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Research Article

Health and Carcass implications of dietary inclusion of graded level of sun-cured Neem (Azadirachta indica, A. juss) leaf meal for broilers

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ABSTRACT

The effects of feeding graded levels (0, 5, 10, 15 and 20%) of Neem leaf meals (NM) to broiler chicks on live weights, carcass and organ weights and blood constituents was investigated. Two hundred and forty (240) 7 days-old unsex broiler chicks (Arbor acres) were randomly allocated to five treatments of 48 birds each, replicated four times with 12 birds each in a completely randomized design. Feed and water were given *ad-libitum* till 56 days. The results of the live weights and carcass and organ weights decreased with increased NM inclusion in the diets. Haematological constituents were not affected (P>0.05) except the packed cell volume while the serum biochemical indices of birds fed 20 % NM diets were decreased (P<0.05) compared with those fed control, 5 %, 10% and 15% NM diets. Inclusion of 15% NM in broiler chicks' diet had no adverse effects on live, carcass and organ weights and immunity responses. NM needs further treatment to improve inclusion levels beyond 15 % in broilers' diets.

INTRODUCTION

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 favorably to areas with severe drought, poor, shallow and even saline soil (Ogbuewu *et al*. (2011b). The utilization of several leaf meals as feed ingredient to reduce production cost in poultry diet is not new but the inclusion levels at various ages and physiological conditions varies (D'Mello and Acamovic, 1987; Udedibie and Opara, 1998; Nworgu, *et al*., 2003; Kakengi, *et al*., 2007; Iheukwumere, *et al*., 2007; Onyimonyi, *et al*.,2009; Ncube, *et al.,* 2012).

The maximum tolerance level of Neem leaf meal (NM) as stated by Obikaonu *et al*. (2012) is 10% in starter broilers but Esonu *et al*. (2006) included 15% in laying birds while Ogbuewu *et al.* (2010a, b; 2011) asserted the use of 15 % in rabbits. The use of Neem leaf meal is limited due to bioactive compounds (Azadirachtin, limonoids and tannin) that have deleterious effects on nutrient utilization (Lale, 2002; Ogbuewu*,* 2008; Akpan *et al*., 2008) of monogastric animals. Siddiqui *et al.* (1986) reported the isolation of a triterpenoid called nimbocinone from fresh neem leaves as well as two steroids identified as sitosterol and stigmasterol.

The detection of a disease in the animal is based on a good history, obvious clinical signs and para clinical experiments. The Para clinical examinations are important for confirmation of a disease and have great value for diagnosis of some chronic or subclinical forms of a disease. An important part of such examination depends on measurements of serum constituents; electrolytes, non-electrolytes and enzymes. Because of the central role of serum constituents in the body's homeostasis and the close relationship between serum constituents and their tissue concentrations, much information regarding the body's response to disease can be obtained by measuring these factors in the blood serum of the animal.

Severe depressive effects in the blood constituents of broiler chicks and laying birds fed NM at 10%, and 15% has been reported by Esonu *et al*. (2006); Onyimonyi *et a*l.(2009); Biu *et al*. (2009) and Obikaonu *et al*. (2012) and at 15% in rabbit diets by Ogbuewu *et al.* (2010a; b; 2011a). The bioactive principles in NM had earlier been reported (Esonu *et al*., 2006; Ogbuewu *et al.,* 2010a; b; 2011; Obikaonu *et al*., 2012) reduced by sun drying . This study was therefore designed to evaluate optimum acceptable limit of Neem leaf meal by finisher broiler chicks on live weights, carcass and organ weights and blood constituents.

MATERIALS AND METHODS

Study Site

This study was conducted at the Poultry Unit of Teaching and Research Farm, Federal College of Wildlife Management, New Bussa, Niger State, Nigeria. New Bussa is located between latitude 7°31^{1}- 10°00^{1}N and longitude 4°30^{1}- 4°33^{1}E (Adewetan *et al*., 1980) in the savanna areas of Niger basin.

Collection and processing of Neem leaf meal

The fresh young Neem leaves were collected within the estate of Federal College of Wildlife Management. The leaves were sun-cured for 3-4 days so as to maintain its greenish coloration and to reduce the bioactive components. The Neem leaves were ground to particle size of 2mm sieve using a hammer mill.

Experimental diets

Five experimental diets were formulated with Neem leaf meals (NM) included at 0%, 5%, 10%, 15% and 20% respectively, replacing groundnut cake and presented as NM0, NM5, NM10, NM15 and NM20 (Tables 1 and 2).

Table 1: Composition of Experimental Starter Diets Fed Broiler Chicks.

Ingredients	Dietary Neem levels				
	NM0	NM5	NM10	NM15	NM20
Maize	48.00	48.00	48.00	48.00	48.00
Wheat bran	10.00	10.00	10.00	10.00	10.00
Fish meal	4.00	4.00	4.00	4.00	4.00
Groundnut cake	31.0	26.00	21.00	16.00	11.00
Neem leaf meal	0.00	5.00	10.00	15.00	20.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Oyster shell	3.50	3.50	3.50	3.50	3.50
*Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Crude Protein	23.00	21.80	20.49	19.21	18.00
ME (Kcal/Kg)	2831.20	2899.12	2867.12	2835.12	2803
Analyzed proximate composition (% DM basis)					
Dry matter	90.12	89.67	90.01	87.05	88.77
Crude protein	22.75	23.10	22.93	23.80	23.45
Crude fibre	4.00	4.05	4.50	6.00	7.50
Ash	5.00	4.00	6.00	7.00	7.00
Ether extracts	5.00	6.00	6.00	5.00	5.00
Nitrogen free extract	53.37	52.47	50.58	45.25	46.82

*Premix to provide the following: Vitamin A 12,000,000,I.U; Vitamin D_3 3,000.000I.U; Vitamin E 30,000mg; Vitamin K 2,500mg; folic acid 1,000mg; Niacin 40, 000mg; Cal Pan 10,000mg; Vitamin B_{12}, 20mg; Vitamin B_1 2,000mg; Vitamin B_6 3,500mg; Biotin 80mg; Antioxidant 125,000mg; Cobalt 250mg; Selenium 250mg; Iodine 1,200mg; Iron 40,000mg; Manganese 70,000mg; Copper 8,000mg; Zinc 60,000mg; Chlorine 200,000mg.

Source of experimental birds and design

Two hundred and forty (240) day-old broiler chicks (Arbor acres) were purchased from Zarm farm Ilemona, in Kwara State. The birds were fed on commercial starter diet for seven days before allocation for the feeding trial. The birds were allocated to five experimental treatments of 48 birds each, replicated four times with 12 birds each in a completely randomized design (CRD).

Table 2: Composition of experimental finisher diets

Ingredients	Dietary Neem levels				
	NM0	NM5	NM10	NM15	NM20
Maize	57.00	55.00	53.00	51.00	49.00
Wheat bran	10.00	9.00	8.00	7.00	6.00
Fish meal	4.00	4.00	4.00	4.00	4.00
Groundnut cake	22.00	20.00	18.00	16.00	14.00
Neem leaf meal	0.00	5.00	10.00	15.00	20.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Oyster shell	3.50	3.50	3.50	3.50	3.50
*Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Crude Protein	19.99	19.60	19.30	19.00	18.70
ME (Kcal/Kg)	2839.16	2755.00	2840.56	2945.23	3009.88
Analyzed proximate composition					
DM	89.56	89.24	90.65	88.88	88.91
CP	20.30	20.13	20.88	20.70	20.75
CF	5.00	5.50	6.00	6.00	7.50
Ash	4.00	5.00	5..50	6.00	6.00
EE	5.00	5.50	6.00	6.00	6.50

*Premix to provide the following: Vitamin A 12,000,000I.U; Vitamin D_3 3,000.000I.U; Vitamin E 30,000mg; Vitamin K 2,500mg; folic acid 1,000mg; Niacin 40, 000mg; Cal Pan 10,000mg; Vitamin B_{12} 20mg; Vitamin B_1 2,000mg; Vitamin B_6 3,500mg; Biotin 80mg; Antioxidant 125,000mg; Cobalt 250mg; Selenium 250mg; Iodine 1,200mg; Iron 40,000mg; Manganese 70,000mg; Copper 8,000mg; Zinc 60,000mg; Chlorine 200,000mg.

Housing of birds and experimental design

The birds were raised in a deep litter system using 2.5× 2.5m pen sizes. The chicken house with a foot dip was disinfected using Dazintol^{R} solution in water 2 weeks prior to stocking of the chicks. Feed and water were supplied *ad-libitum.* At first day of birds' collection, vitalyte^{R} soluble powder was given against stress condition while on the second day; birds were vaccinated using New Castle Disease Vaccine (I/O). Similarly, on day 7 and 14, birds were orally immunized against New Castle Disease (Lasota) and Infectious Bursal Disease (Gumboro), respectively by dissolving 200 doses of each vaccine in 2 litres of chlorine-free water. The birds were also prophylactically treated against bacterial infection at second week using Terramycin (chick formula) soluble powder (50g in 60 litres of water) and against coccidiosis using Embazin forte^{R} at 30g per 50 litres water on the 18th day as recommended by Oluyemi and Roberts (2000). At the end of 28 days, birds were weighed and changed to a finisher diets and were fed for 4 weeks until day 56.

Blood collection and analyses

Approximately 5ml of blood was collected from jugular vein of slaughtered chicken into two sets of five sterilized glass bottles at the conclusion of the feeding trial. For haematology, the blood samples were collected into a set of five sterilized bottles containing ethylene diaminetetra-acetic acid (EDTA). Blood samples for serum biochemical studies were collected into plain sterile bottles (i.e. without anticoagulant) for serum separation, Packed cell volume, red blood cells count , haemoglobin concentration , white blood cell count and differentials leukocyte count (lymphocytes, neutrophils, basophils, monocytes and eosinophils) were analyzed according to the methodology of Schalm *et al.* (1975).

Serum total protein was determined by Kjedahl method as described by Kohn and Allen (1995). Serum albumin was determined using a BCG (bromocresol green) method as described by Peters *et al.* (1982). Creatinine concentration was determined using a commercial kit (Creatinine Liquicolor, Germany). Serum glucose and nitrogen urea were determined spectrophotometrically (Thermo Fisher Scientific Inc., Madison, Wisconsin, USA) using commercial reagent kits (United Diagnostic Industry, Dammam, Saudi Arabia).

Evaluation of carcass and organ weights

At the end of the feeding trial (56 days), three birds per replicate were randomly selected, starved overnight, weighed and slaughtered. The birds were bled and dipped into a warm water (60^{o}C) bath for 5 minutes, feather-plucked after scalding and eviscerated. The carcass, visceral organs (liver, heart, gizzard and pancreas) and offals (head and legs) were removed and expressed as percentage of live weight according to Salsbury and Salsbury (1962) procedure.

Proximate analysis

The experimental diets were analyzed for dry matter (DM), crude protein (CP), crude fibre (CF) ether extracts (EE) and ash according to AOAC (2005) methods.

Statistical Analysis

All data obtained were subjected to analysis of variance (ANOVA) using SPSS 17.0 (Microsoft, Window, 2003 version) and mean differences separated by methods of Duncan Multiple Range Test (1955).

RESULTS

Live, carcass and organ weights of broiler chicks fed Neem leaf meal.

The live weights of broilers fed Neem leaf meal is presented in Table 3. The live weight of birds on NM0 and NM5 were similar (P>0.05) but significantly different among NM10, NM15 and NM20. The plucked and eviscerated weights of birds followed a similar trend as the live weights pattern (Table 3). The progressive decrease in all the carcass, offal and relative organ weights is a reflection of the live weight.

Table 3: Live weights and carcass and organs weight of broilers fed Neem diets expressed in % live body weight.

Parameters	Diets					
	NM0	NM5	NM10	NM15	NM20	SEM
Live body weight (g/bird)	2013.11^{a}	1993.22^{a}	1850.45^{b}	1808.00^{b}	1302.50^{c}	85.52
Plucked weight (g)	1794.11^{a}	1791.22^{a}	1698.50^{b}	1624.50^{b}	1144.50^{c}	80.66
Eviscerated weight (g)	1676.00^{a}	1684.22^{a}	1683.43^{a}	1519.00^{b}	1131.00^{c}	70.31
Carcass weight (%)	79.26	78.01	75.86	76.60	67.27	1.43
Heart (%)	0.47	0.48	0.49	0.49	0.46	0.01
Liver (%)	1.92^{a}	1.88^{a}	1.83^{a}	1.49^{b}	1.38^{b}	0.14
Pancreas (%)	0.29^{a}	0.28^{a}	0.27^{a}	0.24^{a}	0.20^{b}	0.75
Gizzard (%)	2.18^{a}	1.90^{b}	1.85^{b}	1.76^{b}	1.19^{c}	0.11
Head (%)	3.05^{a}	3.00^{a}	2.85^{b}	2.73^{c}	2.26^{d}	0.09
Leg (%)	4.51^{a}	4.23^{ab}	4.03^{ab}	3.80^{b}	2.74^{c}	0.21

SEM=Standard error of mean

abcd Means on the same row with different superscripts are significantly (P<0.05) different

Haematological constituents of broiler chicks fed Neem leaf meal

The haematological results did not show any significant (P>0.05) difference except for the PCV as presented in Table 4. The PCV of birds on NM based diets reduced significantly (P<0.05) while the red blood cell, white blood cell and neutrophil counts of birds were numerically increased with increase NM inclusion. The haemoglobin concentration decreased with increase in NM inclusion in the diets.

Table 4: Haematological constituents of finisher broilers fed experimental diets

Components	Diets					
	NM0	NM5	NM10	NM15	NM20	SEM
PCV (%)	28.50^{a}	22.00^{c}	22.00^{c}	25.00^{b}	25.50^{b}	0.66
RBC (× 10^{6}/mm^{3})	3.74	3.58	4.40	3.60	3.20	0.14
Hb (g/dl)	9.50	7.50	7.33	8.33	6.00	0.66
WBC (× 10^{6}/mm^{3})	10.80	11.22	16.36	13.48	13.28	0.85
Neutrophil (%)	22.50	27.50	29.00	29.00	29.50	0.73
Basophils (%)	0.50	0.00	0.00	0.00	0.00	0.10
Lymphocytes (%)	70.00	68.50	62.50	65.50	65.50	2.00
Eosinophils (%)	3.50	2.50	3.50	2.50	3.00	0.33
Monocytes	4.00	1.50	2.50	3.50	3.00	0.41

abcde Means on the same row with different superscripts are significantly (P<0.05) different.

Biochemical indices of broilers fed experimental finisher diets

The biochemical indices of finisher broilers are presented in Table 5. There were significant differences (P<0.05) among dietary treatments for all the biochemical indices measured. However, serum urea concentrations increase with increasing NLM inclusion levels in the diets. The serum glucose concentration was differed (P<0.05) among the dietary treatments (Table 5). The glucose concentrations in this study increase with increased in NM in the diets except for NM5 diet.

Table 5: Effects of Neem leaf meal based diets on serum biochemical indices

Parameters	Diets					
	NM0	NM5	NM10	NM15	NM20	SEM
Total protein (g/dL)	2.48^{b}	2.80^{b}	2.25^{b}	3.26^{a}	2.07^{c}	0.22
Albumin (g/dL)	1.41^{b}	1.59^{b}	1.54^{b}	1.71^{a}	1.13^{c}	0.33
Urea (mg/dL)	1.68^{c}	2.19^{b}	2.06^{b}	2.14^{b}	2.33^{a}	0.08
Creatinin (mg/dL)	0.89^{c}	0.81^{c}	0.72^{d}	1.47^{b}	1.73^{a}	1.00
Glucose (mg/dL)	144.10^{b}	139.91^{b}	180.97^{a}	189.12^{a}	198.2^{a}	26.66

ab Means within a row with different superscript are significantly different at p<0.05.

DISCUSSION

The decreased in live weights of birds on NM20 based diet could be attributed to high crude fibre and the probable effects of bioactive compounds in the leaf meal. The reduction in live body weight of birds with increasing NM in the diets is in agreement with findings of Esonu *et al* (2006) who observed a similar growth trend with laying birds fed 15% NM. These results confirmed earlier findings by Esonu *et al* (2006); Ogbuewu *et al.* (2010a, b); *Ogbuewu et al.* (2011) and Obikaonu *et al*. (2012) that 15% NLM is the tolerance level for laying birds and adult rabbits.

The percentage carcass weight obtained in this study is similar to those published by Oluyemi and Robert (2000), and Anyoechie and Madubuike (2007) who reported range values of 70-75%. A decrease in the relative organs weights of liver, heart, pancreas and gizzard with increasing NM inclusion in the diets could be an indication of residual bioactive components (Azidirachtins, tannins and linonoids) in the leaf meal which may have depressed these parameters. The variations in absolute values of organs and offals (head and legs) of broilers fed NM0 and NM based diets probably resulted from their heavier live weights which according to Broadbent *et al. (1981*), that the surface area and the live weight determine the amount of feathers and visceral organs required respectively. Butcher *et al.* (1983) opined that the external offal percent tended to increase as slaughter weight of animals increased.

The mild depressive effects of NM on packed cell volume observed in this study is in agreement with findings of Biu *et al*. (2009) but invariance with those reported by Esonu *et al*. (2006) who reported slight increments in values of packed cell volume of laying birds fed NM diets at 0% to15%. The observed differences in the two studies could be attributed to breed differences. The blood indices in this study are in conformity with those reported by Obikaonu *et al.* (2012), who fed starter broilers NM up to 15% in their diets. The numerical reductions in the haemoglobin and RBC contents of the blood of broiler chicks on NM20 are an indication that the oxygen carrying capacity of the animals' blood would be reduced. The neutrophils are concerned with day to day immunological defense against pathogens. The numerical increased in white blood cell and neutrophil counts of birds on NM based diets imply that the ingestion of NM may have increased the production of these blood components against the residual metabolites in the leaf meal. The eosinophils and monocytes results are in agreement with those reported by Esonu *et al*. (2006) for laying hens fed NM. The probable reduction of lymphocyte counts could be an indication that the birds were immunologically challenged against negative antigenic effects associated with the NM bioactive compounds.

The result of the serum protein, albumin, creatinine and urea in birds fed diet NM20 is in agreement with the findings of Eggum (1970); Iyayi and Tewe (1998) and Esonu *et al.* (2001), who reported that serum urea and total protein contents depend on both the quantity and quality of the protein supplied in the diet. 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 birds on NM20 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 increase in glucose concentrations in birds with increase NM in the diets could be due to inhibition of glycolysis by the presence of glyco-proteins and possibly bioactive compounds which may have some adverse effect on regulation of insulin from pancreatic β-cells and on blood sugar. The glucose concentration in this study was within the literature limit by Mitruka and Rawsley (1977). The increased in serum glucose with increased NM inclusion in this study is contrary to decreased values reported by Ogbuewu *et al*. (2010b), who fed rabbits 10% NM.

Glucose is one of the metabolites measured as indicator of the energy status of the animal. Normal glucose levels in birds indicate adequate synthesis in the liver from propionate, a major glucose precursor (Houtert, 1993).

CONCLUSION

Neem leaf meal is tolerated by finisher broilers not exceeding 15% inclusion level in the diet.

The live weights, carcass and organ weights and blood constituents of broilers are safe at 15% NM inclusion.

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