-	-	0.702	-	0.680
HOS		-	-	-	-0.725	-	0.641
Volume	Ml	-	-	-	0.606	-	0.814
Motility	%	0.882	-	-	-	-	0.897
Concentration	$x10^6$	0.811	-	-	-	-	0.837
pH		-	-	-	-	0.530	0.891
Live cells	%	0.892	-	-	-	-	0.895
Dead cells	%	-	-	-	-	0.496	0.909
NCC		0.728	-	-	-	-	0.890
DH	%	0.793	-	-	-	-	0.816
AH		-	-	0.675	-	-	0.813
BT	%	-	-	-	-	-	0.721
CT	%	0.576	-	-	-	-	0.779
NT		-	0.871	-	-	-	0.921
CIR	Cm	-	0.942	-	-	-	0.965
LGT	Cm	-	0.862	-	-	-	0.943
Eigenvalue		5.35	3.22	2.06	1.69	1.30	
Variance %		29.70	17.90	11.45	9.39	7.23	
CV %		29.70	47.60	59.05	68.44	75.67	

HOS-; NCC- number of cells counted; DH- detached head; AH- acrosomal head; BT- bent tail; CT- coiled tail; NT- number of testes; CIR- scrotal circumference; LGT- length of testis

CONCLUSION

Most of the semen quality traits and physical testicular characteristics were similar for the two breeds hence reproductive performance should be similar. Red Sokoto bucks had higher numbers of detached head and curled tail which is an indication of probable lower fertilizing ability compared to the Sahelian bucks. The dietary proteins had no effect on body weight, semen quality and sperm morphological abnormalities of the bucks hence the three dietary proteins can be fed at the levels utilized without a detrimental effect on the bucks' reproductive ability. The higher scrotal circumference and length of scrotum recorded

for bucks fed SBC and CSC than bucks fed GNC did not affect the quality of semen produced. The PC1 will be chosen and others rejected.

RECOMMENDATION

Groundnut cake, cotton seed cake or soyabean meal can be utilized in diets at 20.97, 46.43 and 18.57% respectively without compromising body weight, semen quality, testicular characteristics and sperm morphology of bucks.

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