FULL PAPER

Sokoto Journal of Veterinary Sciences
(ISSN 1595-093X)
Jibril *et al.* /*Sokoto Journal of Veterinary Sciences* (2011). 9(1):22-27.

Effect of graded levels and sources of protein on scrotal circumference and semen profile of Yankasa rams

A Jibril[1]*, IU Ate[2], PI Rekwot[3] & CU Osuhor[3]

[1]*Department of Theriogenology and Animal Production, Usmanu Danfodiyo University, Sokoto*
[2]*Department of Surgery and Medicine Ahmadu Bello University, Zaria*
[3]*National Animal Production Research Institute, Shika-Zaria*

*Correspondence: Tel.: +234-8035998852, E-mail: aliyujibril2002@yahoo.com

Abstract
The effect of graded levels and sources of protein on scrotal circumference and semen profile in Yankasa rams were investigated in a 96 day study. Twenty Yankasa rams aged 18-24months and weighing 21-30 kg with clinically normal genitalia were divided into 4 groups (A, B, C and D) of 5 rams each. All animals were fed *Digitaria* hay as a basal diet *ad libitum* and supplemented with the formulated ration at 2% of their respective live-weights. Iso caloric rations (10.50 MJ/kg DM ME) were formulated using non-conventional protein source (maize offal and dry layer litter) to contain 12.11% CP, 14.96% CP, and 17.94% CP and fed to groups A, B and C respectively. Another ration was formulated using conventional protein source (maize, wheat bran, groundnut cake, bone meal, vitamin premix and salt) to contain 12.26% CP and fed to group D (control group). Semen was collected every two weeks with the aid of a battery powered electro-ejaculator and then evaluated. Rams on 12.11% CP had significantly higher scrotal circumference than those on 17.11% C. and control. Significantly higher ($P<0.05$) semen volume was recorded for group B when compared with control group, but other groups showed no significant difference in volume of semen ($P>0.05$). Rams fed 14.96% CP diet had significantly higher semen concentration when compared with rams on 17.94% CP and control ($P<0.01$). Percentage gross motility, sperm morphology, sperm output and sperm viability were not influenced by level and source of protein ($P>0.05$). Thus, it is evident from this study that dry layer litter and maize offal compete favorably with conventional protein sources in improving scrotal circumference and semen concentration of Yankasa rams.

Keywords: Dry layer litter, protein, rams, semen, scrotal circumference.

Introduction:
Chronic low animal protein intake in developing countries is a basic problem that needs an urgent solution (Attah *et al.*, 2006). The low animal protein intake may be attributed to low livestock productivity and therefore available animal protein are very expensive for a population with a very low per capita income. Successful reproduction as an important factor in livestock production economy (Rasbech, 1984) depends on genetic merit, physical environment, nutrition and management.

Yankasa sheep are the most numerous and most widely distributed among the 22.3 million indigenous sheep population (FDLPCS, 1991) mainly kept for meat in Nigeria and are estimated to constitute 60% of the national flock (Afolayan *et al.*, 2006). Their numerousity, wide distribution, short generation interval and resistance to some diseases are attributes that can be harnessed in using them to alleviate the problem of low animal protein intake.

In Nigeria, a major limitation to animal production is poor reproductive performance (RIMS, 1992). Other factors are skyrocketing prices and scarcity of conventional animal feed rich in protein. Enjalbert (2006) attributed many reproductive health disorders in animals to diet inadequacy. It is well documented that protein deficient feeds reduce semen quality and sexual activity in bulls (Brown, 1994; Rekwot *et al*., 1994), which may likely be the same for rams.

There is paucity of information on nutritional factor (especially protein) as a determinant of reproductive performance in Yankasa rams. Thus, this study was designed to investigate the effect of different levels and sources of proteins in diets of Yankasa rams on their semen characteristics.

Materials and methods

Study area

The study was carried out at the National Animal Production Research Institute (NAPRI) Shika, Ahmadu Bello University Zaria, situated in the Northern Guinea Savannah, and lying between latitudes 11° 11 60 N and between longitude 7° 34 60 E at an elevation of 646 m above sea level.

Experimental animals and management

Twenty healthy Yankasa rams with clinically normal genitalia, aged 18-24 months and weighing 21-30 kg were randomly divided into four groups of five rams each. The rams were managed under intensive system, kept in separate pens and fed individually. They were acclimatized for two weeks during which they were screened for haemoparasites and helminths.

Experimental diets formulation and design

Three isocaloric rations (10.50 MJ/kg DM ME) containing graded level of protein were formulated (12.11%, 14.96% and 17.94% CP) using non-conventional feed stuffs {Maize Offal (MO) and dried layer litter (DLL)}. Group A, B, and C were fed a concentrate mixture of 12.11% CP, 14.96% CP and 17.94%CP respectively composed using DLL and MO, while group D (control) were fed a concentrate mixture of 12.26 % CP and 10.50 MJ/kg DM ME composed using conventional protein sources (Table 1). All rams under study were fed a basal diet of hay (*Digitaria* spp) *ad libitum* and given a supplement ration of concentrate mixture of 2% body weight/head/day. All test diets were subjected to proximate analysis using the method of AOAC (1990). Ingredients and chemical compositions of feeds fed to the different experimental groups are presented (Tables 1 and 2).

Table 1: Ingredients and nutrient composition of experimental diets fed to Yankasa rams

	Groups			
Ingredients/Nutrients (%)	A	B	C	D
Ground Corn	-	-	-	80.05
GNC	-	-	-	6.53
Wheat bran	-	-	-	11.42
Common salt	-	-	-	0.50
Bone meal	-	-	-	1.25
Vitamin premix	-	-	-	0.25
Maize bran	91.68	70.52	49.40	-
Dry layer litter	8.32	29.48	50.60	-
Total	100.00	100.00	100.00	100.00

Table 2: Chemical composition of experimental diets fed to Yankasa rams

Chemical composition (%)	A	B	C	D
DM	94.71	94.16	94.13	93.63
CP	12.11	14.96	17.94	12.26
E E	36.64	31.42	48.39	25.32
CF	11.17	15.22	16.47	32.66
Ash	6.47	13.39	20.92	3.42
Energy MJ/Kg DM ME	10.52	10.48	10.46	10.54

Scrotal measurements
Scrotal circumference was measured weekly in centimetres using a flexible measuring tape at the widest scrotal diameter by applying pressure with a hand above the head of the epididymides, thereby gently forcing the testes into the scrotum, then the flexible measuring tape was placed at the widest scrotal diameter to take the reading.

Semen collection
Semen samples were collected fortnightly from each ram in the morning between 09.00 and 11.00 h using a portable battery powered electro-ejaculator (Lane Manufacturing Inc. No. 72707C). The rams were adequately restrained; the prepuce was washed and dried. The probe of the electro-ejaculator was lubricated using petroleum jelly and inserted into the rectum and switched on, this resulted in erection and subsequently ejaculation. Ejaculated semen was collected in a calibrated tube and placed in a flask with warm water at 37ºC.

Semen evaluation
Semen samples collected were evaluated as described by Zemjanis (1970) with modifications. The volume of semen was measured directly from the calibrated tube used for collection. Microscopic examination for wave pattern (gross sperm motility) was determined by placing a drop of raw undiluted semen on a pre-warmed slide then cover-slipped and viewed using a field microscope at X40 magnification. Sperm concentration was determined using a haemocytometer. Live dead ratio of the sperm cells was determined as described by Esteso *et al.* (2006). A thin smear of the semen sample was made on clean grease free glass slide and stained with eosin-nigrosin stain. Four hundred sperm cells were counted using light microscopy at X40 magnification. Sperm abnormalities were determined by making a thin smear of the semen sample on clean grease free glass slide and fixed with buffered formol saline. Four hundred sperm cells were counted per slide using light microscopy at X40 magnification.

Data analysis
Data collected were expressed as means and their standard error of mean (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). Analysis was conducted using the Graphpad Instat computer programme (GRAPHPAD, 2000).

Results and Discussion
Level of protein in the diets appeared to influence scrotal circumference (Table 3). Rams on 12.11% CP had significantly higher (P<0.05) scrotal circumference than those on 17.11% CP (P<0.01) and control (12.26% CP). This might be attributed to the optimum utilization of dietary protein at about 12% CP level as previously reported (Negesse *et al.*, 2001). This agrees with Fourie *et al*., 2004 who reported increase in SC of rams on a low protein diet of 12.5% CP. Similar findings were also reported by Paérez-Clariget *et al.* (1998) and Elmaz *et al.* (2007). This finding disagrees with that of Rekwot (1987) and Rekwot *et al.* (1988) who found that bulls on high protein diet of 14.45% CP had higher SC than those on 8.51% CP. This disagreement may be explained by the fact that 8.51% CP used by Rekwot *et al*. (1988) is lower than the optimum level of 12% CP (NRC, 1985) for efficient utilization of dietary protein in rams. Also rams in group B (14.96% CP) had significantly higher (P<0.01) SC than those in group C (Table 3).

In this experiment (Table 4), rams on 14.96% CP level had higher semen concentration when compared with the those on 17.94% CP diet and the control on 12.26% CP diet (P<0.01). The increase in semen concentration between group B (14.96% CP) and C (17.94% CP) might be an indication that even though an increased protein intake above the minimum requirement (12% CP) enhanced spermatogenesis, higher levels of CP in diets resulted in excess urea and more available ammonia which might have influenced the physiology and reproduction often associated with decline in fertility as reported by other workers (Jordan & Swanson, 1979; Kaim *et al.*, 1983; Canfield *et al.*, 1990; Elrod & Butler, 1993).

Table 3: Scrotal circumference (SC) of Yankasa rams placed on different levels of protein in the diet (Mean ±SEM).

Group	Scrotal Circumference (cm)
A(12.11% CP)	27.28±0.17[a]
B(14.96% CP)	27.08±0.18[ab]
C(17.94% CP)	25.86±0.19[b]
D(control)	26.40±0.29[b]

[ab]Means in same column with different superscript alphabets are statistically (P<0.05) different

Table 4: Semen characteristics of Yankasa ram fed different levels of protein (Mean±S.E.M.)

Group	Seminal characteristics					
	Volume (ml)	Concentration ($\times10^6$/ml)	Sperm output ($\times10^6$)	Gross sperm motility (%)	Viability (%)	Sperm Morphology (% Normal)
A (12.11% CP)	0.89±0.12	257.91±20.47^{ab}	257.95±49.85	76.94±4.29	83.30±3.17	77.40±1.61
B (14.96% CP)	0.77±0.12	309.91±25.13^{a}	243.08±48.79	76.54±3.70	85.70±3.33	82.14±2.36
C (17.94% CP)	0.91±0.15	200.29±17.91^{b}	189.99±44.07	63.23±5.11	82.90±2.67	75.09±3.28
D (control)	0.82±0.10	199.14±19.52^{b}	172.39±34.16	72.54±5.41	81.59±2.47	78.14±1.85

^{ab} Means in same column with different superscript alphabets are statistically (P<0.05) different

Table 5: Morphological sperm abnormalities of Yankasa rams on different levels of protein in the diets (Mean±SEM)

Group	Morphological Sperm Abnormalities							
	Bent Tail	Coiled Tail	Double Tail	*MAC	*MIC	*PCD	*DCD	*DH
A (12.11%CP)	2.2±0.86^{a}	3.6±1.08	0.00	11.8±2.01^{a}	3.4±0.51^{a}	51.4±3.27^{ab}	7.8±1.16^{a}	11±1.73
B (14.96%CP)	1.2±0.37^{ac}	4.4±0.75	0.00	8.4±1.17^{ac}	2.4±0.51^{abc}	28.2±7.18^{a}	15.4±2.94^{b}	7.2±0.86
C (17.94%CP)	6.2±0.73^{b}	7.2±1.11	0.00	2.2±0.73^{b}	1±0.45^{c}	63.2±10.17^{b}	13.8±1.16^{abc}	7.4±0.81
D (control)	2±1.05^{ac}	5.2±1.16	0.00	5.6±0.75^{bc}	3.4±0.51^{ab}	46.2±3.97^{ab}	16.6±2.54^{bc}	11.4±1.72

^{abc} Means in the same column with different superscript alphabets are statistically (P<0.05) different
*PCD: Proximal cytoplasmic droplets
*DCD: Distal cytoplasmic droplets
*DH: Detached head
*MAC: Macrocephali
*MAC: Microcephali

The statistically significant increase in semen concentration seen in rams placed on 14.96% CP when compared with those on 12.11% CP was attributed to the fact that an increase CP intake above the minimum requirement (12% CP) resulted in improved reproductive function. This finding is in accord with that obtained by Rekwot (1987) who reported increased semen concentration of bulls placed on a high protein diet of 14.45% CP when compared with bulls placed on 8.51% CP. This study is in agreement with Fenandez *et al.* (2004) who found that increased protein supply favours spermatogenesis.

Semen volume was not influenced by level and source of protein. The findings in this study corroborates the works of Rekwot *et al.* (1987) and Abi-saab *et al.* (2008) who reported non-significant differences in the semen volume of young bulls and bucks, respectively when fed two different protein diets. It was also reported in a study by Kheradmand *et al.* (2006) that there were no statistically significant differences in semen volume of rams placed on diet above the maintenance level which was also in harmony with the findings in this study.

Percentage progressive motility (Table 4) was not influenced by level and source of protein (P>0.05) There were no significant differences between treatment groups in progressive motility (P>0.05). The mean progressive motility (%) for all treatments was above 60% with the group fed 17.94% CP having the lowest value of 63.23% and rams on 12.11% CP having the highest value of 76.94%. Similar results were reported by Elmaz *et al.* (2007) who obtained high motility values for rams on different level of protein.

Different levels of protein in diet had no influence on the viability (% live cells) of sperm cells of Yankasa rams. The viability of sperm cells in all groups (Table 4) was above 80% and there were no significant differences in sperm viability among

groups. Rams on 14.96% CP diet had the highest percentage viability (85.70%), while the control had the least viability of 81.59%, with difference of 4.11% between them (P>0.05). This result is in agreement with the findings of Rekwot *et al.* (1987) and Kheradmand *et al.* (2006) who recorded no significant differences in the viability of sperm cells of bulls placed on different protein diets.

Graded levels of protein in diets had no significant effect (P>0.05) on overall sperm morphology (Table 4). Percentage sperm defect was below 25% for all groups with rams on 14.96% CP diet having sperm cells with the lowest defects of 17.86% and those on 17.94% CP diet having the highest percentage sperm defect of 24.92%. This might be partly explained by the fact that percentage of morphologically normal spermatozoa increases as the male ages and reach highest at puberty as reported by Rekwot (1987) which is true for this study where all rams used had attained puberty. The findings in this study were consistent with those of Barth *et al*. (2008) who found that medium or high level of nutrition does not have influence on overall percentage of morphologically normal spermatozoa. However, variations were observed in the individual sperm morphological abnormalities (Table 5). Rams fed diets containing 17.94% CP had significantly higher bent tail than those on 12.11% CP, 14.96% CP and control diet (P<0.01; Table 5). Proximal cytoplasmic droplets (PCD) had the highest proportion of all morphological abnormalities, where rams on 17.94% CP had higher PCD than those on 14.96% CP. This might be due to the negative effect of high NPN in 17.94% CP diet on spermatogenesis. Significantly higher (P<0.05) sperm cells with distal cytoplasmic droplet (DCD) was observed when rams in groups B and D were compared with those in group A (Table 5). Macrocephalic sperm cells were significantly higher (P<0.05) in rams fed 12.11% CP diet when compared with those fed 17.94% CP and control diets (Table 5). Sperm cells that were microcephalic were highest in group A and Control group, while group C had significantly (P<0.05) the lowest value (Table 5). Significantly higher (P<0.05) macrocephalic and microcephalic spermatozoa recorded for rams on 12.11% CP diet might be as a result of high sperm output earlier recorded for the group making the proportions of cells to be higher.

In conclusion, from the findings, the study concludes that dry layer litter (DLL) and maize offal (MO) competes favorably with conventional protein sources to increase scrotal circumference in Yankasa rams. Also feeding 14.96% CP diet formulated using DLL resulted in higher semen quality especially sperm concentration. Feeding high protein diet (17.94% CP) had a negative effect on semen concentration and resulted in lower motility confirming that feeding high level of CP in diet is associated with decline in fertility. Sperm output, sperm morphology, semen volume and sperm viability were not influenced by level and source of protein. Thus, Dry layer litter and maize offal can be used as substitutes for conventional protein sources in the diets of Yankasa rams to improve scrotal circumference and semen characteristics on account of availability and probably lower cost. However the use of dry layer litter in formulating rations for ruminants should be with caution because of risk of drug residue and other contaminants.

References

Abi-saab S, Hajj E, Abi salloum B & Rahme, E (2008). Seasonal and altitudinal variations on adaptation, growth and testicular activity of baladi goats with vertical transhumance in eastern Mediterranean. *Lebanese Science Journal,* **9**(1):99-111.

Afolayan RA, Adeyinka IA & Lakpini CAM (2006). The estimation of live-weight from body measurements in Yankasa sheep. *Czech Journal of Animal Science,* **51**(8):343–348.

AOAC (Association of Official Analytical Chemists) (1990). *Official Methods of Analysis, (15th edition)*. Washington DC. Pp 69-88.

Attah S, Omojola AB & Adesehinwa AOK (2006). Yield and Carcass Composition of Goats as Affected by Breed and Slaughter Weight. *W. Applied Science Journal,* **1**(1):08-11.

Barth AD, Brito LFC & Kastelic JP (2008). The effect of nutrition on sexual development of bulls. *Theriogenology*, **70**:485–494.

Brown BW (1994). A review of nutritional influences on reproduction in boars, bulls and rams. *Reproduction Nutrition Development*, **34**:89-114.

Canfield RW, Sniffen CJ & Butler WR (1990). Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. *Journal of Dairy Science*, **73**:2342–2349.

Elmaz Ö, Cirit Ü, Keser O, Gürbulak K, Güvenç K & Kutay, C (2007). Effect of two dietary protein levels on testosterone, testicular parameters and semen quality in ram lambs during pubertal development. *Medycyna Weterynaryjna,* **63**(10):1177-1180.

Elrod CC & Butler WR (1993). Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. *Journal of Animal Science*, **71**:694–701.

Enjalbert F (2006). *Assessment of nutritional adequacy in dairy cows through diet characteristics and animal responses*. World Buiatrics Congress, 2006 - Nice, France. www.ivis.org/proceedings/wbc/wbc2006/enjalbert.pdf?LA=1, retrieved 2011-05-15.

Esteso MC, Soler A, Ferna´ ndez-santos M, Quintero-moreno AA & GARDE JJ (2006). Functional significance of the sperm head morphometric size and shape for determining freezability in Iberian Red Deer (*Cervus elaphus hispanicus*) epididymal sperm samples. *Journal of Andrology,* **27**(5):662–670.

FDLPCS (1991): Nigerian National Livestock Survey. Federal Department of Livestock and Pest Control Services, Abuja, Vol. 2:89.

Fernandez M, Giralde FJ, Frutos P, Lavin P & Mantecon AR (2004). Effect of undegradable protein supply on testicular size, spermiogram parameters and sexual behavior of mature Assaf rams. Theriogenology, **62**:299-310.

Graphpad (2000). *GraphPad InStat version 3.05 for Windows 95*, GraphPad Software Inc., San Diego California USA. www.graphpad.com, retrieved 2010-04-10.

Jordan ER & Swanson LV (1979). Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. *Journal of Animal Science*, **48**:1154–1158.

Kaim M, Folman J, Neumark H & Kaufmann W (1983). The effect of protein intake and lactation number on postpartum bodyweight loss and reproductive performance of dairy cows. *Animal Production*, **37**:229–235.

Kheradmand A, Babaei HR & Batavani A (2006). Effect of improved diet on semen quality and scrotal circumference in the ram. *Veterinarski Arhiv,* **76**:333-341.

Negesse T, Rodehutscord M & Pfeffer E (2001). The effect of dietary crude protein level on intake, growth, protein retention and utilization of growing male Saanen kids. *Small Ruminant Research,* **39**:243-251.

NRC (National Research Council) (1985): *Nutrient Requirements of Sheep*. (6th Edition) National Academy Press, Washington, DC. Pp 2-25.

Paérez-clariget R, Bermúdez J, Andersson H & Burgueno J (1998). Influence of nutrition on testicular growth in Corriedale rams during spring. *Reproduction Nutrition Development,* **38**:529-538.

Rasbech NO (1984). The male and fertility of domestic animals. In: *The male in farm animal reproduction.* (M Courot editor). Pp 2-23.

Rekwot PI (1987). *Effects of varying levels of protein intake on growth, puberty and semen quality of bunaji and friesian X bunaji crossbred bulls*. MSc thesis, Department of Veterinary Surgery and Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Rekwot PI, Oyedipe EO & Ehoche, OW (1994). The effects of feed restriction and realimentation on the growth and reproductive function of Bokoloji Bulls. *Theriogenology,* **42**:287-295.

Rekwot PI, Oyedipe EO, Akerejola OO, Kumi-diaka J & Umoh JE (1987). The effect of protein intake on the onset of puberty in Bunaji and Friesian × Bunaji crossbred bulls in Nigeria. *Theriogenology*, **28**:427-434.

Rekwot PI, Oyedipe EO, Akerejola OO & Kumi-diaka J (1988). The effect of protein intake on body weight, scrotal circumference and semen production of Bunaji bulls and their Friesian crosses in Nigeria. *Animal Reproduction Science*, **16**:1-9.

RIMS (1992). Nigerian National Livestock Resource Survey. In: *Resource Inventory and Management*. RIM`s report to Federal Department of Livestock and Pest Control Services. Federal Ministry of Agriculture and Natural Resources (FDLPCS), IV Vols. IV. Abuja, Nigeria: Pp 440.

Zemjanis R (1970). *Collection and Evaluation of Semen. In: Diagnostic and Therapeutic Techniques in Animal Reproduction*. (2nd Edition), Williams and Wilkins Co. Baltimore, Pp 139-156.