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TESTICULAR MORPHOMETRY, GONADAL AND EXTRA GONADAL SPERM RESERVE OF RED SOKOTO BUCKS FED VARYING LEVELS OF ENERGY (MOLASSES) AND PROTEIN (GROUNDNUT CAKE) FEEDS IN A MIXED RATION

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ABSTRACT

An experiment was conducted to determine the effect of molasses inclusion on testicular histometry of Red Sokoto bucks. Twenty (20) sexually mature Red Sokoto bucks were randomly allotted to four dietary treatments with four animals per treatment. Treatment compared were T_1 = 0% molasses, T_2 = 10% molasses, T_3 = 20% molasses and T_4 = 30% molasses and $T_{5=}$ 40% molasses. Parameters monitored were testicular dimensions, gonadal and extra gonadal sperm reserves. The results of chemical composition of the diets show that the dry matter (DM) content was higher for all the dietary treatments. The values for DM ranged from 913.60 - 919.00 g kg^{-1} DM. The crude protein (CP) content was observed to decrease with increase in the level of molasses with T_1 (162.20 g kg^{-1} DM) having the highest value while T_5 (140.10 g kg^{-1} DM) had the least. The neutral detergent fibre (NDF), non-fibre carbohydrate and energy values were significantly ($p<0.05$) affected by treatments. The gonadal sperm reserves were significantly different ($p< 0.05$) among the treatments with T_2 having the highest value of 35.15 $x10^6$ and 38.65 $x10^6$ for right and left testes respectively. The right and left testicular weight were significantly ($p<0.05$) higher for T_2 compared to other treatment groups while the right and left testicular length was observed to be higher for T_3 compared to other treatment groups. It can be concluded that the reproductive potential of Red Sokoto bucks fed these diets was affected by the level of inclusion. The goats can be of good genetic resources to support planned breeding programs when such feeds are used in the Guinea Savannah.

Keywords: Goats, Epididymal, Gonadal, Molasses, Groundnut cake, sperm reserves

INTRODUCTION

Red Sokoto goats are known to breed all year round, their fertility characteristics have not been fully documented to facilitate effective genetic improvement by selection and crossbreeding at all levels of production. In the male for instance, there is the need to establish measurable criteria for judging breeding soundness and guiding selection of males for breeding. These criteria include scrotal measurements, libido and semen quality tests (Michelson *et al*., 1981; Ogwuegbu *et al*., 1985) and the relationships between them. Biometric parameters, such as scrotal circumference (SC), testicular weight (TW) and testicular length (TL), are essential measurements in the andrological evaluation of a breeding animal.

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). Evaluation of the morphometric structure of spermatozoa can be an indicator of their fertilization capacity. Studies on sperm morphometry in various mammalian species (Saravia *et al.*, 2007; Brito *et al.* 2011) and Hidalgo *et al.* (2007) have shown a link between sperm dimensions and fertility. Sperm morphometry is evaluated using traditional microscopy with image analysis software, as well as increasingly popular Computer Assisted

Semen Analysis (CASA) (Maroto-Morales *et al.,* 2016; Van Van Der Horst *et al.,* 2018), which are believed to ensure more reliable and repeatable results Yániz *et al.* (2015). However, irrespective of the method used to evaluate sperm morphometry, it is important to take into account the effect of various factors that may influence variability in the results (Waheed *et al.,* 2015). Some authors have indicated that slide preparation and staining methods can significantly affect the morphometric dimensions of sperm (Wysokińska *et al.,* 2019). This effect is due to the means of slide preparation, the type of staining reagents, the temperature, and the type and quality of equipment (Rijsselaere *et al.,* 2004).

The use of molasses and groundnut cake diets is a fundamental component of the feeding strategy in order to balance nutrients for improved performance. Poor nutrition results in low rates of production, often defined by growth and reproduction. It also affects the immune system and the ability of an animal to fight disease. In extreme conditions of malnutrition, death can occur. In many breed production systems, approximately two-thirds of improvements in livestock productivity can be attributed to improved nutrition.

Groundnut cake is a protein rich feed for livestock with crude protein content of 40-45%. It is a good supplement, promotes growth and is palatable in livestock feeds. Groundnut is a valuable source of vitamins E, K and B. It is the richest plant source of thiamine (B_1) and also rich in niacin, which is low in cereal (FAO, 2000). Groundnut cake protein is known to be deficient in lysine and methionine and also has a limited amount of tryptophan and threonine but amino acid quality improves in artificial diets when reinforced with lysine, methionine and tryptophan (Eyo and Olatunde, 1998). Therefore, the study was aimed at determining the effect of molasses and groundnut cake on testicular morphometry, gonadal and extra gonadal sperm reserve in Red Sokoto goats.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the Teaching and Research farm, University of Abuja, Faculty of Agriculture, located in Gwagwalada area council of the Federal capital territory Abuja. It lies between latitude 08^0 51″ and 09^0 37″N and longitude of 70 20″ and 7^0 51″E. The area is characterized by two distinct seasons which are the rainy season from May to October and the dry season from November to April. Rainfall in this area is unimodal with its peak in August and sometimes September. The annual rainfall ranges 1,100 to 1,450 mm (Ishaya and Grace, 2007). Temperature in this area is generally high ranging from 30 to 37^0C (Balogun, 2001).

Experimental Animals and Their Management

Twenty (20) Red Sokoto bucks age between 5 and 6 months with an average weight of 8.6±0.67 kg were used for the experiment. All the animals were treated against external and internal parasites, using ivermectin and albendozole respectively. Terramycin long acting (TLA) injection was used to take care of subclinical bacterial infections. The animals were confined in individual well ventilated raised pens within a common house. The roof of the house was relatively high so as to reduce heat. Proper hygiene of the house was ensured all through the period of the experiment.

Experimental Treatments and Design

The animals were allotted to five (5) dietary treatments in a complete randomized block design (CRBD) with four (4) animals per treatment. The treatments were: T_1 (0% molasses and 40% GNC), T_2 (10% molasses and 30% GNC), T_3 (20% molasses and 20% GNC), T_4 (30% molasses and 10% GNC) and T_5 (40% molasses and 0% GNC).

Table 1: Composition of Experimental Diets (%)

Feed stuff	T_1	T_2	T_3	T_4	T_5
Molasses	0.00	10.00	20.00	30.00	40.00
Groundnut cake	40.00	30.00	20.00	10.00	0.00
Wheat Offal	10.00	10.00	10.00	10.00	10.00
Sorghum Stover	20.00	20.00	20.00	20.00	20.00
Cowpea husk	25.00	25.00	25.00	25.00	25.00
Premix	3.00	3.00	3.00	3.00	3.00
Salt	2.00	2.00	2.00	2.00	2.00
Total	**100**	**100**	**100**	**100**	**100**

T_1 (0% molasses and 40% GNC), T_2 (10% molasses and 30% GNC), T_3 (20% molasses and 20% GNC), T_4 (30% molasses and 10% GNC) and T_5 (40% molasses and 0% GNC).

Chemical analysis

Samples of the experimental diets were collected and dried in an air-draft oven at 60°C for 96 h, ground separately to pass through a 1 mm sieve in a Wiley mill and sampled for chemical analysis using the standard methods of the Association of Official Analytical Chemists [AOAC] (2005). Dry matter was determined by drying at 100°C for 24 h. Ash content was determined after ignition at 550°C for 4 h in a muffle furnace and used to calculate organic matter (OM). Fibre fraction analysis was by the methods of Van Soest *et al*. (1991).

Testicular measurement

The testicular dimensions were measured at the onset and subsequently on weekly basis before semen collection.

Testicular length (TL): This was measured in centimeters with a flexible tape as the distance along the caudal surface of the scrotum, from its point of attachment to the tip of the scrotum as described by Bratte *et al*. (1999) and Akpa *et al*. (2012).

Determination of Gonadal and Extra Gonadal Sperm/Spermatid Reserve

Gonadal and epididymal sperm/spermatid reserve were determined by the method of Rekwot *et al* (1987). Each testis was homogenized in 50 ml of saline that contained the antibiotics (1.h/1m of streptomycin and 100 IU/ml sodium penicillin G); using high-speed blender operated at full speed for two minutes and the homogenate volume was measure after rinsing the blender container with 20 ml of saline and adding the effluent. From the homogenate, 5 ml was transferred into a conical flask and diluted further with 80 ml of saline. The homogenate was stored overnight at 5^0C. The sperm/spermatid concentration was determined with a hemocytometer. The epididymis was carefully separated from each testis with scalpel blade and the lengths and weights of each caput; corpus and cauda portion was measured. The caput, corpus and cauda epididymides were separated and minced in 20 ml of saline with sharp scissors and stored overnight at 5^0C. The minced caput, corpus and cauda epididymides were filtered through gauze and the volume of the filtrate measured. One milliliter of the epididymal filtrate was diluted with 2 ml of saline. The concentration of the spermatozoa was determined with hemocytometer as described for gonadal sperm (Gyang, 1990)

Statistical Analysis

The data was analyzed using the analysis of variance ANOVA procedure of SAS (2002) package. Duncan multiple range test (DMRT) of the same software was used for separating significant differences among means and significance was measured when $p \leq 0.05$.

RESULTS

The results of the chemical composition of the experimental diets fed to Red Sokoto goats is presented in Table 2 Except for neutral detergent fibre, non-fibre carbohydrates and energy, no significant (p>0.05) difference was observed among diets.

Dry matter content ranged between 914.80 to 919.00 g kg^{-1} DM. Crude protein content was observed to decrease with increase in the level of molasses. The values for crude fibre ranged between 127.20 to 138 g Kg^{-1} DM). The results also indicated a level of 56.00 – 67.90 g Kg^{-1} DM for ether extract on diets T_5 and T_1. Acid detergent fibre spanned from 224.10% on diet T_5 to 239.10 g Kg^{-1} DM on diet T_1. The values were observed to decrease with increase in the level of molasses. However, there was a significant difference (P<0.05) in neutral detergent fibre content, with diets T_1 (394.00 g Kg^{-1} DM having the highest and diet T_5 (352.00 g Kg^{-1} DM %) having the lowest.

Table 2: Proximate Composition of Experimental Diets (g kg^{-1} DM)

	TREATMENTS					
Parameters	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**SEM**
Dry matter	919.00	917.80	915.00	913.60	914.80	1.33^{NS}
Crude protein	162.20	150.80	145.70	143.00	140.10	2.15^{NS}
Crude fibre	138.00	129.20	128.50	128.00	127.20	1.92^{NS}
Ether extract	67.90	59.20	58.80	57.10	56.00	0.98^{NS}
Acid Detergent Fibre	239.10	229.70	227.00	225.60	224.10	1.23^{NS}
Neutral Detergent Fibre	394.00^{a}	384.20^{a}	378.20^{ab}	365.00^{c}	352.00^{d}	0.04*
Non-fibre Carbohydrates	301.90^{c}	329.00^{ab}	325.50^{ab}	335.00^{a}	341.00^{a}	1.79*
Ash	81.00	82.20	85.00	86.40	85.20	0.92^{NS}
Lignin	102.20	114.00	116.20	110.60	109.20	1.21^{NS}
Energy (kcal/kg)	2550.20^{e}	2591.00^{d}	2601.40^{c}	2665.30^{b}	2668.70^{a}	0.28*

a, b, c, d, e = Means within the same row with different superscripts differed significantly; * = (P<0.05), SEM = Standard Error of the Mean, NS = Not significant

Non-fibre carbohydrate ranged from T_1 (301.90 g kg^{-1} DM) to T_5 (341.00 g kg^{-1} DM). The values were observed to increase with increase in the level of molasses. There was a significant (P<0.05) difference amongst the treatment group for non-fibre carbohydrate. Ash content of the diets ranged from 81.00 on diet T_1 to 86.40 g kg^{-1} DM on diet T_4. Lignin values ranged from 102.20 to 116.20 g kg^{-1} DM. Metabolizable energy was significantly (P<0.05) higher for diet T_5 (2668.70 kcal/kg) compared to other dietary treatments.

Effect of inclusion of molasses and GNC on gonadal sperm reserve ($x10^6$) of Red Sokoto bucks (Table 3) shows that significant differences (p< 0.05) were observed among the treatments. T_2 having the highest value of 35.15 and 38.65 right and left testes.

TABLE 3: Gonadal Sperm Reserve (×10^6) of Red Sokoto Bucks fed Molasses and Groundnut Cake in a mix Ration.

ITEMS	TREATMENT					SEM
	T_1	T_2	T_3	T_4	T_5	
Right testis	21.50^{c}	35.15^{a}	11.00^{e}	23.00^{b}	20.65^{d}	1.02*
Left testis	$20.5.0^{c}$	38.65^{a}	12.50^{d}	38.60^{a}	34.50^{b}	1.15*

^{abcde} Means within the same row with different superscripts differed significantly; * = (P<0.05), SEM = Standard Error of the Mean, NS = Not significant

Extra Gonadal Sperm Reserve of Red Sokoto Bucks fed Molasses and Groundnut Cake in a mix Ration

The result of extra gonadal sperm reserve is presented in Table 4. The result shows no significant (p> 0.05) effects among the treatments for left carpus epididymides while other parameters show significant (p<0.05) difference among treatments. The left caput epididymides was higher than the right caput. The right corpus was higher than the left corpus while the left caudal was higher than the right caudal epididymides.

Table 4: Extra gonadal sperm reserve (x10^6) of Red Sokoto Bucks fed Molasses and Groundnut Cake in a mix Ration.

PARAMETERS	T_1	T_2	T_3	T_4	T_5	SEM
Left Caput epididymides	1.31^{d}	2.34^{b}	2.22^{c}	2.59^{a}	0.84^{e}	0.44*
Right Caput epididymides	1.58^{c}	2.35^{a}	1.37^{c}	2.08^{b}	0.91^{d}	1.31*
Left Corpus epididymides	0.57	0.62	0.91	0.33	0.64	0.49^{NS}
Right Corpus epididymides	0.40^{c}	0.80^{a}	1.19^{a}	0.59^{b}	0.39^{c}	0.49*
Left Caudal epididymides	1.05^{c}	1.61^{b}	0.95^{d}	1.77^{a}	0.74^{e}	0.07*
Right Caudal epididymides	0.95^{a}	1.25^{b}	1.29^{c}	1.18^{a}	1.76^{d}	0.91*

a, b, c, d, e = Means within the same row with different superscripts differed significantly; * = (P<0.05), SEM = Standard Error of the Mean, NS = Not significant

Testicular morphometry of Red Sokoto bucks fed molasses and groundnut cake in a mixed ration

Table 5 shows the testicular morphometry of Red Sokoto bucks fed molasses and groundnut cake in a mixed ration. The right testicular weight, left testicular weight, right testicular length and the left testicular length ranged between 14.32 – 34.94(g), 14.67 – 34.12(g), 3.90 – 5.80 (cm) and 3.40 – 5.05(cm) respectively. All the parameters observed in this study were significantly (P<0.05) influenced by the dietary treatments.

Table 5: Testicular morphometry of red Sokoto bucks fed molasses and groundnut Cake in a mix Ration.

	TREATMENTS					
Parameters	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**SEM**
Right testicular Weight (g)	21.16^{c}	34.94^{a}	20.76^{c}	29.14^{b}	14.32^{d}	2.11
Left testicular Weight (g)	18.98^{d}	34.12^{a}	21.17^{c}	30.33^{b}	14.67^{d}	1.24
Right testicular Length (cm)	4.00^{b}	5.80^{a}	5.15^{a}	3.90^{b}	4.60^{b}	1.31
Left testicular Length (cm)	4.00^{b}	5.00^{a}	5.05^{a}	3.40^{a}	4.70^{b}	0.05

a, b, c, d, e = Means within the same row with different superscripts differed significantly; * = ($P<0.05$),
SEM = Standard Error of the Mean, NS = Not significant

DISCUSSION

Testicular weights observed in this study had similar trends with the findings of Iliyasu *et al.* (2014). Okwun *et al.* (1996) stated that rams with larger testes tend to produce more spermatozoa, a finding which was similar to what was observed in this study. Oyeyemi *et al.* (2002) 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 for all the groups regardless of the level of inclusion of the experimental diets. This finding is similar to the observations of Raji and Njidda, (2014) 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 Ibrahim *et al.* (2014). Babashani *et al.* (2015) 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 (Obidi *et al.*, 2008). 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 Nasir *et al.* (2014), 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 (Yunusa *et al.*, 2016), that the corpus epididymis had the lowest sperm reserves in goats.

However, the positive relationship between the age of the buck and the extra gonadal motility was a consistent finding in the right and left testes which is similar to previous reports (Maina *et al*., 2006a). The relationship observed with the extra gonadal motility is a direct consequence of the influence of the gonads as positive relationship exists between the gonadal motility and the extra gonadal motility. Age appears to be an important factor, that determines the percentage motility of the Sahel bucks in the humid zone. Moreover, the positive relationship between the age of the buck and the gonadal livability, which in turn determines the percentage of the extra gonadal livability, and the relationship of the latter with the gonadal motility, corroborate the role of age in the determination of the quality of semen produced by the Sahel buck in the humid zone. However, certain spermatozoa defects namely rudimentary tail, coiled tail, curved tail and bent tail abnormalities were observed to increase during the epididymal transit. This is also similar to previous report and the influence of season on scrotal thermoregulation during the spermatozoa storage in the epididymis has been incriminated (Maina *et al*., 2006b).

The significant ($p<0.05$) difference observed in the ratio of left to right testes weight is in strong agreement with the study of Ott *et al*. (1982) who showed that the left testes was 10% larger than the right in rams on subsequent examinations. In buffalo, cattle and goats, similar results have been reported by Ahmad *et al*. (1985), Purbey and Choudhury (1985). Significant ($p<0.05$, $p<0.01$, $p<0.001$) and positive correlations exist between the testes weight**.** This is much similar with the report obtained in some domestic animals such as the goat (Bitto and Egbuinike, 2006). Similarly, scrotal circumference has been shown to correlate ($p<0.05$, $p<0.01$) with epididymal weight, tunica albuginea, testes volume, and all epididymal segments. Similar observation was reported by Tegegne *et al*. (1992), and Bitto and Okpale (2006). Overall, the correlation results indicate that the increase in one testicular trait leads to an increase in the other and vice versa. The good and positive correlations between testes weight, scrotal circumference and morphometric characteristics, indicates the possibility of predicting organs weight, since testes weight is known to be very highly correlated with testicular sperm reserves (Ogwuegbu *et al*., 1985), and males with larger testes tend to produce more sperm (Okwun *et al*., 1996). Also, Ugwu (2009) asserted that a good measurement of circumference, testes length and width would be a reliable predictor of the sperm producing capacity of bucks. More so, Keith *et al*. (2009) suggested the use of scrotal size and testicular measurements to select for improved sperm production and breeding males.

A significantly high correlation was found between sperm reserve of right and left testes with paired testes and daily sperm production of paired testes. However, there was a significantly negative correlation between daily sperm production of right testes with scrotal circumference (r= -0.56, $p<0.05$), corpus sperm reserve and caudal sperm reserved, (r=0.90, $p<0.01$). The significant correlation observed between the testes and scrotal circumference, daily sperm production (DSP) corpus sperm reserve and caudal sperm reserve is in agreement with the observation by Bitto and Egbunike (2006) in West Africa dwarf bucks.

Testicular measurements and live weight have been reported to generally indicate the production of viable spermatozoa by the male (Agga *et al.*, 2011). The morpho-biometrical analysis of testicular development is of great importance since it is significantly correlated with reproductive activity (Emsen, 2005). Testicular sizes of animals are some good indicators for identification of those with adequate sperm production. Testicular traits are important variables directly associated with sperm features and animal fertility. The shape and content of the scrotum are associated with fertility parameters. Scrotal circumference (SC) and testicular consistency (TC) have been extensively used in predicting the reproductive capacity of male domestic animals (Antonio, 2011). This is because scrotal circumference is an indirect measurement of testicular weight and a reliable indicator of testicular growth and spermatogenic capacity of the testis (Daudu, 1984). Likewise, testicular weight (TW) is a reliable variable for estimating the sperm production capacity of males. Animal size and traits such as birth weight (BW) are closely associated with testicular weight (Nsoso, 2003). Keith (2009) reported high and positive correlation between testicular measurement and body conformation traits (HG, HTW and BL) in pubertal Boer bucks while Bitto and Egbunike (2006) recorded high correlations between body weight and testicular morphometry in WAD bucks.

The variation observed in the testicular weight among treatments may be due to the fact that molasses inclusion in the diet had a significant effect on testicular morphometry as observed in this study, while the testicular length values varied from 3.40 – 5.80 cm. T_2 (right testis) having the highest testicular length while T_4 (right testis) had the lowest testicular length. This result was in line with the findings of Nasir *et al* (2014) who recorded high values of right testicular weight and length of 64.10 g and 64.10 cm. Raji *et al.* (2008) reported high

values of left testicular weight and length of 68.00 g and 65.00 cm. However, this variation could be attributed to the differences in environmental factors, management or diet composition.

CONCLUSION

The result indicates that left testes were significantly ($P<0.05$) higher compared to right testis except for T_1 likewise gonadal sperm reserves. Base on the result of this research, it can be concluded that the reproductive capacity of the Red Sokoto buck fed these diets **are of good sires** and can be of genetic resources to support planned breeding programs for the goats in the Guinea Savannah.

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