Evaluation of Toxicological Effects of Leaf Meal of an Ethnomedicinal Plant-Neem on Blood Chemistry of Puberal Chinchilla 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 local feedstuffs. In view of this, there is increased interest by Nigerian livestock farmers to harness unconventional feed ingredients such as neem leaf meals. Neem has been reported to contain several biologically active constituents such as azadirachtin, meliantriol, salanin, nimbin as well as nimbidin. The present study aimed to investigate the effect of long term feeding of neem leaf meal based diets on blood chemistry chinchilla rabbit does. Thirty-six clinically healthy rabbits were divided into four groups. Rabbits on group 1 served as a control whereas those on group 2, 3 & 4 were used for the determination of toxic effect of neem leaf meal on blood chemistry. Blood samples were collected to obtain serum for biochemical studies and heparinized blood for hematological investigations. The neutrophil counts of rabbits on group 2, 3 & 4 were significantly (p<0.05) reduced. Serum cholesterol and serum glucose concentrations of group 4 rabbits were significantly (p<0.05) elevated relative to the group 1 (control) rabbits. These results indicate that neem leaf meal based diets had visible deleterious effects on blood chemistry of chinchilla rabbit does. [Report and Opinion 2010;2(2):29-34]. (ISSN: 1553-9873).

Keywords: rabbits; neem leaf meal; blood chemistry; phytoxicity

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 cereal and vegetable protein sources being used in animal feeds are under pressure of competition through their use in human diets. 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 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 native of India and Burma and adapted favorably to the sub-sahelian Nigeria. The stem bark is an astringent and the root bark and young fruits are used in a similar way (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 Eg bunike, 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 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 tigic acid (5-methyl- 2-butanic acid) responsible for the distinctive odour of the 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 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.
Thereof, this study was designed with specific objectives of investigating the hazards effect of neem leaf meal on blood chemistry of chinchilla 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 4°4’ and 6°3’N and longitude 16°15’ and 8°15’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 – 27.5°C 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 matured neem leaves were harvested in and around the Federal University of Technology, Owerri. The fresh neem leaves were immediately shade dried 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 (Esonu et al., 2006; 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 NLM was sourced as earlier discussed. All 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).

2.4 Experimental design

Thirty six Chinchilla 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 Group 2, Group 3 (10%), Group 4 (15%) and 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 50 cm × 50 cm × 30 cm. 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 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 coccidioses with amprodon according to manufacturer’s instructions. Water and feed were given ad libitum.

2.6 Hematological analysis

The packed cell volume (PCV), red blood cell count (RBC), white blood cell (WBC) and hemoglobin (Hb) 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

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Table 1. The composition of experimental diets.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Group 1 (0% NLM)</th>
<th>Group 2 (5% NLM)</th>
<th>Group 3 (10% NLM)</th>
<th>Group 4 (15% NLM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spent grain</td>
<td>55.00</td>
<td>50.00</td>
<td>45.00</td>
<td>40.00</td>
</tr>
<tr>
<td>Neem leaf meal</td>
<td>0.00</td>
<td>5.00</td>
<td>10.00</td>
<td>15.00</td>
</tr>
<tr>
<td><strong>Calculated analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>18.87</td>
<td>18.70</td>
<td>18.53</td>
<td>18.37</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>10.1</td>
<td>10.78</td>
<td>11.02</td>
<td>11.27</td>
</tr>
<tr>
<td>Ether extract</td>
<td>5.97</td>
<td>5.95</td>
<td>5.93</td>
<td>5.91</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.41</td>
<td>1.39</td>
<td>1.38</td>
<td>1.36</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.66</td>
<td>0.62</td>
<td>0.58</td>
<td>0.53</td>
</tr>
<tr>
<td>ME (MJ/kg)</td>
<td>10.42</td>
<td>10.38</td>
<td>10.33</td>
<td>10.22</td>
</tr>
</tbody>
</table>

Each diet contained 35% maize, 3% local fish meal and groundnut cake each, 2% bone meal, 1% oyster shell, 0.50% vitamin/mineral premix, 0.3% 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; panthenoic 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.

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computed using appropriate formulae as described by Jain (1986).

2.7 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), albumin by Bromocresol green (BCG) method (Peters et al., 1982), creatinine (Boisness and Taussky, 1985), urea nitrogen (Baker and Silverton, 1985), serum glucose (Toro and Ackerman, 1979), Sodium ions and Potassium ions by flame photometry, bicarbonate ions and 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 effect of neem leaf meal diets on the hematological values of rabbit does is shown in Table 2. The PCV, hemoglobin concentration, WBC and RBC count of group 4 rabbits were not significantly (p>0.05) different from the group 1 rabbits. The white blood cell count and hemoglobin concentration were similar (p>0.05) among the groups. The neutrophil counts of rabbits on group 2, 3 & 4 were significantly (p<0.05) reduced. The rabbits on group 2 & 4 had significantly (p<0.05) lower MCV value when compared with rabbit does on group 1.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (0% NLM)</th>
<th>Group 2 (5% NLM)</th>
<th>Group 3 (10% NLM)</th>
<th>Group 4 (15% NLM)</th>
<th>S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.20</td>
<td>12.60</td>
<td>12.40</td>
<td>12.00</td>
<td>0.07</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>36.60</td>
<td>37.00</td>
<td>37.00</td>
<td>35.00</td>
<td>0.32</td>
</tr>
<tr>
<td>RBC (×10^6/mm^3)</td>
<td>3.60</td>
<td>4.20</td>
<td>3.80</td>
<td>4.00</td>
<td>0.07</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>105.6 a</td>
<td>88.00 b</td>
<td>97.40 a</td>
<td>87.50 b</td>
<td>2.16</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>33.90</td>
<td>30.00</td>
<td>32.60</td>
<td>30.00</td>
<td>0.49</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>32.10</td>
<td>34.10</td>
<td>33.50</td>
<td>34.30</td>
<td>0.25</td>
</tr>
<tr>
<td>WBC (×10^9/mm^3)</td>
<td>8.80</td>
<td>9.90</td>
<td>8.70</td>
<td>8.50</td>
<td>0.16</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>61.00 ab</td>
<td>66.00 ab</td>
<td>73.00 a</td>
<td>58.00 b</td>
<td>1.64</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>39.00 ab</td>
<td>30.00 ab</td>
<td>34.00 b</td>
<td>36.00 ab</td>
<td>1.37</td>
</tr>
</tbody>
</table>

Means within a row with different superscripts differ 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.

The data on the effects of neem leaf meal on serum biochemical characteristics of rabbit does are presented in Table 3. The serum total protein concentration of rabbits on the group 4 was numerically reduced. The group 4 rabbits 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 were significantly (p<0.05) reduced. The serum glucose value of rabbits on group 4 was adversely affected (p<0.05) by the neem leaf meal diet. Serum sodium concentration of group 2 (58.20 mmol/l) and group 3 (66.40 mmol/l) were significantly (p<0.05) depressed 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 was significantly (p<0.05) higher than those on group 1. Serum total bilirubin concentration of rabbits 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (0% NLM)</th>
<th>Group 2 (5% NLM)</th>
<th>Group 3 (10% NLM)</th>
<th>Group 4 (15% NLM)</th>
<th>S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>7.50</td>
<td>6.20</td>
<td>5.60</td>
<td>4.10</td>
<td>0.35</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>4.30</td>
<td>2.60 ab</td>
<td>2.40 ab</td>
<td>0.70 c</td>
<td>1.37</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.20</td>
<td>3.60</td>
<td>3.20</td>
<td>3.40</td>
<td>0.05</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>66.40 ab</td>
<td>55.50 b</td>
<td>68.40 ab</td>
<td>77.30 a</td>
<td>2.24</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.10</td>
<td>1.00</td>
<td>1.10</td>
<td>1.20</td>
<td>0.02</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>130.00 a</td>
<td>95.40 b</td>
<td>72.10 c</td>
<td>64.30 d</td>
<td>7.37</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>89.80 a</td>
<td>91.40 a</td>
<td>85.00 a</td>
<td>50.30 b</td>
<td>4.85</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>89.00 a</td>
<td>58.20 b</td>
<td>66.40 b</td>
<td>80.10 a</td>
<td>3.44</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.30</td>
<td>4.40</td>
<td>3.80</td>
<td>3.50</td>
<td>0.11</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>86.50 b</td>
<td>96.40 ab</td>
<td>100.00 a</td>
<td>103.60 a</td>
<td>1.84</td>
</tr>
</tbody>
</table>
4. Discussion

The present study was carried out to establish base line data on the toxicological effect of neem leaf meal based diets on hematological and serum biochemical characteristics of chinchilla rabbit does. Hematological parameters are good indicators of the physiological status of animal and its changes are of value in assessing the response of animals to various physiological situations (Esonu et al., 2006). The results of the present study showed that neem leaf meal had mild depressive effect on the hemoglobin concentration and packed cell volume of female rabbits at 15% inclusion level. This mild depressive effect indicates that these animals were slightly stressed by the test diet (neem leaf meal). The slight reduction in packed cell volume and hemoglobin concentrations observed in the present study was not in support 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% to 15%. The differences observed in the two studies could be attributed to breed 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 may have decreased the production of these blood components. The lymphocyte count of group 3 rabbits was slightly above the normal range of (53.5 - 65.8%) recommended by Kronfield and Mediway (1979) for healthy rabbits raised in the temperate climate. It is probable that the raised lymphocyte count could be an indication that the rabbits were immunologically challenged. The elevated lymphocyte counts of rabbits on group 3 could be a physiological adjustment presented by the animals against depressive effect associated with the neem leaf meal 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 creatinine values of rabbit does on group 4 was an indication that neem leaf meal diet was of poor quality relative to the control diet. This suggests that there was little breakdown of muscle tissues and those animals could be surviving at the expense of its 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 is an indication that the animals were tending towards negative nitrogen balance. This could be attributed to the presence of some of neem leaf bioactive compounds (Azadirachtin, nimbin, salanin) 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 serum cholesterol and serum glucose value were observed to decrease progressively with increasing levels of neem leaf meal in the diets. The reduction in serum cholesterol concentration (130.00 – 64.30 mg/dl) agreed with the hypocholesemic 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 agreed with the report that serum ALP could be influenced by changes in the physiological state of an animal.

4. Conclusion

It appears that rabbit does cannot tolerate the neem bioactive compounds 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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