# Evaluation of Toxicological Effect of Leaf Meal of an Ethnomedicinal Plant -Neem - on Blood Chemistry of Crossbred New Zealand

# Typed Rabbit Does

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**Abstract:** The livestock industry in Nigeria, in the last decade, has been greatly affected by high cost of feed. The provision of feed alone has been reported to account for 60 - 80% of the total cost in most livestock production in Nigeria and this emphasize the interest to develop non conventional feedstuffs. In view of this, there is increased interest by Nigerian livestock farmers to harness unconventional feed ingredients such as neem leaf meal. Neem has been reported to contain several biologically active constituents such as azadirachtin, meliantriol, salanin, nimbin as well as nimbidin. The present study was designed to investigate the effect of long term feeding of neem leaf meal based diets on blood chemistry of crossbred New Zealand white typed rabbit does. Thirty-six healthy rabbit does were divided into four treatment groups. Rabbit does on group 1 served as a control whereas those on group 2, 3 & 4 were used for the determination of the toxic effect of neem leaf meal on blood chemistry. Blood samples were collected to obtain serum for biochemical analysis and heparinized treated blood for hematological analysis. The neutrophil counts of rabbits on group 2, 3 & 4 were significantly (p<0.05) reduced. Serum cholesterol and serum alkaline phosphatase concentrations were significantly (p<0.05) affected by the treatment. The serum globulin and serum glucose concentrations of group 4 rabbits were significantly (p<0.05) lowered relative to the group 1 rabbits. These results indicate that neem leaf meal based diets had visible deleterious effects on blood chemistry of crossbred New Zealand white typed rabbit does. Report and Opinion 2010: 2(1): xx-xx]. (ISSN: 1553-9873).

**Keywords**: rabbits; neem leaf meal; blood chemistry; phytotoxicity.

# 1. Introduction

Scarcity of feed resource has been the main limitation in the production of livestock products to meet the animal protein requirements of human and other industrial needs. The conventional feed ingredients used in animal feed formulation are under pressure of competition via their use in human diets. The conventional feedstuffs such as soybean and groundnut cake are very expensive in developing countries like Nigeria due to high exchange rate as many of them still import these commodities. In view of this, there is increased interest by Nigerian livestock farmers to harness unconventional feed ingredients. One of such unconventional feed ingredient is leaf meal of ethnomedicinal plants such as neem (Esonu *et al*., 2006).

The neem plant is a native of India and Burma and adapted favorably to the sub-sahelian Nigeria. The stem bark is an astringent. The root bark and young fruits are used as an astringent (Ketkar and Ketkar, 1995). Fresh twigs are often used for cleaning teeth. The seed is a stimulant and is also applied externally in the treatment of rheumatism and skin diseases. The leaves can be used in the treatment of abscesses and can also apply topically after castration (Kausik *et al*., 2008). Neem has been identified among the tropical plant that has been used as livestock feed resource (Sokunbi and Egbunike, 2000a, b; Akpan *et al*., 2008; Kausik *et al*., 2008; Ogbuewu *et al*., 2008; Ogbuewu *et al*., 2009). Chemically analysis revealed that neem leaf meal is

relatively high in crude protein (20.69%) (Esonu *et al*.,

2006) and low in metabolisable energy (0.34KJ / Kg DM) (Bakshi *et al*., 2006).

Biologically active compounds isolated from different parts of the neem plant include: azadirachtin, meliacin, gedunin, salanin, nimbin, valassin and many other derivatives of these principles. Azadirachtin forms the bitter principles of neem leaf. Azadirachtin has also shown direct detrimental and histopathological effects on most insect tissues e.g. muscles, body fat and gut epithelial cells (Mordue (Luntz) and Blackwell, 1993). The seed also contain tignic acid (5-methyl- 2-butanic acid) which was responsible for the distincitive odour of the neem seed oil (Akpan *et al*., 2008). These compounds belong to natural products called triterpenoids (Limonoids). The active principles are slightly hydrophilic, but freely lipophilic and highly soluble in organic solvents like, hydrocarbon, alcohols, ketones and esters (Akpan *et al*., 2008).

# Neem leaves like most tropical tree leaves contain bioactive compounds (Kausik *et al*., 2008; Akpan *et al*., 2008) which may also affect nutrient utilization. These bioactive compounds may also alter the hematological and serum biochemical parameters of animals. The blood contains a myriad of metabolites and other constituents which provide a valuable medium for clinical investigation and nutritional status of an individual hence (WHO, 1963) recommended the use of blood parameters for medical assessment

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Therefore, this study was designed with the main objectives of investigating the hazardous effects of neem leaf meal on blood chemistry of crossbred New Zealand white typed rabbit does.

**2. Materials and Methods**

**2.1 Experimental location**

An investigation was conducted at the Rabbit Unit of the Teaching and Research Farm, Department of Animal Science and Technology, Federal University of Technology, Owerri, Imo State. Imo State lies between latitude 4o4' and 6o3'N and longitude 16o15' and 8o15'E. Owerri, the capital city of Imo State is located in the south-eastern agro-ecological zone of Nigeria. Owerri is about 91m above sea level with annual rainfall, temperature and humidity ranging from 2300 - 2700 mm, 26.5 – 27.5oC and 80 - 90% respectively. Owerri has a three month dry season duration (i.e. month with < 65mm rainfall) and this covers December – February (MLS, 1984; Ibeawuchi *et al*., 2005; Ibeawuchi *et al*., 2007).

**2.2 Collection and preparation of neem leaf meal**

Fresh maturedneem leaves were harvested in and around the Federal University of Technology, Owerri. The fresh neem leaves were immediately sun dried for about 9 hours in an open clean concrete floor space until moisture content became constant at 13%. The sun-dried leaves were later milled using a commercial feed milling machine (Artec, model 20) into neem leaf meal according to the procedures described by Esonu *et al*., 2006 and Herbert *et al*., 2005.

**2.3 Experimental ration formulation**

The feed ingredients used in ration formulation were purchased locally from reputable commercial feed ingredient dealers in Owerri, Imo State. The Neem leaf meal was sourced as earlier discussed. All the diets were formulated to contain identical crude protein content (iso-nitrogenous) and metabolisable energy (isocaloric). Group1 (control diet) was formulated without neem leaf meal. Group 2, 3, and 4 were formulated such that they contained 5%, 10% and 15% levels of neem leaf meal respectively (Table 1).

**Table 1. The composition of experimental diets.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ingredients** | **Diets (% Neem leaf meal)** | | | |
| **Group1 (0% NLM)** | **Group 2 (5% NLM)** | **Group 3 (10% NLM)** | **Group 4 (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 | 10.1 | 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. Vitamin / mineral premix contributed the following per kg of feed: vitamin A, 10,000iu; vitamin D3, 2000iu; vitamin E, 5iu; vitamin K, 2mg; riboflavin, 4.2mg; vitamin B12, 0.01mg; panthothenic acid, 5mg; nicotinic acid, 20mg; folic acid, 0.5mg; Choline, 3mg; magnesium, 56mg; iron, 20mg; copper, 1.0mg; zinc, 5.0mg; cobalt, 1.25mg; iodine, 0.8mg.

**2.4 Experimental design**

Thirty six crossbred New Zealand white typed rabbit does were randomly divided into four treatment groups of nine animals each. Each group was further divided into 3 replicates of three animals. Each treatment group was randomly assigned to one of the four neem leaf meal based diets at 5% (Group 2), 10% (Group 3), 15% (Group 4) and 0% (Group 1; control) in a completely randomized designed (CRD) experiment.

**2.5 Management of experimental rabbits**

The experimental rabbits were housed in a 2 - tier rabbit hutches measuring 1.5m × 1m × 1cm. The hutches are made up of wooden frame with wire gauze of 0.3 mm covering both sides of the hutch. The experimental rabbits were stabilized for two weeks using starter broiler ration (Vital feed) before the commencement of the actual experiment. During this

period, the rabbits were dewormed subcutaneously with Ivomectin at the dose of 0.2 ml / Kg and also treated prophylactically against coccidiosis with amprodon according to manufacturer’s instructions. Water and feed were given *ad libitum.*

**2.6 Blood collection**

On the end of the 16th week of the feeding trial, between the hours of 9.00am and 10.00am, 3 rabbit does were randomly selected from each treatment group for blood collection. Blood was taken from their marginal ear vein. The rabbit was first removed from the cage by holding the securely on the scruff, held by the hind and fore limbs. The ear from which the blood was to be

drawn held upright, shaved with shaving stick to remove the furs so as to reveal the vein more clearly. The shaved ear was swabbed thoroughly with a clean cotton wool dipped in methylated spirit. The blood vessel was engorged by gentle tapping of the ear after which the hypodermic needle was inserted into the largest auricular vein. About 12 mL of blood was collected from the auricular vein. The blood was quickly aspirated into a vial bottles treated with or without heparin. Samples were transported in an ice pack immediately to the Federal Medical Centre Owerri, Nigeria for hematological and serum biochemical test.

**2.7 Hematological analysis**

The Packed Cell volume (PCV), Red Blood Cell (RBC) count, White Blood Cell (WBC) count and Hemoglobin (Hb) were determined were determined as outlined by Schalm *et al*. (1975) and Kelly (1979). The blood indices: Mean Cell Hemoglobin (MCH), Mean Cell Volume (MCV) and Mean Cell Hemoglobin Concentration (MCHC) were computed using appropriate formulae as described by Jain (1986).

**2.8 Serum biochemical analysis**

The serum biochemical assay were carried out using standard chemical procedures: Total Serum Protein by Golgberg refractometer method (Kohn and Allen, 1995), Serum albumin by Bromocresol green (BCG) method (Peters *et al*., 1982), Serum creatinine

(Boisness and Taussky, 1985), Serum urea nitrogen (Baker and Silverton, 1985), Serum glucose (Toro and Ackerman, 1979), Serum sodium ions and Serum potassium ions by flame photometry, Serum bicarbonate ions and Serum chloride ions (Schales and Schales, 1941), Serum enzymes by spectrophotometric method (Rej and Hoder, 1983).

**2.8 Data analysis**

Data collected were subjected to one way analysis of variance (Steel and Torrie, 1980) and means were separated using the Duncan’s New Multiple Range Test (1990).

**3. Results**

The data on the effect of neem leaf meal based diets on the hematological characteristics of rabbit does is shown in Table 2. The PCV, Hemoglobin and RBC count of rabbit does on group 2, 3 & 4 were not significantly (p>0.05) different from the control rabbit does (group 1). The white blood cell (WBC) count was similar (p> 0.05) among the four treatment groups. The neutrophil counts of rabbit does on group 2 & 3 were significantly (p<0.05) reduced relative to those on group 1. The rabbit does on group 2 & 4 were significantly (p<0.05) lower MCV value when compared with rabbit does on control diet (group 1).

**Table 2. Hematological values of female rabbits on neem leaf meal based diets**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **Inclusion levels of Neem leaf meal (NLM)** | | | | **S.E.M** |
|  | | | |
| **Group 1 (0% NLM)** | **Group 2 (5% NLM)** | **Group 3 (10% NLM)** | **Group 4 (15% NLM)** |
|  |  |  |  |  |  |
| Hemoglobin (g/dl) | 12.20 | 12.60 | 12.40 | 12.00 | 0.07 |
| PCV (%) | 36.00 | 37.00 | 37.00 | 35.00 | 0.32 |
| RBC (×106/mm3) | 3.60 | 4.20 | 3.80 | 4.00 | 0.07 |
| MCV (fl) | 105.6a | 88.00b | 97.40a | 87.50b | 2.16 |
| MCH (pg) | 33.90 | 30.00 | 32.60 | 30.00 | 0.49 |
| MCHC (%) | 32.10 | 34.10 | 33.50 | 34.30 | 0.25 |
| WBC(×109/mm3) | 8.80 | 9.90 | 8.70 | 8.50 | 0.16 |
| Lymphocytes (%) | 61.00ab | 66.00ab | 73.00a | 58.00b | 1.64 |
| Neutrophil (%) | 39.00a | 30.00b | 34.00b | 36.00ab | 1.37 |

abc Means within a row with different superscripts differ significantly (p<0.05); PCV - Packed Cell Volume; RBC - Red Blood Cell; WBC - White Blood Cell; MCV - Mean Cell Volume; MCH - Mean Cell Hemoglobin; MCHC -Mean Cell Hemoglobin Concentration.

The data on the effects of neem leaf meal on serum biochemical characteristics of rabbit does is presented in Table 3. The serum total protein concentrations of rabbit does were similar (p<0.05) among the treatment groups. The rabbit does on group 4 had significantly (p<0.05) lower serum globulin value when compared with the control rabbits (group 1). The serum cholesterol value for the group 2, 3 and 4 rabbits were significantly (p<0.05) reduced. The serum glucose value of rabbit does on group 4 was adversely affected

(p<0.05) by the treatment diets. Serum sodium value of

rabbit does on group 2 (58.20 mmol/l) and group 3 (66.40 mmol/l) were significantly (p<0.05) reduced by the treatment diets. The serum chloride values of rabbits on group 3 and 4 were significantly (p<0.05) elevated. The serum alkaline phosphatase values of rabbit does on group 2, 3 and 4 were significantly (p<0.05) higher than the control does (group 1). Serum total bilirubin concentration of the rabbit does on group 3 was significantly (p<0.05) higher than the control rabbits.

**Table 3: Serum biochemical values of rabbit does fed neem leaf meal**.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **Inclusion levels of Neem leaf meal** | | | | **S.E.M** |
| **Group 1 (0% NLM)** | **Group 2 (5% NLM)** | **Group 3 (10% NLM)** | **Group 4 (15% NLM)** |
| Total protein (g/dl) | 7.50 | 6.20 | 5.60 | 4.10 | 0.35 |
| Globulin (g/dl) | 4.30a | 2.60ab | 2.40ab | 0.70c | 1.37 |
| Albumin (g/dl) | 3.20 | 3.60 | 3.20 | 3.40 | 0.05 |
| Urea (mg/dl) | 66.40ab | 55.50b | 68.40ab | 77.30a | 2.24 |
| Creatinine (mg/dl) | 1.10 | 1.00 | 1.10 | 1.20 | 0.02 |
| Cholesterol (mg/dl) | 130.00a | 95.40b | 72.10c | 64.30d | 7.37 |
| Glucose (mg/dl) | 89.80a | 91.40a | 85.00a | 50.30b | 4.85 |
| Sodium (mmol/l) | 89.00a | 58.20b | 66.40b | 80.10a | 3.44 |
| Potassium (mmol/l) | 4.30 | 4.40 | 3.80 | 3.50 | 0.11 |
| Chloride (mmol/l) | 86.50b | 96.40ab | 100.00a | 103.60a | 1.84 |
| Bicarbonate (mmol/l) | 25.80 | 26.40 | 24.00 | 22.10 | 0.49 |
| Total bilirubin (mg/dl) | 0.30b | 0.30b | 0.50a | 0.40ab | 0.04 |
| Conj. bilirubin (mg/dl) | 0.20 | 0.20 | 0.30 | 0.30 | 0.01 |
| ALT (µl) | 6.00 | 8.00 | 8.00 | 7.00 | 0.24 |
| AST (µl) | 8.00 | 11.00 | 13.00 | 9.00 | 0.55 |
| ALP (µl) | 52.10d | 81.60b | 93.70a | 65.80c | 4.54 |

a,b,c Mean within a row with different superscripts differs significantly (p<0.05), AST- Aspartate Transferase, ALT- Alanine transferase, ALP – Alkaline phosphatase.

**4. Discussion**

The present study was carried out to establish the base line data on the toxicological effect of neem leaf meal based diets on hematological and serum biochemical characteristics of crossbred New Zealand white typed rabbit does. Hematological parameters are good indicators of the physiological state of an animal and its changes are of value in assessing the response of animal to various physiological situations (Esonu *et al*., 2006). The results of the present study implied that neem leaf meal based diets had mild depressive effect on the hemoglobin concentration and packed cell volume of female rabbits at 15% inclusion level. This mild depressive effect could be an indication that these animals were slightly stressed by the test diet (neem leaf meal). The slight reduction in packed cell volume and hemoglobin concentrations as the dietary inclusion levels of neem leaf meal was increased as observed in the present study disagreed with Esonu *et al*. (2006) who reported slight increments in the values of hemoglobin and packed cell volume of laying birds fed neem leaf meal diets at 0% to15%. The differences observed in the two studies could be attributed to genetic differences (WHO, 1963).

The neutrophils are concerned with day to day immunological defense against pathogens. The significant reduction in neutrophil counts of rabbits on group 2 and 3 implies that the ingestion of neem leaf meal based diets could decrease the production of these blood components. The lymphocyte count of group 3 rabbit does was slightly above the normal range of (53.5 - 65.8%) recommended by Kronfield and Mediway (1979) for clinically healthy rabbits raised in the temperate climate. It is probable that the elevation of lymphocyte count could be an indication that these rabbits were immunologically challenged. The elevated lymphocyte counts of rabbits on group 3 could be a physiological adjustment presented by these animals

against negative antigenic effect associated with the ingestion of neem bioactive compounds.

Serum biochemical investigations have been explored extensively to distinguish normal state from stress and disease conditions in animals. Dietary components have also been shown to have measurable effects on blood components (Awosanya *et al*., 2000) hence the serum biochemical metabolites are used to detect the existence of heart attack, liver damage and to evaluate protein quality and amino acid requirements in animals (Harper *et al*., 1999). The slight increment in serum urea and serum creatinine value of rabbit does on group 4 was an indication that neem leaf meal diet could be of poor quality relative to the control diet. The slight increment on serum urea and serum creatinine suggests little breakdown of muscle tissues and those animals could be surviving at the expense of their body reserve. The relative increase in serum urea at group 3 and 4 observed as the dietary level of neem leaf meal was increased in the ration could be an indication that these animals were tending towards negative nitrogen balance. This could be attributed to the presence of some of neem bioactive compounds which have been reported to block the energy metabolic pathway in animals, thus making it difficult for the animals to meet their energy requirement (Ogbuewu *et al*., 2008).

The reduction in serum cholesterol concentration (130.00 – 64.30 mg/dl) agreed with the hypocholesmic effects of neem earlier reported by Ogbuewu *et al*. (2008) in rabbit bucks and Oforjindu (2006) in broiler birds. It appeared that neem leaf meal indirectly inhibit HMG - COA reductase, a key enzyme in cholesterol biosynthesis. The hypoglycaemic activity of neem leaf meal observed in the present study was in agreement with the values reported by Ogbuewu *et al*. (2008) in buck rabbits. The significant changes in the serum alkaline phosphatase (ALP) concentrations rabbits fed neem leaf meal diets relative to those on

control diet did agree with the report that serum ALP could be influenced by changes in the physiological state of an animal.

**4. Conclusion**

It appeared that rabbit does could not tolerate the neem leaf meal based diets for a long period. It therefore concluded that neem leaf meal had visible deleterious effect on blood chemistry of female rabbits. However, the observed alterations in blood chemistry of rabbits receiving neem leaf meal are a source of concern, and therefore should be given adequate attention while incorporating neem leaf meal in the ration of rabbit does.

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