Effect of neem (*Azadirachta indica*) leaf meal on serum metabolite profiles of male rabbits

Efecto de la harina de hojas de neem (*Azadirachta indica*) en los perfiles metabólicos séricos de conejos machos

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ABSTRACT

This study was undertaken to determine the effect of neem leaf meal (NLM) supplementation on metabolic of male rabbits. Male rabbits (36) with mean body weights of 2025 g were randomly allotted to four treatment groups (n = 9/group). Rabbits in CD_1, CD_2, CD_3 and CD_4 groups were fed diets containing 0% (control), 5%, 10% and 15% NLM, respectively in a completely randomized design.. The feeding trial lasted 16 weeks inclusive of a two week acclimatization period. At the end of the trial, the animals were starved for 12 hours and blood samples taken from the marginal ear vein. The serum globulin values of bucks on CD_2 and CD_3 groups were significantly ($p<0.05$) lower than those on CD_4. The serum sodium levels of bucks on CD_2 and CD_4 groups were significantly ($p<0.05$) different from the bucks on control group (CD_1). The bucks on CD_3 and CD_4 groups had significantly ($p<0.05$) elevated serum urea as compared to bucks on control group. The bucks on CD_2, CD_3 and CD_4 groups had significantly ($p<0.05$) lower serum glucose and cholesterol values relative to control group. It may be concluded that inclusion of NLM up to 15% in the ration of breeding male rabbits resulted a significant reduction in serum glucose and cholesterol values.

Key words: Neem leaf meal, serum, metabolites, male rabbits, biochemical profile

RESUMEN

El objetivo fue determinar el efecto de la harina de hojas de neem (HHN) en los perfiles metabólicos séricos de conejos machos. Conejos con un peso corporal promedio de 2025g se asignaron aleatoriamente a cuatro grupos de tratamiento (n = 9/grupo). Los conejos en los grupos CD1, CD2, CD3 y CD4 se alimentaron con dietas de HHN a 0% (control), 5%, 10% y 15%, respectivamente, en un experimento con un diseño completamente aleatorizado. El ensayo de alimentación duró 16 semanas incluyendo un período de aclimatación de dos semanas. Al final del ensayo, los animales ayunaron durante 12 horas y se le tomaron muestras de sangre de la vena marginal de la oreja. Las muestras de sangre se transfirieron inmediatamente dentro de botellas estériles de plástico sin anticoagulante para la prueba bioquímica sérica. Los valores séricos de globulina de los conejos en los grupos CD2 y CD3 fueron significativamente ($p<0,05$) menores que en el grupo CD4. Los niveles séricos de sodio de los conejos en los grupos CD2 y CD4 fueron significativamente ($p<0,05$) diferentes de los conejos en el grupo control (CD1). Los conejos en los grupos CD3 y CD4 tuvieron significativamente ($p <0,05$) un valor sérico de urea elevado en comparación con los conejos en el grupo control. Los conejos en los grupos CD2, CD3 y CD4 tuvieron significativamente ($p<0,05$) menores valores séricos de glucosa y colesterol en relación a aquellos del grupo control. Estos resultados sugieren que la inclusión hasta 15% HHN en la ración de conejos machos reproductores pudieran causar un efecto depresivo severo en los parámetros sanguíneos, especialmente los valores séricos de glucosa y colesterol.

Palabras clave: Harina de hojas de neem, conejos, pruebas bioquímicas del suero.

INTRODUCTION

Blood profiles are important indices of the physiological state of animals (Khan and Zafar, 2005). The ability to interpret the state of blood profiles in normal and diseased conditions is a primary objectives of haematological and serum biochemical studies. Research has proved that definite changes occur in the blood throughout life (Khan *et al*. 1987). The serum biochemical and haematological features have attracted many workers to look at their indices in order to make clinical predictions of the health status of a particular animal. The blood picture varies with certain conditions such as stress, infections and toxicity (Khan and Zafar, 2005).

Blood constituents provide valuable media for clinical investigations and nutritional evaluations of an animal (Aderemi, 2004). The ingestion of numerous dietary materials has been reported by Church *et al*. (1984) to have measurable effects on blood constituents. Thus, blood provides proximate measures for long term nutritional status of animals (Kerr *et al*., 1982). Consequently, blood sampling for the assay of biochemical constituents and haematological traits are frequently employed in nutritional studies.

With nutritional role of leaf meals in mind and its concomitant significance to animal health. Therefore, the present study was designed with the main objective of determining the effect of neem leaf meal based diets on serum biochemistry of breeding male rabbits.

MATERIALS AND METHODS

Experimental location

The 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, Nigeria. The project site lies between latitude 4°4' and 6°3'N and longitude 16°15' and 8°15'E. It 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 (< 65mm rainfall) and this covers December-February (Ibeawuchi *et al*., 2007).

Experimental animals

Thirty six male rabbit bucks weighing 1025 g were procured from Shongai farm limited, Owerri. The experiment lasted for 16 weeks including the 14 days acclimatization period. These rabbits were randomly separated on the basis of their weight into four treatment groups of nine rabbits each (CD_1, CD_2, CD_3, CD_4). 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°C and 70%, respectively. They were fed with starter broiler ration (Vital feed) for the two weeks of acclimatization. Feed and water were given *ad libitum*.

Processing of neem leaf meal

Fresh matured neem leaves were harvested in and around the Federal University of Technology, Owerri. The chopped leaves were sun 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 later milled using electric grinding machine to produce the neem leaf meal (NLM).

Experimental diets

The neem leaf meal (NLM) was used in the formulation of four rabbit diets (CD_1, CD_2, CD_3 and CD_4 containing NLM at 0, 5, 10 and 15%, respectively). The chemical composition of the experimental diets has been shown in Table 1.

Table 1. Ingredient composition of experimental diets fed to male rabbits.

Ingredients*	Diets (%)			
	CD_1	CD_2	CD_3	CD_4
Brewer spent grain	55.00	-	-	-
Neem leaf meal	-	5	10	15
Calculated nutrient composition				
Crude protein (%)	18.87	18.70	18.53	18.37
Crude fibre (%)	10.10	10.78	11.02	10.27
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)	10.42	10.38	10.33	10.22

* Each diet contained white maize (35%), local fishmeal (3%), groundnut cake (3%), bone meal (2%), oyster shell (1.5%) and common salt (0.5%).

The daily consumption of neem leaf meal was 0.0, 2.1, 5.94 and 11.05 g for CD_1, CD_2, CD_3 and CD_4 groups, respectively. The total amount of neem leaf meal consumed by each animal over the 16 weeks feeding trial was 0.0, 234.98, 665.06 and 1237.60 g in CD_1, CD_2, CD_3 and CD_4 groups, respectively.

Blood collection

The blood collection was done at the end of the feeding trial. The animals were starved for 12 hours and bled between 9.00 to 10.30 a-m. Blood was randomly collected from the marginal ear vein of three selected rabbits per treatment group. The 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 and blood was aspirated. This was rhen drained into a set of sterile plastic bottles without anti-coagulant to harvest serum for biochemical tests.

Serum biochemical analysis

The serum biochemical assay 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 and chloride ions (Schales and Schales, 1941) and serum enzymes (AST, ALT, ALP) by spectrophotometric method (Rej and Hoder, 1983).

Data 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 New Multiple Range Test (Obi, 1990).

RESULTS

Data on the effects of neem leaf meal on serum biochemical constituents of rabbit bucks are presented in Table 2. The serum creatinine, albumin,

Table 2. Effect of graded levels of neem leaf based diets on serum biochemical values of male rabbits

Parameters	CD_1 (0% NLM)	CD_2 (5%NLM)	CD_3 (10%NLM)	CD_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
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
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 superscripts are significantly different at $p<0.05$;
NLM: Neem leaf meal, SEM: Standard error of the mean AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline Phosphatase

total protein, $HC0^{-}_{3}$, K^{+}, Cl^{-}, total bilirubin and conjugated bilirubin, alanine aminotransferase and aspartate aminotransferase values were similar ($p>0.05$) among the various treatment groups. The serum urea level of bucks on CD_3 and CD_4 groups were significantly ($p<0.05$) different from the bucks on CD_1 and CD_2 groups. Serum globulin values of bucks on CD_2 and CD_3 groups were significantly ($p<0.05$) lower than the groups on CD_1 and CD_4. The serum sodium value of the bucks on the control group was significantly ($p<0.05$) different from the groups on CD_2 and CD_4. The bucks on group CD_3 and CD_4 had significantly ($p<0.05$) elevated serum urea values relative to the control group. The bucks fed on various treatment groups had significantly ($p<0.05$) lower serum glucose and serum cholesterol values except CD_2 which had higher ($p<0.05$) glucose level as compared to control bucks.

DISCUSSION

The reduction in serum glucose value in the present study 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). The non comparable serum urea value of bucks on control and those on CD_3 and CD_4 are in agreement with the findings of 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 urea levels and the corresponding decrease in serum glucose levels suggest that serum (urea and creatinine) and serum glucose levels were negatively correlated in the present study. This is in support of Esonu *et al*. (2001) that animals will normally fall back on the stored energy in the muscles when there is reduction in blood glucose level.

The urea and creatinine concentrations in the blood were used as kidney function test (Davis and Berdt, 1994). The non significant differences observed in blood proteins and creatinine in this experiment could be compared with earlier report of protein retained in animals (Akintola and Abiola, 1999). Iyayi and Tewe (1998) and Awosanya *et al.* (2000) reported the dependence of blood proteins and creatinine on the quality and quantity of dietary proteins.

The serum cholesterol level was found to be decreased progressively with increasing dietary levels of neem leaf meal in rabbit bucks. This observation probably suggests a general decrease in lipid mobilization. It is possible that NLM has 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 decreased with increasing levels of neem leaf meal. Serum electrolytes are used in maintaining cellular tonicity, fluid balance, pH and regulation of neural and muscular functions (Cheesbrough, 2000). The results of the serum electrolyte tended to show an improvement in the uptake of sodium ions and chloride ions while bicarbonate and potassium ions uptake decreased with increased levels of NLM diets. This suggests that, with up to 15 % inclusion of NLM diets, the ability of the kidney in boosting these cations and anions is not impaired.

The serum conjugated bilirubin and serum total bilirubin values were similar among the treatment groups. The non-elevated values of total bilirubin and conjugated bilirubin suggest no liver damage which is usually associated with increased serum conjugated bilirubin and total bilirubin (Cheesbrough, 2000). Serum alanine aminotransferase values obtained in this study were below the normal range of 12 - 18 µL while the serum aspartate and serum transferase values were higher than the normal range of 9.0 - 12 µL as reported by Mitruka and Rawnsley (1977). The non significant decrease in serum AST and alanine aminotransferase (ALT) activities of animals on group CD_3 and CD_4 could indicate an improvement in liver function due to hepatoprotective activity of neem (Chattopadhyay *et al*., 2000). The serum alkaline phosphatase values were 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 ALT, serum aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP) could be attributed to environmental and sex differences.

CONCLUSION

It may be concluded that inclusion of neem leaf meal up to 15% in the diets of rabbits resulted in significant reductions in serum cholesterol and

glucose levels. The reduction in serum cholesterol value of the rabbit bucks fed neem leaf meal based diets is an indication that neem leaves could reduce the deposition of cholesterol in the skin and muscles. The reduction in serum cholesterol is a positive development since low cholesterol meats command high market price.

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