PROTEIN QUALITY AND TOXICITY OF FULL-FAT NEEM (*AZADIRACHTA INDICA* A. JUSS) SEED KERNEL*

CALIDAD DE LA PROTEÍNA Y TOXICIDAD DE LA SEMILLA SIN DESGRASAR DE *AZADIRACHTA INDICA* A. JUSS

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ADDITIONAL KEYWORDS

Heating. Nutrient content. Cockerel chicks.

PALABRAS CLAVE ADICIONALES

Calentamiento. Contenido nutritivo. Pollos.

SUMMARY

Autoclaving (30 min) unextracted neem seed kernel (AFK) lowered protein and amino acid but elevated fat contents. Weight gain of chicks fed on test diets (75,150 or 225 gAFK/kg and 75g raw neem seek kernel (RFK)/kg) offered as mash for 35 days was slower to that of chicks offered reference diet. Comsuption of the test diets, except RFK diet was inferior. The slower weight gain of chicks was recorded on 225 g AFK diet. Water ingestion followed the same trend of food consumption. Nutrients from reference diet, except carbohydrates, were more retained. Also, nutrient metabolizity declined with increased levels of AFK. At 150 and 225 g AFK dietary concentrations, carcass yield apparently improved compared to that of the chicks fed on the other diets, whereas only 225 g AFK diet induced enlargement of the lungs and kidneys. Liver size increased considerably with AFK at all levels of dietary concentration. Histology revealed hyperplasia of Kupffer cells and hypertrophy of hepatocytes of chicks on the AFK diets. Renal tubular cells of the chicks on 150 or 225 g AFK diets similarly increased in size and secretary activity. Vascular congestion and mild haemorrhages in the liver and kidneys as well as catarrhal enteritis were seen with RFK diet. RFK induced anaemia, leucocytosis, increased aspartate aminotransferase activity and decreased plasma cholesterol and triglyceride concentrations. No differences were found among the dietary groups on chicks' mortality.

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RESUMEN

El tratamiento (30 min) en autoclave de semilla de neem, no extraída (AFK), bajó la proteína y el nivel de aminoácidos, y elevó los contenidos de grasa. La ganancia de peso en pollos alimentados con la dieta de referencia ofrecida como

harina durante 35 días fue mayor que en los que comían dietas con 75, 150 o 225 gAFK/kg, o 75g/kg de la semilla en bruto (RFK), a causa del menor (salvo en la dieta RFK) consumo. El incremento de peso de los pollos fue más bajo con 225 g AFK. La ingestión de agua siguió la misma tendencia del consumo de alimento. La retención de sustancias nutritivas de la dieta de referencia, excepto carbohidratos, fue mejor. La metabolización de nutrientes declinó al aumentar los niveles de AFK. En las concentraciones de 150 y 225 g AFK, el rendimiento de la canal aumentó comparado con otras dietas; sólo la dieta con 225 g AFK inducía aumento de tamaño de los pulmones y riñones. El tamaño de hígado, sin embargo, aumentó bastante con AFK en todos los niveles de concentración dietética. La histología reveló hiperplasia de células Kupffer e hipertrofia de hepatocitos de los pollos alimentados con las dietas AFK. Las células renales tubulares de los pollos comiendo dietas de 150 o 225 g AFK/kg incrementaron de modo similar el tamaño y actividad secretora. Al contrario, se observó congestión vascular y hemorragias suaves en el hígado y riñones así como enteritis catarral con la dieta RFK. RFK indujo anemia, leucocitosis, aumento de la actividad de aspartato aminotransferasa y disminución del colesterol y triglíceridos del plasma. Sin embargo, no fueron encontradas diferencias de mortalidad de los pollos entre los grupos dietéticos.

INTRODUCTION

Emphasis of current research efforts on neem kernel meal is the assessment of its nutritive value (Gowda *et al.*, 1996; Nagalakshmi *et al.*, 1996). Extracted neem kernel meal contains high protein (47.9 percent) (James *et al*., 1997) similar to that of groundnut meal (46.0 percent)(Oyenuga, 1968) and should, theoretically, make a good protein supplement for poultry. Of many methods available to denature neem kernel toxins and improve its feeding value, the chemical methods are of limited use in the developing countries due to high costs; cheaper and readily adoptable methods of denaturation that would not lead to loss of substantial quantity of neem kernel are needed.

Heat treatment has been found useful in processing rapeseed (Jensen *et al.*, 1995) and soybean (Kaankuka *et al*., 1995). Offiong and Olomu (1990) recorded faster growth of broiler chickens fed on cooked or autoclaved soybean than those fed on raw or roasted soybean diets. The duration of heating recommended for soybean (Offiong and Olomu, 1990), rapeseed (Jensen *et al.*, 1995) and Jackbean (Ologhobo *et al.*, 1999) to substantially remove antinutritional factors and minimize the destruction of amino acids, especially lysine, was 30 min. Although no presence of heat-labile trypsin-inhibitor, glucosinolates and canavanine, has been reported in neem kernel, some toxic compounds of neem could be removed by heating. The aim of this study was, to assess the effect of heat on proximate composition, amino acid profile and toxic implications of feeding full-fat neem seed kernel to cockerel chicks.

MATERIAL AND METHODS

Neem seeds collected in Sokoto, Nigeria, were air-dried and mechanically dehulled. Portions of the raw full-fat neem kernel (RFK) were processed by autoclaving (AFK) at 121°C for 30 min and dried in the sun to constant weight. The RFK or AFK

were ground in a harmer mill and analyzed for chemical constituents and amino acid contents. Protein (N x 6.25) was determined by the macro-Kjeldahl method; ash as the residue remaining after incinerating samples at 600°C for 3 h in a muffle furnace; and crude fibre and ether extract using the AOAC (1990) methods. Assay for amino acid was done as described by Spackman *et al.* (1958). The hydrolysates were run on a Technicon TSM-1 amino acid auto-analyzer (DNA 0209 Technicon Ireland Ltd.) with integrator and printer.

The study comprised of five isoproteic (220 g/kg) diets **(table I)** formulated from corn-peanut reference diet with AFK included at rates of 75, 150 and 225 g/kg, largely at the expense of peanut meal. Low rate (75 g/kg) of RFK was incorporated into the reference diet in place of peanut as negative control. The amounts of blood and fishmeals used were adjusted to maintain comparable crude protein and amino acid levels, as used by Fetuga *et al*. (1977).

The toxicity of RFK or AFK was assessed using two hundred, 7-day old, cockerel chicks of Shika-Brown strain. All the chicks were weighed individually and four replicate cages of 10 chicks each, arranged in a completely randomized design, were assigned to each of the five dietary groups on similar weight basis. The replicates

Table I. *Composition of diets (g/kg).* (Composición de las dietas).

Diets	Control	AFK	AFK	AFK	RFK
Feed ingredients					
Neem kernel meal	-	75.0	150.0	225.0	75.0
Peanut meal	250.2	164.6	85.8	9.0	165.3
Fish meal	85.0	100.0	115.0	130.0	98.0
Blood meal	-	12.3	21.7	30.1	10.8
Corn	546.6	529.9	509.3	487.7	532.7
Wheat bran	81.7	81.7	81.7	81.7	81.7
Bone meal	20.0	20.0	20.0	20.0	20.0
Limestone (ground)	10.0	10.0	10.0	10.0	10.0
Sodium chloride	3.0	3.0	3.0	3.0	3.0
Premix*	2.5	2.5	2.5	2.5	2.5
Choline chloride	1.0	1.0	1.0	1.0	1.0
Determined analysis (dry matter)					
Crude protein(percent)	22.70	22.58	22.50	22.43	22.59
ME (MJ/Kg)[1]	11.84	12.20	12.34	12.48	12.20
Methionine + cystine[1](percent)	0.725	0.722	0.720	0.718	0.723
Lysine (percent) (calculated)	1.060	1.062	1.074	1.083	1.063

*Zoodry (Roche) provide mg/kg of diet: retinol acetate, 3.0; cholecalciferol, 0.055; α-Tocopherol, 12.0; menadione, 2.0; thiamine, 3.0; riboflavin, 6.0; pyridoxine, 3.5; cyanocobalamin, 8.0; biotin, 0.05; folic acid, 0.1, nicotinic acid, 24.0; calcium pantothenate, 12.0; ascorbic acid, 20.0; Mn, 80.0; Fe, 50.0; Zn, 46.0; Cu, 5.0; I, 1.6; Co, 1.8; Se, 0.15.

were housed separately in electrically heated cages with wire screen floors. Room lighting was continuous. Routine management and vaccinations were carried out during the rearing period. Diets as mash and fresh water were supplied *ad libitum*. Body weight of individual cockerel and food consumption on replicate basis were determined weekly whilst water intake and mortality of chicks recorded daily. The study lasted 35 days.

Apparent nutrient metabolizity study was done from day 29 to 35 of feeding trial using 16 cockerels per treatment (4 per replicate). The birds were transferred to metabolism cages where 3 days of adjustment was allowed before the commencement of faecal collection during the last 4 days. Throughout the collection period, food allocation was restricted to 90 percent of the observed voluntary consumption to avoid excessive gastrointestinal motility and food refusal, but chicks had free access to water. Daily faecal outputs were dried at 55°C, ground and stored for analysis. The diets and excreta were analyzed for their chemical constituents (AOAC, 1990).

Eight, 42-day old, cockerels per treatment from the rearing cages were randomly selected and sacrificed by cervical dislocation following food withdrawal for 6 h. Evisceration was done immediately after bleeding and the lungs, heart, proventriculus, gizzard, intestines, pancreas, kidney and liver were isolated and weighed. Sections of liver, pancreas, kidney and segment of small intestine were fixed in 10 percent formal saline for histopathology. The fixed tissues were trimmed, embedded in paraffin wax and 5mm thick sections cut and stained with haematoxylin and eosin for light microscopy. The carcasses were defeathered and weighed for the determination of carcass yield.

For haematology, blood samples were collected into bottles containing ethylene diamine tetra-acetic acid (1mg/ml blood) and those for plasma chemistry into heparinized bottles (0.2 mg/ml blood) from 8 birds per group at 42-day old. The blood samples for haematology were stored under refrigeration temperature (4°C) and analyzed within 3 h of collection. Packed cell volume (PCV), haemoglobin concentration and red and white blood cell counts were determined using Wintrobes' microhaematocrit, cyanomethaemoglobin and improved Neubauer haemocytometer methods, respectively. The erythrocytic indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and MCH concentration (MCHC) were computed using established formulae (Swenson, 1996).

Plasma samples were obtained following centrifugation of heparinised blood and stored at -80°C until analysis. Total protein, albumin, uric acid, creatinine, cholesterol, tryglycerides, total and conjugated bilirubin, and alanine and aspartate aminotransferases were determined using Sigma assay kits. The metabolites were measured in a Hitachi 704 serum autoanalyzer (Mountain View, C.A).

All data from the experiments were subjected to one-way analysis of variance. Treatment means were compared using Duncan's multiple range tests where significant diffe-rences were detected (Steel and Torrie, 1980).

RESULTS

The chemical content and amino acid profile of the AFK and RFK are shown in **table II**. Autoclaving for 30 min lowered crude protein and fibre but elevated fat and NFE contents of neem kernel. The decrease in protein value resulted in slight decrease in almost all the amino acids.

The growth performances of cockerel on the reference or test diets are presented in **table III**. Chicks fed on reference diet had higher ($p<0.01$) live weight and average daily weight gain than chicks fed on AFK or RFK diets. Weight gain of chicks decreased progressively as AFK content of diets increased. At 225g AFK/kg diet, the gain was significantly ($p<0.05$) lesser than that of chicks on lower levels of neem kernels. Autoclaving markedly reduced ($p<0.01$) palatability of neem diets whereas consumption of RFK diet did not differ from that of the reference diet. However, the high intake of RFK diet did not necessarily improve weight gain of chicks. The thirst of chicks followed the same pattern as food ingestion, and efficiency of water utilization (gain: water) did not differ significantly among the dietary groups. Incorporation of 225 g AFK or 75 g RFK/kg diet declined ($p<0.05$) gain: food and gain: protein ratios. The influence of diets on mortality of chicks was not discernible; although increased content of AFK tended to increase the incidence **(table III)**.

Table IV summarizes the nutrient metabolizity coefficients. Neem kernel diet proved to be less digested than the reference diet; and while dry matter, ether extract and ash metabolizity from the test diets lagged ($p<0.05$) behind that from the reference diets, this lag was most apparent ($p<0.01$) for crude protein. Among the diets containing AFK, the effect of increasing levels of neem kernel on nutrient metabolizity was not obvious until the 225 g/kg dietary level, at which the metabolizity

Table II. *Chemical (g/kg) and amino acid contents[1] of raw (RFK) and autoclaved (AFK) neem kernel meals.* (Composición química de harina de granos de neem en bruto (RFK) o tratados en autoclave (AFK)).

	RFK	AFK	RFK -AFK	Diff. percent
Crude protein	302.1	275.6	26.5	8.77
Crude fat	453.2	487.2	-34.0	-7.50
Crude fibre	103.6	88.7	14.9	14.38
Minerals (ash)	46.1	40.5	5.6	12.15
NFE	91.0	108.0	-17.0	-18.68
Amino acids[2]				
Alanine	6.01	5.09	0.92	15.31
Arginine	1.70	1.34	0.36	21.18
Aspartic acid	8.66	8.82	-0.16	-1.84
Cystine	0.76	0.72	0.04	5.25
Glycine	9.66	7.28	2.38	24.64
Glutamic acid	13.06	13.04	0.02	0.15
Histidine	2.72	2.72	0	0
Isoleucine	3.50	2.46	1.04	29.71
Leucine	7.72	6.74	0.98	12.69
Lysine	4.06	3.71	0.35	8.62
Methionine	0.58	0.48	0.12	20.69
Phenylalanine	4.60	4.27	0.33	7.73
Proline	1.44	1.37	0.07	4.86
Serine	6.50	4.12	2.38	36.62
Threonine	4.08	3.42	0.66	16.18
Tyrosine	4.30	3.71	0.59	13.72
Valine	2.99	3.71	-0.72	-24.08

[1]Mean of two determinations; on air dry basis. [2]Solvent extracted neem kernel meals were used (g/16 g N). NFE: Nitrogen-free extract.

Table III. *Body weight, food efficiency and survival rate of cockerel chicks fed experimental diets.* (Peso, eficiencia alimenticia y tasa de supervivencia de pollos alimentados con las dietas).

Diets Performance traits	Control	AFK	AFK	AFK	RFK	SEM
	-	75.0	150.0	225.0	75.0	
Live weight (g/chick)	291.4[a]	229.8[b]	217.5[b]	149.7[b]	227.2[b]	21.24**
Weight gain (g/chick/d)	7.5[a]	5.8[b]	5.3[b]	4.1[c]	5.7[b]	0.50**
Food consumption (g/chick/d)	25.5[a]	16.4[b]	16.5[b]	16.3[b]	23.4[a]	2.40**
Water intake (ml/chick/d)	60.9[a]	41.8[b]	43.7[b]	41.0[b]	54.5[a]	2.75**
Gain: food ratio	0.30[a]	0.34[a]	0.31[a]	0.25[b]	0.25[b]	0.037*
Gain: protein ratio	1.35[a]	1.53[a]	1.45[a]	1.16[b]	1.08[b]	0.222*
Gain: water ratio	0.12	0.13	0.12	0.09	0.10	0.015
Survivors (no./40)	38	39	37	36	38	-
Mortality (percent)	5.0	2.50	7.5	10.0	5.0	0.16

[abc]different superscripts in a row differ significantly. *p<0.05; **p<0.01; SEM: standard error of means.

coefficient for dry matter, protein and ash had all reduced significantly. Similarly, RFK significantly depressed protein and oil metabolizity more than the same level (75 g/kg) of AFK. However, no differences existed among the dietary groups on metabolizity of crude fibre and NFE.

The data on carcass characteristics and organ weight **(table V)** showed that eviscerated carcass weight decreased and its yield increased significantly (p<0.01) with increased levels of neem kernel. However, neither the 75 g AFK nor 75 g RFK/kg diet ostensibly affected the carcass yield. The relative weights of organs were not influenced by the dietary

Table IV. *Apparent metabolizity coefficients (g/kg) of nutrients by chicks on control or neem diets (39-42 d).* (Coeficientes aparentes de metabolización de nutrientes por los pollos alimentados con dietas control y con neem (39-42 d)).

Diets	Control	AFK	AFK	AFK	RFK	SEM
Nutrients	-	75	150	225	75	
Dry matter	703.6[a]	667.4[b]	636.0[ab]	631.0[c]	647.4[bc]	45.08*
Protein (N x 6.25)	734.1[a]	687.4[b]	686.1[b]	456.4[d]	641.1[c]	54.33**
Ether extract	776.9[a]	685.3[b]	704.7[b]	523.6[c]	505.3[c]	46.29*
Minerals (ash)	558.8[a]	505.0[b]	473.4[b]	452.8[c]	468.2[b]	30.41*
Crude fibre	374.9	380.4	368.3	364.5	351.2	22.80
Nitrogen-free extract	713.8	728.2	730.2	729.1	701.8	61.45

[abcd]Means in a row with different superscripts differ significantly. *p<0.05; **p<0.01.

Table V. *Carcass and organ measurements of cockerels offered control or neem diets for 35 days.* (Medidas de la canal y de los órganos de pollos con la dieta control o con neem durante 35 días).

Diets	Control	AFK	AFK	AFK	RFK	SEM
Measurements	-	75.0	150.0	225.0	75.0	
Carcass weight (g/chick)	187.1[a]	167.7[b]	144.4[b]	110.6[c]	166.8[b]	11.57**
Carcass yield (percent)	73.5[b]	73.6[b]	76.9[b]	81.8[a]	74.7[b]	1.69**
Organ weights (g/kg carcass weight)						
Lungs	8.5[b]	8.5[b]	9.3[b]	11.8[a]	11.0[ab]	0.90*
Heart	10.4	10.9	10.3	11.8	10.8	1.38
Liver	41.2[b]	49.0[a]	48.4[ab]	55.8[a]	41.3[b]	3.10**
Proventriculus	9.6	11.1	10.9	12.0	9.7	1.50
Gizzard	42.6	52.8	44.7	52.7	47.4	4.0
Intestines	318.9	408.8	403.3	440.8	346.6	20.70
Pancreas	5.4	7.2	6.6	6.7	6.0	0.80
Kidney	16.9[b]	17.0[b]	18.1[b]	23.7[a]	19.9[ab]	2.40*

[abc]Means in a row with different superscripts differ significantly. *p<0.05; **p<0.01.

treatments except that of lungs, liver and kidneys. Whereas only 225 g AFK diet increased (p<0.05) the weight of lungs and kidneys, the liver of chicks on all AFK diets were markedly heavier (p<0.01) than those of chicks on the control diets.

Table VI, shows data on haematological values of chicks. Haematocrit, haemoglobin value and erythrocyte counts **(table VI)** were severely depressed (p<0.01) by RFK whereas autoclaving of neem kernel, irrespective of its rate of inclusion into

Table VI. *Haematological indices of chicks fed on control or neem diets for 35 days.* (Índices hematológicos de pollos alimentados con las dietas control o con neem durante 35 días).

Diets	Control	AFK	AFK	AFK	RFK	SEM
Haematological traits	-	75	150	225	75	
Packed cell volume (percent)	21.3[a]	21.3[a]	21.5[a]	21.6[a]	18.6[b]	0.73**
Haemoglobin (g/dl)	7.5[a]	7.4[a]	7.5[a]	7.6[a]	6.2[b]	0.24**
Erythrocytes (x10^6/ul)	1.94[a]	1.92[a]	1.96[a]	2.01[a]	1.72[b]	0.60*
Leucocytes (x10^3/ul)	20.5[b]	21.4[b]	22.0[b]	25.4[a]	26.2[a]	1.86*
MCV (fl)	109.8	110.9	109.7	107.5	108.1	2.53
MCH (pg)	38.6	38.5	38.3	37.3	36.0	1.31
MCHC (g/dl)	35.2	34.7	34.9	34.7	33.3	1.20

[ab]Means in a row with different superscripts differ significantly. *p<0.05; **p<0.01.

Table VII. *Plasma chemistry of chicks fed on control or neem diets for 35 days.* (Química plasmática de pollos alimentados con las dietas control o con neem durante 35 días).

Diets	Control	AFK	AFK	AFK	RFK	SEM
Plasma constituents	-	75	150	225	75	
Total protein (g/dl)	5.57	5.30	5.07	4.50	4.67	0.551
Albumin (g/dl)	2.54	2.45	2.27	2.03	2.07	0.264
Globulins (g/dl)	3.03	2.85	2.80	2.47	2.60	0.272
Albumin/globulin	0.84	0.86	0.81	0.82	0.80	0.072
Uric acid (mg/dl)	3.90	4.0	3.50	3.10	3.76	0.584
Creatinine (mg/dl)	1.43	1.53	1.60	1.67	1.40	0.219
Alanine transferase (iu/l)	8.33	8.0	7.67	7.0	7.76	0.812
Aspartate transferase (iu/l)	6.62^{b}	6.45^{b}	6.67^{b}	6.346^{b}	12.31^{a}	1.120*
Cholesterol (mg/dl)	194.3^{a}	181.0^{ab}	183.7^{ab}	185.5^{ab}	142.4^{b}	18.91*
Triglycerides (mg/dl)	130.5^{a}	137.1^{a}	134.4^{a}	140.3^{a}	86.2^{b}	18.91*
Total bilirubin (mg/dl)	0.63	0.64	0.60	0.66	0.63	0.043
Conjugated bilirubin (mg.dl)	0.23	0.24	0.23	0.22	0.27	0.006
Unconjugatged bilirubin (mg/dl)	0.40	0.40	0.43	0.44	0.36	0.005

^{ab}Means in the same row bearing different superscripts differ ($p<0.05$). *$p<0.05$.

diets, ameliorated the depressive effect of RFK. The erythrocytic indices (MCV, MCH and MCHC) among the dietary groups did not differ significantly. Leucocyte number tended to rise with high rates of neem kernel in diets, and at the 225 g AFK or 75 g RFK/kg diet, the rise became higher ($p<0.05$) than that induced by the reference diet or lower levels of AFK.

Results of blood chemistry are shown in **table VII.** No differences ($p>0.05$) were found among chicks fed on the reference or test diets for total protein, albumin, and albumin: globulin ratio; uric acid, creatinine, alanine aminotransferase activity and total and conjugated bilirubin concentrations. Conversely, a significant ($p<0.05$) increase in aspartate aminotransferase activity and decrease ($p<0.05$) in plasma cholesterol and triglyceride concentrations were observed in chicks fed on diet containing RFK compared to values from chicks placed on other diets.

Grossly there was dose-related emaciation of neem-treated chicks. Liver, kidneys and lungs of chicks on AFK diets were slightly enlarged but only the carcasses of cockerels fed on RFK diet appeared pale. Microscopically, hepatocytes of chicks on AFK diets were hypertrophic and kupffer cells hyperplastic, otherwise the liver was normal. Similarly, renal tubular cells of these birds increased in size and in secretory activity especially at 150-225 g AFK/kg dietary concentrations. Pancreas and small intestine were normal. In contrast, liver and kidneys of chicks on RFK diet were moderately congested with few haemorrhagic spots and necrotic foci.

Catarrhal enteritis with inflammatory cell infiltration of the bowel was common.

DISCUSSION

Attempts to inactivate the triterpenoids and other toxicants in raw neem kernel to improve its feeding value have been a continuing process. Some nitrogenous substances including amino acids were lost during autoclaving perhaps, through volatilization or solubilization. Carbohydrates, especially the reducing ones are known to react actively with the nucleophilic amino group, particularly the ∈-amino group, of lysine (Maillard reaction) when feeds are subjected to heat treatment (Alaise and Linden, 1999). It is therefore assumed that the decrease in lysine and some other amino acid contents, at least in part, was caused by the reaction. Autoclaving may have disrupted cellular structures leading to release of more oil from neem kernel which explains the rise in ether extract. This is in accord with the reports of Shires *et al.* (1981) on rapeseed.

The growth performance with the reference diet was superior to the neem kernel diets. The difference is explainable on the combined effects of higher feed and water intake, and better metabolizity of nutrients in the reference diet compared with the others. Furthermore, the superior metabolizity of nutrients, particularly the protein fraction, would result in a greater availability of amino acids from the diet since all diets were formulated to give similar total amino acid concentrations. In previous studies, poor feed consumption with consequent severe growth retardation was reported in chicks fed on diets containing deoiled neem kernel meal (Sadagopan *et al.*, 1982; Reddy *et al.*, 1988).

Autoclaving seems to have further exposed the bitter constituents of neem kernel, which reduced the palatability of diets compared to that of RFK diet. The intensity of bitter taste of AFK diets increased with increasing levels of AFK and that would enhance consumption of the less bitter diets. On the contrary, similar quantities of the AFK diets were consumed. Perhaps the antinutritional constituents in 75 g AFK/kg diet had reached threshold value and higher contents did not have a stronger depressive effect on the appetite of chicks. Diagayete and Hub (1982) reported similar observation on the negative influence of tannins in an *in vitro* digestibility study in which higher tannin content beyond threshold level did not produce further decline in enzymatic attackability of protein. A decrease in weight gain occurred in chicks fed on RFK diet without a decrease in food consumption. Previous studies also recorded weight loss with no concomitant reduction in food ingestion by chicks on diets containing high content of glucosinolates or epiprogoitrin (Wight *et al.*, 1987; Kloss *et al.*, 1996). It seems that concentrations of bitter compounds in RFK were not exposed enough to reduce palatability of the diet but toxic enough to impair the metabolizity of nutrients.

Water ingestion was influenced by the food consumption as recently observed (Uko *et al.*, 1999a). This explains the similarity among the dietary

groups in water utilization efficiency. However, the poor efficiency of dry matter and protein utilization at 75 g RFK or 225 g AFK/kg inclusion rates resulted in depressed growth rate due to marked elimination of the nutrients in faecal droppings in accordance with the report of Reddy and Rao (1988). The low nutrient metabolizity from neem kernel diets compared to that from the reference diet is attributed to the presence in neem kernel of toxic triterpenoids such as azadirachtin, meliantriol, salanin, and nimbin (Devakumar and Sukh Dev, 1993). This was corroborated with the work of Nagalakshmi *et al.* (1996) and that of Nath and Vijjan (1975) which reported decreased metabolizity of fat and NFE in broiler chicks or impaired digestibility of nutrients from feeds in cattle, respectively, offered alkali-treated neem kernel. It is difficult to adequately explain the observed disparity between poor nutrient metabolizity and a fairly good efficiency of their utilization from neem kernel diets. Nevertheless Uko *et al.* (1999a) observed lower ingested nutrients being more efficiently utilized.

The rise in carcass yield with decrease in body weight of chicks offered diets high in AFK (150 or 225 g/kg) was surprising and at variance with the known strong positive relationship between body size and carcass dressing out percentage (Hohenboken, 1977; Uko *et al.*, 1999b). A plausible explanation was the loss in weight of chicks on the high neem diets during the 5th week of feeding trial. While the soft tissues were involved in weight loss, bony structures seemed not to have been affected and that considerably accounted for the false improved carcass yield.

The relative increase in weight of the liver, kidneys and lungs is believed to be a compensatory reaction to inactivate and excrete the absorbed toxic neem substances from the body in conformity with the report of Alumot and Nitzan (1961) on raw soybean. This is supported by the histologic findings of the hypertrophy of hepatocytes and hyperplasia of phagocytic Kupffer cells and tendency towards hypertrophy with increased secretory activity of renal tubular cells of birds on AFK diets. The increase in weight of the lungs is suggestive of the involvement of pulmonary route in the excretion of volatile fraction(s) of AFK. The observed inflammatory and degenerative changes in chicks on RFK diet was an indication of toxicity in line with the report of Christopher *et al.* (1976) on deoiled neem kernel cake. Moreover, the elevation in aspartate aminotransferase activity in chicks lends credence to toxicity as reported on crambe meal diets (Kloss *et al.*, 1996). However, the damages appeared insufficient to cause high mortality in contrast with the reported severe mortality of chicks on 50 g RFK/kg diet (Sadagopan *et al.*, 1982). The discrepancy may be due to different duration of feeding which was short in this study.

Haemorrhagic anaemia has been observed in chicks fed diets containing RFK (Uko, 2003), with toxic constituents of neem being implicated in the toxicity (Devakumar and Sukh Dev, 1993). In the current study, features of anaemia were found only in chicks on the RFK diet. The leucocytosis

associated with neem diets is consistent with the reports of other workers (Christopher *et al.*, 1976; Nagalakshmi *et al.*, 1996), which was a response to the toxic challenges from neem kernel. Sai Ram *et al.* (1997) reported that neem oil stimulates cell mediated immunity especially increased macrophageal activity and lymphocyte proliferative response. However, differential leucocyte count was not done in the present study. The marked decline in plasma cholesterol and tri-glyceride concentrations with RFK was unexpected. The hypolipidaemic effect of RFK may be credited to two factors: (I) poor metabolizity of dietary fats and (2) direct damage to hepatocytes, although the degree to which each factor contributed to the low lipid levels is unknown.

CONCLUSION

Autoclaving for 30 min apparently reduced protein content and quality of neem kernel. The heat treatment did not necessarily improve the feeding value of neem kernel. The slower growth rate of birds fed neem diets was attributed to both lower ingestion and poorer digestion of the diets. However, mortality of birds was not significantly affected by the dietary treatments. Before neem kernel can be used as protein supplement in diets for cockerel chicks, therefore, it would require further processing to remove its toxins.

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