Original Research Article

Semen characteristics and testosterone profiles of Yankasa rams fed graded levels of dietary protein using cotton seed and palm kernel cakes

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Abstract

The increasing demand of the feed market for protein has necessitated the need to look for and use other sources of this nutrient. Cottonseed cake and palm kernel cakes are used extensively for supplementing ruminant rations to increase their productivity. The efficiency of sperm production, libido and quality of spermatozoa tend to remain uniform throughout the reproductive life of an animal but may be significantly altered by nutrition. The aim of this study was to assess the effects of graded levels of protein on semen characteristics, and serum testosterone profile in Yankasa rams. Fifteen rams aged between 18–24 months and weighing 15–25 kg with good body condition scores (3.5) were used in this study. They were divided into three treatment groups (A, B and C) according to the dietary protein level. Group A (n = 5) received 10% , group B (n = 5) received 15% while group C (n = 5) received 20% combined crude protein of cotton seed and palm kernel cakes, respectively. Semen samples were collected weekly using battery-controlled electro ejaculator. Serum samples were harvested using a Pasteur pipette into serum vials and stored at –20 °C for analysis. These samples were from a representative animal in each group for determination of testosterone profiles using ELISA technique at weeks 1, 6 and 12. Data collected were expressed as means and standard error of the mean (± SEM). Significance of differences between treatments means were estimated at $P \leq 0.05$ with Tukey-Kramer multiple comparison test of repeated measures analysis of variance (ANOVA). From this study, it was concluded that rams fed concentrate of 15% crude protein using cotton seed and palm kernel cakes combined had improved semen characteristics in terms of semen concentration, motility, live sperm cells and morphology, but the crude protein levels had no effects on testosterone concentrations.

Keywords: concentrate; crude protein; hormone; nutrition; semen quality

INTRODUCTION

The most important source of protein in livestock and poultry nutrition worldwide is soybean meal. However, because of the increasing demand of the feed market for protein, there is a need to look for and use other sources of this nutrient (Swiatkiewicz et al., 2016). Cottonseed and palm kernel cakes are used extensively for supplementing ruminant rations to increase productivity (Babashani et al., 2015). Reproduction as an important index in livestock production depends on factors which include genetic merit, physical environment, nutrition and management (Rasbech, 1984). Nutrition plays a vital role in enhancing reproductive efficiency in all animals (Bindari et al., 2013). Previous studies have demonstrated the luteinizing hormone (LH) to be secreted during early gonadotropin rise, and it can be sustained for a longer period if calves receive improved nutrition (Cheah and Yang, 2011). The efficiency of sperm production, libido and quality of spermatozoa tend to remain uniform throughout the reproductive life of an animal but may be significantly altered by age, nutrition, environment, health status, drugs and chemicals (Togun and Egbunike, 2006). Nutrition may affect the efficiency of related hormone production and the growth of reproductive organs (Almeida et al., 2007). The fertility of males and females in a particular herd/flock, as well as the level of nutrition to a large extent, determines the rate of production in any livestock industry (Abdulrashid and Darren, 2016).

Table 1. Ingredients and nutrient composition of experimental diets

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

With the foregoing, it was hypothesised that various levels of dietary protein using cotton seed and palm kernel cakes combined would have effects on the semen characteristics and testosterone concentrations in adult Yankasa rams. This study was conducted to investigate if feeding graded levels of protein diet using combined cotton seed and palm kernel cakes would have effects on semen characteristics and testosterone profiles in Yankasa rams.

MATERIALS AND METHODS

Experimental animals: A total of 15 rams of the Biotechnology Research Programme of National Animal Production Research Institute, Nigeria, aged between 18–24 months and weighing 15–25 kg with good body condition scores (3.5) were used for this study. The experiment was laid out in Completely Randomised Design (CRD) and replicated 5 times. The experimental treatments were divided into three treatment groups (A, B and C) according to the dietary protein level. The animals in group A (n = 5) received 10% crude protein, group B (n = 5) received 15% crude protein while group C (n = 5) received 20% crude protein. The rams were screened for blood and helminth parasites, appropriate treatment carried out and the approval of the Animal Welfare Committee was sought before the research was started.

The rams were managed under intensive system, kept in separate pens, fed individually, water was provided *ad libitum* and the rams were allowed a two-week adjustment period. All rams under study were fed a basal diet of hay (*Digitaria* spp.) *ad libitum* and given a supplement ration of concentrate mixture at 2% body weight/head/day. All test diets were subjected to proximate analysis using the method of (AOAC, 1990). The animals were fed for a period of 3 months.

Blood collection

Whole blood (3 ml) was collected aseptically (hourly from 08:00 to 18:00 h) from a representative animal of each group at the start of the experiment, middle of the experiment and end of the experiment. This was done via the jugular venipucture using 5 ml sterile 21-gauge hypodermic needles into vacutainer tubes. The blood samples were allowed to clot by leaving them undisturbed at room temperature.

Serum collection for testosterone evaluation

Serum samples were immediately harvested from the clotted blood samples using a Pasteur pipette into serum vials and stored at –20 °C until analysis. These samples were from a representative animal in each group for determination of testosterone profiles using ELISA technique. Testosterone assay was done using testosterone kits (Accu-bind®) by ELISA technique. The reagent was constituted as described by the manufacturer.

Semen collection

Semen was collected from rams adequately restrained in standing position. The samples were collected with the aid of a battery-operated Bailey electro-ejaculator (MOD2, Western Instrument Company, Denver, Colorado USA). The Bailey ejaculator is powered by a 6-volt battery. The probe was lubricated with petroleum jelly for easy insertion into the rectum and

Table 2. Proximate analysis of feed

Group	% DM	% Ash	% CF	% N	% CP	Energy (MJ/kg DM ME)
A	96.21	11.94	35.69	1.67	10.44	2060
B	95.57	9.26	31.15	2.43	15.19	2120
C	96.05	6.70	30.50	3.30	20.63	2210

DM = dry matter; CF =crude fibre; CP = crude protein; ME = metabolisable energy; N = nitrogen

pushed forward slowly and a series of short electrical stimulation was done intermittently for approximately 2–5 seconds, until erection and ejaculation were achieved. Ejaculates were placed in a water bath at 37 °C before microscopic evaluations. Semen samples were collected once weekly from each ram for 3 months using electro-ejaculator.

Semen evaluation

Semen samples were evaluated according to the method described by Zemjanis (1970). The gross semen characteristics that were examined include volume, motility, colour, and presence or absence of foreign bodies as described by (Maina et al., 2006).

Volume and motility

The semen was collected and read from the graduated collecting tubes immediately. Microscopic examination for gross sperm motility was determined as described by Oyeyemi et al. (2009) using Celestron Penta View microscope (LCD-44348 by RoHS, China). A drop of raw undiluted semen on a pre-warmed slide was covered by a slip and viewed under a field microscope at ×10 magnification.

Sperm concentration

This was determined by using the haemocytometer method. A semen sample was sucked into the red blood cell diluting pipette up to 0.1 mark and the volume made up to 101 mark with 10% formal saline and mixed thoroughly. The mixture was dropped and allowed to spread under the cover-slip placed tightly on the haemocytometer after discarding few drops. The cells were allowed to settle before counting under ×40 magnification. Sperm cells were counted diagonally from top left to right bottom in five small squares of the improved Neubauer haemocytometer. Sperm output of individual ram was calculated as described by Verstegen et al. (2002) using the following equation: Concentration (sperm cells/ml) = Number of sperm cells counted in the twenty-five small squares × dilution factor × 10^4.

Live-dead ratio of the sperm cells

This was determined as described by Esteso et al. (2006). A thin smear of the semen sample was made on clean, grease-free glass slides and stained with Eosin-Nigrosin stain. The dead sperm cells were identified as those that absorbed the stain. At least 400 sperm cells were counted using a light microscope at ×40 magnification. Sperm abnormalities were determined as described by Esteso et al. (2006). A thin smear of the semen sample was made on a clean grease-free glass slide and fixed with buffered formal saline. The preparation was examined and abnormal sperm cells counted in a regular sequence using light microscope at ×100 magnification with oil immersion. A total of 400 well-spaced spermatozoa were carefully examined in each preparation and the percentage of head, midpiece and tail sperm abnormalities were determined as described by Melrose and Laing (1970).

Data Analyses

Data collected were expressed as means and standard error of the 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). Statistical analysis was conducted using the Graphpad Instat computer programme (GRAPHPAD for Windows, Inc., version 3.05 of 2000).

Table 3. Sperm concentrations (×10^6/ml) of Yankasa rams fed graded levels of dietary protein

Weeks	Group A (10% CP) (n = 5)	Group B (15% CP) (n = 5)	Group C (20% CP) (n = 5)
1	107.6 ± 51.0	145.3 ± 29.6	125.5 ± 80.2
2	156.8 ± 84.2	129.3 ± 43.4	130.6 ± 54.5
3	130.3 ± 91.0	261.3 ± 55.8	117.5 ± 66.4
4	29.2 ± 24.5^a	264.8 ± 16.6^b	64.7 ± 11.2^{ab}
5	42.8 ± 37.1^a	163.5 ± 41.6^b	115.8 ± 46.3^{ab}
6	169.4 ± 71.9	200.5 ± 28.0	115.5 ± 62.2
7	145.8 ± 49.9	124.0 ± 60.0	86.4 ± 25.6
8	63.5 ± 40.9	110.8 ± 53.8	95.4 ± 33.8
9	21.8 ± 14.7^a	85.7 ± 27.6^b	104.2 ± 28.6^b
10	62.8 ± 21.0	91.7 ± 42.9	36.2 ± 23.3
11	130.2 ± 37.3	117.5 ± 51.4	95.2 ± 38. 0
12	145.8 ± 49.8	125.0 ± 59.9	115.7 ± 46.3
Mean ± SEM	**96.4 ± 16.3^a**	**163.1 ± 20.6^b**	**98.8 ± 8.5^a**

^{ab} = Means in the same row with different letters are statistically significantly ($P \leq 0.05$) different; CP = Crude protein

Table 4. Sperm motility (%) of Yankasa rams fed graded levels of dietary protein

Weeks	Group A (10% CP) (n = 5)	Group B (15% CP) (n = 5)	Group C (20% CP) (n = 5)
1	43.0 ± 17.4	42.0 ± 5.4	40.0 ± 15.5
2	57.0 ± 17.2	72.5 ± 4.8	53.0 ± 21.9
3	52.5 ± 22.6	82.5 ± 3.2	57.5 ± 19.8
4	55.0 ± 13.8	88.7 ± 3.1	28.3 ± 28.3
5	47.0 ± 15.2	76.2 ± 8.3	67.0 ± 10.7
6	53.0 ± 15.5[a]	90.0 ± 3.5[b]	44.0 ± 15.9[a]
7	78.0 ± 12.3	63.7 ± 14.6	58.0 ± 7.5
8	42.5 ± 18.8	66.2 ± 15.7	63.0 ± 16.1
9	35.0 ± 16.3	70.0 ± 17.0	61.0 ± 16.3
10	64.0 ± 12.9	78.7 ± 7.5	54.0 ± 22.2
11	69.0 ± 6.2[a]	90.0 ± 2.0[b]	61.0 ± 12.6[a]
12	64.0 ± 12.9	85.0 ± 3.2	61.0 ± 16.1
Mean ± SEM	**54.7 ± 3.8[a]**	**75.1 ± 3.0[b]**	**53.91 ± 3.9[a]**

[ab] = Means in the same row with different letters are statistically significantly ($P \leq 0.05$) different; CP = Crude Protein

Table 5. Percentage live spermatozoa (%) of Yankasa rams fed graded levels of dietary protein

Weeks	Group A (10% CP) (n = 5)	Group B (15% CP) (n = 5)	Group C (20% CP) (n = 5)
1	69.0 ± 8.3	71.5 ± 2.5	70.0 ± 0.0
2	56.0 ± 8.1[a]	81.2 ± 4.3[b]	78.0 ± 6.0[ab]
3	72.0 ± 8.0	80.0 ± 4.1	73.0 ± 5.4
4	78.0 ± 5.8[a]	83.7 ± 2.4[a]	64.0 ± 4.8[b]
5	70.0 ± 5.5	72.5 ± 7.5	76.0 ± 4.0
6	70.0 ± 7.6	87.5 ± 4.3	75.0 ± 5.2
7	81.0 ± 5.6[a]	71.2 ± 8.3[a]	66.0 ± 1.9[b]
8	69.0 ± 8.0[a]	75.0 ± 8.9[b]	78.0 ± 3.4[b]
9	73.0 ± 7.0	83.7 ± 8.0	77.0 ± 4.6
10	72.0 ± 6.4	78.7 ± 4.3	67.0 ± 6.2
11	73.0 ± 6.2	85.0 ± 2.9	70.0 ± 5.5
12	69.0 ± 5.6[a]	83.7 ± 8.0[b]	73.0 ± 5.4[ab]
Mean ± SEM	**71.0 ± 1.9[a]**	**80.1 ± 1.5[b]**	**72.2 ± 1.4[a]**

[ab] = Means in the same row with different letters are statistically significantly ($P \leq 0.05$) different; CP = Crude protein

Table 6. Reaction times (minutes) of Yankasa rams fed graded levels of dietary protein

Weeks	Group A (10% CP) (n = 5)	Group B (15% CP) (n = 5)	Group C (20% CP) (n = 5)
1	1.4 ± 0.5	1.4 ± 0.5	1.3 ± 0.5
2	1.1 ± 0.2[a]	0.5 ± 0.1[b]	1.0 ± 0.5[a]
3	1.1 ± 0.4	0.8 ± 0.2	0.7 ± 0.2
4	1.1 ± 0.2[a]	0.5 ± 0.1[b]	0.8 ± 0.4[a]
5	1.5 ± 0.5[a]	0.6 ± 0.2[b]	0.9 ± 0.3[ab]
6	0.8 ± 0.2	1.0 ± 0.0	1.0 ± 0.2
7	1.0 ± 0.3	1.0 ± 0.2	1.1 ± 0.2
8	1.1 ± 0.4	1.4 ± 0.4	1.6 ± 0.3
9	1.3 ± 0.2	1.2 ± 0.3	1.5 ± 0.53
10	0.4 ± 0.1[a]	1.0 ± 0.3[b]	1.3 ± 0.3[b]
11	1.0 ± 0.3[a]	0.6 ± 0.2[a]	0.4 ± 0.0[b]
12	1.5 ± 0.5[a]	0.6 ± 0.2[b]	1.5 ± 0.5[a]
Mean ± SEM	**1.1 ± 0.1**	**0.9 ± 0.1**	**1.0 ± 0.1**

[ab] = Means in the same row with different letters are statistically significantly ($P \leq 0.05$) different; CP = Crude protein

Table 7. Semen volume (ml) of Yankasa rams fed graded levels of dietary protein

Weeks	Group A (n = 5)	Group B (n = 5)	Group C (n = 5)
1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
2	0.5 ± 0.3	0.4 ± 0.0	0.3 ± 0.1
3	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.0
4	0.1 ± 0.0	0.4 ± 0.1	0.4 ± 0.0
5	0.1 ± 0.0^{a}	0.5 ± 0.1^{b}	0.5 ± 0.1^{b}
6	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
7	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.0
8	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
9	0.1 ± 0.1^{a}	0.4 ± 0.1^{b}	0.4 ± 0.2^{b}
10	0.3 ± 0.2	0.4 ± 0.1	0.4 ± 0.1
11	0.2 ± 0.0^{a}	0.5 ± 0.0^{b}	0.4 ± 0.1^{b}
12	0.3 ± 0.0^{a}	0.5 ± 0.1^{b}	0.4 ± 0.1^{b}
Mean ± SEM	**0.2 ± 0.01^{a}**	**0.4 ± 0.01^{b}**	**0.4 ± 0.01^{b}**

^{ab} = Means in the same row with different letters are statistically significantly ($P \leq 0.05$) different

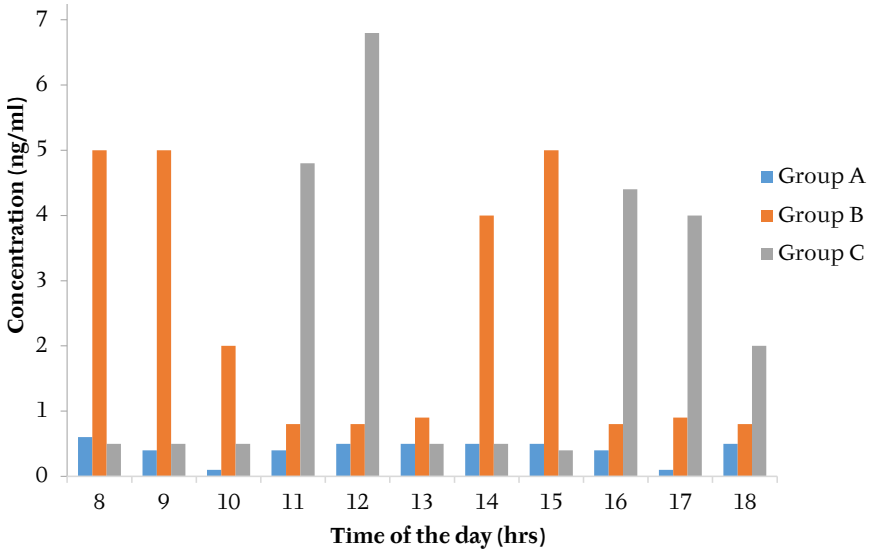

Figure 1. Testosterone profiles of one Yankasa ram fed three protein diets at week one

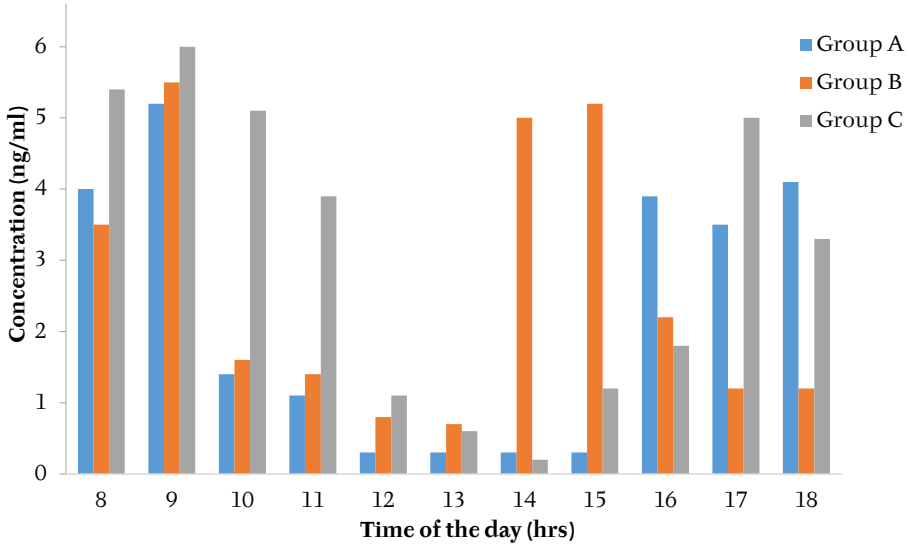

Figure 2. Testosterone profiles of one Yankasa ram per group fed three protein diets at week six

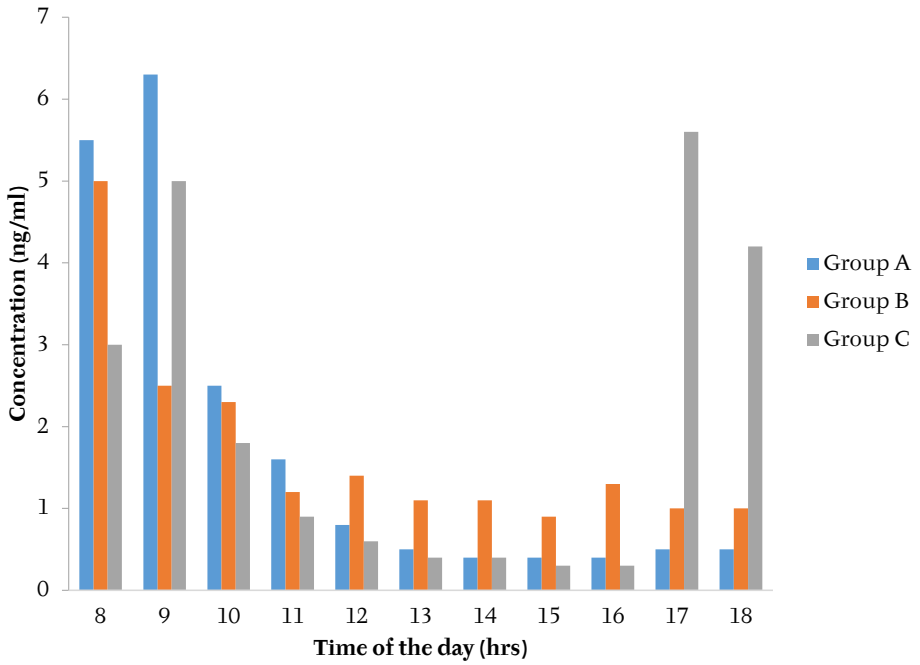

Figure 3. Testosterone profiles of one Yankasa ram fed three protein diets at week twelve

Table 8. Sperm abnormalities (%) of Yankasa rams fed graded levels of dietary protein.

Abnormalities (%)	Group A (10% CP)	Group B (15% CP)	Group C (20% CP)
Normal cells	84.3 ± 5.6	88.0 ± 4.6	78.7 ± 6.2
Detached heads	4.9 ± 2.6	4.7 ± 1.1	6.5 ± 1.9
Folded tails	4.1 ± 1.2	3.3 ± 1.5	4.0 ± 1.3
Coiled tails	2.8 ± 1.2	2.3 ± 0.6	3.0 ± 1.3
Bent tails	3.4 ± 1.4	3.0 ± 1.2	3.7 ± 1.4

RESULTS

Rams fed 10% crude protein had the least semen concentration and rams fed 15% crude protein having the highest semen concentration during the study period as shown in Table 3.

Rams fed 10% and 20% crude protein had lower ($P < 0.05$) sperm motility than rams fed 15% crude protein during the study period (Table 4).

Again, rams fed 10% and 20% crude protein showed lower ($P > 0.05$) values for percentage live sperm than rams fed 15% crude protein during the study period (Table 5).

Reaction time is the time interval between stimulation using electroejaculator and ejaculation. Overall reaction time was observed to be similar ($P > 0.05$) in all rams irrespective of dietary crude protein level (Table 6).

Semen volumes recorded during the study revealed rams fed 15% and 20% crude protein having significantly higher ($P > 0.05$) semen volumes than that of rams fed 10% (Table 7).

Sperm abnormalities recorded include detached heads, folded tails, coiled tails and bent tails. These values were not significantly different (Table 8). However, the rams fed 20% crude protein had the least normal sperm cells, whereas the rams fed 15% crude protein had the highest values for normal cells.

Figures 1, 2 and 3 show the testosterone concentrations on weeks 1, 6 and 12, respectively. Highest concentrations were usually recorded in the early morning period and late evening across all groups. The pulsatility of testosterone is evident in the figures as the concentration differs with time. These are values for a representative animal from each group thus serving as a basic orientation showing the hourly fluctuations of testosterone.

DISCUSSION

The ejaculate volume was highest in rams fed 15% CP (Table 7) and this is similar to the report by Kheradmand et al. (2006) that the ejaculate volume tends to be higher with improvement of nutrition in Bakhtary rams. The ejaculate volume in the present study corroborates the report of Suhair and Abdalla (2010) where ejaculate volume in desert rams was significantly lower with low level of nutrition. The low

ejaculate volume obtained in group A rams (10% CP) could be attributed to the decreased function of the pituitary gland and testis due to decrease in their sizes because of the decreased production of GnRH (Alkass et al., 1982). Consequently, the secretions of androgen-dependent organs (epididymis, testis and accessory glands) are expected to decrease, resulting in a low ejaculate volume (Suhair and Abdalla, 2010). Rams fed 15% and 20% CP had higher semen volumes than rams fed 10% CP (Table 7) corroborating the finding of Ososanya et al. (2014), but disagrees with that of Abi-Saab et al. (2008) who reported non-significant differences in semen volume of young bucks fed two different (8.6% and 15.2%) protein diets. There was no significant difference in semen volume between rams fed 15% and 20% CP, and this agrees with the findings of Kheradmand et al. (2006). In this study, rams on 15% CP had a significantly higher semen concentration when compared to those fed 10% and 20% CP (Table 3). This highest increase in semen concentration might be an indication that though an increased protein intake above 10% enhanced spermatogenesis, higher levels of CP in diets could result in excess urea and more available ammonia, which could alter the physiology of reproduction (Butler et al., 1996). This is similar to the reports of Rekwot et al. (1987) and Fernandez et al. (2004) who reported that increased protein supply up to 14% CP favours spermatogenesis, but lower protein levels below 14% did not enhance it. The rams fed 15% CP had a higher sperm motility than those fed 10% and 20% (Table 4), supporting the report by Ososanya et al. (2014), who reported that West African Dwarf goats fed 14% CP had greater semen motility than those given 12% CP. Kumar et al. (2015) also reported that feed restriction reduced the total motile spermatozoa, and slow spermatozoa were significantly higher in the nutritionally stressed rams. Sperm motility was significantly lower with poor feeding. This is clearly related to decline in the nutritional status of rams, which could have induced low fructose level in seminal plasma and consequently decreased semen motility as these parameters are positively correlated (Amir and Volcani, 1965). Low sperm motility might be related to low plane of nutrition and protein intake, resulting in relatively low concentration of seminal plasma metabolites (Chandrasekhar et al., 1986). The result from this study differs from what was reported by Jibril et al. (2011), in which there was no significant difference between groups fed different (12.11% , 12.26% , 14.96%, 17.94%) levels of CP. The study revealed significantly lower live sperm values in rams maintained on low level of protein (10% CP) (Table 5). The group fed 20% CP also had high live sperm values (72.2 %) and this could be related to the high nutrition, resulting in increased metabolic activity of testicular cells thus causing testicular hypoxia (Suhair and Abdalla, 2010). This is similar to the study conducted by Suhair and Abdalla, (2010) in desert rams and Ososanya et al. (2014) in West African Dwarf rams. Rams that were fed 15% CP had highest values for percentage live sperm cells of 80.1 % (Table 5). Rams fed 15% CP had the lowest ejaculation time, which was an indication of high libido (Ososanya et al., 2014), although there were no significant differences between all groups. It was observed that rams fed 20% CP had the highest percentage of abnormal spermatozoa (Table 8). Similar reports were made by Alabi (2005) who observed higher numbers of sperm defects in bulls fed on both high and low energy diets than those on medium energy diet. Jibril et al. (2013) reported similar findings in rams fed high protein diet. The rams fed 20% CP had higher level of cotton seed cake in their diet (Table 1). Cotton seed cake has a higher amount of bound gossypol, which could affect sperm morphology (Babashani et al., 2015). This occurs through gossypol-induced inhibition of the synthesis of sperm cells histones and other nuclear proteins that stabilise the structure of DNA (Ye et al., 1989). Testosterone is a key mediator in the expression of numerous morphological and behavioral traits in mammals, but the factors underlying individual variation in circulating testosterone levels are poorly understood (Preston et al., 2012). From this study, it was observed that the testosterone concentration was pulsatile in nature and varied with time (Figure 1–3). This could be because the LH that is a precursor to testosterone is released in pulses. It is therefore expected that testosterone concentration too would vary. This trend is similar to what was reported by Maksimovic et al. (2016) who observed pronounced variability of testosterone in rams during their study. In this study, it was observed that the concentration of testosterone was independent of diet type, as animals from the different groups had different testosterone profiles. This is similar to reports by Elmaz et al. (2007) who stated that testosterone concentration had a fluctuating trend independent of diet type. This is similar to reports by Martini and Walkden-Brown (1995) who stated that the energetic components of diet, rather than the protein content, seem to be responsible for affecting gonadotropin secretion in rams. It was observed in this study that all rams sampled usually had high testosterone concentration during the morning period, then a few hours of low concentration which increased usually a few hours after 12:00 h (Figure 1–3). This trend might be due to thermal stress, as atmospheric temperature usually peaks within the period the period low testosterone concentrations were recorded. Higher concentrations were recorded when the atmospheric temperature is usually lower. Lincoln et al. (1982) reported that hormone fluctuations are possibly influenced by changes in ambient temperature or the timing of feeding. From this study it could be concluded that variations in testosterone production could be due to individual variations,

similar to what was reported by Elmaz et al. (2007). This author also stated that variation in serum testosterone profiles could be caused by various internal and external factors.

CONCLUSION AND RECOMMENDATION

From this study, it was concluded that rams fed concentrate of 15% crude protein using cotton seed and palm kernel cakes combined had improved semen characteristics in terms of semen concentration, motility, live sperm cells and morphology. However, the crude protein levels had no effects on their blood testosterone concentrations. The above concentration of protein is thus recommended in order to enhance the reproductive characteristics of rams.

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Received: December 17, 2019
Accepted after revisions: September 15, 2020