Toxicological Effects of Leaf Meal of Ethnomedicinal Plant -Neem- on Serum Biochemistry of Crossbred New Zealand White Typed Rabbit Bucks

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Abstract: Due to high cost of feeding livestock in general and rabbits in particular with conventional feed ingredients in Nigeria. Research efforts are now geared towards identifying and exploiting novel feed ingredients which are not in strict competition with man's dietary need. This novel feed ingredient includes leaf meals of ethnomedicinal plants such as neem. Although neem leaf meal may have performed well as a nutrient source not much has been reported on its effect on serum biochemistry of crossbred New Zealand white typed rabbit bucks. Therefore the present study investigated the hazardous effects of neem leaf meal on serum biochemical characteristics of crossbred New Zealand white typed rabbit bucks. Rabbits with mean body weights of 1644.43gm were randomly assigned to four treatment groups (T_1, T_2, T_3 and T_4) on weight basis and fed neem leaf meal at 0%, 5%, 10% and 15% respectively in a completely randomized design experiment. Serum globulin values of bucks on T_2 and T_3 groups were significantly ($p<0.05$) lower than the T_4 bucks. The serum sodium levels of bucks on T_2 and T_4 groups were significantly ($p<0.05$) different from the bucks on control group. The T_3 and T_4 bucks had significantly ($p<0.05$) elevated serum urea value compared to bucks on T_1 and T_2 groups. Serum alkaline phosphatase values of bucks on T_2 and T_3 groups were significantly ($p<0.05$) affected by the treatment. The serum cholesterol and serum glucose levels of were significantly ($p<0.05$) reduced by treatment. All the other parameters were similar ($p>0.05$) among the treatment groups. It is therefore concluded that neem leaf meal based diets had severe depressive effects on serum cholesterol and serum glucose synthesis. [Report and Opinion 2010;2(2):54-57]. (ISSN: 1553-9873).

Keywords: rabbit bucks; neem leaf meal; serum biochemistry; phytotoxicity

1. Introduction

The worldwide shortage of animal protein sources particularly in developing countries in Africa has necessitated investigations of several novel feed ingredient sources for possible incorporation into animal feeds as replacements for the expensive conventional sources such as fish meal, maize, groundnut cake and soybeans. The acute shortage of animal protein has been attributed to the phenomenal rise in the prices of animal feeds which account for about 75-85% of the recurrent production inputs in intensive monogastric animal production (Fetuga, 1977).

The conventional feed ingredient sources such as maize, 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. The ban on the use of animal by-products in monogastric diets by the European Union has further put pressure on these conventional feed ingredient sources (Ravindran and Ravindran, 1988). Hence the need for research into non conventional feed ingredient sources such as leaf meal of ethnomedicinal plants as neem (*Azadirachta. Indica A. Juss*).

The neem plant, Family *milliaceae* is native of India and Burma and adapted favorably to the sub-sahelian Nigeria with severe drought, poor, shallow and even saline soil. The fruit yield is variable ranging from 10 - 50 kg per tree with an average of 20 kg (Schmutterer, 1995). Neem based products have been under-exploited despite abundance of the plant in northern Nigeria.

Biologically active principles isolated from different parts of the neem plant include: Azadirachtin, meliacin, gedunin, salanin, nimbin, valassin and many other derivatives of these principles. Miliacin forms the bitter principles of neem seed oil; the seed also contain tignic acid (5-methyl- 2-butanic acid) responsible for the distinctive odour of the oil (Lale, 2002). These compounds belong to natural products called triterpenoids. The active principles are slightly hydrophilic, but freely lipophilic and highly soluble in organic solvents like, hydrocarbon, alcohols, ketones and esters (Schmutterer, 1995).

The pesticidal activity of neem span a wide spectrum, having repellent, phagodeterrent (anti-

feedants), insect growth regulatory (IGR), anti-ovipositional, fecundity and fitness reducing properties on insects. These principles act as ecdysteroid analogues, which affect corpus cardiacum and block reproductive and growth processes in most insects causing sterility in females and degenerative changes in male testis due to disturbance in insect metabolism (Krauss *et al.*, 1987). Formulations like: Margosan O(R), Neemix (™), Azatin(R), NIM-20 and NIM-76, gave negative result with respect to toxicity effect on mammals (Govindachari *et al.*, 2000).

Despite the bitter components, livestock consume diets containing varied percentage of neem cake. However, nutritional efficiency and feed utilization were not achieved hence severe growth depression and about 50% mortality. Alkali treatment of neem cake with caustic soda yields palatable product, by removing the toxicant triterpenoids (Devakumar and Dev, 1993). Nagalakshmi *et al.* (1996) reported beneficial effect of alkali treated neem kernel cake incorporated into poultry feeds, in giving increased feeding value and protein utilization with spectacular growth. No significant difference was observed among the different dietary groups in feed intake, egg production, egg quality, fertility and hatchability.

The neem leaf extracts and NIM-76 act as powerful spermicide and significantly inhibited spermatogenesis, decreased sperm motility, count and cessation of fertility (Ogbuewu *et al*., 2009). These conditions were reversed by the withdrawal of neem products 4 - 6 weeks later (Sadre *et al.*, 1983). No significant effect on loss of libido or potency (Ogbuewu *et al*., 2009). Furthermore, neem seed oil possesses anti-implantation and abortifacient properties. Sinha *et al.* (1984) found spermatozoa of human and Rhesus monkey were immotile and die within 30 minutes of contact with neem seed oil in an intravaginal dose of 1.0 ml. Vaginal biopsy revealed no side effect, while radio-isotope studies indicate non-absorption in the vagina and non-antiovulatory (Sinha *et al.*, 1984). These findings enabled the use of neem oil formulation as a powerful contraceptive.

Although the beneficial effect of incorporating some neem products into livestock feeds in terms of increased feeding value and protein utilization has been reported. However, data on the effect neem leaf meal on serum biochemistry of male rabbits are lacking. Therefore, the present study was designed with the main objective of determining the toxicological effect of graded levels of neem leaf meal on serum biochemistry of rabbit bucks.

2. Materials and Methods

This study was carried out 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 is situated in south-eastern agro-ecological zone of Nigeria, and lies between latitude 4^o 4' and 6^o3' N and longitude 6^o15' and 8^o15'E.

Thirty - six crossbred New Zealand white typed rabbit bucks weighing 1644.43gms were procured from Shongai farm limited, Owerri. The trial lasted for 14 weeks inclusive of a 14 day stabilization period. These rabbits were randomly assigned on weight basis into four treatment groups of nine rabbits each. All the rabbits in this study were housed individually in wooden hutch placed in a naturally ventilated experimental room with temperature and relative humidity of about 30^oC and 70% respectively. They were fed with starter broiler ration for a two week during the acclimatization period. Feed and water were given at *ad libitum*.

Fresh matured neem leaves were harvested in and around the Federal University of Technology, Owerri. The chopped leaves were shade dried for about 9 hours every day for 3-4 days until they became crispy while retaining the greenish colouration. The sun dried leaves were milled using electric grinding machine to produce the neem leaf meal.

The ingredient compositions of the experimental buck diets on T_1 group were brewers spent grain (55%), white maize (35%), local fishmeal (3%), groundnut cake (3%), bone meal (2%), oyster shell (1.5%) and common salt (0.5%) with the following macronutrient compositions (as % of dry weight): crude protein (18.87), crude fibre (10.10), ether extract (5.97), calcium (1.41), phosphorus (0.66) and metabolisable energy (10.42MJ/Kg). The diets of bucks on T_2, T_3 and T_4 groups were also formulated by replacing brewer spent grain of T_1 diet with 5%, 10% and 15% Neem leaf meal (NLM) respectively. The T_2 group had the following macronutrient compositions (as % of dry weight): crude protein (18.70), crude fibre (10.78), ether extract (5.95), calcium (1.39), phosphorus (0.62) and metabolisable energy (10.38MJ/Kg). The T_3 group had the following macronutrient compositions (as % of dry weight): crude protein (18.53), crude fibre (11.02), ether extract (5.93), calcium (1.38), phosphorus (0.58) and metabolisable energy (10.33MJ/Kg) whereas the T_4 group were crude protein (18.37%), crude fibre (10.27%), ether extract (5.91%), calcium (1.36%), phosphorus (0.53%) and metabolisable energy (10.22MJ/Kg).

The blood collection was done at the end of the feeding trial. The animals were starved for 12 hours and bled between 08.00 h and 09.00h. Blood was randomly collected from the marginal ear vein of the three selected rabbits per treatment group. The selected rabbit was first removed from the hutch by holding it

securely on the scruff and the hind quarter supported underneath with the left hand. The ear from which the blood was to be drawn was 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. The blood was then collected immediately into a set of sterile plastic bottles without anti-coagulant for serum biochemical tests.

The serum biochemical analysis was 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 by procedures of Schales and Schales (1941) and serum enzymes (AST, ALT, ALP) by spectrophotometric method (Rej and Hoder, 1983).

Statistical differences between treatment means were determined with the one-way- analysis of variance for completely randomized design (Steel and Torrie, 1980) using computerized statistical analysis of SAS (1999). The experimental model used was completely randomized design (CRD) experiment ($Y_{ij} = \mu + T_i + e_{ij}$). Where significant differences were detected between treatment means and mean separation was carried out using Duncan's New Multiple Range Test as outlined by Steel and Torrie, (1980).

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3. Results

The total quantities of test ingredient consumed by each bucks and the data on serum biochemical constituents of rabbit bucks fed neem leaf meal are presented in figure 1 and Table 1 respectively. The body weights of the animals at before slaughter were found within the range of 1636.58 -1653.73g (Figure 2). The serum creatinine, serum albumin, and serum total protein value were similar ($p>0.05$) among the various treatment groups. The serum urea level of T_3 and T_4 bucks were significantly ($p<0.05$) different from the other two treatment groups. The serum globulin values of bucks on T_1 and T_3 groups were significantly ($p<0.05$) lower relative to T_1 and T_4 bucks

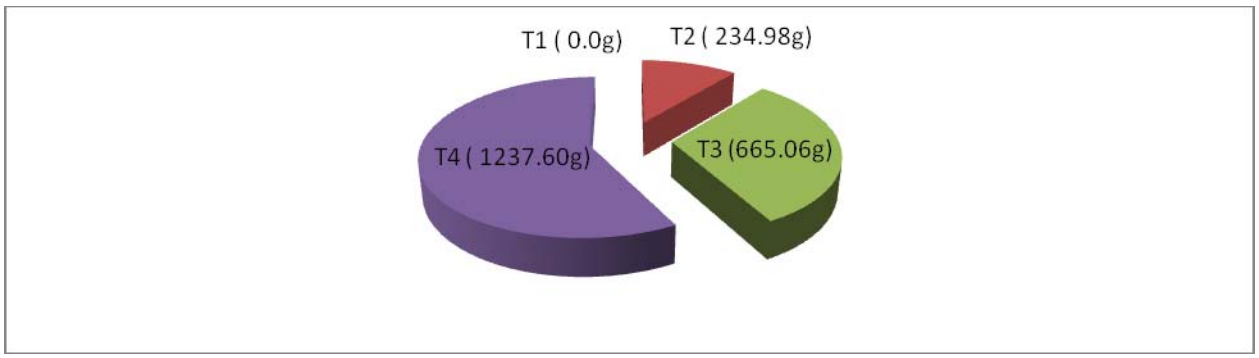

Figure 1. The total quantity of neem leaf meal consumed per rabbit for a duration of 112 days

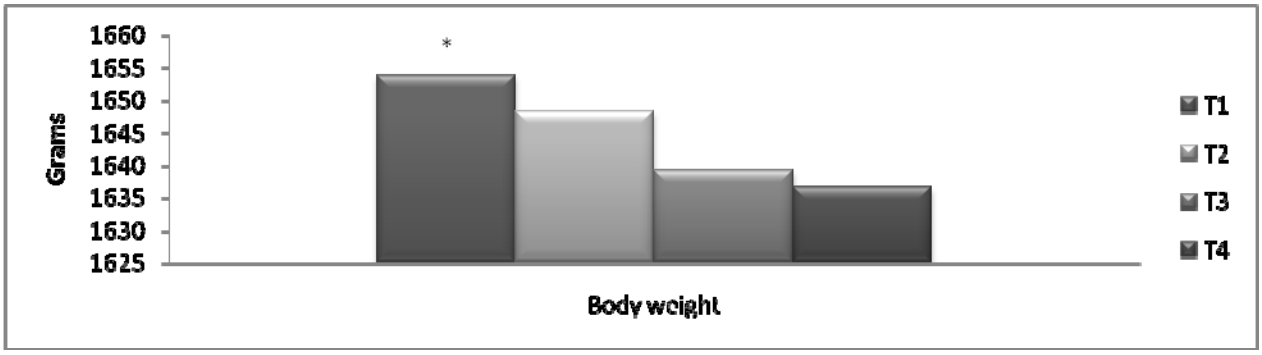

Figure 2. Effects of graded levels of neem leaf meal based diets on body weight of rabbit bucks; T_1 = **0% NLM (Control); T_2 = 5% NLM; T_3 = 10% NLM; T_4 = 15% NLM Post-treatment. Each bar represents mean ± SE. Significance: a = P < 0.05; compared with control.**

Table 1. Effect of neem leaf meal based diets on serum protein, serum urea and serum creatinine characteristics of crossbred New Zealand white typed rabbit bucks.

Parameters	T_1 (0% NLM)	T_2 (5%NLM)	T_3 (10%NLM)	T_4 (15% NLM)	S.E.M
Total protein (g/dl)	6.10	3.00	3.20	6.90	0.50
Globulin (g/dl)	4.70^{a}	2.10^{b}	1.50^{b}	5.10^{a}	0.38
Albumin (g/dl)	1.40^{ab}	0.90^{b}	1.70^{a}	1.80^{a}	2.10
Urea (mg/dl)	46.50^{b}	41.00^{b}	57.20^{a}	64.80^{a}	2.67
Creatinine (mg/dl)	0.80	0.70	1.20	1.20	0.07

^{ab} Means within a row with different superscript are significantly different at $p<0.05$.

The T_2, T_3 and T_4 bucks had significantly ($p<0.05$) lower serum glucose and serum cholesterol values when compared with the control bucks (Table 2). The serum bicarbonate, potassium and chloride value were similar ($p>0.05$) among the various treatment groups. The serum sodium value of the bucks on the control group was significantly ($p<0.05$) different from bucks on T_2 and T_4 groups.

Table 2. Effect of neem leaf meal based diets on serum glucose, cholesterol and serum electrolyte values crossbred New Zealand white typed rabbit bucks.

Parameters	T_1 (0% NLM)	T_2 (5%NLM)	T_3 (10%NLM)	T_4 (15% NLM)	S.E.M
Cholesterol (mg/dl)	174.60^{a}	115.20^{b}	95.40^{c}	56.50^{d}	12.31
Glucose (mg/dl)	63.50^{b}	75.80^{a}	48.30^{c}	18.00^{d}	6.24
Sodium (mmol/l)	198.60^{b}	155.50^{c}	203.40^{b}	269.20^{a}	11.73
Potassium (mmol/l)	4.40^{ab}	5.30^{a}	3.10^{b}	3.53^{a}	0.24
Chloride (mmol/l)	117.10^{b}	112.00^{b}	119.20^{b}	134.50^{a}	2.42
Bicarbonate (mmol/l)	26.40^{ab}	33.00^{a}	19.60^{b}	20.20^{b}	1.57

^{abcd} Means within a row with different superscript are significantly different at $p<0.05$.

The serum total bilirubin and conjugated bilirubin, alanine aminotransferase and aspartate aminotransferase were similar ($p>0.05$) among the treatment groups (Table 3). Rabbit bucks on control (T_1) group recorded the highest conjugated bilirubin value (0.30mg/dl) although not significantly ($p>0.05$) different from the other three treatment groups. Serum alkaline phosphatase value of 117.90 µ/l was obtained from rabbit bucks fed control differed significantly ($p<0.05$) from the other treatment groups.

Table 3. Effect of neem leaf meal based diets on serum bilirubin and serum enzyme characteristics of crossbred New Zealand white typed rabbit bucks.

Parameters	T_1 (0% NLM)	T_2 (5%NLM)	T_3 (10%NLM)	T_4 (15% NLM)	S.E.M
Total bilirubin (mg/dl)	0.40	0.40	0.30	0.40	0.01
Conj. bilirubin (mg/dl)	0.30	0.20	0.20	0.20	0.01
ALT (µ/l)	10.00	11.00	9.00	7.00	0.42
AST (µ/l)	15.00	17.00	13.00	11.00	0.65
ALP (µ/l)	117.90^{b}	97.70^{c}	130.90^{a}	105.10^{bc}	13.67

^{abc}Means within a row with different superscript are significantly different at $p<0.05$. AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, ALP- Alkaline phosphatase

4. Discussion

The total amount of neem leaf meal consumed by each animal during the 16 weeks feeding trial for the groups fed 0%, 5%, 10% and 15% neem leaf meal treatment was 0.00g, 234.98g, 665.06g and 1237.60g respectively. The reduction in the live weight of these animals beyond 5% NLM diet as observed in this study implied a reduction in growth rate. This growth reduction could be due to the presence of biologically active compounds present neem leaf which includes Azadirachtin, meliacin, gedunin, salanin, nimbin, valassin. It appears that these neem bioactive compounds are responsible for depression in nutrient utilization and growth in rabbits.

The reduction in the serum glucose value in the present study could be attributed to the presence of bioactive compounds contained in neem leaves which has the ability to block the energy metabolic pathway (Chattopadhyay, 1999; Ogbuewu, 2008), thus making it difficult for the animals to meet their energy requirements (Dutta *et al*., 1986). The non comparable serum urea value of bucks on control and those on 10%

NLM and 15% NLM was in line with Kenneth and Saladin (1998) who reported that in a state of negative nitrogen balance, muscle proteins are being broken down and used as energy. The increase in serum creatinine and serum urea level and corresponding decrease in serum glucose levels suggest that serum (urea and creatinine) and serum glucose level were negatively correlated in the present study. This is in with the report of Esonu *et al*. (2001) that animal will normally fall back to the stored energy in the muscles when there is a reduction in blood glucose level.

The presence of increasing urea and creatinine concentration in the blood is used in the evaluation of the effects of chemicals on the kidney (Davis and Berdt, 1994). The numerical increase in the value in serum creatinine of rabbit bucks on10% NLM and 15% NLM diet was in consonance with the findings of Omole and Sonaiya (1981) suggesting that there was wasting or catabolism of muscle tissues. The increase in serum urea implies an increase in rate of deamination in the liver. The significant reduction serum glucose value of rabbit bucks in all the groups support the possibility of increased rate of deamination of amino acids by the liver.

The serum cholesterol level was observed to decrease progressively with increasing dietary levels of neem leaf meal in this present study. This fall in serum cholesterol level of rabbit bucks on neem leaf meal diets probably suggest a general decrease in lipid mobilization. This could be that bioactive compounds in neem leaf meal have indirect inhibitory effects exerted at the levels of HMG-CoA reductase, a key enzyme in cholesterol biosynthesis. The results of serum electrolyte tended to show an improvement in the uptake of serum sodium and chloride while serum potassium and bicarbonates decreases with increasing levels of neem leaf meal. The serum electrolytes are used in maintaining the cellular tonicity, fluid balance, pH and regulation of neural and muscular functions (Cheesbrough, 2000). The results of the serum electrolyte tended to show an increase in the uptake of sodium ions and chloride ions with decreasing serum bicarbonate and serum potassium ions uptake. This implies that inclusion of up to 15% inclusion of NLM in rabbit buck's diet, the integrity of the kidney in boosting these cations and anions may have not been impaired severely at macro-anatomical level.

The serum conjugated bilirubin and serum total bilirubin value were similar among the treatment groups. The non-elevated values of total bilirubin and conjugated bilirubin risked out the possibility liver cell (hepatocytes) damage which is usually associated with increased serum conjugated bilirubin and total bilirubin (Cheesbrough, 2000). The serum alanine aminotransferase values obtained in this study were below the normal range of 12.0 – 18.0 µL while the serum aspartate serum transferase values were higher than the normal range of 9.0 – 12.0 µL as reported by Mitruka and Rawnsley (1977). The non significant decrease in serum aspartate aminotransferase and alanine aminotransferase (ALT) activities of animals on group T_3 and T_4 could indicate an improvement in liver function due to hepatoprotective activity of neem (Chattopadhyay *et al*., 2000). The serum alkaline phosphatase values were found within the standard range (17 - 192 µL) reported by Mitruka and Rawnsley (1977) for clinically healthy rabbits in the temperate climate. The observed variations in serum alanine aminotransferase, serum aspartate aminotransferase and serum alkaline phosphatase could be attributed to the active ingredients in neem leaf meal.

5. Conclusion

The association of neem leaf meal with severe reductions in the biosynthesis of serum cholesterol and glucose levels is a source of concern. Therefore detailed research effort should be directed towards the determination of histopathology of the liver, kidney and reproductive organs of male rabbits fed effect neem leaf meal.

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