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HAEMATOLOGICALPARAMETERS AND WEIGHT CHANGES OF COCKERELS FED RAW OR AUTOCLAVED NEEM SEED KERNELS IN DIETS

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SUMMARY

Neem seed kernels were used in feeds either raw or autoclaved for 10, 20 and 30 min and each soaked in water (1:2; wt/vol.) for 24 hours. The four-neem kernel portions were ground and incorporated into standard basal diet at 150 and 225 g/kg, respectively, as substitutes for groundnut cake (GNC). The nine experimental diets were fed to 270 cockerel chicks (30/diet) for 49 days to evaluate haematology, weight changes and organ weight in response to the supplements. At 150 g/kg, autoclaved neem kernels supported weight gain and food efficiency as GNC; higher level of heat-treated and both levels of raw neem kernels depressed ($P<0.05$) food efficiency. Duration of heating had no effect ($P>0.05$) on the economic traits. Autoclaving improved ($P<0.05$) erythrocyte (RBC) production and cockerels fed diets with 150 g/kg heat-treated neem kernels had superior ($P<0.05$) packed cell volume (PCV), RBC number and haemogblobin concentration compared to those of birds on basal diet. Neem diets generally induced ($P<0.05$) lymphocytosis and high level (225 g/kg) of the kernels decreased ($P<0.05$) alkaline phosphatase (ALP) activity; otherwise neem kernels did not significantly alter the plasma metabolites. Carcass yield and organ weights were similar among the experimental groups save the greater ($P<0.05$) hepatic, renal and pancreatic weights in birds fed neem diets. The data revealed some beneficial effects of autoclaving on the economic traits of cockerels and possible presence of heat-resistant haemopoietic factor(s) in neem seed kernel.

KEYWORDS: Neem kernels; Autoclaving; Growth rate, Haematology, Cockerels.

INTRODUCTION

The quest for reduction in the cost of livestock production will continue to necessitate the use of unfamiliar plant protein feeds. Many of them do contain toxic agents that may negate the health status of the animals without necessarily affecting the economic traits in the short-run. Therefore, haematology should be a routine procedure in nutritional studies to provide a quick assessment of the health of animals. This is supported by earlier report that haematological and biochemical estimations are valuable aids to diagnosis in veterinary medicine (Ross *et al.,* 1976). Apart from toxicants that could affect haematological values, diets also have strong influence on blood parameters (Hackbarth *et al.,* 1983). Moreover, it is well known that the size, number and the haemoglobin content of red blood cells (RBC) depend on the amount of vitamins and trace elements

available to animal from diets (Vulterinova, 1981). Radomska *et al.* (1975) also found a significant influence of diets on haematological traits, which differed in protein and cellulose content, although Clapp (1980) observed no dietary effect at all on these variables. Diets with toxic agents may also elicit responses of body organs; especially those that are directly involved in detoxification process. The response may include increase or decrease in organ size and secretion of cellular metabolites (Alumot and Nitzan, 1961). In spite of the overwhelming evidence in support of the dietary effects on blood components and integrity of body organs, most animal nutritional studies using unconventional feeds are confined to the growth performance. Therefore, this study has been conducted to compare the effects of feeding raw or autoclaved and water-washed neem seed kernels versus groundnut cake (GNC) in diets on haematolgoical parameters and weight changes of cockerels.

MATERIALS AND METHODS

Diets and birds

Intact neem seed kernels were used either raw or autoclaved for 10, 20 and 30 min (Neem 10, 20, and 30, respectively) and separately soaked in water at a ratio of 1 to 2 (wt/Vol.) for 24 hours. They were rinsed three times, dried in the sun and ground. Each of the four-neem products were examined for the proximate constituents (AOAC, 1990) and incorporated into standard cockerel chicks' diets at two levels (150 and 225 g/kg diet). GNC was used as control protein in a completely randomised experiment with nine treatments (2x4+1) and three replicates per treatment. The diets were similar in crude protein (22%) and metabolisable energy (12 MJ/kg) content. Two hundred and seventy, one-day-old, cockerel chicks of Hubbard strain with initial mean weight of 42.9 ± 4.3 g were equally distributed to the 27 replicate groups of 10 chicks, each, on weight equalization basis. The chicks were fed their respective diets as mash *ad libitum* for 49 days. Lighting regimen of 24 hours daily was maintained for the first 35 days, but switched on to natural light of about 12 hours per day subsequently. Weekly data on weight changes; feed consumption and computation of efficiency of weight gains were recorded.

Blood analysis

Blood samples were drawn from the heart of 6 chickens per group (2 per replicate) on day 49 of the study and dispensed into ethylenediamine tetra-acetate-coated bottles for blood counts. Samples without anticoagulant were allowed to clot at room temperature, centrifuged and sera stored at -80°C before use for the determination of serum biochemical constituents. However, glucose concentration and enzyme activities were determined immediately. Whole blood samples were analyzed for PCV, Hb (Benjamin, 1985), RBC and leucocyte (WBC) counts (Natt and Herrick, 1952). Thin blood smears were made and stained with Wright-Leishman stain for WBC differential count. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (Swenson, 1996). The following blood metabolites were also determined: total protein, based on Coomassie blue binding reaction (Bradford, 1976); albumin, based on dye-binding reaction with bromocresol green (Doumas *et al.*, 1971); uric acid, according to the method of Henry *et al.* (1957); creatinine, according to Slot (1965); glucose, by the glucose oxidase procedure of Mattenheimer (1970); ALP (Wootton, 1964); alanine (ALT) and aspartate (AST)

aminotransferases, by colorimetric method (Reitman and Frankel, 1957); total cholesterol, by enzymatic method (Allain *et al.*, 1974); triglycerides, by the method of Kessler and Lederer (1965) as modified by Noble and Campbell (1970); and total and conjugated bilirubin by the methods of Michaelson (1961) using commercially available reagents (Sigma-Aldrich Fine Chemicals GmbH, St. Louis, MO). The tests were measured using auto-analyzer (SMA. 12/60 Technicon Autoanalyser, Terry-town, N. Y).

Organ measurements

Six cockerels from each group were selected at random for slaughter at 49th day of age. Food was withdrawn 6 h before slaughter but birds had free access to water. Each bird was weighed (slaughter weight) just prior to slaughter, killed by decapitation and manually eviscerated. Visceral organs were isolated and weighed. The carcasses were scalded, plucked and carcass yield computed (carcass wt/live wt.)

Statistics

Based on the experimental design, data were statistically analysed by the GLM procedure and means compared by the Ducan's multiple range test (SAS Institute, 1992).

RESULTS

Weight changes

Neem kernel is rich in protein and much more in fat content; although autoclaving slightly lowered these values. The mineral content as ash is low but was not affected by heat (Table I). High level (225 g/kg) of autoclaved neem kernel significantly ($P<0.01$) depressed appetite and slowed ($P<0.01$) weight gain of cockerels. However, inclusion rate of 150 g neem kernel/kg diet stimulated similar food ingestion and weight gain as GNC. On the contrary, increased ($P<0.01$) intake of raw neem diet did not necessarily support superior weight gain. Efficiency of food utilization worsened ($P<0.05$) with high level of autoclaved and both levels of raw neem kernels. Autoclaving beyond 10 min. did not produce any beneficial effect on the economic traits (Table II).

TABLE I: Protein, oil and mineral content of neem seed kernels

Nutrients (g/kg)	Raw neem	Duration of heat treatment (min.)		
		Neem 10	Neem 20	Neem 30
Crude protein	282	278	274	269
Ether extract	517	508	489	486
Ash	46	46	46	46

TABLE II: Weight changes and feed efficiency of cockerels fed raw or autoclaved neem diets

Parameters	Diets									SEM
	Control (g/kg) -	Raw Neem 150	Raw Neem 225	Neem 10 150	Neem 10 225	Neem 20 150	Neem 20 225	Neem 30 150	Neem 30 225	
Weight gain (g/bird)	375.3^{a}	365.0^{a}	300.8^{b}	369.9^{a}	255.8^{b}	376.3^{a}	268.0^{b}	359.7^{a}	270.9^{b}	18.59**
Feed intake (g/bird)	1107.4^{b}	1347.5^{a}	1043.7^{b}	1092.7^{b}	862.4^{c}	1141.7^{b}	872.2^{c}	1058.4^{b}	857.5^{c}	58.31**
Feed efficiency (feed/gain)	2.94^{b}	3.70^{a}	3.45^{a}	2.94^{a}	3.35^{b}	3.03^{b}	3.23^{a}	2.94^{b}	3.25^{a}	0.784*

^{abc} Means in a row with different superscripts differ (*P<0.05; **P<0.01).

Blood composition

The haemogram of chickens are presented in Table III. Birds fed on diets containing Neem 30 or diets with 150 g/kg Neem 10 and 20, respectively, had higher (P<0.05) PCV values, RBC counts and Hb concentrations than chickens fed reference diet or diets with higher level of Neem 10 and 20, respectively. On the other hand, the two levels of raw neem kernels lowered (P<0.05) the blood traits compared to GNC or autoclaved neem kernels. The RBC indices (MCV, MCH and MCHC), however, were remarkably similar among the groups. Similarly, no significant differences were observed in WBC and differential counts among chickens on any of the neem diets but these (total WBC and lymphocytes) were higher (P<0.05) than the cells of birds on reference diet. As shown in Table IV, the blood metabolites were not significantly altered by the dietary treatment except that ALP activity was elevated (P<0.05) by reference or 150 g/kg neem diets.

Carcass and organ weights

The data on carcass and organ weights (Table V) showed that neem supplementation did not affect (P>0.05) relative weights of carcass and organs except those of the liver, kidneys and pancreas. Carcass yield marginally improved with control diet or diets containing low than with high levels of neem kernels. The reverse was noticed for the heart, proventriculus, small intestine, ceca, colorectum and gizzard weights save the smaller (P>0.05) weights of the gizzards of birds on 225 g/kg raw neem diet. There was no consistent pattern of weights of lungs and spleen as influenced by the dietary treatments. Neem diets significantly (P<0.05) increased the weights of liver and kidneys than control diet, and the high level of neem kernels had a more pronounced (P>0.05) effect. However, only raw neem diets or 225 g/kg autoclaved neem diets stimulated the production of heavier (P<0.05) pancreas.

TABLE III: Haematology of cockerels on diets with raw or heat-treated neem kernels for 49 days

Nutrients	Ref. (GNC)	Raw Neem	Neem	Neem 10	Neem	Neem 20	Neem	Neem 30	SEM
	-	150	225	150	225	150	225	150	225	
Packed cell Vol. (%)	20.0^{b}	18.13^{c}	18.05^{c}	23.75^{a}	19.25^{b}	22.43^{a}	19.50^{b}	23.03^{a}	22.50^{a}	1.391
Erythrocytes (x10^{6}/µl)	1.788^{b}	1.620^{c}	1.615^{c}	2.125^{a}	1.661^{bc}	2.035^{a}	1.770^{b}	2.060^{a}	2.002^{a}	0.123
Haemoglobin (g/dl)	4.69^{b}	4.24^{c}	4.24^{c}	5.54^{a}	4.49^{bc}	5.26^{a}	4.58^{b}	4.56^{a}	5.25^{a}	0.478
MCV (fl)	111.8	112,0	113.8	111.7	115.8	110.1	110.2	111.9	112.5	2.58
MCH (Pg)	26.23	26.17	26.25	26.07	27.03	25.86	25.87	27.01	26.22	1.675
MCHC (g/dl)	23.45	23.39	23.50	23.42	23.32	23.48	23.48	24.14	23.42	1.099
Leucocytes (x10^{3}/µl)	20.123^{b}	24.342^{a}	23.114^{a}	25.573^{a}	24.37^{3}	25.463^{a}	23.076^{a}	25.315^{a}	25.176^{a}	1.08
Lymphocytes (%)	51.3^{b}	58.4^{a}	55.1^{a}	57.3^{a}	54.0^{a}	55.3^{a}	59.9^{a}	56.7^{a}	59.3^{a}	3.12
Heterophils (%)	37.0	30.2	33.6	30.5	33.8	32.7	27.4	34.1	27.3	2.01
Monocytes (%)	8.0	9.2	11.1	10.0	10.1	6.7	8.2	7.2	9.4	0.64
Eosinophils (%)	3.7	2.2	0.2	2.2	2.1	4.0	3.3	2.0	3.5	0.23
Basophils (%)	0	0	0	0	0	1.3	1.2	0	0.5	-

[a,b,c] Means in a row without the same superscripts differ (P<0.05).

TABLE IV: Blood chemistry of cockerels on diets containing raw or heat-treated neem kernels for 49 days

Blood metabolites	Ref.	Raw	neem	Neem	10	Neem	20	Neem	30	SEM
	-	150	225	150	225	150	225	150	225	
Total protein (g/dl)	4.10	4.30	3.74	4.0	4.05	4.20	4.10	4.05	3.65	0.44
Albumin (g/dl)	1.80	1.95	1.79	1.70	1.82	1.87	1.84	1.80	1.62	0.231
Globulin (g/dl)	2.30	2.35	2.04	2.22	2.23	2.33	2.26	2.25	2.03	0.301
Albumin: glubulin	0.78	0.82	0.83	0.81	0.82	0.80	0.81	0.80	0.80	0.087
Uric acid (mg/dl)	2.50	2.64	2.75	2.65	2.60	2.63	2.76	2.56	2.45	0.107
Creatinine (mg/dl)	0.40	0.52	0.48	0.45	0.50	0.51	0.41	0.47	0.50	0.026
Glucose (mg/dl)	7.20	7.50	8.15	7.25	7.75	7.68	8.20	7.46	7.05	1.192
Total bilirubin (mg/dl)	0.54	0.47	0.50	0.48	0.49	0.45	0.55	0.48	0.51	0.035
Conj. bilirubin (mg/dl)	0.22	0.19	0.21	0.21	0.22	0.18	0.23	0.20	0.24	0.015
Unconj. Bilirubin (mg/dl)	0.33	0.27	0.29	0.26	0.27	0.28	0.31	0.29	0.31	0.047
Alkaline phosph. (iu/l)	201.3[a]	185.7[a]	148.3[b]	185.6[a]	153.0[b]	189.4[a]	164.6[b]	183.6[a]	196.4[a]	351.58
Aspartate trans. (iu/l)	27.5	26.1	24.8	27.5	25.0	26.2	22.5	24.8	27.5	3.745
Alanine trans. (iu/l)	14.5	15.3	16.0	15.0	15.0	14.8	15.5	16.0	15.0	1.387
Cholesterol (mg/dl)	128.8	131.4	127.5	146.4	112.1	127.3	113.6	119.8	136.6	29.71
Triglycerides (mg/dl)	118.3	113.0	114.9	111.1	110.3	108.7	120.2	115.1	111.4	24.08

[ab] Means in a row without the same superscripts differ (P<0.05).

TABLE V: Carcass yield and organ weights (%) of cockerels on diets with raw or heat-treated neem kernels for 49 days

Carcass/Organ weights	Ref.	Raw	Neem	Neem	(Duration of autoclaving (mins.)					SEM
					10	Neem	20	Neem	30	
	-	150	225	150	225	150	225	150	225	
Carcass yield	73.8	74.2	72.5	75.7	72.5	73.0	71.4	74.5	73.0	2.60
Heart	0.79	0.84	0.96	0.90	1.03	0.78	0.88	0.87	0.74	0.083
Lungs	0.68	0.67	0.68	0.69	0.65	0.64	0.66	0.65	0.68	0.055
Liver	2.78[b]	3.42[a]	4.01[a]	3.31[a]	4.17[a]	3.53[a]	3.70[a]	3.75[a]	3.67[a]	0.503
Kidneys	1.16[b]	1.36[a]	1.44[a]	1.31[a]	1.38[a]	1.32[a]	1.43[a]	1.36[a]	1.47[a]	0.095
Spleen	0.23	0.21	0.23	0.20	0.19	0.22	0.23	0.18	0.23	0.043
Pancreas	0.30[b]	0.38[a]	0.46[a]	0.32[b]	0.45[a]	0.30[b]	0.39[a]	0.31[b]	0.38[a]	0.051
Proventriculus	0.89	0.80	0.87	0.79	0.88	0.78	0.84	0.81	0.83	0.113
Gizard	4.98	5.10	4.92	4.96	5.19	4.73	4.98	4.77	4.96	0.54
Small intestine	8.75	8.39	8.84	8.19	9.38	8.12	8.77	8.46	8.65	1.054
Ceca	0.93	0.88	0.92	0.90	0.94	0.87	0.94	0.91	0.95	0.126
Colorectum	0.28	0.30	0.33	0.28	0.31	0.27	0.34	0.31	0.36	0.059

Organ weights were expressed as % of carcass weight. a, b Means in a row with different superscripts differ (P<0.05).

DISCUSSION

The improvement in weight gain and feed efficiency as promoted by diets low in autoclaved neem kernels was consequent upon their higher consumption. The increased palatability of these diets is explicable in the light of lower content of neem bitters. Although, the exact stimulus for the marked increase in the ingestion of raw neem diets is yet to be identified, it is believed that autoclaving further exposed neem bitters. Also, heat-labile toxicant(s) in raw neem kernel appeared to have 'tied-down' nutrients; therefore birds had to ingest larger quantity of diets to meet their nutrient requirements. The above view is consistent with that of Alumot and Nitzan (1961) on the relationship between some anti-nutritional factors and feed intake by animals. The insufficient availability of nutrients accounted for the poorer feed efficiency of the raw neem diets.

The higher erythrocytic traits with reference, 150 g/kg autoclaved neem or 225 g/kg Neem 30 diets than the other diets evinced the nutritional superiority of the former diets. The influence of diets on haematological traits is strong (Hackbarth *et al.*, 1983), and PCV and Hb have been shown to indicate nutritional status of subjects (Church *et al.*, 1984). The high blood values supported by 225 g/kg Neem 30 diet appears confounding since birds on this diet did not grow as fast as those on reference diet. It is possible that heating for 30 min destroyed most toxins responsible for suppressing blood production. The highest blood values elicited by 150 g/kg autoclaved neem diets suggests the presence of heat-resistant haemopoietic agent(s) in neem kernel, the effect of which could not be suppressed by the low level of neem toxins. The leucocytosis induced by neem diets was characterized by lymphocyte-proliferative response, which is in accordance with the report of immunomodulatory activities of NIM-76, a fraction of neem oil, and other neem extracts (SaiRam *et al.*, 1997, 2000). The known rise in serum ALP activity during active growth (Wootten, 1964, Nagalakshmi *et al.*, 1996) was demonstrated in the current study by an increase in the enzyme activity of birds that had higher growth rate. The low bilirubin level in birds on raw neem diets is suggestive of non-haemolytic anaemia. This agrees with findings in an earlier study (Uko and Kamalu, 2002) in which raw, unprocessed neem kernels induced severe haemorrhagic anaemia.

The heavier relative weights of liver, kidneys and pancreas of neem-fed birds suggest hypertrophic response. Visceral organ hypertrophy is common when monogastrics are fed insufficiently processed plant proteins (Simovic *et al.*, 1972; Babatunde and Pond, 1987). It is usually associated with increased enzyme secretions by the organs in response to presence of enzyme inhibitors from the plants (Alumot and Nitzan, 1961),

In conclusion, water-washed full-fat raw neem kernel is detrimental to feed efficiency and erythrocytic traits in cockerels. Autoclaving partially alleviated these detrimental effects; and heat treatment for 30 min stimulated production of RBC better than GNC. Moreover, the results clearly showed that autoclaved full-fat neem kernel may be safely incorporated up to 150 g/kg in rations for cockerels.

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