



Effect of neem leaf (*Azadirachta indica*) meal on growth performance and haematology of rabbits

C. R. Unigwe^{1,*}, F. A. Balogun¹, U. P. Okorafor¹, I. S. Odah¹, F. O. Abonyi²
and J. F. Olona¹

¹Federal College of Animal Health and Production Technology, Moor Plantation,
Ibadan, Oyo State, Nigeria

²Department of Veterinary Animal Health and Production, University of Nigeria, Nsukka, Nigeria

*E-mail address: robinsonunigwe@gmail.com

ABSTRACT

A ten-week experiment using twenty four (24) weaner rabbits (Chinchila x New Zealand White) aged 8 to 9 weeks with an average initial body weight of 431.20 ± 0.74 g were randomly allocated to four treatment diets of T₁(control), T₂(5% NLM), T₃(10% NLM) and T₄(15% NLM) in a completely randomized design. They were fed for 10 weeks during which data on growth and haematology were collected and analysed using analysis of variance (ANOVA) and means separated using Duncan Multiple Range Test. The results showed that the average total body weight gains were 739.60g (T₁), 717.85g (T₂), 740.18g (T₃) and 729.45g (T₄). There was no significant difference ($p > 0.05$) when T₁ and T₃ as well as T₂ and T₄ were compared but significant ($p < 0.05$) when T₁ and T₂ as well as T₃ and T₄ were compared. Also the average weekly feed intake showed that T₁, T₂, T₃ and T₄ consumed 313.91g, 313.24g, 312.48g and 314.69g respectively. However, there was significant difference ($p < 0.05$) when all the treatments were compared in this respect. The feed conversion ratio (FCR) showed that T₃ (4.22) was the best followed by T₁ (4.24), T₄ (4.31) and T₂ (4.36) with significant differences ($p < 0.05$) among them except ($p > 0.05$) between T₁ and T₃. The haematological indices showed that though all the parameters fell within the normal physiologic ranges, the PCV was 37.62% (T₁), 38.42% (T₂), 39.60% (T₃) and 39.03% (T₄) and when compared, were all significantly different ($p < 0.05$) except ($P > 0.05$) for T₃ and T₄ while the haemoglobin concentration showed that T₁(13.47g/dl) was significantly different ($p < 0.05$) from T₂(14.18g/dl), T₃(14.34g/dl), and T₄(13.97g/dl). The white blood cell count showed that T₃($10.62 \times 10^9/L$) had the highest value followed by T₁($10.12 \times 10^9/L$), T₄($9.34 \times 10^9/L$) and T₂($9.18 \times 10^9/L$) with a significant difference ($p < 0.05$) occurring when T₁ and T₃ were compared to T₂ and T₄ while the red blood cell counts indicated that T₁ had the highest value of 4.92

$\times 10^6/L$ followed by $T_3(4.89 \times 10^6/L)$, $T_2(4.73 \times 10^6/L)$ and $T_4(4.65 \times 10^6/L)$ without any significant difference among the treatments. All the values fell within the normal range. It is therefore recommended that inclusion of neem leaf meal in the diets of rabbits up to 10% is not detrimental since it improved feed conversion ratio (FCR), growth performance and had no negative effect on haematological values.

Keywords: Rabbits; Neem leaf meal; growth performance; haematology; feed conversion ratio

1. INTRODUCTION

Scarcity of feed resources has been the main limitation in the production of livestock products to meet the animal protein requirements of human and other industrial needs. Due to serious problems posed by stiff competition for feed stuffs' energy and protein between humans and livestock, other available but neglected cheaper novel feed resources have been focused areas of recent research (Mahmud *et al.*, 2015). The conventional cereal and vegetable protein sources being used in animal feeds are under pressure of competition through their use in human diets (Ogbuewu *et al.*, 2010c). The conventional vegetable protein sources such as soybean and groundnut cake are very expensive in developing countries like Nigeria due to high exchange rate as a lot is still being imported (Esonu *et al.*, 2006). Inadequate supply of protein from traditional livestock such as cattle, sheep, goat, pig, and poultry has led to a shift of emphasis toward enhanced productivity of this animal, in the light of this, there is increased interest in Nigeria livestock farmers to substitute conventional feed ingredients with the non-conventional types, which one of such unconventional feed ingredient is leaf meal of ethno medical plant such as neem (Esonu *et al.*, 2005).

Rabbit production is a veritable way of alleviating animal protein deficiency in Nigeria (Unigwe *et al.*, 2009). The prolific nature of rabbit coupled with its short gestation and generation intervals makes it the animal of choice for multiplication (Olubanjo, 1976). The growing acceptability of rabbit meat of late, for its whiteness, juiciness and low cholesterol should be keyed into to expand the enterprise. In spite of all these advantages over other livestock, rabbit production has not achieved its potential as cheap animal protein source in the tropics (Herbert and Adejumo, 1995).

Azadirachta indica belongs to the family meliaceae and is widely distributed in Asia, Africa and other tropical parts of the world (Sombatsiri *et al.*, 1995). Neem leaf contains approximately 20.69% crude protein and 4.1% fat after processing into neem meal via drying and milling (Oforjindu, 2006). More than one hundred and thirty five compounds have been isolated from different neem trees. Traditionally, neem was used by the Indians for treatment of a number of health conditions such as parasitic infections and reduction of plasma cholesterol levels.

Research has shown that neem will boost the immune system by stimulating the production of T-cells when challenged with infections (Upadhyay, 1990). The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents, usually associated to a wide range of amphipathic molecules, broadly termed polyphenolic compounds (Demiray *et al.*, 2009).

The bark of the neem has been reported to have higher phenolic and antioxidant activity compared to the leaf (Ghimera *et al.*, 2009; Olabinri *et al.*, 2009).

Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control diseases like leprosy, intestinal helminthiasis, respiratory disorders, constipation, and skin infections (Biswas *et al.*, 2002). The neem tree contains more than 100 bioactive ingredients and the most important bioactive compound is azadirachtin (Nahak and Sahu, 2010). The Neem leaves, neem oil and de-oiled neem seed cake are used as animal feeds [Ogbuewu *et al.*, 2010a]. The neem leaves contain appreciable amounts of proteins, minerals, carotene and adequate amount of trace minerals [Ogbuewu *et al.*, 2010b]. Neem tree as one of the most researched tree in the world has attracted world-wide prominence due to its vast range of medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepatoprotective and various other properties without showing any adverse effects (Kale *et al.*, 2003).

Nutritional status of an individual is related to dietary intake and effectiveness of metabolic processes and it can be determined by either or combinations of clinical anthropometric, biochemical or dietary methods (Bamishaiye *et al.*, 2009). Dietary contents affect the blood profile of healthy animals (Odunsi *et al.*, 1999; Yeong, 1999; Iheukwumere and Herbert, 2002; Kurtoglu *et al.*, 2005). According to Oyewoye and Ogunkunle (2004) and Isaac *et al.* (2013), haematological components are valuable in measuring toxicity, especially with feed constituents that affect the blood as well as the physiological and health status of farm animals.

Afolabi *et al.* (2010) posited that changes in haematological parameters are often used to determine stresses due to nutrition and other factors. Recently numerous research efforts have been directed at the optimal utilization of neem leaf in the feeding and medication of farm animals (Sokunbi and Egbunike, 2000; Oforjindu, 2006; Esonu *et al.*, 2006). Therefore, this research was designed to unravel the consequences or otherwise, of supplementing neem leaf meal in the diet of rabbits with respect to their growth performance and haematology.

2. MATERIALS AND METHODS

Experimental site

This research was carried out at rabbitry section of the Federal College of Animal Health and Production Technology, Moor Plantation, Oyo State.

Source and processing of neem leaves

Fresh neem leaves were harvested from neem trees around the experimental site, immediately sundried using open clean concrete floor space, to a constant weight. The sun dried leaves were milled using a commercial milling machine into neem leaf meal (NLM) according to the procedure described by Esonu *et al.* (2006) and stored in air-tight container.

Experimental animals, design, duration and management

Twenty four (24) unsexed weaner rabbits, being a progeny of cross between Chinchila and New Zealand White, aged between 8 and 9 weeks with an average initial body weight of 431.20 ± 0.74 g were used in the experiment. The rabbits were randomly assigned to four treatments in a completely randomized design as stated below:

T₁ = normal feed 0% NLM (control)

T₂ = feed compounded with 5% NLM

T₃ = feed compounded with 10% NLM

T₄ = feed compounded with 15% NLM

Each treatment was replicated twice, with two rabbits each. The feeding trial lasted for ten weeks. The rabbits were housed in wooden hutches netted with wire gauze of 0.3mm covering both sides of the hutch. The hutches were disinfected using IZAL^R and diazintol before stocking. They were acclimatized for two weeks during which they were given conventional chicken grower diet and as well treated against coccidiosis, parasitism and microbial infections using Embazin forte^R (oral), Ivermectin (s/c) and Oxytetracycline (LA) (i/m) respectively, according to the manufacturer's prescription. Water and feed were given at *ad-libitum* by 7am and 5pm daily and proper biosecurity including washing of the feed and water troughs on daily basis, cleaning the environment and regular foot-dip program were all observed.

Data collection

Weight Gain: The weight of each individual rabbit was measured with a 5kg measuring scale, at first week of experimental procedure, and subsequently at seven days interval until the ten weeks of the experiment was completed.

Feed Intake: Daily feed intake was gotten through weigh-back mechanism by subtracting left over feed from feed served.

Feed Conversion Ratio: This was calculated by dividing the average total feed intake by the average total weight gain.

Blood collection: The blood collection was carried out at the end of the tenth week of the feeding trial, bleeding was done via the ear vein aseptically. The blood was then transferred into a sterile EDTA bottle for hematological analysis for packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC) and red blood cell (RBC).

Statistical Analysis

Data obtained were subjected to one way analysis of variance for completely randomized design (Steel and Torrie, 1980) using computerized statistical analysis of SAS (2000). Treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955).

3. RESULTS AND DISCUSSIONS

Performance characteristics of rabbits given diet with neem leaf meal

Table 3 shows the performance characteristic of weaner rabbits fed diets supplemented with neem leaf meal (NLM). The initial body weights showed that T₁, T₂, T₃ and T₄ were 431.62g, 432.19g, 430.31g and 430.67g respectively without any significant difference ($p > 0.05$) among them. It further revealed the final body weights to be 1171.22g, 1150.04g, 1170.49g, and 1160.12g for T₁, T₂, T₃ and T₄ respectively. In the same vein, the total weight

gains were 739.60g, 717.85g, 740.18 and 729.45g while the weekly weight gains were 73.96g, 71.79g, 74.02g and 72.95g for T₁, T₂, T₃ and T₄ respectively. There was significant difference ($p < 0.05$) when T₁ and T₃ were compared with T₂ and T₄ whereas T₁ and T₃ as well as T₂ and T₄ were not significantly different ($p > 0.05$) with respect to final body weight and total weight gains. There was however a significant difference ($p < 0.05$) when T₁ and T₃ were compared to T₂ and T₄ in terms of weekly weight gains while no significant difference ($p > 0.05$) comparing T₁ and T₃ as well as T₂ and T₄. The weekly feed intakes were 313.91g, 313.24g, 312.48g and 314.69g whereas the feed conversion ratios (FCR) were equally 4.24, 4.36, 4.22 and 4.31 for T₁, T₂, T₃ and T₄ respectively. The weekly feed intake had significant difference ($p < 0.05$) across the treatments and similar to the FCR except between T₁ and T₃ ($p > 0.05$). Numerically, the weekly weight gains resemble although the T₃ was superior but not significantly different from T₁. The comparable weight gain of the birds fed Neem leaf meal and the control is an indication that quantity of toxic factors such as terpenes and limonoids (Kabeh and Jalingo, 2007; Ogbuewu *et al.*, 2011) was minimal to have depressed the growth.

The results of present study disagree in part with the findings of Chakraverty and Parsad (1991) and Durrani *et al.* (2008), who reported that boilers fed on diet containing Neem (*Azadirachta indica*) leaves and Neem leaf infusion respectively, had higher body weight gain except off course, with respect to T₃ (10% NLM). The increased weight gain of T₃ could also be associated with anti-microbial activities of neem in which case the competition for nutrients by the microbes was put to a halt by their death as well as their subsequent digestion and absorption. Kale *et al.* (2003) reported that neem has antibacterial, antiviral, antifungal, antiprotozoal, hepatoprotective and various other properties without showing any adverse effects. Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control diseases like leprosy, intestinal helminthiasis, respiratory disorders, constipation, and skin infections (Biswas *et al.*, 2002).

The increased weight could have resulted from the anti-oxidant property of neem (Ghimeray *et al.*, 2009; Olabinri *et al.*, 2009; Demiray *et al.*, 2009) as well as the extra nutrients it supplied since Ogbuewu *et al.* (2010b) reported that neem leaves contain appreciable amounts of proteins, minerals, carotene and adequate amount of trace minerals. Reduction in weight gain beyond 10% NLM (T₃) implies reduction in growth. Dagbir *et al.* (1980) reported that bulkiness of feed makes animals unable to meet their energy and protein requirements. This decrease agrees with the work of Dagbir *et al.* (1980). It could also be due to the increased presence of anti-nutritional factor with increase in the quantity of NLM as supported by Dutta *et al.* (1986).

The feed intake was smallest in T₃ and highest in T₄ possibly because T₄ consumed more and gained less weight since there was possible reduction in serum glucose value in the present study which could be attributed to the presence of bioactive compounds contained in neem leaves which have the ability to block the energy metabolic pathway (Chattopadhyay, 1996), thus making it difficult for the animals to meet their energy requirement (Dutta *et al.*, 1986). Also, Opende *et al.* (2004) reported that NLM directly or indirectly inhibits the secretion of trypsin by the enzyme secreting cells of the gut. This result agrees with those obtained using broilers (D'Mello *et al.*, 1987; Udedibie and Opara, 1996; Gowda and Sastry, 2000; Esonu *et al.*, 2005; Obikaonu *et al.*, 2011) and quail (Mahmud *et al.*, 2015) where there was increased consumption of feed with increase in NLM. However, the FCR was in favor of T₃ similar to other works (Onu and Aniebo, 2013; Mahmud *et al.*, 2015).

Haematological indices of rabbits fed diets with neem leaf meal

Table 4 shows the haematological indices of weaner rabbits fed diets supplemented with neem leaf meal. The PCV values were 37.62%, 38.42%, 39.60% and 39.03%, haemoglobin values of 13.47g/dl, 14.18g/dl, 14.34g/dl and 13.97g/dl, red blood cell (RBC) counts of $4.92 \times 10^6/\mu\text{L}$, $4.73 \times 10^6/\mu\text{L}$, $4.89 \times 10^6/\mu\text{L}$ and $4.65 \times 10^6/\mu\text{L}$ and white blood cell (WBC) counts of $10.12 \times 10^9/\mu\text{L}$, $9.18 \times 10^9/\mu\text{L}$, $10.62 \times 10^9/\mu\text{L}$ and $9.34 \times 10^9/\mu\text{L}$ for T₁, T₂, T₃ and T₄ respectively.

With respect to PCV, there was no significant difference ($p>0.05$) when T₃ and T₄ were compared but a significant difference ($p<0.05$) when compared with T₁ and T₂. Similarly, a significant difference ($p<0.05$) was noted when T₁ and T₂ were compared. With respect to Hb, a significant difference ($p<0.05$) occurred when T₂, T₃ and T₄ were compared to T₁ but not the same ($p>0.05$) among T₂, T₃ and T₄.

However, there was no significant difference ($p>0.05$) among treatments with respect to RBC. The WBC showed that there was a significant difference ($p<0.05$) comparing T₁ and T₃ with T₂ and T₄ but no difference ($p>0.05$) between either T₁ and T₃ or T₂ and T₄. The MCV, MCH and MCHC were not significantly different ($p>0.05$) among the treatments. The blood indices show normal physiological ranges as established by Kronfield and Mediway (1975); Mitruka and Rawnsley (1977) and Hewitt *et al.* (1989).

Meanwhile, T₃ showed superiority in terms of PCV, Hb and RBC. Okoli *et al.* (2002) reported that neem leaf extract was traditionally used as human blood building tonic especially for weak toddlers by the Igbos in South-Eastern Nigeria. The results of present study disagree with the findings of Sadre *et al.* (1984), Gowda *et al.* (1998) and Biu *et al.* (2009) that neem preparations fed to laying hens significantly reduced the content of haemoglobin, erythrocyte count and packed cell volume.

The decrease in Hb and RBC observed in this study beyond 10% NLM (T₃) could suggest that NLM at high quantity might not support erythropoiesis. There was a slight increase beyond normal range (Kronfield and Mediway, 1975; Mitruka and Rawnsley, 1977; Hewitt *et al.*, 1989) in the MCV of T₂, T₃ and T₄ suggesting increased erythropoiesis, thus, blood provides proximate measures for long term nutritional status of animals (Kerr *et al.*, 1982).

Though the MCH was slightly higher in T₂ and T₄, the MCHC fell within normal range (Kronfield and Mediway, 1975; Mitruka and Rawnsley, 1977; Hewitt *et al.*, 1989) and it has been shown that MCHC is the most accurate and absolute value that indicates anaemic condition in animals (Merck Manual, 1998; Thompson, 2006). Therefore, the results of this experiment support that 10% NLM could substitute protein source to enhance growth performance and haematological values in rabbits without endangering the animal productive potentials.

4. CONCLUSION

It is therefore recommended that feeding rabbits with 10% neem leaf meal is not detrimental to growth and haematological indices. At this level, it helps to improve the conversion of feed to meat better than higher inclusions.

Table 1. Proximate composition of neem leaf meal

Nutrient	Dry matter (%)
Dry matter	92.42
Crude protein	20.68
Crude fiber	16.60
Ash	7.10
Ether extract	4.13
Nitrogen free extract	43.91

Source: Esonu *et al.* (2006)

Table 2. Composition of experimental diet

Ingredient	T ₁	T ₂	T ₃	T ₄
Maize	42	42	42	42
G.N.C	8	8	8	8
P.K.C	11	11	11	11
Wheat offal	25	20	15	10
B.D.G	10	10	10	10
NLM	-	5	10	15
Bone	2.5	2.5	2.5	2.5
Salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Lysine	1.0	1.0	1.0	1.0
Total	100	100	100	100
CP (%)	15.83	16.01	16.2	16.38
ME (Kcal/Kg)	2558.65	2464.89	2371.14	2277.39

Key; CP = Crude Protein, ME = Metabolisable Energy, BDG = Brewer's dried grain, GNC = Groundnut cake, PKC = Palm kernel cake. NLM = neem leaf meal

Table 3. Performance characteristics of weaner rabbits fed diet with NLM.

Parameter	T ₁ (Control)	T ₂ (5%NLM)	T ₃ (10%NLM)	T ₄ (15%NLM)	SEM
Av initial wt (g)	431.62	432.19	430.31	430.67	0.39
Av final wt (g)	1171.22 ^a	1150.04 ^c	1170.49 ^a	1160.12 ^b	4.08
Av total wt gain (g)	739.60 ^a	717.85 ^c	740.18 ^a	729.45 ^b	4.26
Av wkly wt gain (g)	73.96 ^a	71.79 ^b	74.02 ^a	72.95 ^b	0.43
Av total feed intake (g)	3139.12 ^b	3132.44 ^c	3124.83 ^d	3146.89 ^a	3.09
Av wkly feed intake (g)	313.91 ^b	313.24 ^c	312.48 ^d	314.69 ^a	0.31
FCR	4.24 ^c	4.36 ^a	4.22 ^c	4.31 ^b	0.27

abcd: means with different superscripts on the same row are significantly different (P < 0.05)

Table 4. Haematological indices of rabbits fed diets supplemented with neem leaf meal

Parameter	T ₁ (Control)	T ₂ (5%NML)	T ₃ (10%NML)	T ₄ (15%NML)	SEM
PCV (%)	37.62 ^c	38.42 ^b	39.60 ^a	39.03 ^a	0.28
Hb (g/dl)	13.47 ^b	14.18 ^a	14.34 ^a	13.97 ^a	1.13
RBC (x 10 ⁶ μL)	4.92	4.73	4.89	4.65	0.13
WBC (x 10 ⁹ μL)	10.12 ^a	9.18 ^b	10.62 ^a	9.34 ^b	0.23
MCV (fl)	76.46	81.23	80.98	83.94	2.76
MCH (pg)	27.38	29.97	29.33	30.04	1.43
MCHC (pg)	35.81	36.91	36.21	35.79	0.68

abc: means with different superscripts on the same row are significantly different (P < 0.05)

Key: PCV = packed cell volume, Hb = haemoglobin, RBC = red blood cell, WBC = white blood cell, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration

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