Pre and post-gestation blood indices of rabbit does fed graded levels of neem (*Azadirachta indica*) leaf meal

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Abstract

This study was conducted to investigate the effect of neem leaf meal on the blood status of rabbit does before and after gestation. A total of twenty-four (24) rabbit does of mixed breeds, with initial average live weight of 1975g were randomly allocated into four (4) experimental dietary treatments; each of which was replicated three (3) times with two (2) rabbits per replicate. The rabbits were fed diets containing 0%, 2.5%, 5.0%, and 7.5% neem (*Azadirachta indica*) leaf meal. Parameters measured included packed cell volume (PCV), haemoglobin, red blood cells (RBC), white blood cells (WBC) and its differentials for haematology while total protein, globulin, albumin, cholesterol, alkaline phosphate (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured for biochemical indices. All data generated were subjected to one-way Analysis of Variance in a Completely Randomized Design. The findings from this study at pre-gestation showed that neem leaf meal (NLM) significantly ($p < 0.05$) influenced basophil and serum total protein, globulin and glucose. Basophil was significantly reduced in rabbits fed 5.0% NLM (0.33%) while the serum components were affected in rabbits fed 2.5% NLM. The result at post-gestation showed no significant ($p > 0.05$) difference in all the parameters measured except neutrophils. Neutrophils were significantly ($p < 0.05$) reduced in rabbits on the control diet (26.67%) while rabbits on NLM diets recorded similar values. From the serum component, cholesterol was significantly ($p < 0.05$) highest in rabbits fed 5.0% NLM (62.33mg/dL) and lowest in those fed 2.5% NLM (49.67mg/dL). It was, therefore, concluded that inclusion of NLM in rabbit does' diet up to 7.5% had no adverse effect on the health status of the animals as the values obtained for neutrophils and cholesterol were still within the normal range of rabbit blood profile.

Keywords: Rabbit does, Neem leaf meal, gestation, haematology, serum biochemical indices

Introduction

In recent time, the interest of Nigerian animal producers has been centred mostly on the search for cheaper feed ingredients that are always available and have no competition with man's dietary demands (Esmail, 2002). Some agro-industrial by-products and plants have been used with varied levels of success; thereby increasing the raw material base for livestock feed formulation. *Azadirachta indica* is a hardy plant from the family Milliaceae. It is popularly known as Neem tree and is native of India and Burma, and is adapted

favourably to areas with severe drought, poor, shallow and even saline soil (Ogbuewu *et al.*, 2011a). Neem tree (*Azadirachta indica*) is one of the tropical plants that have attracted attention of animal nutritionists (Esonu *et al.*, 2005). Neem leaves contain approximately 20.68% crude protein, 16.60% crude fibre and 4.13% fat after processing into leaf meal via drying and milling (Oforjindu, 2006). Rabbit as micro-livestock is socially acceptable on the combined basis of space requirement and absence of religious taboos as well as peculiar digestive physiology which

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permits the use of forages and agro-industrial by products, thus making it non-competitive species with man for cereals and legume grains (Ahemen *et al.*, 2013). Most emphasis by animal nutritionist is usually on nutrient digestibility, utilization and performance of the farm animals, but the future health status of the animal used in feeding trials either with conventional or non-conventional feedstuffs should also be considered. Blood contains a myriad of metabolites and other constituents, which provide a valuable medium for clinical investigation and assessment of nutritional status of human beings and animals. Dietary components have measurable effects on blood components; hence, blood constituents are widely used in nutritional evaluation and survey of animals (Olorode *et al.*, 1995). Hence, Aderemi (2004) reported that blood examination gives the opportunity to investigate the presence of several metabolites and other constituents and helps detect conditions of stress, which can be nutritional, environmental or physical. It is generally acknowledged that gestation induces progressive alterations in several haematology and blood biochemistry parameters in animals. Various reports involving animals fed Neem leaf meals indicate some negative effects on reproductive performances such as age at first kindling, litter size at weaning, weaning weight, re-mating interval, and semen quality (Ogbuewu *et al.*, 2011a; 2011b). Herbert (1998) reported a depressed reproductive performance in rabbits following inclusion of high level of leucaena and gliricidia leaf meal in the diet, especially during gestation and lactation. This study, therefore, investigated the pre and post-gestation blood indices of rabbit does fed diets containing graded levels of neem (*Azadirachta indica*) leaf meal.

Materials and methods

Experimental site

The experiment was carried out at the Rabbitary Unit of the Directorate of University Farms (DUFARMS) of the Federal University of Agriculture, Abeokuta in Odeda Local Government Area of Ogun State, Nigeria. The site falls within the savanna agro-ecological transition zone, Southwest Nigeria, on latitude 7° 10' N and longitude 3° 2' E, it received a mean precipitation of 1037mm per annum an average temperature of 34.7°C and an average relative humidity of 82% throughout the year (Google Earth, 2015).

Experimental animals and management

The experiment was carried out using 24 rabbit does with initial average live weight of 1975g. These were divided into 4 dietary treatments having 6 rabbits per treatment and were housed individually. The rabbits were fed experimental diets supplemented with forages after a week of adaptation. Fresh water was provided for them in the morning and late in the evening. They were mated after six weeks of feeding the experimental diets. All other routine management such as disinfection of the hutches and stable, cleaning of feeders and drinkers, etc. were observed and medication was administered when needed. Kindling boxes were also placed inside the hutches on the 28th day of gestation. The experiment was carried out for a period of 14 weeks.

Experimental diets

Neem (*Azadirachta indica*) leaves were harvested by hand-plucking from the trees within Abeokuta metropolis. The leaves were air-dried and milled to form neem leaf meal (NLM) while other feed ingredients were sourced from a reputable feed mill. After this, experimental diets were formulated such that NLM were included at 0% (control), 2.5%, 5% and 7.5% levels for diets T1, T2, T3 and T4, respectively as shown on Table 1.

Table 1: Percentage composition of experimental diets

	T1	T2	T3	T4
	Level of Inclusion of NLM (%)			
Ingredients	0	2.5	5.0	7.5
Maize	40.00	40.00	40.00	40.00
Rice husk	30.00	27.50	25.00	22.50
NLM	0.00	2.50	5.00	7.50
Soya bean meal	24.00	24.00	24.00	24.00
Fish meal	1.00	1.00	1.00	1.00
Bone meal	3.00	3.00	3.00	3.00
Oyster shell	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25
Vit. Prix	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated Analysis				
ME (MJ/kg)	9.89	10.03	10.80	10.31
Crude protein (%)	15.93	16.02	16.11	16.20
Fibre (%)	12.71	12.19	11.67	11.15

NLM: Neem leaf meal

Blood Analyses: At the 6th (before mating) and 11th week of the experiment (a week after kindling), 2.5mL of blood sample each was collected from three rabbits per treatment into EDTA (ethylene diamine tetra acetic acid) bottle for haematological parameters such as packed cell volume (PCV), red blood cells (erythrocyte) count (RBC), haemoglobin (Hb), white blood cell (leukocyte) count and the differentials while for serum biochemical parameters, plain bottle was used for the blood sample collection to determine serum metabolites which include: total serum protein, glucose cholesterol, alkaline phosphate (ALP), aspartate transferase (AST), albumin and globulin.

Statistical analysis

Data generated were subjected to one-way Analysis of Variance in a Completely Randomized Design using the statistical package (SAS, 2007) while the significant means among treatments were separated using Duncan Multiple Range Test of the statistical package at 5% significant level.

Results and discussion

Pre-gestation haematological parameters of

rabbit does fed diets containing graded levels of neem leaf meal (NLM) are shown on Table 2. The packed cell volume, haemoglobin, red blood cells, white blood cells, neutrophils, lymphocytes and eosinophils, MCV, MCH, and MCHC of rabbits fed test diets and the control were not significantly influenced by the dietary treatments and were within the normal physiological ranges reported for rabbits (Jenkins, 1993; Hillyer, 1994), except basophil which was significantly ($p < 0.05$) reduced in rabbits fed diet containing 5.0% NLM. The value (0.33%) was below the lower limit of the normal range for rabbits which is an indication that the animals were predisposed to high risk of infection. Madubuike and Ekenyem (2006) indicated that haematological characteristics of livestock suggested their physiological disposition to the plane of nutrition. The Red Blood cells indices (MCH, MCV, and MCHC) are important morphological characteristics of anaemia (Campbell, 1988). This is perhaps an indication that the rabbits were not anaemic before gestation.

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Table 2: Pre-gestation haematological parameters of rabbit does fed neem leaf meal diets

Parameter	Level of Inclusion of NLM (%)				SEM
	0	2.5	5.0	7.5	
Pack Cell Volume (%)	30.67	32.00	30.33	32.00	1.19
Haemoglobin (g/dl)	10.30	11.13	9.57	9.90	0.36
Red Blood Cell ($\times 10^{12}/L$)	5.80	5.47	5.70	5.83	0.26
White Blood Cell ($\times 10^9/l$)	5.43	6.57	6.40	6.83	0.27
Neutrophil (%)	30.00	27.67	33.33	31.00	1.42
Lymphocyte (%)	67.33	67.00	64.67	66.33	1.45
Eosinophil (%)	0.33	1.00	0.33	0.67	0.19
Monocytes (%)	1.00	1.67	1.33	1.00	0.29
Basophil (%)	1.33 ^{ab}	2.67 ^a	0.33 ^b	1.00 ^{ab}	0.38
MCH (pg)	18.70	20.37	17.27	17.83	1.30
MCHC(g/dl)	34.33	34.80	31.67	31.00	0.99
MCV (fl)	56.27	58.53	54.03	57.17	3.85

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

MCH- Mean corpuscular haemoglobin, MCHC-Mean corpuscular haemoglobin concentration, MCV- Mean corpuscular volume

Significant effect of diet was not observed on all the parameters studied for serum biochemical components at pre-gestation except total protein, globulin and serum glucose that were significantly ($p < 0.05$) reduced in rabbits fed 2.5% NLM diet. These reduced values at 2.5% NLM are an indication that NLM will not be able to supply same level of protein and energy for the animals as the control at 2.5% but at a

higher level above 2.5%. The increase in globulin of rabbits on the control and trial diets above 2.5% in this study may suggest increased immunity which would predispose the animals for better performance. According to Keeney and Finlay (2011), stimulation of immune response is among the mechanisms established for improvements in animal performance.

Table 3: Pre-gestation serum biochemical indices of rabbit does fed neem leaf meal diets

Parameter	Level of Inclusion of NLM (%)				SEM
	0	2.5	5.0	7.5	
Total Protein (g/dL)	8.70 ^a	5.34 ^b	9.70 ^a	9.70 ^a	0.17
Albumin (g/dL)	4.77	3.67	4.80	4.97	0.26
Globulin (g/dL)	3.93 ^a	1.67 ^b	4.90 ^a	4.73 ^a	0.45
Alkaline phosphate (U/L)	35.33	39.67	40.00	38.33	1.37
Alkaline aminotransefrase (U/L)	21.67	18.67	23.67	26.33	1.59
Aspartate aminotransferase (U/L)	36.67	33.00	52.67	40.33	4.09
Cholesterol (mg/dL)	64.33	78.00	88.67	72.00	4.92
Glucose (mg/dL)	102.00 ^a	52.00 ^b	93.00 ^a	84.67 ^{ab}	7.49

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

The effect of NLM on the haematological parameters of post-gestational rabbit does is presented in Table 4. Haematological indices are an index and a reflection of the effects of dietary treatments on animals in terms of the quality of feed ingested and nutrients available to an animal to meet its physiological requirements (Olorode *et al.*,

1995). The values recorded for haematological parameters at post-gestation were not significantly ($P > 0.05$) affected by the different levels of inclusion of NLM except neutrophil and were within the normal ranges reported for healthy rabbit. This non-significant difference could be an indication of the wellness of the

animals throughout the gestation period as normal haematological parameters of an animal are direct indications of absence of disease (Olafadehan *et al.*, 2010). The neutrophil values of the does on NLM diets were significantly ($p < 0.05$) higher than the mean value of those on the control (0% NLM) diet but were within the ranges 20-75% (Merck Manual, 2012) and 35 - 43.2% (Kronfield and Mediway, 1975; Mitruka and Rawnsley, 1977; Hewitt *et al.*, 1989), respectively, reported for clinically healthy rabbits. This could be explained by the intrigues that occur during gestation. White blood cell count is increased in pregnancy due to the physiologic stress induced by the pregnant state (Fleming,

1975). According to Gatti *et al.* (1994) and Konijnenberg and Stokkers (1997), neutrophils are the major type of leucocytes on differential counts; this is likely due to impaired neutrophilic apoptosis in pregnancy. The neutrophil cytoplasm shows toxic granulation. Neutrophil chemotaxis and phagocytic activity are depressed, especially due to inhibitory factors present in the serum of a pregnant female (Jessica *et al.*, 2007). There is also evidence of increased oxidative metabolism in neutrophils during pregnancy. The neutrophils are concerned with day to day immunological defense against pathogens. This implies that the ingestion of NLM did not trigger the production of this blood component.

Table 4: Post-gestation haematological parameters of rabbit does fed neem leaf meal diets

Parameter	Level of Inclusion of NLM (%)				
	0	2.5	5.0	7.5	SEM
Pack Cell Volume (%)	36.00	31.67	32.00	29.33	1.44
Haemoglobin (g/dl)	10.30	9.03	8.63	8.43	0.42
Red Blood Cell ($\times 10^{12}/L$)	6.00	5.33	5.77	5.33	0.21
White Blood Cell ($\times 10^9/l$)	7.57	7.60	8.30	7.60	0.30
Neutrophil (%)	26.67 ^b	32.67 ^a	31.33 ^a	31.67 ^a	0.89
Lymphocyte (%)	69.67	65.67	66.33	66.33	0.73
Eosinophil (%)	1.00	0.33	0.33	0.33	0.15
Monocytes (%)	2.00	1.00	1.00	0.33	0.29
MCH (pg)	17.23	16.90	14.93	15.87	0.50
MCHC(g/dl)	28.53	28.53	26.90	28.97	0.41
MCV (fl)	60.17	59.17	55.57	54.90	1.27

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

MCH- Mean corpuscular haemoglobin, MCHC-Mean corpuscular haemoglobin concentration, MCV- Mean corpuscular volume

The result obtained on the effect of NLM on the serum biochemical components is shown on Table 3. Serum biochemical analysis is used to determine the level of liver damage and to evaluate protein quality and amino acid requirements in animals (Harper *et al.*, 1979). Significant effect of diet was not observed in all the parameters studied except serum cholesterol. The serum cholesterol value of the rabbits fed 2.5% NLM was significantly ($p < 0.05$) lower than the mean value of those fed 5.0% NLM diet, while does on control (0%) and 7.5% NLM diets recorded similar mean

values. This might be an indication that NLM could reduce and not increase serum cholesterol beyond there commended range for clinically healthy rabbits. This was in agreement with the earlier findings of Oforjindu (2006) that NLM in the diets of broiler birds resulted to a decrease in the cholesterol and liver lipid levels. The serum enzymes activities assessed (ALT, AST and ALP) were within the normal range reported by CCAC (1980). This result corroborated the report of Ewuola *et al.* (2011) who observed that serum enzyme activities of gestating and lactating rabbits

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administered crude moringa extract were not significantly different from the control rabbits. This implied that animals fed test

diets were not adversely affected because of no indication of organ toxicity from the serum enzymes assessed.

Table 5: Post-gestation serum biochemical indices of rabbit does fed neem leaf meal diets

Parameter	Level of Inclusion of NLM (%)				SEM
	0	2.5	5.0	7.5	
Total Protein (g/dL)	5.43	5.60	5.53	5.23	0.17
Albumin (g/dl)	3.13	3.17	3.10	3.00	0.11
Globulin (g/dl)	2.30	2.43	2.43	2.23	0.07
Alakaline phosphate (U/L)	45.67	53.00	55.33	59.33	3.06
Alkaline amino transefrase (U/L)	22.00	22.67	28.00	29.67	1.41
Aspartate aminotransferase (U/L)	46.67	51.67	49.67	48.00	1.67
Cholesterol (mg/dl)	53.33 ^{ab}	49.67 ^b	62.33 ^a	58.33 ^{ab}	1.97
Glucose (mg/dL)	83.33	90.33	93.00	99.00	4.07

^{a,b}: Means with different superscripts along the same row are significantly (p<0.05) different.

Conclusion

Inclusion of NLM in rabbit does' diet up to 7.5% had no adverse effect on the health status of the animals at pre and post-gestation, as the discrepancy observed were still within the normal range of rabbit blood profile.

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