

ASSESSMENT OF BLOOD CHEMISTRY, WEIGHT GAIN AND LINEAR BODY MEASUREMENTS OF PRE-PUBERAL BUCK RABBITS FED DIFFERENT LEVELS OF NEEM (*Azadirachta indica* A. Juss.) LEAF MEALS

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ABSTRACT

A 16 week feeding trial was carried out to investigate the effect of dietary Neem (*Azadirachta indica* A. Juss.) leaf meal (NLM) on body weight gain, linear body measurements and blood chemistry of pre-puberal buck rabbits. Four treatment diets were formulated to contain the NLM at inclusion levels of 0 (control), 5, 10 and 15%. Thirty six crossbred New Zealand white × Chinchilla pre-puberal buck rabbits aged 5 to 6 mo were divided into four groups of nine rabbits and each group was further replicated into three of three rabbits each. The rabbits were randomly assigned to the four dietary treatments. Lymphocyte count of rabbits fed control diet ($8.32 \times 10^9 \text{ mm}^{-3}$) was significantly higher than the group fed 15% NLM ($4.60 \times 10^9 \text{ mm}^{-3}$). The mean cell hemoglobin (MCH) and mean cell volume (MCV) of the control bucks were significantly ($p < 0.05$) higher than those fed 5% NLM. The neutrophils count of bucks fed 15% NLM ($0.95 \times 10^9 \text{ mm}^{-3}$) was significantly ($p < 0.05$) different from the groups fed 0% NLM ($2.08 \times 10^9 \text{ mm}^{-3}$), 5% NLM ($2.69 \times 10^9 \text{ mm}^{-3}$) and 10% ($2.13 \times 10^9 \text{ mm}^{-3}$). All the other parameters measured were similar ($p > 0.05$) among the treatment groups. The results suggest that buck rabbits could tolerate up to 15% dietary inclusion of NLM without deleterious effects on body weight gain, linear body measurements and some hematological parameters.

Key words: Neem leaf meal, body weight gain, blood chemistry, linear body measurements, buck rabbits.

INTRODUCTION

Due to high cost of conventional feedstuff, nutritionists and other related workers in developing countries such as Nigeria have seriously advocated the use of non-conventional feed ingredients such as leaf meals of tropical browse plants and forages which are not in competition with man's dietary needs. Omoikhoje *et al.* (2006) reported that rabbit has greater ability to efficiently convert leaf meals and agro-industrial by-products into meat than other livestock.

Leaf meal made from fodder shrubs, leguminous crops and trees are currently helping small-scale farmers in many tropical countries to boost their yields (Esonu *et al.*, 2002; Nworgu and Fapohunda, 2002). Different authors have reported the nutritional values (Omoikhoje *et al.*, 2006; Ogbuewu, 2008) of different leaf meals that

are in use in Nigeria. Some of these leaf meals have been found to have high crude protein content. One of such leaf meal is velvet beans (*Mucuna pruriens* (L.) DC. var. *utilis* (Wight) Burck) and neem (*Azadirachta indica* A. Juss.).

Neem is an indigenous tropical plant predominant in Nigeria. The neem leaf meal has the nutrient composition of 92.42% dry matter (DM), 7.58% crude protein (CP); 16.60% crude fibre; 4.13% ether extract; 7.10% ash and 43.91% N free extract (Esonu *et al.*, 2006). However, neem leaf meal like most leaf meals contains anti-nutritional factors (Opende *et al.*, 2004) which may affect nutrient utilization. These anti-nutritional factors may also alter the blood profiles and also affect the linear growth of animals fed this leaf meal.

The evaluation of linear growth performance and blood profiles of buck rabbits fed different levels of the leaf meal will provide valuable information for its assessment and use as feed ingredient in rabbit diets. The present study was therefore designed to determine the growth performance, linear body measurements and blood chemistry of pre-buck rabbits fed graded levels of neem leaf meal.

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MATERIALS AND METHODS

Location of study. This research was carried out in the Rabbit Section of the Teaching and Research Farm of the Department of Animal Science and Technology, Federal University of Technology, Owerri, Imo State. Imo state (4°4' -6°3' N, 6°15' -8°15' E) is situated in South-Eastern agro-ecological zone of Nigeria. The mean annual rainfall, temperature range and humidity range of the area were 2500 mm, 26.5-27.5 °C and 70-80%, respectively.

Processing of leaf meals. Fresh matured neem leaves were harvested in and around the Federal University of Technology, Owerri, Nigeria. The leaves were sun dried for about 9 h every day for 3 to 4 d until they became crispy while retaining its greenish coloration. The dry leaves were milled using a hammer mill to produce neem leaf meal (NLM).

Experimental diets. Four experimental rations were formulated such that the diet contained 0%, 5%, 10% and 15% dietary levels of NLM, respectively. The chemical compositions of the formulated rations are shown in Table 1.

Experimental animals and feeding trials. Thirty-six New Zealand white × Chinchilla pre-puberal buck rabbits with initial weight ranging 950 to 1100 g were randomly allocated on the weight basis to four experimental groups of nine rabbits each and each group replicated into three in a completely randomized design (CRD) experiment. The groups were randomly assigned the diets containing the control (0%), 5, 10 and 15% NLM respectively (Table 1). Feed and water were given *ad libitum*. The feeding trial lasted for 16 wk.

Sanitation and health management. The hutches and its surroundings were kept clean using Izal disinfectant. Routine practices such as sweeping and washing of floor and feeding troughs were done regularly. The incidence of diarrhea was combated with anti-coccidial drug Farmavet (Amprodon). To reduce the incidence of parasites, rabbits were treated with 1% ivermectin (Ivomec®) injection.

Blood collection. At the end of the trial, three rabbits from each treatment group were randomly selected and bled between 09:00 and 10:30 h from the marginal ear vein using 5 mL hypodermic needles. The blood was then collected immediately into a set of sterile plastic tube containing EDTA for hematological assay. Hematological measurements were determined using the methods outlined by Schalm *et al.* (1975) and Kelly (1979). Linear body measurements and body weight were taken on 2 wk basis for 16 wk. The body weight was taken using weighing scale (Kenwood) whereas linear measurements were taken with the aid of a measuring tape in the morning before feeding the animal. Each animal was gently restrained in an unforced position while taking the measurements. The linear traits studied were ear length (EL), length of fore limb (LFL), length of hind limb (LHL), tail length (TL), heart girth (HG), head to shoulder (HTS) and body length (BL). The description of the measurements is as follows: Ear length - measured from the tip of the ear to the junction of the ear and the skull. Length of fore and hind limb- this was measurement taken in centimeter from the shoulder and pelvic joints to the tips of the paws respectively. Head to shoulder- measured from the tip of the nose to the end of the neck bone. Heart girth- this was determined by measuring the circumference of the chest region directly below the fore arms whereas the tail length

Table 1. The composition of experimental diets fed to New Zealand white × Chinchilla buck rabbits.

Ingredients	Diets			
	0%NLM	5% NLM	10%NLM	15%NLM
Spent grain	55.00	50.00	45.00	40.00
Neem leaf meal	0.00	5.00	10.00	15.00
Calculated analysis				
Crude protein	18.87	18.70	18.53	18.37
Crude fibre	10.10	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
Metabolizable energy, MJ kg ⁻¹	10.42	10.38	10.33	10.22

NLM: neem leaf meal; 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.

Vitamin and mineral premix contributed the following to each kilogram of diet: vit. A 500 IU; Vit. D₂ 1500 IU; Vit. E 3 IU; Vit. K 2 mg; riboflavin 3 mg; pantothenic acid 6 mg; niacin 15 mg; Vit B₁₂ 0.8 mg; choline, 3 mg; folic acid 4 mg; Mn 8 mg; Zn 0.5 mg; iodine 1.0 mg; Co 1.2 mg.

was taken from the junction of the hip to the apex of the tail.

Data analysis. Data collected were subjected to one way ANOVA (Steel and Torrie, 1980). The treatment values were tested for significant differences by Duncan's new multiple range test (DNMRT) of SAS (2000) Package.

RESULTS AND DISCUSSION

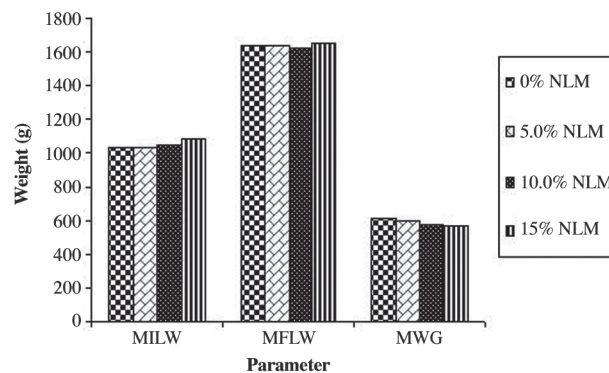
The results indicate that mean initial live weight (MILW), mean final live weight (MFLW) and mean weight gain (MWG) were similar ($p > 0.05$) among the treatment groups. The non significant reduction in the body weight gain of groups fed 10% and 15% NLM diets as observed in this study (Figure 1) implied a reduction in growth rate. This decrease is similar to that reported by Dagbir *et al.* (1980), that bulkiness of feed results in animals not being able to satisfy their energy and protein requirements. Dutta *et al.* (1986) reported that growth reduction could be attributed to the presence of anti-nutritional factor contained in leaf meals. It appears that different bio-active components of NLM may be responsible for depression in nutrient utilization and growth in rabbits. This is in view of the fact that all the diets adequately met the recommended nutrient requirements for a growing male rabbits (Esonu, 2006). Similarly, it has been reported that odor, taste, texture and color of finished feeds influence intake in animals (Arnold *et al.*, 1980; Farinu *et al.*, 2005; Olayeni, 2005).

The non significant differences observed (Figure 2) in the linear body growth parameters of rabbits fed graded levels of NLM are indication that the inclusion of the test ingredient up to 15% level does not have deleterious effects on bone growth especially the long bones. The live body weight and linear traits contributes significantly

to the life time performance of the animal. Research on linear body measurements have been used to evaluate breed performance, predict live weight gain, and to examine reproductive performance (Chineke *et al.*, 2000).

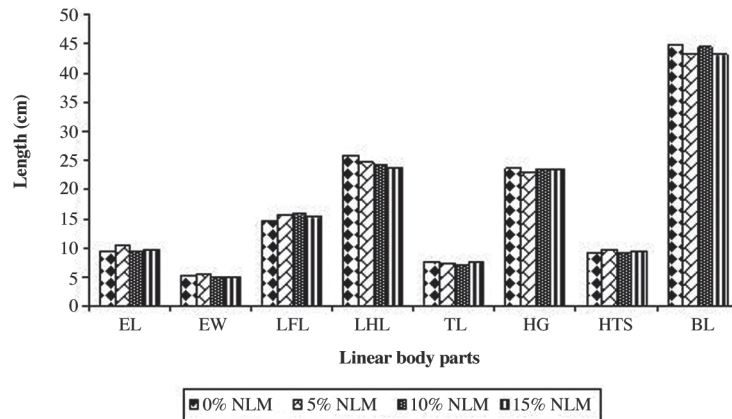
The hematological values obtained in this study (Table 2), however, are within the standard range recommended for clinically healthy rabbits (Mitruka and Rawnsley, 1977). The mean cell hemoglobin concentration (MCHC) of the treatment bucks were not significantly ($p > 0.05$) different from the control group. The 32.30-35.90% range of MCHC obtained in this study is within the 31.1-37.0% reported by Mitruka and Rawnsley (1977) for clinically healthy rabbits. The MCHC values have been shown to be the most accurate and absolute values that indicate anemic condition in animals (Thompson, 2006). The MCV values observed in this study were above the normal 58.0-79.6 fL (femtoliters) reported for healthy rabbits in temperate climate (Mitruka and Rawnsley, 1977). The higher mean cell volume (MCV) value recorded in the present study when compared to that reported by Mitruka and Rawnsley (1977) in the temperate climate could be attributed to climate and breed differences. The MCH values of all treatment groups except those on 5% NLM diet (38.9×10^{-12} g) were within the range of 19.2×10^{-12} to 29.5×10^{-12} g reported for healthy rabbits by Mitruka and Rawnsley (1977).

The non significant value of red blood cells (RBC), packed cell volume (PCV) and hemoglobin (Hb) of the bucks on NLM diets relative to the control group is an indication that the animals were not anemic. The PCV and Hb values of rabbits on test diets were not significantly ($p > 0.05$) different from the control group. This tends to confirm the report of Talebi *et al.* (2005) that nutrition affect the blood profiles of animal and this implies that up to 15% inclusion of NLM had a positive effect on the relative quantity of blood cell as well as total volume of blood.



MILW: mean initial live weight; MFLW: mean final live weight; MWG: mean weight gain.

Figure 1. Initial, final weight and body weight change of New Zealand white \times Chinchilla pre-pubertal buck rabbits fed graded level of neem leaf meal (NLM) based diet.



EL: ear length; LFL: length of fore limb; LHL: length of hind limb; TL: tail length; HG: heart girth; HTS: head to shoulder; and BL: body length.

Figure 2. Effects of neem leaf (*Azadirachta indica*) meal (NLM) on linear body measurements of New Zealand white x Chinchilla pre-pubertal buck rabbits.

The values of white blood cells (WBC) and their differential counts were within normal range reported for healthy rabbits (Mitruka and Rawnsley, 1977). White blood cell in animal possesses phagocytic function (Campbell and Coles, 1986) and differential WBC counts were used as an indicator of stress response and sensitive biomarkers crucial to immune function (Graczyk *et al.*, 2003). The results of the WBC in the present study clearly points to the fact that the rabbits on 15% NLM diet were stressed hence the significant ($p > 0.05$) reduction in leukocyte counts.

The significant decrease in blood lymphocyte level of the group on 15% NLM diets relative to the control group is not in line with earlier finding that neem leaves stimulates lymphocyte proliferation responses by selectively activating T helper (TH₁) cells of the T-cell

(Upadhyay, 1990). The significance higher value of the WBC counts of rabbits on control diet to those on 15% NLM diet was indication that these animals were not in a healthy state.

CONCLUSION

The experiment showed that up to 15% neem leaf meal could be included in the diet of buck rabbits without deleterious effects. This was based on the findings that body weight gain, linear body measurements and some hematological values were comparable to those rabbits fed control diet. Furthermore, it is recommended that a detailed research on pathophysiology of bucks fed *ad libitum* of the same dietary levels to be conducted to ascertain its effects at micro-anatomy.

Table 2. Effects of dietary inclusion of neem leaf meal on hematological characteristics of New Zealand white x Chinchilla buck rabbit.

Parameters	Inclusion levels of neem leaf meal (%)				SEM
	T _{0.0}	T _{5.0}	T _{10.0}	T _{15.0}	
Hemoglobin, g dL ⁻¹	12.90	14.00	13.90	13.20	0.18
PCV, %	38.00	39.00	43.00	40.00	0.63
RBC, ×10 ⁶ mm ⁻³	4.60	3.60	5.20	4.70	0.17
MCV, fL ¹	80.40b	108.30a	82.70b	85.10b	3.23
MCH, 10 ⁻¹² g	28.00b	38.90a	26.70b	28.10b	1.42
MCHC, %	34.90	35.90	32.30	33.00	0.42
WBC, ×10 ⁹ mm ⁻³	10.40a	11.20a	8.20ab	7.30b	0.46
Lymphocytes, ×10 ⁹ mm ⁻³	8.32a	8.51a	6.07a	4.60b	2.14
Neutrophils, ×10 ⁹ mm ⁻³	2.08a	2.69a	2.13a	0.95b	0.04

Means within a row with different letters differs significantly ($p < 0.05$).

PCV: packed cell volume; RBC: red blood cells; WBC: white blood cells; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; SEM: standard error mean.

¹fL: femtoliters.

RESUMEN

Evaluación de química sanguínea, ganancia de peso y mediciones corporales lineales de conejos pre-púberes alimentados con diferentes niveles de harina de hojas de neem (*Azadirachta indica* A. Juss.). Se realizó un ensayo de alimentación de 16 semanas para investigar el efecto de harina de hojas de neem *Azadirachta indica* A. Juss.) (NLM) sobre ganancia de peso, mediciones corporales lineales y química sanguínea de conejos machos pre-púberes. Se formularon cuatro dietas con niveles de inclusión de NLM de 0 (control), 5, 10, y 15%. Treinta y seis conejos híbridos New Zealand white × Chinchilla, pre-púberes, de 5 a 6 meses, se distribuyeron en cuatro grupos de nueve conejos, y cada grupo fue repetido en tres grupos de tres conejos cada uno. Los conejos se asignaron aleatoriamente a las cuatro dietas tratamiento. El recuento de linfocitos de los conejos alimentados con la dieta control ($8,32 \times 10^9 \text{ mm}^{-3}$) fue significativamente mayor que el grupo alimentado con 15% NLM ($4,60 \times 10^9 \text{ mm}^{-3}$). La hemoglobina celular media (MCH) y el volumen celular medio (MCV) de los conejos control fueron significativamente ($p < 0,05$) mayores que aquellos alimentados con 15% NLM. El recuento de neutrófilos de conejos alimentados con 15% NLM ($0,95 \times 10^9 \text{ mm}^{-3}$) fue significativamente ($p < 0,05$) diferente de los grupos alimentados con 0% NLM ($2,08 \times 10^9 \text{ mm}^{-3}$), 5% NLM ($2,69 \times 10^9 \text{ mm}^{-3}$) y 10% ($2,13 \times 10^9 \text{ mm}^{-3}$). Todos los otros parámetros medidos fueron similares ($p > 0,05$) entre grupos tratamiento. Los resultados sugieren que los conejos pudieron tolerar hasta 15% de inclusión dietaria de NLM sin efectos deletéreos en ganancia de peso corporal, mediciones corporales lineales y algunos parámetros hematológicos.

Palabras clave: harina de hoja de neem, ganancia de peso corporal, química sanguínea, mediciones corporales lineales, conejos macho.

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