Blood chemistry and relative organ weights of rabbits fed neem leaf meal based diets

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Abstract

Two experiments were conducted to determine the effect of neem leaf meal (NLM) based diets on blood and relative organ weight characteristics of rabbits. In the first experiment, 36 rabbit bucks aged 7 to 8 months were distributed into four dietary groups, T_1 , T_2 , T_3 and T_4 of 9 rabbits using completely randomized design. Each group was replicated 3 times with 3 rabbits per replicate. Rabbits in T_1 group were fed diet that contained 0% NLM while those in T_2 , T_3 and T_4 groups were fed diets that contained NLM at 5%, 10% and 15%, respectively for 112 days. In the second experiment, 36 rabbit does were used and experimental procedures were laid out as described in experiment 1. Blood samples for analysis, live weight and organ weights were collected from each replicate and data obtained analysed statistically. Higher ($p < 0.05$) kidney weight was obtained for rabbit does on diets T_2 , T_3 and T_4 . Bucks on diet T_2 recorded significantly ($p < 0.05$) higher pancreas weight than those on diet T_1 diet. No significant effect ($p > 0.05$) on haematological parameters was observed among all treatments except for MCV and neutrophils which were significantly ($p < 0.05$) decreased in the groups fed diets T_2 and T_3 and diets T_3 and T_4 groups respectively than others. Rabbit bucks and does on diet T_1 recorded significantly ($p < 0.05$) higher serum cholesterol values than those on the other diets. Serum glucose value of rabbits on diet T_1 was significantly ($p < 0.05$) different from those on the other diets. Serum ALP value of rabbits on diet T_1 differed significantly ($p < 0.05$) from those on the other 3 diets with the exception of bucks on diet T_1 that had similar value with those on diet T_2 . Addition of neem leaf meal in rabbit diets increased serum AST and ALT values but the differences were not significant ($p > 0.05$). It was therefore concluded that up to 5% of neem leaf meal can be included in the diet of pubertal rabbits without any deleterious effects on their organs and blood characteristics.

Keywords: Rabbits, neem leaf meal, haematology, serum biochemistry, organs

Introduction

Livestock production occupies approximately 30% of global arable land. With fast depletion of natural resources, ever increasing population pressure and rising living standards, it has become extremely important to diversify the present day animal agriculture to meet increasing demand for animal products. From 2000 to 2020, the global meat and milk consumption is expected to rise from 233 to 300 million tonnes and from 568 to 700

million tonnes, respectively. Egg production will also increase by 30%. An area of livestock production that calls for critical examination is the availability of feed resources.

The cost of livestock feed alone account for about 70% of the total cost of production of monogastric animals and this has led to unprofitability livestock production (Esonu *et al.*, 2006). There is the need to use unconventional feed ingredients that are cheaper, affordable and easily obtainable in

order to reduce feed production for monogastric animals. One of such feedstuff is neem [*Azadirachta indica* A Juss] leaf. Neem is a medicinal plant that is widely grown in Nigeria. It is known by names such as *Ogwu-Iba* in Ibo land and *Dogonyaro* in Hausa (Ogbuewu *et al.*, 2011). Neem belongs to the family *Maliaceae* and is the most versatile, multifarious trees of tropics, with immense potentials (Esonu *et al.*, 2006; Ogbuewu *et al.*, 2011). It has proximate compositions of 92.42% dry matter, 7.58% moisture, 20.68% crude protein, 16.60% crude fibre, 4.13% ether extract, 7.10% ash and 43.91% nitrogen free extract (Obikaonu *et al.*, 2011). Neem leaves like most tropical tree leaves contain bioactive compounds (Akpan *et al.*, 2008; Ogbuewu *et al.*, 2011) which may affect nutrient utilization and hematochemical constituents of animals.

Blood is an important index of the physiological state of animals (Ogbuewu *et al.*, 2009; Ogbuewu *et al.*, 2010). Haematochemical constituents provide valuable media for clinical investigations and nutritional evaluations of an animal (Esonu *et al.*, 2006). The ingestion of numerous dietary materials has been reported by Ogbuewu (2011) to have measurable effects on blood constituents. Thus, blood provides accurate measures for long term determination of the nutritional status of an animal (Esonu *et al.*, 2006; Ogbuewu *et al.*, 2013). Hence, this study was aimed at determining the optimal inclusion levels of neem leaf meal in rabbits using such physiological parameters as internal organ weight and blood characteristics of rabbits.

Materials and Methods

The study was conducted at the Rabbit Unit of the Teaching and Research Farm, School of Agriculture and Agricultural

Technology, Department of Animal Science and Technology, Federal University of Technology, Owerri. Owerri is located in the tropical rainforest zone of southeastern Nigeria within latitude 4°4' and 6°3'N and longitude 16°15' and 8°15'E. Fresh matured neem leaves were harvested in and around the Federal University of Technology, Owerri, Imo - State, Nigeria. The harvested leaves without petioles were shade dried for 5 days on a platform, milled according to the procedures described by Esonu *et al.* (2006) and stored in unused feed bags.

Four experimental diets were formulated such that diet T₁ (control) contained no NLM while diets T₂, T₃ and T₄ contained 5, 10 and 15% NLM, respectively. Seventy two, 6 - 8 months old sexed New Zealand White × Chinchila rabbits were procured from Emii Farms Limited, in Owerri for the experiment. Male and female rabbits were divided into two groups of 36 males (Experiment 1) and 36 females (Experiment 2). Each group was further divided into 4 treatments of 9 animals with each group subdivided into three replicates of 3 rabbits each on weight equalization basis. The mean initial weights were ranged between 1033.33 - 1081.25g and 675.00 - 700.00g for the males and females respectively. Each treatment group was assigned to one of the experimental diets (Table 1) in a completely randomized design. Rabbits were housed in individual cells measuring 50 cm × 50 cm × 30 cm in a 2 - tier hutch. The experimental rabbits were stabilized for two weeks before the commencement of the actual experiment. The hutches and its surroundings were kept clean using Izal disinfectant. Routine practices such as sweeping and washing of floor and feeding troughs were done regularly. Water and feed were given *ad libitum*.

At the end of the 16 weeks, between the hours of 9am and 10am, one rabbit whose weight was close to the average was selected from each replicate, tagged and deprived of feed but not water for 24 hours. They were slaughtered and eviscerated to determine the internal organ weights. In addition, 8 mL of blood was collected from each of the slaughtered animals. Afterwards, 5 ml of the collected blood was transferred into sterile ethylene diamine tetra-acetic acid (EDTA) treated Bijou bottles for haematological assays while 3 ml was transferred into a Bijou bottle without EDTA for serum biochemical analysis. Haematological parameters such as packed cell volume, red blood cell, white blood cell and haemoglobin were determined (Schalm, 1975). Mean cell haemoglobin, Mean cell volume and Mean cell haemoglobin concentration were computed using appropriate formulae. Serum biochemical parameters such as serum protein (total serum protein, albumin and globulin), urea, creatinine, glucose, cholesterol, electrolyte (sodium, potassium, chloride and bicarbonate), total bilirubin, conjugated bilirubin and serum enzymes (AST, ALT and ALP) were determined using standard methods (Mitruka and Rawnsley, 1977).

All the data collected were analysed statistically using one way Analysis of variance (ANOVA). Significant means were separated using the Duncan's New Multiple Range Test (Obi, 1990).

Results and Discussion

The results of relative organ weights characteristics of female rabbits are presented in Table 2. Final live weight was not affected by the treatments ($p>0.05$). There were no significant differences ($p>0.05$) in their lungs, heart and pancreas

weights. Rabbit does on diet T_1 recorded significantly lower ($p<0.05$) percentage kidney weight than those on diets T_2 , T_3 and T_4 . There was no significant difference ($p>0.05$) in the liver weight of rabbit does on diet T_1 and those on diets T_2 , T_3 and T_4 but those on diet T_2 differed significantly ($p<0.05$) from those on diet T_3 . Data on relative organ weights characteristics of rabbit bucks are presented in Table 3. Results indicated that live weights were not significantly affected by the treatments ($p>0.05$). Heart and lungs weights were not affected by the treatments ($p>0.05$). Rabbit bucks on diets T_1 , T_3 and T_4 had similar ($p>0.05$) pancreas weight than those on diet T_2 , while those on diet T_2 had significantly ($p<0.05$) higher weight. There was no significant difference ($p>0.05$) in liver weight of bucks on diets T_2 , T_3 and T_4 and those on diet T_2 but those on diet T_1 differed significantly ($p<0.05$) from those on diet T_4 .

It is a common practice in feeding studies to use weights of some internal organs like liver and kidneys as indicators of toxicity. Examinations of organ weights and histopathological findings are considered the very important aspects of any routine studies because they reveal many of the age related, naturally occurring lesions of the animals used (Ogbuenu, 2011). Our results revealed that T_2 (male) and T_4 (females) data across all parameters measured remained within the superior brackets, thus indicating higher percentage yields of these organs by animals in this group. The significant increase in the weight of the kidney of rabbit does at on diets T_1 , T_3 and T_4 relative to the control rabbits could be attributed in part to increased metabolic activity carried out in the kidney (Cheesbrough, 2000), which corroborates Bawa *et al.* (2006) and Abu and Tanimowo (2012), who reported

Table 1. Composition of the experimental rabbit diets with graded levels of neem leaf meal

Ingredients**	Inclusion levels of Neem leaf meal (NLM)			
	T ₁ (0%)	T ₂ (5%)	T ₃ (10%)	T ₄ (15%)
Maize	35.00	35.00	35.00	35.00
Neem leaf meal	0.00	5.00	10.00	15.00
Spent grains	55.00	50.00	45.00	40.00
Local fishmeal	3.00	3.00	3.00	3.00
Groundnut cake	3.00	3.00	3.00	3.00
Common salt	0.50	0.50	0.50	0.50
Bone meal	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00
Vitamin / mineral premix	0.50	0.50	0.50	0.50
Calculated chemical 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

Vitamin and mineral premix contributed the following to each kilogram of diet: Vit. A 500 IU; Vit. D₂ 1500 IU; Vit. E 3 IU; Vit. K₂ 2 mg; Riboflavin 3 mg; Pantothenic acid 6 mg; Niacin 15 mg; Vit B₁₂ 0.8 mg; Choline, 3 mg; Folic acid 4 mg; Mn 8 mg; Zn 0.5 mg; Iodine 1.0 mg; Co 1.2 mg

that larger organs are attributed to anti-nutritional factors. The non significant differences in liver weight across the treatments could be attributed to the hepatoprotective ability of some phytochemical constituents contained in neem (Ogbuewu *et al.*, 2011).

The results of haematological values of rabbit does fed neem leaf meal based diets are presented in Table 4. All the haematological parameters measured were similar ($p>0.05$) among the rabbit does with the exception of neutrophils and MCV. Similar ($p>0.05$) mean MCV values were obtained for rabbit does on diet T₁ and those on diets T₂ but differed significantly ($p<0.05$) from those on T₃ and T₄. The mild depressive effect of NLM on PCV and Hb concentrations observed in this study is in agreement with the findings of Bawa *et al.* (2006) but at variance with the observation of Ogbuewu *et al.* (2010) who reported slight increase in values of Hb and PCV of

female rabbits fed NLM at 5%, 10% and 15%. The result show that the neutrophil value followed a downward trend as the inclusion levels of NLM increased from 5% to 10% but rose up at 15% inclusion level. Neutrophil count of rabbits on diet T₁ differed significantly ($p<0.05$) from those on diets T₂ and T₃ but similar to those on diet T₄. The neutrophil results (27.00 – 39.00%) fall within the normal range (35.02 - 43.2) reported by Mitruka and Rawnsley (1997) for healthy rabbits. The observed significant reduction in neutrophil in the current study could be an indication that inclusion of NLM at 5% and 10% do not support the production of this major blood component. Lymphocyte count of rabbits on diets T₂ and T₃ was slightly above the normal range of 53.5 - 65.8% reported by Mitruka and Rawnsley (1977) for clinically healthy rabbits. The raised lymphocyte count of rabbit does on diets T₂ and T₃ was an indication that these rabbits were

Table 2. Live weight and relative organs weight characteristics of rabbit does fed neem leaf meal diets

Parameters	Inclusion levels of Neem leaf meal (NLM)				S.E.M
	T ₁ (0%)	T ₂ (5%)	T ₃ (10%)	T ₄ (15%)	
Initial live weight (g)	700.00	675.00	687.50	693.75	6.450
Final live weight (g)	1210.91 ^a	1213.44 ^a	1192.80 ^{ab}	1147.26 ^{ab}	3.659
Heart weight (% live weight)	0.18	0.21	0.20	0.22	0.003
Lungs weight (% live weight)	0.72 ^a	0.59 ^a	0.45 ^{ab}	0.48 ^{ab}	0.030
Kidney weight (% live weight)	0.53 ^b	0.81 ^a	0.71 ^a	0.73 ^a	0.030
Pancreas weight (% live weight)	0.02	0.04	0.02	0.04	0.003
Liver weight (% live weight)	2.80 ^{ab}	2.34 ^b	3.43 ^a	2.78 ^{ab}	0.112

^{a,b}Means on the same row with different superscripts are significantly ($p < 0.05$) different, S.E.M – Standard error of the mean

Table 3. Live weight and relative organ weight characteristics of rabbit bucks fed neem leaf meal diets

Parameters	Inclusion levels of Neem leaf meal (NLM)				S.E.M
	T ₁ (0%)	T ₂ (5%)	T ₃ (10%)	T ₄ (15%)	
Initial live weight (g)	1033.33	1037.50	1050.00	1041.25	9.55
Final live weight (g)	1643.73 ^a	1639.29 ^{ab}	1623.21 ^{ab}	1647.56 ^a	10.66
Heart weight (% live weight)	0.18	0.18	0.22	0.21	0.005
Lungs weight (% live weight)	0.60 ^a	0.65 ^a	0.42 ^{ab}	0.39 ^{ab}	0.032
Kidney weight (% live weight)	0.75	0.69	0.63	0.77	0.016
Pancreas weight (% live weight)	0.03 ^b	0.06 ^a	0.04 ^{ab}	0.05 ^{ab}	0.003
Liver weight (% live weight)	2.58 ^{ab}	2.72 ^{ab}	2.21 ^b	2.94 ^a	0.077

^{a,b}Means on the same row with different superscripts are significantly ($p < 0.05$) different, S.E.M – Standard error of mean

immunologically challenged and this could be attributed to anti-nutritional factors (Ogbuenu, 2008) present in the experimental diet. The elevated blood

lymphocyte counts of rabbit does on diets T₂ and T₃ corroborated the earlier findings of Upadhyay *et al.* (1999) that neem leaves stimulates lymphocyte proliferation

Table 4: Haematological constituents of rabbit does fed neem leaf meal based diets

Parameters	Inclusion levels of Neem leaf meal (NLM)				S.E.M
	T ₁ (0%)	T ₂ (5%)	T ₃ (10%)	T ₄ (15%)	
Hb (g/dl)	12.20	12.60	12.40	12.00	0.07
PCV (%)	36.00	37.00	37.00	35.00	0.32
RBC ($\times 10^6/\text{mm}^3$)	3.60	4.20	3.80	4.00	0.07
MCV (fl)	105.6 ^a	88.00 ^b	97.40 ^a	87.50 ^b	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 ($\times 10^3/\text{mm}^3$)	8.80	9.90	8.70	8.50	0.16
Lymphocytes (%)	61.00 ^{ab}	66.00 ^{ab}	73.00 ^a	58.00 ^b	1.64
Neutrophils (%)	39.00 ^a	30.00 ^b	27.00 ^b	36.00 ^{ab}	1.37

^{a,b}Means on the same row with different superscripts are significantly ($p < 0.05$) different; Hb:

Haemoglobin; PCV - Packed Cell Volume; RBC - Red Blood Cells; WBC - White Blood Cells; MCV - Mean Cell Volume; MCH - Mean Cell Haemoglobin; MCHC - Mean Cell Haemoglobin Concentrations

responses by selectively activating the T-cells.

Data on serum biochemical characteristics of female and male rabbits fed neem leaf meal based diets are presented in Table 5 and Table 6, respectively. Serum protein, albumin and creatinine values of rabbits on diet T₁ were not significantly different ($p>0.05$) with those on the other diets. Serum globulin values of rabbit does on diet T₁ and bucks on diets T₂ and T₃ differed significantly ($p<0.05$) from those on diet T₁. Serum proteins have been explored extensively in nutritional studies to distinguish normal state from abnormal conditions in animals. Dietary components had shown to have measurable effects on blood protein components (Harper *et al.*, 1999; Awosanya *et al.*, 2000). The significantly low serum globulin value recorded for rabbit does on diet T₁ and

bucks on diets T₂ and T₃ indicate low ability of the diet to support production of this blood component. Serum creatinine values were within the normal range of 0.14 - 1.65 mg/dl reported by Mitraka and Rawnsley (1977). Bell *et al.* (1978) reported that in the cases of muscle wasting, serum creatinine values is characteristically higher than the recommended range. This is not supported in the present study, thus ruling out the possibility of muscle wasting (Mitraka and Rawnsley, 1977). Serum urea values of rabbit does were not significantly affected by the treatments ($p>0.05$). Bucks on diets T₂ and T₃ had significantly ($p<0.05$) higher serum urea values than those on diets T₁ and T₄. The significantly high serum urea values recorded for bucks on diets T₂ and T₃ could be attributed to poor nutrient utilization of the experimental as a result of anti-nutritional factors contained in the test

Table 5: Serum biochemical characteristics of female rabbits fed Neem leaf meal based diets

Parameters	Inclusion levels of Neem leaf meal (NLM)				S.E.M
	T ₀ (0%)	T ₅ (5%)	T ₁₀ (10%)	T ₁₅ (15%)	
Total protein (g/dl)	7.50	6.20	5.60	4.10	0.35
Globulin (g/dl)	4.30 ^a	2.60 ^{ab}	2.40 ^{ab}	0.70 ^c	1.37
Albumin (g/dl)	3.20	3.60	3.20	3.40	0.05
Urea (mg/dl)	66.40 ^{ab}	55.50 ^b	68.40 ^{ab}	77.30 ^a	2.24
Creatinine (mg/dl)	1.10	1.00	1.10	1.20	0.02
Cholesterol (mg/dl)	130.00 ^a	95.40 ^b	72.10 ^c	64.30 ^d	7.37
Glucose (mg/dl)	89.80 ^a	91.40 ^a	85.00 ^a	50.30 ^b	4.85
Sodium (mmol/l)	89.00 ^a	58.20 ^b	66.40 ^b	80.10 ^a	3.44
Potassium (mmol/l)	4.30	4.40	3.80	3.50	0.11
Chloride (mmol/l)	86.50 ^b	96.40 ^{ab}	100.00 ^a	103.60 ^a	1.84
Bicarbonate (mmol/l)	25.80	26.40	24.00	22.10	0.49
Total bilirubin (mg/dl)	0.30 ^b	0.30 ^b	0.50 ^a	0.40 ^{ab}	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)	52.10 ^d	81.60 ^b	93.70 ^a	65.80 ^c	4.54

^{abc} Means on the row with different superscripts are significantly ($p<0.05$) different; AST - Aspartate aminotransferase; ALT- Alanine aminotransferase; ALP - Alkaline phosphates; Conj. Bilirubin - Conjugated bilirubin.

Table 6. Serum biochemical characteristics of male rabbits fed neem leaf meal based diets

Parameters	Inclusion levels of Neem leaf meal (NLM)				S.E.M
	T ₁ (0%)	T ₂ (5%)	BT ₃ (10%)	T ₄ (15%)	
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 (μl)	10.00	11.00	9.00	7.00	0.42
AST (μl)	15.00	17.00	13.00	11.00	0.65
ALP (μl)	117.90 ^b	97.70 ^c	130.90 ^a	105.10 ^{bc}	13.67

^{abc} Means on the same row with different superscripts are significantly ($p < 0.05$) different; AST - Aspartate aminotransferase; ALT - Alanine aminotransferase; ALP - Alkaline phosphates

feedstuff (Ogbuewu, 2008). This is in agreement with the range of 60 - 70 mg/dl reported by Bawa *et al.* (2006) for growing rabbits fed differently processed neem seed meal that. Serum glucose value of rabbit bucks on diet T₁ was significantly higher ($p < 0.05$) than those on diets T₂, T₃ and T₄ while rabbit does on diet T₄ had significantly lower value than those on diet T₁. The significant reduction in serum glucose values may be attributed in part to the presence of some bioactive principles contained in plants that have the ability to block the energy metabolic pathway in animals (Dutta *et al.*, 1986).

Serum cholesterol values for the rabbit bucks and does were significantly affected by the treatments ($p < 0.05$). Serum cholesterol value decreased progressively with increasing levels of neem leaf meal in the diets. Clinical data strongly support a relationship between diets and serum cholesterol level (Konjucfa *et al.*, 1997).

This is supported in the current study. This finding agreed with the results of Obikaonu *et al.* (2011), who observed similar trend for the aforementioned parameters in a similar study. The significant reduction in serum cholesterol level of rabbits fed neem leaf meal diets probably suggest a hypocholesmic activity of neem (Chattopadhyay, 1996).

Serum sodium values for males on diet T₂ and females on diets T₂ and T₁ were significantly ($p < 0.05$) reduced compared to those on control diet (T₁). The results show that serum sodium values were higher than the range of 130 - 155 mmol/l reported by Mitruka and Rawnsley (1977) for healthy rabbits. The observed relative hypernatremia (high level of sodium) may be due to reduction in the fluid component of the blood from insufficient fluid intake (Aiello and Mays, 1998; Tiez, 1979). Neem leaf meal has high fibre (Ogbuewu, 2008) and sodium content

(Amhandin, 2010) which may result in dehydration. Values for serum potassium ranged from 3.10 – 3.50 mmol/l for males and 3.50 – 4.40 mmol/l for females; with males on diet T_3 having the lowest value which differ significantly ($p < 0.05$) from those on diets T_1 and T_4 . There was no significant difference ($p > 0.05$) in serum bicarbonate value of rabbits on diet T_1 with those on the other diets. Serum chloride values for females in all the treatments maintained an increasing trend; with rabbits on diets T_3 and T_4 differing significantly ($p < 0.05$) from those on control diet (T_1). Males on diet T_4 had significantly higher serum chloride value ($p < 0.05$) compared to those on the 3 other diets. Elevated serum chloride results in dehydration and hyperventilation; elevated sodium level is also found in diarrhoea and metabolic acidosis (Tietz, 1976). The elevated serum chloride level (hyperchloremia) observed on T_1 males may be due to slightly hepatomegaly (Schmidt-Nielsen and Duke, 1995) and high level of serum sodium (Aiello and Mays, 1998). This means that rabbits on diet T_1 contained toxic substances at a level enough to alter sodium and chloride levels in rabbits.

Serum conjugated bilirubin, AST and ALT values were similar ($p > 0.05$) among the dietary groups. The conjugated bilirubin values reported here were within the recommended range of 0 – 1.0 mg/dl reported by Mitruka and Rawnsley (1977). Serum total bilirubin concentration for female rabbits on T_3 treatment was significantly

($p < 0.05$) higher than those on T_1 and T_2 diets but similar to those in T_4 dietary groups. The significantly higher total bilirubin value recorded for female rabbits at 10% inclusion level suggests possibility of hepatocellular injury, which could be as a result of anti-nutritional factors (Ogbuewu, 2008) contained in the experimental diets. This could be the cause of significantly increased liver weight. Serum alkaline phosphatase values differed significantly ($p < 0.05$) among the treatment groups except for T_4 males that had similar values with those in T_1 and T_2 treatment groups. However, the observed difference in serum alkaline phosphatase values fall within the normal range (17 – 192 μ L) recommended by Mitruka and Rawnsley (1977) for clinically healthy rabbits. The serum ALT values were below the normal range of 12.0 – 18.0 μ L, while the serum AST values were higher than the normal range of 9.0 – 12.0 μ L as reported by Mitruka and Rawnsley (1977). Ellis *et al.* (1978) and Enemor *et al.* (2005) reported that in the cases of toxicity, serum alanine aminotransferase (ALT) is characteristically higher than the serum aspartate aminotransferase (AST). This is not supported in the present study, suggesting that anti-nutritional factors present in the experimental diets did not reached a threshold level beyond the tolerance level of the rabbits.

Conclusion

Dietary inclusion of neem leaf meal up to 5% in bucks and 15% in does tended to improve organ weight, and some

blood characteristics. From our results, neem leaf meal has demonstrated good potentials as immune booster as well as blood builder. However, the decline in serum glucose and cholesterol, and corresponding increase in serum urea as observed in this present study merit further research.

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