EFFECT OF MORINGA OLEIFERA LEAF MEAL BASED DIET ON SEMEN CHARACTERISTICS AND TESTICULAR MORPHOMETRY OF KANO BROWN BUCKS

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ABSTRACT

Effect of Moringa oleifera leaf meal based diet on semen characteristics and testicular morphometry of Kano Brown bucks. Twenty (20) Kano Brown bucks aged 10-12 months old with average weight of 12.5 ± 1.07kg were used for the study. The bucks were allotted to five dietary treatments in a completely randomized design with four animals per treatment. Animals on treatment one (T_1) were fed the control diet. Animals on treatments two, three, four and five were offered 5, 10, 15 and 20% Moringa oleifera leaf meal designated as T_2, T_3, T_4 and T_5 respectively. Result indicated that feeding of Moringa oleifera leaf meal based diet had significant ($P<0.05$) negative effects on the semen quantitative parameters measured. M. oleifera fed bucks at 0% yielded the best result: ejaculate motility (65.84%), Concentration (146.19 x 10^6/ml), live sperm (63.33%) and dead sperm cells (36.67%). Morphometry results of the testes (weight and length of testes, epididymis, epididymal head and body) were affected by increased level of Moringa oleifera except for the epididymal tail length where the control group had higher value (60.98 and 38.12) than the other groups in both left and right testes. From this study, it was concluded that feeding Moringa oleifera leaf meal based diet up to 20% inclusion level may have an alarming effect on semen characteristic and testicular morphometry of Kano Brown bucks; hence, it is recommended that Bucks could be fed up to 5% Moringa oleifera leaf meal inclusion level without deleterious effects.

INTRODUCTION

Reproductive performance of the male is an essential economic trait in the management of breeder stock and the evaluation of the ejaculate is an important aspect of the determination of the reproductive status of male animals (Nwoko and Ibe, 2005). It is important to understand and improve the reproductive performance of small ruminants in Nigeria, in order to select bucks of high fertility and for the purpose of using ejaculate that can be relied upon to give a high conception rate for artificial insemination program and increased productivity. Reproduction is one of the main factors which determine the overall productivity of the goat enterprise. It is important that goat keepers are able to manage the reproduction of their goats according to their own objectives, the availability of feed, and the demands of the market. Reproduction dictates not only the rate of expansion of the flock and the number of excess stock for sale, but also the availability of milk for home consumption and sale. It should be possible for goat farmers to exert enough control to determine when their goats start breeding, how often they breed and, if required, what season they give birth.

A large number of plants have been tested throughout the world for possible fertility regulatory properties (Bhatia *et al.,* 2010). Some medicinal plants are extensively used as aphrodisiac (agents that arouse sexual desires) to relieve sexual dysfunction, or as fertility enhancing agents. They provide a boost of nutritional value thereby improving sexual performance and libido (Yakubu *et al.,* 2007; Sumalatha *et al*., 2010).Therefore, the need to feed and evaluate *Moringa oleifera* leaves in Kano Brown bucks with a view to improve the nutritional status thereby leading to improved reproductive performance. Hence, the study was conducted to evaluate the effect of *Moringa oleifera* leaf on semen characteristics, testicular morphometry and histology of Kano Brown bucks.

MATERIALS AND METHODS

Study area

The study was conducted at Teaching and Research Farm of the Department of Animal Science Kano University of Science and Technology, Wudil, Kano State. Wudil is located on the latitude 11° 37'N and longitude 8° 58'E and at an altitude of 403 m above sea level within the Sudan savannah region of Nigeria. The annual rainfall ranges from 850 mm – 870 mm with minimum and maximum temperature of 26°C and 36°C, respectively. The relative humidity of the region is always low and ranges between 40% - 51% (Olofin *et al.,* 2008).

Experimental animals and their managements

Twenty (20) Kano Brown bucks aged 10-12 months old with an average weight of 12.5 ± 1.07 kg was used for the study. The age was determined by the use of dental eruption pattern as described by Gillespie (2004), the animals were obtained from Wudil weekly local livestock market in Kano State.

The animals were treated for endo and ecto parasites using albendazole (3ml/head) and acaricide (Asuntol), respectively. Antibiotic prophylaxis using oxytetracycline (long acting at 10mg/kg body weight) was given. Animals were housed in clean and disinfected pens and fed their experimental diets in groups for two weeks adaptation period. They were fed with the experimental diets in the morning at 08 hours and in the afternoon at 14 hrs. Basal diet of *Cynodon dactylon* (Kirikiri) and water were provided *ad libitum*. The feeding trials lasted for 84 days (12 weeks). The experimental diets were made of *Moringa oleifera* leaves, cowpea husk, wheat offal, maize offal, bone meal and salt.

Experimental diets

The *Moringa oleifera (Mo)* leaves were sourced from the local markets; air dried and evaluated for proximate composition prior to feed formulation. The other ingredients were sourced from Kurmi market around Kano. Five diets were formulated as follows T1=0% Mo leaf meal, T2=5% Mo leaf meal, T3=10% Mo leaf meal, T4=15% Mo leaf meal and T5=20% Mo leaf meal inclusion levels respectively. The animals were allotted to five dietary treatments (Table 1),in a complete randomized design with four animals per treatment; each animal within a group is housed individually

Experimental procedure

Semen samples were collected after the bucks were well restrained to resist any violent reactions arising from the electric shocks and evaluated weekly using electro-ejaculator (Lane Manufacturing),The bucks were given semen collection preliminary training for two weeks . The semen volume was determined and recorded immediately after collecting the semen using graduated tubes (Hassan *et al*., 2016). The color of the ejaculate was determined by visual observation immediately after semen collection and coded as 1 = bloody, 2 = watery, 3 = milky and 4 = creamy. Semen pH was determined using pH paper. A drop of semen sample was placed on the pH paper which was later on matched with the corresponding value on the pH chart and then recorded (Hassan *et al*., 2016). Sperm motility was determined by placing a drop of semen on pre warmed (34˚C to 37˚C) glass slide and covered with 22 x 32mm cover slip and examined at x10 eye piece objective magnification. The motility score was defined as good when the sperm cells showed a vigorous forward movement and poor when weak, slow and spasmodic movement of the sperm cells was noted (Kemp *et al.,* 1989). Semen concentration was determined using hemocytometer following semen dilution at a ratio of 1:200. Sperm concentration is expressed at $x10^6$/ ml and calculated using the formula (Hassan *et al*., 2016).

$$C = 50{,}000 \times N \times D.$$

Where C = sperm concentration, N = sperm count, D = dilution ratio.

Live - dead ratio was determined by examining semen smear stained with eosin-nigrosin solution by counting 200 sperm cells and estimated in the ratio of 100. Dead sperm cells pick the colour of the stain (pinkish) while live sperm cells remained colourless (Kemp *et al.,* 1989).

Testicular morphology determination

At the end of the trial, two bucks from each treatment were randomly selected, restrained and orchidectomized for testicular morphology and histopathology as described by (Hassan *et al*., 2016). The orchidectomy was done after the animals were placed on dorsal recumbency and restrained by an assistant. The testes were held firmly and liberal area of the caudal part of the scrotum were cleaned with soap and water, shaved and disinfected with chlorhexidine. Two ml of local anesthesia (lidocaine) was infiltrated longitudinally to block the nerve sensation. An incision was made on the scrotum through the tunica albuginea; the testicles were milked out of the incision site. The spermatic cord was exposed, ligated and incised. The subcutaneous tissues were closed with hemostatic forceps and then ligated with size 2/0 chromic catgut and the skin was sutured with nylon. Two ml of antibiotic (pen-strep) was given intra muscular; diclofenac sodium sulphate and multivitamin were administered at 10 mg per kg body weight while oxytetracycline blue spray was sprayed on the suture immediately and daily for 5 days. The black silk was removed one-week post orchidectomy. Both right and left paired testis were measured immediately; right and left epididymis were also measured.

The length and weight of the testis, length and weight of epididymis were measured, length and weight of epididymal head, body and tail were measured, weight of testis alone and weight of epididymis alone were determined.

RESULTS AND DISCUSSION

Semen characteristics

The overall result from this study (Table 2), showed that *Mo* leaf meal based diet has no effects on the semen volume but when the volume was observed on weekly basis, animal in treatment 3 and 4 (10% and 20% *Mo* leaf meal based diet) showed improvement compared to the control treatment at the beginning, mid and toward the end of the trial. The increases in semen volume with higher dosage of *Mo* leaf meal (up to 20%) agrees with the findings of Fatoba *et al.* (2013) who recorded significant increase in semen volume of albino rats treated with *Moringa* root extract. Semen concentration decreased with increased level of *Mo* leaf meal in the diet across the treatments, where the control group (0%) had the highest value of 146.19 x 10^6. This study is contrary with the report of Ogunsola *et al.* (2017) who suggested that the consumption of Moringa leaves increases reproductive function in male as evidenced by the increase in the level of reproductive hormones that helps in sperm cells production and maturation. The study is consistent with the findings of Awodele *et al.* (2012), who reported a decrease in sperm count following *Moringa oleifera* administration. The result of sperm motility (Table 2) showed that sperm motility in all treatments was above the standard (50% motility) but the control had the highest value of 65.84% motility. This result is in agreement with that of Abu *et al* (2013) who observed that *Moringa oleifera* did not significantly influence sperm active motility.

The result (Table 2) shows that there was a significant increase in semen pH of animals in treatment 2 (5%) and 3 (10%) *Mo* leaf meal based diet having value of 8.00 and 8.11, respectively compared to the control (0%) having a value of 7.97, which is greater than that of treatment 4(15%) and 5 (20%) having 7.86 and 7.75 respectively, even though all the treatments with the exception of treatment 3 (8.11) had normal semen pH range of 7.1 and 8.0. According to Kathleen and Timothy, (2013) normal pH range is 7.1 - 8.0. WHO (1992) specified normal semen pH as 7.2- 7.8. The study (Table 2) revealed that in terms of live and dead sperm cells, animals on the control diet (0%) *Mo* leaf meal based diet had the highest percentage of live sperm cells compared to the other treatments, while for dead sperm cells, animals on treatment 4 (15%) *Mo* leaf meal based diet had the highest percentage of dead sperm cells. This result disagree with earlier findings of Fatoba *et al*. (2013) on *Moringa* root extract treated rats having higher mass activity and sperm cell livability than the control.

Testicular morphometry

The results shows that the weight and length of both testes (left and right) increased with increased level of *Mo* leaf meal based diet compared to the control group (0%) with value of 27.39g and 27.55g for testis weight and 31.54mm and 27.54mm for testis length, respectively. This concurred with the report of Abu *et al.* (2013) who observed enhanced organ weights and sperm output in rabbits fed *Moringa oleifera* leaf meal. The epididymal weight for both testes also shows progressive change compared to the control, while for the length of the epididymis only animals under treatment 4 (15%) and 5 (20%) in the right testis and treatment 5 (20%) in the left testis. Abu *et al.* (2013) evaluated the effect of *M. oleifera* leaf meal (MOLM) on testicular morphometry and sperm quality, no significant effect of the diets was observed on all the parameters studied. For the weight and length of caput, corpus and cauda epididymides of both testes, significant differences exist ($P < 0.05$) between the treatment, this is similar to the finding of Njidda *et al.* (1984) who reported that; the left caput epididymides weights were significantly ($P < 0.05$) different among the treatments.

CONCLUSION AND RECOMMENDATION

The result obtained from the study indicated that feeding *Moringa oleifera* leaf meal based diet up to 20% inclusion level have a detrimental effect on semen characteristics of Kano Brown buck at early age; hence it adversely affect reproductive performances, also at pubertal age have negative effects on testicular morphometry. Hence, it is recommended that Bucks could be fed up to 5% Moringa oleifera leaf meal inclusion level without deleterious effect

Table 1: Gross ingredient composition of experimental diets

Ingredients	Treatments (kg)				
	T1(0%)	T2(5%)	T3(10%)	T4(15%)	T5(20%)
Moringa oleifera leaf meal	0	5	10	15	20
Wheat offal	50	45	40	35	30
Cowpea husk	27	27	27	27	27
Groundnut haulms	20	20	20	20	20
Cotton seed cake	2.0	2.0	2.0	2.0	2.0
Bone meal	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**	**100**

Table 2: Effect of *Moringa oleifera* leaf meal based diet on semen characteristics of Kano Brown bucks (overall)

	Treatments					
Weeks	T1(0%)	T2(5%)	T3(10%)	T4(15%)	T5(20%)	SEM
Semen volume(ml)	0.34	0.33	0.41	0.34	0.36	0.035
Semen conc. ($x10^6$)	146.19^a	125.10^b	114.19^b	129.23^b	128.33^b	7.645
Motility (%)	65.84^a	61.39^{ab}	61.25^{ab}	57.50^b	63.47^a	2.650
Colour	3.31	3.17	3.19	3.19	3.28	0.090
Live sperm cell (%)	63.33^a	61.30^{ab}	58.48^b	54.17^b	59.20^{ab}	2.373
Dead sperm cell (%)	36.67^c	38.70^{bc}	41.52^{ab}	45.83^a	40.80^{bc}	2.373
pH	7.97^{ab}	8.00^{ab}	8.11^a	7.86^{ab}	7.75^b	0.130

^{abc}Means within the same row with different superscript are significantly different ($P < 0.05$), SEM = Standard Error of means

Table 3: Testicular Morphometry for Kano Brown bucks fed with *Moringa Oleifera* leaf meal based diet (left testis)

	Treatments					
Parameters	T1(0%)	T2(5%)	T3(10%)	T4(15%)	T5(20%)	SEM
Testicular weight (g)	27.55^e	36.55^a	30.07^d	35.52^b	34.23^c	0.05
Testicular length (mm)	27.54^e	43.22^c	81.40^a	36.30^d	70.06^b	0.06
Epi. weight (g)	5.24^d	7.11^b	5.11^e	7.94^a	6.43^c	0.01
Epi. length (mm)	97.34^d	159.78^a	104.18^c	121.72^b	80.99^e	0.26
Epi. weight head (g)	3.34^b	4.39^a	2.36^e	3.02^c	2.66^d	0.02
Epi. length head (mm)	55.46^b	38.04^e	46.66^d	50.26^c	58.18^a	0.01
Epi. weight body (g)	0.57^e	1.70^a	1.11^b	0.86^c	0.69^d	0.05
Epi. length body (mm)	3.66^d	5.27^b	3.72^c	8.96^a	2.27^e	0.01
Epi. weight tail (g)	2.32^e	4.48^a	2.54^d	3.86^b	2.64^c	0.01
Epi. length tail (mm)	38.12^a	31.51^b	33.48^b	33.71^b	27.28^c	1.42

^{abcde}means within the same row with different superscript are significantly difference ($P < 0.05$), SEM = Standard Error of means

Epi. = Epididymal

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