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Original article

Live weight changes, gonadal and epididymal sperm reserves of Yankasa Rams fed different levels of dried layer litter in their diets

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ABSTRACT

The effects of feeding different levels of dried layer litter as a protein source on live weight, testicular weight, gonadal and epididymal sperm reserves of Yankasa rams was investigated in a 98-day study. Twenty healthy Yankasa rams with clinically normal genitalia were divided randomly into 4 groups (A, B, C and D) of 5 rams each. Diets containing 12.11% CP, 14.96% CP and 17.94% CP were formulated using dry layer litter (DLL) and maize offal (MO) were fed to groups A, B and C respectively. Group D (control) was fed 12.26% CP diet from conventional protein sources. All animals were fed *Digitaria* hay as a basal diet *ad libitum* and a supplement at 2% body weight/animal/day. Group A gained more live-weight when compared with group C and the control ($P<0.05$). Also the same group had the highest testicular weight. Significantly higher gonadal sperm and epididymal reserves were observed in group A when compared with the control. This is attributed to the significantly higher increase in live-weight observed in group A. The increase in testicular size in rams on 12.11% CP indicated a large volume of testicular parenchyma and increase in the volume of seminiferous epithelium and diameter of seminiferous tubules where spermatogenesis occurs. It was concluded from the study that dried layer litter can serve as a dietary protein source used to improve live

weight and gonadal reserves especially when given at low levels.

1. Introduction

Chronic low animal protein is a basic problem that needs an urgent solution especially in developing countries which constitutes approximately 60% of the global populace, most of who are suffering from animal protein deficiency (Attah *et al*., 2006). This animal protein deficiency results from the low level of livestock productivity and/or a lack of affordability. Reproduction is one of the most important factors affecting livestock production and its success greatly depends on a mixture of factors including genetic merit, physical environment, nutrition and management (Rasbech, 1984). It is well documented that reproductive well-being and performance of farm animals is largely dependent on their nutritional status. Evidence from the literature suggests that nutritional factors are the most crucial in terms of their direct effect on reproductive phenomenon and have the potential to moderate the effects of other factors (Rekwot *et al*., 1987; Alabi, 2005; Kheradmand *et al*., 2006). Improvement in the nutritional status of livestock will lead to improve reproductive performance which in turn results in improved livestock production. Yankasa sheep being the most numerous and widely distributed breed of sheep in Nigeria (FDLPCS, 1991) can play a vital role in alleviating the problem of animal protein deficiency. The effect of nutrition, particularly underfeeding and flush feeding on the female fertility has been extensively studied (Helali *et al*., 1990; Kleemann *et al*., 1991; Lozano *et al*., 2003). However nutritional factor as a determinant of reproductive performance has received little attention in the male, thus makes it necessary to investigate the effect of nutrition on reproductive performance using gonadal sperm reserves as a fertility index in Yankasa rams.

2. Materials and methods

Twenty healthy Yankasa rams with clinically normal genitalia, aged 18-24 months and weighing 21-30 kg were selected for the study. The rams were randomly divided into four groups of five each. They were managed under intensive system, kept in separate pens and fed individually. Before the commencement of the feeding trials, they were acclimatized for two weeks. During acclimatization, they were screened for hemoparasites and helminths. All the rams 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. Three isocaloric rations (10MJ/Kg DM ME) varying in protein level were formulated to contain 12.11%, 14.96% and 17.94% crude protein (CP) using non-conventional feed stuffs {Maize Offal (MO) and dry 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. Group D (control) were fed a concentrate mixture of 12.26 % CP composed using conventional protein sources (Table 1). All test diets were subjected to proximate analysis using the method of AOAC (1990); (Table 2). Live-weight and testicular measurements were taken weekly in the morning before feeding. Scrotal circumference was measured 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. At day 98, eight (8) rams, two (2) from each group were surgically castrated under local anaesthesia and the testis removed intact, then dissected free from any extraneous tissue. Tunica albugenia was removed using a scalpel blade and the testicular parenchyma weighed. The left and right epididymis were weighed, separated into caput, corpus and cauda based on gross anatomy and weighed. They were placed in normal saline for onward determination of epididymal sperm reserves. Gonadal and epididymal sperm reserves were determined as described by Coulter *et al.,* (1987); Alabi, (2005); Ogunlade *et al.* (2006). The testicular parenchyma was weighed, sliced and homogenized with a high speed blender for two minutes with 50 mls of 0.9% NaCl containing antibiotics (sodium penicillin G, 100 IU/ml and streptomycin sulphate, 1 mg/ml) to prevent bacterial growth. In determining the epididymal sperm reserves, caput, corpus and cauda epididymides were isolated, weighed and minced with a pair of scissors separately in 20 mls of 0.9% NaCl solution. All tissues were homogenized 2-6 hours after castration. Testicular homogenates and epididymal samples were refrigerated

(4^{o}C) overnight. After 24 hours the samples were filtered through gauze. The filtrate volume was measured and recorded. One ml each of epididymal and gonadal filtrates were diluted separately with 2 mls of saline solution. Sperm concentrations of the testicular and epididymal samples were determined using a haemocytometer and light microscopy.

Data collected were expressed as means and their standard error of mean (SEM). Significance of differences between treatments means were estimated at P≤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).

3. Results and discussion

Results from the study showed that rams on 12.11% CP from non conventional sources had significantly higher live-weights (kg) than those on 17.94% CP and those on control diet (P<0.01; Table 3). This can be explained by tolerance and effective utilization of non protein nitrogen (NPN) at lower levels than at higher levels. Dietary nitrogen was utilized most efficiently at 12% CP with optimum weight gain at 13.6% CP (Negesse *et al.*, 2001). Highest weight gain at 13% CP level in male kids was also observed (Atti *et al.,* 2004). Findings in this study contradicts the results recorded earlier, where bulls and rams on a higher protein diet had significant increase in live-weight when compared to those on low level protein diet (Rekwot *et al.,* 1988 and Elmaz *et al.,* 2007). The animals placed on a high protein diet by Rekwot *et al.* (1988) were fed 14.5% CP which was close to CP level fed to Group C in this study while those placed on a low protein diet were lower than those used in this experiment. Our findings also agrees with Fourie *et al.* (2004) who reported an increase of 26% in the live-weight of Dorper race male lambs fed diets containing low levels of protein (12.5% CP) compared to those on high protein diet (16% CP) with an increase of 8% live-weight.

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

	Group of rams			
Ingredients (%)	**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 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

Table 3
Live weight, Testicular weight, gonadal and epidydimal sperm reserves of Yankasa rams fed different levels of protein in the diet (Mean ±SEM).

Group	**Live weight (Kg)**	**Testicular weight (g)**	**Gonadal sperm reserves (×10^6/gm testis)**	**Epididymal sperm reserves (×10^6/gm testis)**		
				Caput	Corpus	Cauda
A (12.11% CP)	27.69±0.25^{a}	186.25±11.16^{a}	124.75±16.22^{a}	294.00±24.65^{a}	182.25±37.77	2960.00±194.98
B (14.96% CP)	26.28±0.46^{ab}	140.00±4.42^{b}	89.50±12.68^{ab}	286.50±40.19^{ab}	83.75±22.43	3340.00±142.30
C (17.94% CP)	24.79±0.52^{c}	157.25±12.46^{ab}	81.75±12.36^{ab}	209.75±12.43^{ab}	114.25±24.71	2880.00±311.15
D (control)	25.06±0.40^{bc}	147.75±3.73^{ab}	66.25±8.77^{b}	170.00±15.93^{b}	156.00±27.33	2197.50±308.37

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

This study also showed that animals fed the 12.11% CP from non-conventional sources gained more live-weight than those on the same plane of nutrition but from conventional sources, which agree with Saleh *et al*., (2002) who found that daily weight gain was higher in group fed poultry litter when compared with control. The lack of significant increase in live-weight of rams in group C (17.94% CP) may be because of the production of high levels of ammonia associated with the high rumen digestible protein and nitrogen retention. The high content of dried layer litter used in the formulation of 17.94% CP diet probably resulted in high nitrogen content in the diet, which may be toxic to the rumen microflora affecting the conversion of rumen digestible protein to amino acids as such making it unavailable for uptake by the body as reported in the literature (Al-Haboby *et al.,* 1999).

The rams on 14.96% CP diet had a significantly higher live_weight than rams on control diet ($P<0.05$; Table 3). Rams on 14.96% CP diet had a live weight of 1.49 kg significant higher than rams on 17.94% CP which ($P<0.05$; Table 3). The findings in this study showed that there was an increase in live-weights in the group A fed 12.11% CP and B (14.96% CP) when compared with the control (12.26% CP) and 17.94% CP. Level of protein in diet had effect on testicular weight ($P<0.05$; Table 3). The rams on 12.11% CP diet had significantly higher testicular weight than those on 14.96% CP ($P<0.05$; Table 3).

Rams fed 12.11% CP diet had significantly higher gonadal reserves of 124.55×10^6/ml than rams on control diet with gonadal reserve of 66.25×10^6/ml ($P<0.05$; Table 3).

The result showed that diets with equal energy levels but different protein levels had effect on epidydimal and gonadal sperm reserves. Rams on 12.11% CP level showed significantly higher gonadal sperm reserves when compared with those on control diet (12.26% CP). This is attributed to the significantly higher increase in live-weight and scrotal circumference observed in the former group. The increase in testicular size in rams on 12.11% CP indicated a large volume of testicular parenchyma and increase in the volume of seminiferous epithelium and diameter of seminiferous tubules where spermatogenesis occurs (Abi-Saab *et al.,* 1997).

The difference observed in the epidydimal sperm reserves of rams on 14.96% CP and control is in relation to the increase in semen concentration recorded for the former, owing to the fact that improved nutrition above the normal maintenance requirement favoured spermatogenesis. The relatively higher percentage of live sperm cells in rams fed 14.96% CP might have also resulted in more cells moving from the testicular parenchyma to the epidydimis. This finding is in accord with that obtained by Rekwot (1987) who found increase in semen concentration in bulls placed on a high protein diet of 14.5% CP when compared to bulls placed on a lower protein diet. It is also in agreement with the work of Paérez-Clariget *et al.* (1998) who found that improved nutrition accelerated testicular growth in Corriedale rams, which is a reflection of sperm output and reserves.

4. Conclusion

This study concluded that lower levels of dry layer litter in diets (12%CP) when used in Yankasa rams increased their live weights and sperm reserves, consequently improved the fertility. Thus dry layer litter and maize offal are recommended as substitutes for conventional feed stuff.

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