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DOSE RESPONSE EFFECT OF PERCENTAGE NEEM LEAF MEAL INCLUSION LEVEL ON SPERMATOGENESIS IN RABBITS

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ABSTRACT

A total of 36 crossbred male rabbits aged 7-8 months were randomly assigned to four dietary treatments designated as T0 (0 %), T1 (5%), T2 (10%) and T3 (15%) of neem (Azadirachta indica) leaf meal (NLM) levels with three replications, each having three rabbits for 16 weeks. Semen volume, sperm motility, sperm concentration, total sperm production, abnormal sperm percent and reaction time were assessed at week 4, and thereafter at weeks 6, 8, 10, 12, 14 and 16. Duncan's test for multiple comparisons was used to test the significant difference between treatment means ($p < 0.05$). A quadratic equation was used to determine dietary NLM inclusion levels for optimum parameters which were significantly different. NLM inclusion level of 0.71% supported optimum sperm concentration ($p = 0.02$). This was higher than the levels of 0.24% and 0.69% that supported optimum sperm motility ($p = 0.01$) and total sperm concentration ($p = 0.01$), respectively in in male rabbits. On the other hand, NLM level of 0.85% gave the least abnormal sperm percent ($p = 0.01$). It is concluded that dietary NLM inclusion levels of 0.24 - 0.85% supported spermatogenesis in male rabbits. Thus, optimizing the NLM inclusion level in the diet of male rabbits could help enhance their reproductive efficiency.

Keywords: Rabbits, neem leaf, sperm production, dose response, quadratic equation

INTRODUCTION

Major features that promote rabbit farming include high reproductive efficiency and the ability to convert quality feedstuffs to meat. Despite these attributes, rabbit farming in Nigeria like in other developing countries is confronted with myriads of problems including unavailability of quality feedstuffs and harsh production environment all culminating in poor reproductive efficiency. Hence, suggesting that more research is needed to improve the productivity of rabbits reared in a humid tropical environment. One likely method of increasing the reproductive efficiency of rabbits is to formulate diets that would meet the nutrient requirements of rabbits using leaf meals of tropical plants such as neem that are readily available. Neem is native to India and belongs to the family Meliaceae. It is widely grown in Nigeria and its leaf meal is high in fibre, protein and minerals (Muhammad *et al*., 2015). Ogbuewu *et al*. (2009) showed that the inclusion of up to 15% neem leaf meal in the diets of male rabbits had a negative effect of spermatogenesis. A quadratic optimization model is increasingly being used in the animal science discipline to determine the inclusion levels of feedstuffs for optimal productivity (Ogbuewu and Mbajiorgu, 2020; Anyanwu *et al*., 2021). The knowledge will help in the formulation of a diet to optimise spermatogenesis in breeding male rabbits. The objective of this study was to determine the optimal inclusion levels of neem leaf meal that supports spermatogenesis in rabbits using a quadratic regression model

MATERIALS AND METHODS

This research was performed at Rabbit Unit of Teaching and Research farm, School of Agriculture and Agriculture Technology, Federal University of Technology Owerri, Imo State (latitude 4° 4' and 6°3' N and longitude 6°15' and 8°15'E). The mean temperature around the experimental site is ranged between 26.5 °C and 27.5 °C. This investigation followed the guidelines of the Institution's Research Policy. Fresh neem leaves were air-dried at room temperature for 4-5 days and then milled to produce neem leaf meal. Four treatment diets were compounded; diet T0 contains 0% NLM (control), while diets T1, T2 and T3 contain 5.0%, 10.0% and 15.0% NLM, respectively as shown in Table 1. Experimental animals were stabilized for 2 weeks to the experimental conditions. Thirty-six crossbred rabbit bucks

were distributed to four treatment groups of three replicates of three rabbits. Rabbits in each treatment group were allotted to one of the 4 diets for 16 weeks in a completely randomised design. Duncan's test for multiple comparisons was used to test the significant difference between treatment means ($p < 0.05$). A quadratic equation was used to determine dietary NLM inclusion levels for optimum parameters which were significantly different at 5% probability level. Feed and water were offered to the experimental rabbits *ad libitum*.

Table 1. Ingredients and chemical composition of experimental diets (%)

Ingredients	Diets (% Neem leaf meal)			
	T0	T1	T2	T3
Maize	35.0	35.0	35.0	35.0
Spent grain	55.0	50.0	45.0	40.0
Neem leaf meal	0.00	5.00	10.0	15.0
Vitamin/mineral premix*	0.50	0.50	0.50	0.50
Fish meal	3.00	3.0	3.00	3.00
Groundnut cake	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00
Common salt	0.50	0.50	0.50	0.50
Total	100.0	100.0	100.0	100.0
Chemical analysis				
Crude protein	18.87	18.70	18.53	18.37
Crude fibre	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

*Composition per Kg: Vitamin A 10, 000 IU, Vitamin D_2 1500 IU, Vitamin E 3 IU, Vitamin K 2 mg, Riboflavin 3 mg, Panthothenic acid 6 mg, Niacin 15 mg, Vitamin B_{12} 0.8 mg, Choline, 3 mg, Folic acid 4 mg, Manganese 8 mg, Zinc 0.5 mg, Iodine 1.0 mg, Cobalt 1.2 mg.

Semen quality and reaction time were determined at week 4, and thereafter at weeks 6, 8, 10, 12, 14 and 16 following the method described by Ogbuewu et al. (2009). In this study, reaction time was considered as an indication of libido. It is the time in seconds it took for the rabbit bucks to sniff, groom and mount the female was recorded with a stop watch. Ejaculate volume was measured using calibrated collection tubes to the nearest 0.1 ml, and pH was determined using a pH meter (Hanna instruments®, Portugal). Sperm motility and abnormal sperm percent were measured using the procedures as outlined by Jimoh and Ayedun (2020). Sperm concentration per ml of semen was assessed using a visual count under the microscope. Total sperm concentration per ejaculate was calculated as sperm concentration per ml × volume per ejaculate The inclusion related responses in reproductive outcomes of breeding male rabbits to NLM were modeled using quadratic regression equation: $Y = a + b_1x + b_2x^2$, where Y = reproduction variables (reaction time and semen quality); a = the Y-intercept; b = coefficient of quadratic equation; x = NLM inclusion levels and $-b_1/2b_2 = x$ value for optimum response. The quadratic type equation was fitted to the experimental data using the nonlinear model procedure of SAS (2008) and the choice of quadratic function was based on the fact that it gave the best fit.

RESULTS AND DISCUSSION

Data on ideal neem leaf meal levels for optimal variable responses in rabbits are scarce. Figures 1 and 2 revealed that NLM influenced semen quality in rabbits. Our results (Table 2) indicate that the inclusion of NLM at 0.24% in the diet

of breeding male rabbits supported optimum sperm motility. The NLM inclusion level of 0.69% required to optimize total sperm concentration was higher than the level of 0.24% that optimizes sperm motility but lower than the level of 0.71% that optimises sperm concentration. The physiological explanation for this difference is not well understood and requires further study. However, it may be that these sperm parameters require different dietary components and levels for their production as corroborated by Ogundun et al. (2013) that optimum inclusion-response value of Uromalt supplementation doses for optimising semen quality traits in goats is dynamic. Results showed that no single NLM inclusion level optimised all the sperm quality parameters in the present study. Ogundun et al. (2013) and Ogbuewu et al. (2020) recorded similar results in animals other than rabbits. The results of the current study supported the results of Ogbuewu and Mbajiorgu (2020) who found that no single feed supplement level optimised all the health markers in growing rabbits. This indicates that the nutrient requirements of breeding rabbits for optimal spermatogenesis are dynamic and depend on the sperm variable under investigation and this should be factored in when formulating feed for male rabbits. Results of the present study indicate that abnormal sperm percent has a dose-response value of with the optimum NLM inclusion level being 0.85%. The quadratic calculation indicated that sperm quality parameters had high R^2 values of 97.7- 99.9% which indicates higher strength of associations between sperm parameters and NLM. This suggests that sperm quality traits can be predicted at any given level of NLM added to the diets of breeding male rabbits.

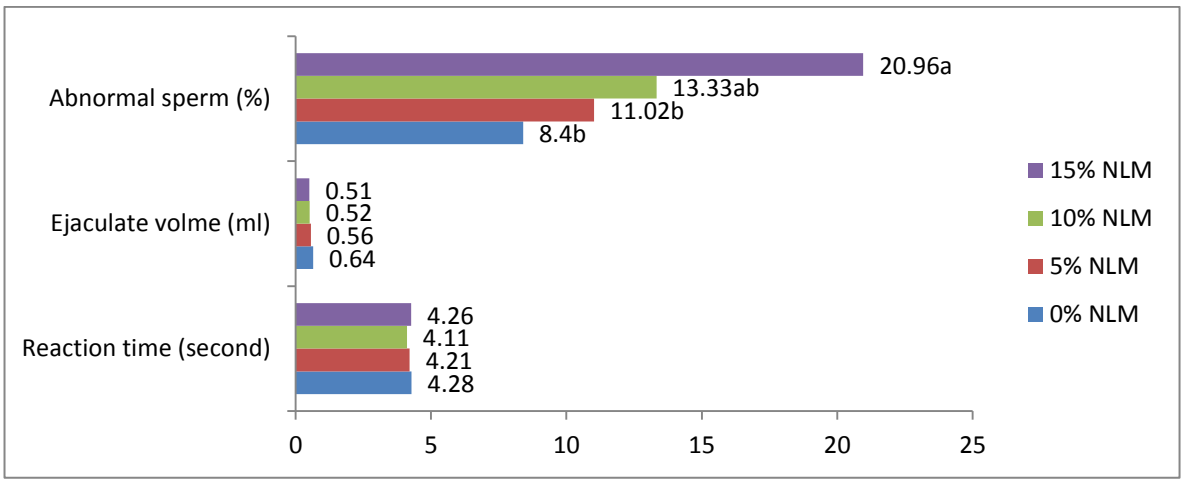


Figure 1. Effect of NLM on reaction time, ejaculate volume and abnormal sperm in male rabbits

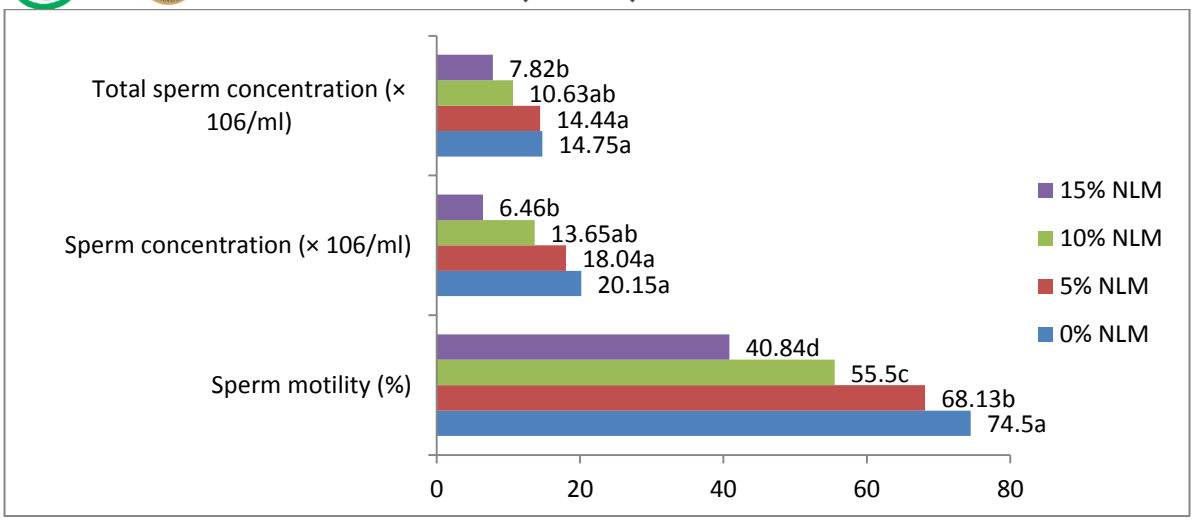


Figure 2. Effect of NLM on sperm motility, sperm concentration and total sperm concentration in male rabbits

Table 2. NLM inclusion level for optimal semen quality in breeding male rabbits

Variables	Formula	R^2	X	Y	P-value
Sperm concentration ($\times 10^6$/mL)	$Y = 12.58 + 1.80\ NLM - 1.27\ NLM^2$	0.99	0.71	13.22	0.02
Sperm motility (%)	$Y = 77.78 - 0.99\ NLM - 2.07\ NLM^2$	0.99	0.24	77.90	0.01
Total sperm concentration ($\times 10^6$/mL)	$Y = 14.60 + 0.94\ NLM - 0.68\ NLM^2$	0.98	0.69	14.92	0.01
Abnormal spermatozoa (%)	$Y = 11.43 - 2.06\ NLM + 1.22\ NLM^2$	0.98	0.85	10.56	0.01

R^2 = Coefficient of determination; X = Optimal NLM value; Y = optimal value

CONCLUSION

The study indicates that inclusion of NLM at 0.71, 0.24, 0.69 and 0.85% supported optimum sperm concentration, sperm motility, total sperm concentration and minimum sperm abnormality, respectively in breeding male rabbits. Results also revealed that neem leaf meal levels for optimal response are dependent on the semen parameter in question and this has implications on feed formulation for male rabbits. It is therefore concluded that optimizing neem leaf meal inclusion level in the diet of male rabbits could aid in improving their reproductive efficiency.

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