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Prediction of optimal inclusion levels of neem leaf meal that supports spermatogenesis in rabbits using quadratic optimisation model

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Abstract This study was designed to assess the effect of neem (*Azadirachta indica* A. Juss) leaf meal (NLM) on reaction time and semen quality characteristics of male rabbits. A total of 36 New Zealand white × Chinchilla male rabbits aged 7-8 months were randomly assigned to four dietary treatments designated 0, 5, 10 and 15% NLM 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. Results indicate that addition of NLM at 0.71% supported optimum sperm concentration ($12.58 + 1.80$ NLM $- 1.27$ NLM^2; $p = 0.02$). This was higher than the levels of 0.24% and 0.69% needed to improve sperm motility ($77.78 – 0.99$ NLM $– 2.07$ NLM^2; $p = 0.01$) and total sperm concentration ($14.60 + 0.94$ NLM $–$ 0.68 NLM^2; $p = 0.01$), respectively in male rabbits. However, a value of 0.85% NLM gave the least abnormal sperm percent ($11.43 – 2.06$ NLM $+ 1.22$ NLM^2; $p = 0.01$). It is suggested 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 to enhance their reproductive efficiency.

Keywords: Bucks, Medicinal plant**,** Sperm production, Prediction equation, Optimization

Introduction

To the resource-poor farmers, backyard rabbits provide an instant source of meat and revenue. Major features promoting rabbits 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. Thus, more research effort is required to improve the performance of rabbits reared in a humid tropical environment. One likely method of increasing the reproductive performance of rabbits

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is to formulate diets that would meet the nutrient requirements of rabbits using leaf meals of tropical plants such as neem leaves that are cheap and readily available. Neem is native to India and belongs to the family Meliaceae. It is widely grown in Nigeria and its leaf meal contains 92.42% dry matter, 20.68% crude protein, 16.60% crude fibre, 7.10% ash 4.13% ether extract and 43.91% nitrogen-free extracts (Sokunbi and Egbunike, 2000; Esonu *et al*., 2006; Muhammad *et al*., 2015). Neem leaves are high in beneficial compounds such as phenols, flavonoids, saponins, steroids, alkaloids, among others (Al-Hashemi *et al*., 2016). Studies have revealed a dose-dependent negative effect of neem leaves on male reproduction (Mishra and Singh, 2005). Similarly, Ogbuewu *et al*. (2009) showed that inclusion of up to 15% neem leaf meal in the diets of male rabbits had a negative effect of spermatogenesis. As a follow up of their work, the present study is attempted by the same authors to predict the dose-response effect of percentage neem leaf meal inclusion level on individual reproductive outcomes, since it is known that inclusion of high levels of neem leaf meal in the diets of rabbits reduces fertility (Mishra and Singh, 2005; Ogbuewu *et al*., 2009). Although the inclusion of high levels of neem leaf meal in male rabbit diets reduces fertility, there is need to establish the safe level in male rabbit diets. In addition, economic analysis revealed that addition of neem leaf meal in rabbit diets resulted in higher profit and lower cost of production (Ayuba *et al*., 2021). Therefore, knowing the neem leaf meal inclusion levels for optimal fertility in breeding male rabbits using a prediction equation will help in the formulation of diets to optimise spermatogenesis in breeding male rabbits.

Quadratic optimization model is increasingly being used in the animal science discipline to determine the inclusion levels of feedstuffs for optimal productivity (Alabi *et al*., 2013; Ogundun *et al*., 2013; Jafari, 2014; Sebola *et al*., 2015). The objective of this study was to determine the optimal inclusion levels of neem leaf meal that support spermatogenesis in rabbits using a quadratic optimisation model.

Materials and methods

Experimental site and ethics

This research was performed at Rabbit Unit of Teaching and Research, School of Agriculture and Agriculture Technology, Federal University of Technology Owerri, Imo State (latitude 4^{o} 4' and 6^{o}3' N and longitude 6^{o}15' and 8^{o}15'E). The mean temperature around the experimental site is ranged between 26.5 ℃ and 27.5 ℃. This investigation followed the guidelines of the Institution's Research Policy.

Preparation of neem leaf meal, experimental design and diet

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 1 contains 0% NLM (control), while diets 2, 3 and 4 contain 5.0%, 10.0% and 15.0% NLM, respectively as shown in Tables 1. Experimental animals were stabilized for 2 weeks to the experimental conditions. Thirty-six crossbred rabbit bucks (New Zealand white × Chinchilla) 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. The experimental animals were housed in a wooden cage with in-built feeders and drinkers. 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)**			
	0.0	5.0	10.0	15.0
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 collection and analysis

Semen and reaction time were determined at week 4, and thereafter at weeks 6, 8, 10, 12, 14 and 16. Cycling female rabbits were brought to the bucks every 14 days to record their reaction time which was considered as an indicator of libido. Bucks were trained to serve an improvised artificial vagina. Semen was collected from 3 bucks per treatment using an artificial vagina (AV) designed and constructed for rabbits by Herbert and Adejumo (1995). Bucks were trained to ejaculate using the AV and the training lasted for 3 weeks. Semen was collected during the earlier hours of the day (08.00 am – 11.00 am). Upon semen collection, 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, sperm concentration and abnormal sperm percent were measured using the procedures as outlined by Jimoh and Ayedun (2020).

Statistical analysis

Data generated on reaction time and sperm quality parameters were subjected to one-way analysis of variance in SAS (2008) software. The difference between the mean values was considered significant at $P < 0.05$, and significant means were separated by Duncan's test for multiple comparisons in SAS (2008) software. The inclusion related responses in reproductive outcomes of breeding male rabbits to NLM were modeled using quadratic 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).

Results

The impact of NLM on sperm production and quality in male rabbits is shown in Figures 1 and 2. Results indicated differences among means for different sperm quality parameters under consideration in male rabbits following NLM inclusion level. However, NLM inclusion levels had a quadratic effect on the sperm quality parameters in male rabbits (Table 2). Our results indicated that the inclusion of NLM at 0.24% in the diet of breeding male rabbits supported optimum sperm motility ($77.78 - 0.99$ NLM - 2.07 NLM^2). In addition, NLM inclusion levels of 0.71% and 0.69%, respectively supported optimum sperm concentration ($12.58 + 1.80$ NLM - 1.27 NLM^2) and total sperm concentration ($14.60 + 0.94$ NLM - 0.68 NLM^2; $p = 0.01$) in male rabbits. Results indicated that abnormal sperm percent had a dose-response value of ($11.43 - 2.06$ NLM + 1.22 NLM^2; $p = 0.01$) with the optimum NLM inclusion level being 0.85%. The quadratic calculation indicated that sperm quality parameters had high R^2 values (98 - 99%).

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

Variables	Formular	R^2	X	Y	P-value
Sperm concentration ($\times10^6$/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 conc ($\times10^6$/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

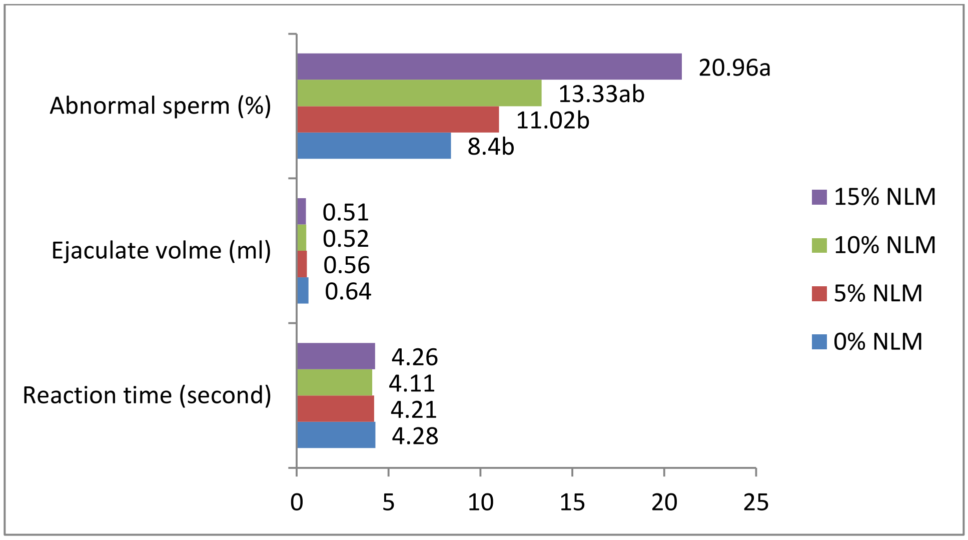

Figure 1. Effect of NLM on reaction time, ejaculate volume and abnormal sperm in male rabbits

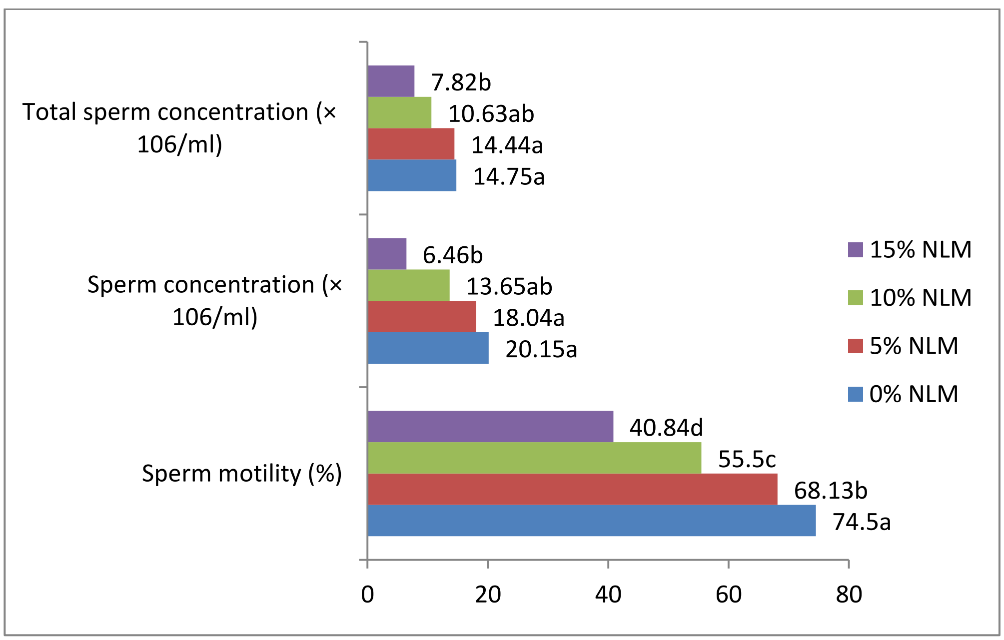

Figure 2. Effect of NLM on sperm motility, sperm concentration and total sperm concentration in male rabbits

Discussion

The rate of sperm production tended to be constant throughout the reproductive life of an animal but may be seriously influenced by nutrition among other factors. The result of the current study revealed that dietary NLM inclusion levels had a quadratic effect on sperm quality in breeding male rabbits. Data on neem leaf meal levels for optimal variable responses in rabbits are scarced. However, our results indicated that the inclusion of NLM at 0.24% in the diet of breeding male rabbits supported optimum sperm motility. In addition, a value of 0.71% supported optimum sperm concentration in male rabbits. Total sperm concentration which is a product of ejaculate volume and sperm concentration is a vital semen quality feature related to fertility. It was also a reflection of testicular volume, which is directly linked to the chances of pregnancy after mating. The NLM inclusion level of 0.69% required to optimize total sperm concentration was higher than the level of 0.24% that optimized sperm motility, but lower than the level of 0.71% that optimised sperm concentration. Results showed that no single NLM inclusion level optimised all the sperm quality parameters in the present study. Ogundun *et al*. (2013) recorded similar results in animals other than rabbits. Quadratic calculations suggested that the NLM level for optimal sperm concentration was higher than the level needed to optimise sperm motility. The physiological explanation for this difference is not clear and required further study. However, it may be that these sperm parameters required different dietary components and levels for their production as corroborated by Ogundun *et al.* (2013), who noticed that optimum inclusion-response value of Uromalt supplementation doses for optimising semen quality traits in goats was dynamic.

The quadratic calculation indicated that sperm quality parameters had high R^2 values of 97.7- 99.9% which shows higher strength of associations between sperm parameters and NLM. This suggested that sperm quality traits can be predicted at any given level of NLM added to the diets of breeding male rabbits. Few authors have employed a quadratic function to determine the inclusion levels of non-conventional feedstuffs that gave the best productivity in male rabbits (Ogbuewu and Mbajiorgu, 2020). Moreover, there is little or no data on the using a quadratic optimization model to determine the NLM level for optimal sperm quality traits in breeding male 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. It also indicated that nutrient requirements of breeding rabbits for optimal spermatogenesis were dynamic and depend on the sperm variable under investigation and should be factored in when formulating feed for male rabbits. The present study showed that diets containing NLM at the range of 0.24 - 0.85% supported optimum sperm quality in male rabbits offered diets having NLM at 0, 5, 10 and 15%, indicating that for optimal sperm production in male rabbits, NLM should be included in the diet at a level less than 1%.

It concluded that inclusion of NLM at the levels of 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. The results also revealed that neem leaf meal levels for optimal response are dependent on the semen parameter in question which has implications on diet formulation for male rabbits. This study also indicated that sperm quality traits were influenced by the inclusion of neem leaf meal in the diet of male rabbits and this could be ascribed to the anti-nutrient factors present in the diet (Al-Hashemi *et al.* 2016; Ubua *et al*., 2019) which increased with increasing levels of neem leaf meal in the diet. 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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