Iranian Journal of Reproductive Medicine Vol.7. No.1. pp: 23-28, Winter 2009

Semen quality characteristics, reaction time, testis weight and seminiferous tubule diameter of buck rabbits fed neem (*Azadirachta indica A. Juss*) leaf meal based diets

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Received: 5 October 2008; accepted: 15 March 2009

Abstract

Background: To ascertain the effects of tropical leaf meals on semen production and semen quality.

Objective: This study was conducted with the main objective of investigating the effect of neem leaf meal on physiological responses of rabbit bucks fed graded levels of neem leaf (*Azadirachta indica A. Juss*) meal.

Materials and Methods: The varying levels of neem leaf meal (NLM) in the different experimental diets were 0, 5, 10 and 15% respectively. Four groups of nine crossbred New Zealand type rabbit bucks each, aged 7-8 months were randomly assigned to four diets containing neem leaf meal (NLM) at 0% (control) (CD_0), 5% (CD_1), 10% (CD_2) and 15% (CD_3) respectively for 16 weeks.

Results: The sperm concentration values obtained were 20.15 $\times 10^6$ /ml, 18.04$\times 10^6$ /ml, 13.65$\times 10^6$ /ml, 6.46 $\times 10^6$ /ml for the CD_0, CD_1, CD_2 and CD_3 groups respectively. The results obtained indicate that sperm motility were lowest ($p<0.05$) in the treatment groups than the control group. Total sperm per ejaculate was similar ($p>0.05$) between the control and those on 5–10 %NLM dietary groups however, the value for the 15%NLM group was significantly ($p<0.05$) lower than that of the control. Abnormal sperm percentage of the bucks fed 15% NLM was significantly ($p<0.05$) higher than those bucks on CD_1, CD_2 and CD_3 groups. The seminiferous tubule diameters were significantly smaller in the 15% NLM (203µm) than the other 3 dietary groups. All the other variables measured including semen volume, weight of testis and reaction time did not differ ($p>0.05$) among the experimental group.

Conclusion: The results of the study indicate that the inclusion of neem leaf meal up to 15% in the ration of matured rabbit bucks could cause mild depressive effect on the spermatogenesis, semen quality and seminiferous tubule diameter.

Key words*: Rabbits, Neem, leaf, Histology, Semen*.

Introduction

Reproductive inefficiency is the most limiting constraint to efficient rabbit production in the tropics (1).

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The efficiency of sperm production, libido and quality of sperm 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 (2). Among these factors nutrition is the most prominent. The survival of an animal is dependent on the availability of feedstuff.

The unavailability of grains and high cost of imported feed ingredients have made the price of commercial animal feed to go up. These problems

remain the most important constraints to the expansion of commercial livestock production in Nigeria.

The need to exploit other available but neglected cheaper novel feed resources such as leaf meals of tropical trees which are indigenous to our environment is urgently necessary. The neem leaf meal, a novel feed resource has a proximate composition of 92.42% dry matter, 7.58% moisture, 20.68% crude protein, 16.60% crude fibre, 4.13% ether extract, 7.10% ash and 43.91% nitrogen free extract (3). Earlier studies on rabbits fed neem leaf meal based diet have been focused on its feeding value. Nothing has been reported so far on the effect of feeding the leaf meal on reproductive performance of buck rabbits.

The good reproductive performance is necessary for optimal production and profitability and in addition the need to reduce feed cost is fundamental. Therefore this study attempts to investigate the effect of graded levels of neem leaf meal on semen quality, libido and testicular morphometry of New Zealand × chinchilla buck rabbits raised in a humid tropical environment.

Materials and methods

Experimental location

This research was carried out in the Rabbit section, of Teaching and Research Farm of the Department of Animal Science and Technology, Federal University of Technology, Owerri, Imo state. Imo state is situated in south-eastern agro-ecological zone of Nigeria. Imo state lies between latitude 4^o 4' and 6^o3' N and longitude 6^o15' and 8^o15'E. Owerri is about 100m above sea level. The climatic data of Owerri as summarized in Ministry of Lands and Survey Atlas of Imo state is as follows: Mean annual rainfall, 2500mm; temperature range, 26.5-27.5^{o}C and humidity range of 70-80%. Dry season duration (i.e. months with less than 65mm rainfall) is 3 months. The annual evapotranspiration is 1450mm and the soil type is essentially sandy loam with average pH of 5.5.

Preparation of neem leaf meal and experimental diet formulation

Fresh matured neem leaves were harvested in and around the Federal University of Technology, Owerri, Nigeria. The leaves were chopped for effective drying. The chopped leaves were sun dried for about 9hours every day for 3-4 days until they became crispy while retaining its greenish colouration. The neem leaves were processed according to the procedure described (3, 4). The dry leaves were milled using a hammer mill to produce leaf meal before they were incorporated into the rations. Four experimental rations were formulated such that they contain 0%, 5.0%, 10.0% and 15.0% neem leaf meal expressed as weight of the formulated rations. It means that in 100kg feed formulated there are 0.0kg, 5.0kg, 10kg and 15kg of neem leaf meal for the CD_0, CD_1, CD_2 and CD_3 groups respectively. The chemical compositions of the formulated rations were analyzed (Table I).

Table I. Composition of experimental diets fed to New Zealand White × Chinchilla buck rabbits.

Ingredients**	Diets (% Neem leaf meal)			
	0.0% NLM	5.0% NLM	10.0% NLM	15.0% NLM
Spent grain	55.00	50.00	45.00	40.00
Neem leaf meal	-	5.00	10.00	15.00
Calculated analysis				
Crude protein	18.87	18.70	18.53	18.37
Crude fiber	10.1	10.78	11.02	11.27
Ether extract	5.97	5.95	5.93	5.91
Calcium	1.41	1.39	1.38	1.36
Phosphorus	0.66	0.62	0.58	0.53
ME (MJ/kg)	10.42	10.38	10.33	10.22

**Each diet contained 35% maize, 3% local fish meal and groundnut cake each, 2% bone meal, 1% oyster shell, 0.50% vitamin./mineral premix*, 0.5% common salt; *contributed the following to each kilogram of diet: Vit. A 500 IU; Vit. D_2 1500 IU; Vit. E 3 IU; Vit. K 2mg; Riboflavin 3mg; panthothenic acid 6mg; Niacin 15mg; Vit B_{12} 0.8mg; choline, 3mg; Folic acid 4mg; Mn 8mg; Zn 0.5mg; iodine 1.0mg; Co 1.2mg, NLM- neem leaf meal.

Experimental animal and management

Thirty-six New Zealand white × Chinchilla rabbits buck with initial weight ranging from 950g to 1100g were randomly allocated on the weight basis to four experimental groups (CD_1, CD_2, CD_3 and CD_4) of nine rabbit bucks each and fed diets containing, 5, 10 and 15 Neem leaf meal expressed as percentage of the total diets.

The choice of 5% to 15% inclusion levels of neem leaf meal in the present study was based on previous study conducted by Bawa *et al* (5) which showed that adult rabbits can handle up to 20%

neem diet without any deleterious effect on their performance, carcass and hematological characteristics.

The daily consumption rates of neem leaf meal for each buck were 0.0g, 2.1g, 5.94g and 11.05g for groups fed 0%, 5%, 10% and 15% neem leaf meal respectively. The total amount of neem leaf meal consumed by each animal during the 16 weeks feeding trial were 0.0g for buck on 0%, 234.98g for buck on 5%, 665.06 for buck on 10% and 1237.60g for buck on 15% neem leaf meal inclusion level.

Care was taken when placing the animals into groups in order to balance the groups such that there were no significant differences between them on basis of weight. The animals were housed in individual hutches measuring 1.5m × 1m × 1m with wire mesh floor with wooden frames. They were fed with starter broiler ration (vital feed) for a two week of stabilization. Feed and water were given *ad libitum*. Starting from the end of second week of the feeding trial, the general conditions of the experimental animals and their hutches were monitored. The animals were weighed and re-weighed at every 2 weeks interval. The feeding trial lasted for 16 weeks.

Semen collection

The semen was collected twice a week between 9.00am and 10.00am. This was to ensure that optimum quality semen was obtained. A matured cyclic doe was used to tease the buck. Semen collection was done using artificial vagina that was locally fabricated as described by Herbert (6) and Herbert and Adejumo (7). .Prior to semen collection, the artificial vagina (AV) was warmed by allowing it to stay for 10-15 minutes in warm water at 60^{o}c from thermo flask thereafter blotted dry with serviette. The AV was then lubricated with glycerol which is a heavy organic solvent that does not contaminate semen when in contact with it, and at the same time retains heat. Care was however, taken not to allow the AV to get too hot as this would result in decreased efficiency of semen collection and a possible contamination of the semen with urine.

The animals were ejaculated twice weekly for 2 months. Semen volume was read off the collection tube and recorded in milliliters. Sperm motility was determined on freshly collected semen placed on a warm stage at 37^{o}c. The samples were diluted with a physiological saline solution and observations were made at ×400 magnification. Total spermatozoa per ejaculate were derived by calculation.

Reaction time (libido)

A matured cyclic doe (teaser) was introduced to the buck every 2 weeks interval to monitor their sex drive. In this study, reaction time was considered as an indication of libido. The time in seconds it took for the rabbit bucks to sniff, groom and mount the female was recorded with a stop watch, and libido scored using the scoring pattern of 1-5 (no libido - high libido) described by Chibundu (8).

Testicular morphometry

The animals were weighed and slaughtered at the end of the 16^{th} week. At slaughter, the pairs of testes were quickly milked-out, weighed and recorded in grams after the epididymis has been trimmed off. These testicular measurements were taken at the Animal Physiology Laboratory of Department of Animal Science and Technology, Federal University of Technology, Owerri.

Testicular histomorphometry

The testes were fixed in aqueous Bouin fluid. Tissue samples were taken from the equatorial regions of the testis, washed in 50% and 70% alcohol before being embedded in paraffin wax. After embedding, tissue samples were cut at 5µm using a microtome. Staining was done with haematoxylin-eosin. Two slides were prepared per testis. Histometric measurements were taken on selected slide to generate data on seminiferous tubule diameter. This was carried out using a Zeiss microscope fitted with an ocular micrometer according to the procedure described by Majumdar (9). Measurements on tubules were taken twice on each testis, the second been perpendicular to the first. The tubular diameter was calculated as the average of the two measurements.

Statistical analysis

Statistical differences between treatment groups were determined with the analysis of variance test (10) using the computerized statistical analysis of SAS (11).The experimental model was completely randomized design (CRD) experiment ($Y_{ij} = \mu + T_i + e_{ij}$). Where differences were observed between treatments, the means were compared using Duncan's New Multiple Range Test (DNMRT).

Results

The results of buck rabbits fed Neem leaf meal based diets are shown in table II. The treatment means were compared using Duncan's New Multiple Range Test (DNMRT) at 5% level of

probability. The DNMRT = SSR × [MSE/ r] $^{1/2}$. The abbreviation SSR, MSE and r stands for significant studentized range, error mean square and number of replicate respectively. Whereas the square of (MSE/ r) constitute the standard error of the mean.

The body weights of the animals at pre-slaughter were found within the range of 1636.58 - 1653.73g. In this study, reaction time was considered as an indication of libido. The reaction time obtained in this study was found within the range of 4.11-4.28 seconds. The reaction time of bucks on control diet was similar to those on test diets.

The paired testis weights obtained in this study were found within the range of 4.41–4.61 g. The testis weight of bucks on control diet was similar to those on test diets.

From the result of present study there appeared the tendency that as the neem leaf meal inclusion rate increased, the testicular size decreases. The diameters of the seminiferous tubules of bucks on the control and 5.0% NLM diets were significantly ($p<0.05$) larger than those on the other two treatment diets.

The semen volume of bucks on control diet was relatively higher when compared to those that received the test diets. The 15%NLM diet had the least sperm concentration (6.46 $\times 10^6$/ml) which was significantly ($p<0.05$) lower compared to those on control diet (20.15×10^6/ml). The sperm motility of bucks on control diet was significantly ($p<0.05$) higher when compared to those on neem leaf meal based diets.

Table II. Semen quality characteristics, reaction time, testis weight and seminiferous tubule diameter of rabbit fed neem leaf meal based diets.

Parameters	Inclusion levels of Neem leaf meal				S.E.M
	0% NLM	5% NLM	10% NLM	15% NLM	
Body weight (g)	1653.73	1648.29	1639.12	1636.58	2.566
Reaction Time (Libido)	4.28	4.21	4.11	4.26	0.03
Semen volume (ml)	0.64	0.56	0.52	0.51	0.02
Sperm concentration($\times 10^6$/ml)	20.15^b	18.04^b	13.65^{ab}	6.46^a	2.51
Sperm motility (%)	74.50^a	68.13^b	55.50^c	40.84^d	3.72
Total sperm / ejaculate($\times 10^6$/ml)	14.75^a	14.44^a	10.63^{ab}	7.82^b	3.82
Abnormal spermatozoa (%)	8.40^a	11.02^a	13.33^{ab}	20.96^b	2.36
Paired testes weight (g)	4.61	4.53	4.46	4.41	0.02
STD (um)	215^a	210^a	203^{ab}	194^b	6.28

^{a,b,c,d}:Means within a row with different superscripts differs significantly ($p<0.05$); Neem leaf meal; STD- seminiferous tubule diameter; S.E.M-Standard error of mean.

The bucks on 15% NLM diet had the least total sperm per ejaculate (7.82×10^6/ml) while those on control group returned the highest total spermatozoa per ejaculate (14.75×10^6/ml).The total sperm per ejaculate of the bucks on control diet differed significantly ($p<0.05$) relative those fed 15% NLM diet.

Discussion

The total amount of neem leaf meal consumed by each animal during the 16 weeks feeding trial for the groups on 0%, 5%, 10% and 15% neem leaf meal treatment was 0.00g, 234.98g, 665.06g and 1237.60g respectively.

In this study, all the semen quality parameters except abnormal sperm percentages tend to follow a down ward trend as the inclusion rate of neem leaf meal increases. The depression in the reproductive performance of bucks neem leaf meal fed diets as observed in this study have been reported in male monkey, man and albino rats (12-16). Administered aqueous neem leaf extract. Herbert *et al* (4). Has reported similar results on rabbits fed *Leucaena* and *Gliricidia* leaf meal diets. According to Herbert *et al* (4) feeding 20% *Leucaena* and *Gliricidia* leaf meals to rabbits had a depressive effect on the diameter of the seminiferous tubule, sperm motility, semen volume and sperm concentration. Rahman (17) and Rahman *et al* (18) observed the disappearance of spermatozoa in the seminiferous tubules of rats fed *Leucaena* leaves. He equally observed that the epithelia of the seminiferous tubule of rats fed *Leucaena leucocephala* leaf meal diets were devoid of spermatozoa.

The anti-androgenic and anti-spermatogenic properties of neem leaves had been reported to reduce the fertilizing ability of the spermatozoa (14). The result of the decreased sperm output was accompanied with increased proportion of abnormal sperm and this shows that the treatment did adversely affects the ultra structure of the spermatogenic cells during the process of spermatogenesis or ejaculation. The percentage of abnormal sperm cell was observed to exceed the upper limit of 20% recommended as the minimum for good reproductive potential and fertility in

either normal mating or in artificial insemination (19,20).

This is in agreement with significant increase in abnormal sperm morphology of rats fed neem leaves (21). Neem leaf extracts have been reported to impair spermatogenesis and increases the number of headless spermatozoa and significantly decreased motility of the caudal spermatozoa leading to decline in fertility index (22).

The low sperm concentration witnessed in buck fed the test diet could be attributed to atrophy or decreased secretory activity in the lumen of the epididymis and seminiferous that normally goes with feeding of neem leaves in male animals (14, 23). The sperm motility was observed to decrease significantly as the inclusion rate of neem leaf meal in the rations increases. The decreased sperm motility as observed in this study may be opined to the earlier findings of blockage in the energy metabolic route in animals administered Neem leaves (15, 24).

Aqueous extracts of neem leaf has been reported (13) to have adverse effects on motility, morphology and number of spermatozoa in caudal epididymis, levels of fructose in the seminal vesicles and on litter size. The total sperm per ejaculate is an important semen variable related to fertility. It reflects testicular volume (25, 26), and thus is a measure of total testicular sperm output (27) which is directly related to the chances of pregnancy after mating.

The non significance value in reaction time (libido) observed in this study was in line with the earlier findings (15, 24) that neem leaf extract reduces fertility in male monkey (*Macaca fascicularis*) without reducing libido. The increase in spermatozoa abnormality following inclusion of neem leaf meal in the diet of buck rabbits as recorded in this experiment indicated that neem leaf meal the diets could produce a suppressive effect in the maturation of sperm cells.

The knowledge of the ability of the testes to store spermatozoa is of immense importance in rabbit breeding program. The reduction in some of the testicular parameters weighed in the present study with the introduction of neem leaf meal diet is a pointer that these neem leaf meals do not promote testicular growth.

These indicate that inclusion of neem leaf meal is detrimental to the development of spermatogenic potentials of the buck as it has been observed in the present study. Testis size is a good indicator of the present and future spermatozoa production of an animal (2, 28-30). The knowledge of basic morphometric characteristics of the reproductive organs have been found to provide valuable information in the evaluation of breeding and fertility potential of the animals (31). The fact that the diet with 15% NLM resulted in testes weight which was only 35% of the control confirms the poor quality of these testes relative to those on the control diet in relation to spermatogenesis. Larger testes without any abnormality have been reported to produce more spermatozoa than smaller testes (20, 32-33).

Morton (34) reported that in sacrificed animals, decreased weight of the testes indicates widespread or diffuse lost of seminiferous epithelial cells. The testes which possess greater number of sertoli cells were heavier and produced more spermatozoa than testes with fewer sertoli cells (35).

The small testes weight of the bucks on 15% neem leaf diets would mean that these testes would contain fewer seminiferous tubules (the environment where spermatogenesis takes place), fewer leydig cells, fewer sertoli cells and fewer spermatogenic cells.

The slight decline in the size of the testis reported in this study was in consonance with earlier findings of Anonymous (36) that oral administration of 50% ethanol extract of neem leaves caused a marked reduction in the size of the testes and epididymis of male rats, by arresting spermatogenesis.

The reported alterations induced in the reproductive organs of Parkes (P) strain mice by administration of aqueous neem leaf extract recovered to the control levels after 42 days withdrawal of treatment (13). This biochemical and histological changes in the testis after treatment with neem leaves and its pattern of recovery revealed its possible reversible anti-androgenic property (37).

Conclusion

The sperm quality of the bucks fed neem leaf meal based diets was observed to be of poor quality relative to those on control diet. The diameter of seminiferous tubule and testicular size of bucks on neem leaf meal were smaller compared to those on control diet. The 5 -15% inclusion of test diet in the present study do not improved the sexual drive of the bucks.

The association of neem leaf meal with depressed spermatozoa production and semen output in the bucks receiving the leaf is a source of concern and should be given adequate attention, while recommending neem leaf meal for the diet of breeding buck rabbits.

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