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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

The Effects of Dietary Graded *Leucaena leucocephala* Seeds on Performance and Blood Parameters in Broiler Chicks

Khadiga Abbas Abdelati¹, Mohamed Elamin Ahmed², Hasabelrasoul Hussein Mohamed³

¹Department of Animal Nutrition, Faculty of Animal Production,
University of Khartoum, P.O. Box: 71, Postal Code 13314, Khartoum, Sudan

²Department of Animal Production, Faculty of Agricultural Technology and Fish Sciences,
Alneelain University, P.O. Box: 12702, Postal Code 11121, Sudan

³Department of Animal Science, Faculty of Health, Agriculture and Natural Sciences,
Tshwane University of Technology, Pretoria, South Africa

Abstract: The core objective of this study was to assess the effects of different dietary *Leucaena leucocephala* seed on the performance and some blood parameters in broiler chick. Seeds were included at 0, 3, 6, 9 and 12% in the diet of one hundred twenty one-day-old unsexed broiler chicks. The results showed that, feed intake, body weight gain and PER were significantly ($p \leq 0.01$) depressed with the inclusion of 6, 9 and 12% leucaena seeds in the diet. Feed intake, body weight gain and PER were negatively correlated ($p \leq 0.05$) with levels of inclusion of leucaena seeds. However, overall FCR, haematological parameters, plasma albumin, glucose and mortality rate were not influenced ($p \geq 0.05$) by dietary treatments. Inclusion of dietary leucaena significantly decreased plasma Ca, Na, total protein and cholesterol. While plasma Glutamic oxaloacetic transaminase GOT, alkaline phosphatase ALP and uric acid were increased with the increase in levels of leucaena in the broiler diets.

Key words: *Leucaena leucocephala*, broiler, amino acids and cholesterol

Introduction

Leucaena leucocephala is a drought-resistant, leguminous tree found throughout the tropics and subtropics (Devendra, 1993). *Leucaena* leaves are readily consumed and nutritious; however, leucaena contains toxic compounds such as mimosine (Hammond, 1994). Seed legumes are potential substitute for soybean meal because of the similarity in their amino acids and energy profile (Wiryawan, 1997). Babiker (1997) used leucaena seeds at 5, 10, 15 and 20% in broiler diets. Although feed intake was not affected by inclusion of leucaena seeds, weight gain showed a significant depression. Lee and Yang (1981) investigated the use of leucaena seeds (soaked, boiled and dried) to supply 0, 5, 10, 15 and 20% of dietary protein for young chicks. It was found a marked decrease in gain and feed conversion ratio. Although there are differences in amino acid concentrations between legumes, there may be much variation between samples of one legume (Evans, 1985). The amino acids content of grain legumes varies according to cultivar and environment (Igbasan and Guenter, 1996). It is well established that the composition of grain legumes varies between and within species (Wiryawan, 1997). Various reports indicated the significant contribution of leucaena to small ruminants and pig's performance and production (Kanani *et al.*, 2006). However the data on poultry is scarce and fragmented. The *Leucaena leucocephala* tree has a high annual biomass yield with

a high protein content, which makes it one of the forages with a high potential for feeding non-ruminants in tropical countries (Whiteman, 1980). However, the utilization of *Leucaena* in non-ruminant has been limited due to its detrimental effect on productive performance. The objective of this experiment is to assess the dietary effect of leucaena seeds on broiler performance.

Materials and Methods

Chickens and treatments: One hundred twenty one-day-old unsexed broiler chicks of a commercial strain (Ross 308) were purchased from Coral hatcheries after being vaccinated against Marek's disease. The chicks were divided into five treatment groups of twenty four birds each and randomly assigned to the dietary treatments. Each group was further divided into four replicates of six birds each. The chicks were reared from one-day-old to the seventh week of age in 20 pens (1x1x1 m) with wood shavings litter. The experiment was conducted in an open-sided poultry house (25X5 meter) in the poultry unit, Faculty of Animal Production, University of Khartoum.

Five isocaloric and isonitrogenous starter and finisher diets were formulated according to nutrient specifications of the standards published by National Research Council (NRC, 1994). Proximate and amino acids composition *Leucaena leucocephala* seeds were analyzed according to the methods outlined in the AOAC (1990). Diet A was the control with 0% of *Leucaena*

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Table 1: Composition of experimental broiler starter diets containing graded levels of *Leucaena leucocephala*

Ingredients, %	Levels of <i>Leucaena leucocephala</i> , %				
	0 (A)	3 (B)	6 (C)	9 (D)	12 (E)
Sorghum	59.30	59.00	58.00	58.00	58.00
Ground nut meal	17.20	12.40	10.00	7.00	5.90
Sesame meal	12.50	14.20	15.00	16.80	14.80
Wheat bran	2.00	2.20	1.90	0.00	0.00
Super concentrates*	5.00	5.00	5.00	5.00	5.00
Bone meal	2.00	2.20	2.10	2.20	2.30
NaCl	0.30	0.30	0.30	0.30	0.30
Vegetable oil	1.70	1.70	1.70	1.70	1.70
Calculated analysis					
ME (kcal/kg)	3171.09	3160.23	3152.50	3162.97	3155.86
CP%	23.39	22.98	22.99	23.07	22.72
Crude fiber%	4.57	4.66	4.83	4.83	4.96
Ca%	1.35	1.42	1.41	1.46	1.45
Av. Phosphorous%	0.42	0.44	0.43	0.44	0.44
Lysine%	1.07	1.07	1.09	1.09	1.10
Methionine%	0.47	0.48	0.49	0.51	0.49
Meth. +Cystine%	0.75	0.76	0.77	0.78	0.76
Determined analysis					
CP%	25.03	24.40	24.31	23.96	24.11
Crude fiber%	4.66	4.75	4.88	4.86	5.15
EE%	4.96	5.03	5.10	5.13	3.88
Ash%	9.12	8.15	7.44	8.8	7.86
NFE%	49.67	50.81	52.34	51.05	51.87
Ca%	1.38	1.40	1.48	1.51	1.54
Total phosphorous%	1.42	1.47	1.44	1.49	1.50

* Cp 40%, ME 2000 kcal/kg, C.fiber 3%, EE 3%, Ash 34%, Ca 8%, Av. P 1.38%, Lysine 12%, Methionine 3%, Methionine+Cystine 3.5%.
 Vitamin A 250000 IU/Kg, Vitamin D₃ 50000 IU/Kg, Vitamin E 500Mg/Kg, Vitamin K₃ 60 Mg/Kg, Vitamin B₁/ Thiamin 20 Mg/Kg, Vitamin B₂/ Riboflavin 100 Mg/Kg, Niacin Vitamin PP 600 Mg/Kg, Pantothenic acid/ Vitamin B₅ 160 Mg/Kg, Vitamin B₆/ Pyridoxine 40 Mg/Kg, Vitamin B₁₂ 300 Mcg/Kg, Biotin/ Vitamin H 2000 Mcg/Kg, Choline 10000 Mg/Kg, Vitamin C 4000 Mg/Kg, Folic Acid 30 Mg/Kg, Iron 800 Mg/Kg, Manganese 1400 Mg/Kg, Copper 120 Mg/Kg, Zinc 1000 Mg/Kg, Iodine 6 Mg/Kg, Cobalt 12 Mg/Kg, Selenium 3 Mg/Kg.

leucocephala seeds. *Leucaena leucocephala* seeds were supplemented at 3%, 6%, 9% and 12% in diets B, C, D and E, respectively (Tables 1 and 2.). For the first 3 weeks, the chicks were fed starter diets and afterward they were placed on finisher experimental diets.

The birds had continuous access to water and the experimental diets were fed *ad libitum*. Feed samples were analyzed for proximate composition according to AOAC methods of analysis (1990). Continuous light was provided throughout the experimental period by a combination of natural daylight and artificial light. Artificial light was (12 hours) provided by incandescent bulb lamp of 60 watts. All chicks were vaccinated against infectious bursal disease (Gumboro) at two weeks of age. Vitamins were offered via drinking water at first three days. Feed intake and body weight were recorded weekly. Mortality was recorded daily as it occurred.

Sample collection and analysis: Blood samples were taken from jugular vein during slaughtering of two birds per pen. The blood was received in a 10 ml test tube containing ETDA. Hematological indices examined include packed cell volume (PCV); hemoglobin

Table 2: Composition of experimental Broiler finisher diets containing graded levels of *Leucaena leucocephala*

Ingredients, %	Levels of <i>Leucaena leucocephala</i> , %				
	0 (A)	3 (B)	6 (C)	9 (D)	12 (E)
Sorghum	64.80	64.80	64.80	64.80	64.80
Ground nut meal	5.90	5.90	5.90	5.90	5.90
Sesame meal	7.40	7.40	7.40	7.40	7.40
Wheat bran	13.30	10.30	7.60	4.90	2.20
Super concentrates*	5.00	5.00	5.00	5.00	5.00
Bone meal	1.00	1.00	1.00	1.00	1.00
NaCl	0.30	0.30	0.30	0.30	0.30
Vegetable oil	2.30	2.30	2.00	1.70	1.40
Calculated analysis					
ME (kcal/kg)	3161.73	3182.21	3181.97	3181.72	3181.48
CP%	18.72	19.15	19.62	20.10	20.58
Crude fiber%	4.66	4.67	4.71	4.76	4.81
Ca%	0.92	0.93	0.93	0.94	0.95
Av. Phosphorous%	0.30	0.30	0.29	0.29	0.29
Lysine%	0.97	0.99	1.01	1.04	1.06
Methionine%	0.39	0.40	0.40	0.41	0.41
Meth. +Cystine%	0.62	0.62	0.63	0.64	0.64
Determined analysis					
CP%	19.11	19.77	19.97	20.13	20.45
Crude fiber%	4.77	4.72	4.8	4.82	4.97
EE%	3.90	4.10	4.14	4.21	4.32
Ash%	7.15	6.88	8.10	7.45	6.93
NFE%	56.62	56.89	56.11	57.44	56.48
Ca%	0.93	0.96	0.95	0.97	0.99
Total Phosphorous%	1.10	1.07	1.00	1.00	1.00

* As shown in Table 1.

concentration (Hb); red blood cells (RBCs). The percentages of lymphocytes, neutrophils, monocytes, eosinophils and basophils were determined microscopically. The blood film was examined using immersion lens (X100) magnification in the ideal area of the film. Examination of the blood film was done by Battlement method. The differential leukocyte count was also measured. Plasma total protein was determined as described by (King and Wooton, 1965). Plasma albumin, plasma globulin, plasma Na and plasma K were the determined by calorimetric method of (Baratholmew and Delaney, 1966). Plasma glucose and plasma cholesterol were determined by enzymatic calorimetric methods using kit GOD-PAP (Randox laboratory Ltd. Lodon). Plasma uric acid was measured by an enzymatic method using a kit (Plasmetec Laboratory Products Ltd., U.K). Plasma calcium was determined by calorimetric method described by (Trinder, 1967). Inorganic plasma phosphorus was determined by the method described by (Gomori, 1942). Plasma GOT was assayed by the method described by Reitman and Frankel (1957).

Statistical analysis: The experiment was arranged in a complete randomized design. Statistical analysis of the data was carried out using one-way analysis of variance (Steel and Torrie, 1980). In SAS (1985) version 6.12, Duncan's Multiple Range Test (DMRT) was used to detect significant differences between treatment means. Furthermore, regression analysis was used to

Table 3: Overall Performance of Broiler Chicks in Relation to Dietary *Leucaena leucocephala* Seeds

Parameter	Dietary levels of <i>Leucaena leucocephala</i> seeds %					± SEM
	0	3	6	9	12	
Feed intake (g/bird)	2708.36 ^{ab} ±295.32	2818.30 ^{ab} ±237.53	2371.46 ^{bc} ±207.14	2132.90 ^{cd} ±300.29	1863.71 ^{de} ±107.18	120.04
Body weight gain (g/bird)	1182.50 ^{ab} ±76.67	1258.25 ^{ab} ±98.17	1035.00 ^{bc} ±48.81	901.21 ^{cd} ±107.57	770.42 ^{de} ±18.59	38.61
FCR (g feed /g Bwt gain)	2.29±0.16	2.24±0.05	2.29±0.09	2.37±0.17	2.42±0.13	0.06
PER (Bwt gain/ protein consumed)	2.23 ^{ab} ±0.16	2.25 ^{ab} ±0.06	2.17 ^{bc} ±0.08	2.05 ^{cd} ±0.14	1.97 ^{de} ±0.10	0.06
Mortality	4±1.15	1±0.5	0±0.0	5±0.96	0±0.0	0.35

Values are means (±SD) of 4 replicates per treatment. abcdMeans with different superscripts in the same row were significantly different (P= 0.05) SEM: Standard error of the means from ANOVA d.f 15

Table 4: Linear regression equations relating levels of inclusion of *Leucaena leucocephala* seeds to performance parameters of broiler chicks

Parameter	Equation	R ²
Feed intake	- 7915.7X+2853.9	0.8985*
Body weight gain	- 3937.3X+1265.7	0.8756*
Feed conversion ratio	1.3X+2.244	0.7355 ^{ns}
Protein efficiency ratio	- 2.4X+2.278	0.8950*

* p<0.05, NS = Not Significant.

determine the possible effect of leucaena levels on feed intake, body weight gain, FCR and PER.

Results and Discussion

Overall performance as affected by inclusion of graded levels of leucaena seeds is shown in Table 3. Feed intake was significantly (p<0.01) affected by the dietary treatments. Birds fed the control diet and 3% leucaena seeds consumed significantly (p<0.05) higher feed than those fed 9, 12% while birds on 6% did not differ (p>0.05) from either those on control or on 9%. Regression equation (Table 4.) showed a significant and negative correlation (p<0.05) between levels of inclusion of leucaena seeds and feed intake. Reduction of feed intake of broiler chicks fed leucaena had been reported by El-Harith *et al.* (1979) and Sethi and Kulkarni (1995), who attributed the effect to mimosine which inhibits the appetite. Growth curve of broiler chicks as affected by different levels of dietary leucaena seeds was shown in Fig. 1. Higher body weight was observed for chicks fed the control diet and 3% leucaena. However, the lowest body weight was reported for birds given 12% leucaena seeds. The body weight gain was significantly (p<0.01) influenced by the dietary treatments. Highest weight gain was observed in chicks fed the control diet and 3% leucaena seeds (p<0.05). Regression equation relating levels of inclusion of leucaena seeds to body weight gain is given in Table 4. It revealed a significant and negative correlation (p<0.05). These results are in agreement with the findings of Sethi and Kulkarni (1995), who reported that body weight gain decreased with the increased leucaena seeds in the diet. The decrease in body weight gain for groups fed on leucaena seed diets is possibly related to the inadequate intake of necessary essential

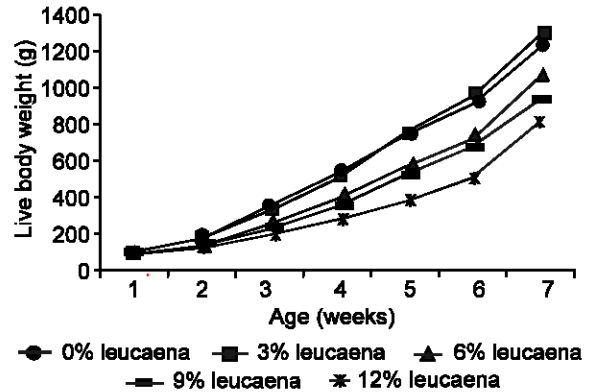


Fig.1: Growth curve of broiler chicks fed different levels of *Leucaena leucocephala* seeds.

nutrients, proteins and essential amino acids. This is coincided with the finding of (Scott *et al.*, 1982), who attributed the reduced weight gain to inadequate protein and essential amino acids. Furthermore, those authors ascribed the reduced growth of chicks fed legumes seeds to trypsin and chymotrypsin inhibitors, which interfere with digestion of proteins in the gastrointestinal tract of the animal. Overall FCR was not affected (p>0.05) by the dietary treatments. Moreover regression analysis (Table 4.) indicated that FCR was not correlated (p>0.05) with levels of inclusion of leucaena seeds. Overall PER was significantly (p<0.05) affected by inclusion of graded levels of leucaena seeds. It was similar for birds fed the control diet compared to the other groups except that for birds fed 12% leucaena seeds, which was considered as the poorest one. Regression analysis (Table 4) showed that PER is negatively (p<0.05) correlated with levels of inclusion of leucaena seeds. The better improvement in the performance of broiler chicks fed 3% leucaena seeds could be attributed to the quality of dietary protein, which enhanced as a result of the combination of more than one source of protein (Scott *et al.*, 1982). The observed growth depression, reduced feed intake and PER of broiler chicks given leucaena seeds diets higher than 3% may also, in part, be attributed to the higher levels of the residual anti nutritional factors, particularly

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Table 5: Some haematological parameters of broiler chicks as affected by dietary *Leucaena leucocephala* seeds

Parameters	Dietary levels of <i>Leucaena leucocephala</i> seeds, %					± SEM
	0	3	6	9	12	
PCV%	27.33±2.31	28.67±1.15	27.00±2.65	27.33±2.08	28.67±1.15	1.14
Hb g/dL	13.57±0.62	12.68±1.12	13.39±1.61	12.86±1.42	12.86±0.54	0.66
RBC (x10 ⁹ /mm ³)	2.87±0.16	2.78±0.63	2.59±0.19	2.38±0.20	2.27±0.42	0.21
MCV (fl)1	95.72±13.43	107.06±25.57	105.19±18.81	115.05±7.75	129.05±22.97	10.88
MCH (pg) ²	47.40±3.75	47.04±9.51	52.16±9.91	54.23±7.01	58.03±11.61	5.08
MCHC% ³	49.82±3.59	44.18±2.17	49.63±4.13	47.01±2.94	44.86±0.97	1.72
Neutrophils%	34.33±1.15	36.67±1.53	36.00±3.61	38.67±3.21	41.67±2.89	1.53
Lymphocytes%	40.00±0.00	40.00±0.00	41.67±2.89	40.00±0.00	40.00±0.00	0.75
Monocytes%	10.33±3.21	8.00±2.65	8.33±5.03	7.00±1.73	6.33±2.08	1.83
Eosinophils%	11.33±1.53	11.33±0.58	12.00±2.65	13.00±1.00	10.67±0.58	0.86
Basophils%	4.00±2.65	4.00±2.65	2.00±1.73	1.33±0.58	1.33±0.58	1.09

Values are means (±SD) of 3 replicates per treatment. SEM: Standard error of the means from ANOVA d.f 10.

Table 6: Plasma constituents of broiler chicks as affected by dietary *Leucaena leucocephala* seeds

Constituents	Dietary levels of <i>Leucaena leucocephala</i> seeds, %					± SEM
	0	3	6	9	12	
GOT U/L	16.00 ^a ±1.00	19.00 ^a ±1.00	22.33 ^b ±2.08	25.33 ^b ±0.58	29.00 ^c ±1.00	0.71
ALP U/L	79.00 ^a ±1.00	80.67 ^a ±1.15	84.00 ^a ±1.00	86.33 ^a ±0.58	88.33 ^a ±0.58	0.52
Ca mg/dL	8.17 ^a ±0.21	7.97 ^a ±0.06	7.80 ^a ±0.10	7.60 ^a ±0.10	7.50 ^a ±0.10	0.07
Na mEq/L	127.33 ^a ±2.52	124.33 ^b ±0.58	122.00 ^b ±1.00	120.67 ^b ±0.58	118.67 ^b ±0.58	0.75
K mEq/L	5.10±0.10	5.10±0.10	5.10±0.10	4.80±0.10	4.57±0.06	0.05
Pi mg/100ml	4.00±0.10	3.87±0.06	4.00±0.10	3.53±0.06	3.43±0.06	0.04
Total protein g/dL	4.23±0.25	4.03 ^a ±0.06	4.00 ^a ±0.10	3.90 ^a ±0.10	3.70 ^a ±0.10	0.08
Albumin g/dL	2.27±0.31	2.03±0.06	1.97±0.06	2.03±0.12	1.97±0.15	0.10
Globulin g/dL	1.97 ^a ±0.06	2.00±0.00	2.03±0.06	1.87 ^a ±0.06	1.73 ^a ±0.12	0.04
Cholesterol mg/dL	194.33 ^a ±5.03	180.67 ^b ±1.15	167.67 ^b ±10.02	153.67 ^b ±1.53	150.00 ^b ±2.00	2.98
Uric acid mg/dL	1.30 ^a ±0.10	1.47 ^a ±0.06	1.63 ^a ±0.06	1.77 ^a ±0.06	2.03 ^b ±0.15	0.05
Glucose mg/dL	255.00±8.66	273.33±2.89	266.67±30.55	273.33±25.17	270.00±17.32	11.40

Values are means (±SD) of 3 replicates per treatment. abcde Means with different superscripts in the same row were significantly different ($P \leq 0.05$) SEM: Standard error of the means from ANOVA d.f 10

mimosine, protease inhibitors, haemagglutinins, tannin and phytin with the attendant adverse cumulative effect on the performance. These anti nutritional factors may exert their deleterious effects through depressed nutrient absorption as a result of extensive structural and functional disruption of intestinal microvilli. They could have led to shedding of brush-border membranes and decreased villus length with consequent reduction in the surface area for absorption in the small intestine as suggested by (Lorenzson and Olsen, 1982).

Haematology results as shown in Table 5 indicated that none of the experimental diets had a significant effect on haematological parameters. Agbede and Aletor (2003) obtained similar findings. Moreover, similar findings concerning PCV% are reported by Martinez *et al.* (1992), while results of RBC and MCV disagree with those researchers, who noted a significant reduction in RBC count and MCV in mice fed raw field beans.

Results of the effect of the inclusion of graded levels of leucaena seeds on plasma constituents are shown in Table 6. Plasma Ca, Na, total protein and cholesterol were significantly depressed as the level of leucaena seeds increased, while uric acid was significantly ($p \leq 0.01$) increased. The lowest levels of plasma electrolytes: Na and K were observed in birds fed 12% leucaena seeds. Plasma Pi was significantly ($p \leq 0.01$)

affected by inclusion of leucaena seeds. It was lowest for birds fed 9 and 12% leucaena seeds. Plasma GOT and ALP were significantly ($p \leq 0.01$) increased as dietary inclusion of leucaena seeds increased. Similar findings reported by Oyelola *et al.* (2004) who attributed high levels of GOT for birds fed on raw melon seed to negative effect of antinutrients. This also in agreement with Adam (1974) and Benno (1963) who ascribed the release of GOT in blood plasma to liver cell necrosis, altered cell membrane permeability, hepatotoxin and circularity disturbances of the liver. Decreasing plasma cholesterol with the increase of leucaena seed levels may be due liver necrosis as liver is one of the primary sites of cholesterol biosynthesis in poultry (Kurtoglu *et al.*, 2004). GOT and ALP are used to detect pathology of the liver (Oyelola *et al.*, 2004).

Plasma uric acid was significantly increased as the level of leucaena seeds increased. Similar response was reported by Oyelola *et al.* (2004) in broiler fed raw melon seed and fermented melon seed diets. These effects may be due to inefficient utilization of the protein as a result of antinutritional factors found in these diets. Whereas, Iheukwumere and Herber (2003) attributed low plasma uric acid observed in broilers to good quality protein utilization, which was shown by high plasma total protein. With reference to plasma total protein, it may

indicate the good quality dietary protein of the control, 3 and 6% leucaena seeds compared to the others. Plasma protein may be used as an indirect measurement of dietary protein quality (Tewe, 1985). Plasma albumin and glucose were not significantly affected by the inclusion of leucaena seeds.

Diet affected ($P < 0.05$) plasma concentrations of urea, threonine, arginine, valine, phenylalanine, isoleucine, leucine and lysine, with concentrations increasing as dietary level of leucaena increased. Diets of moderate to high levels (e.g., 45%) of leucaena with 0.75% mimosine can be fed to goats without adverse effects on BW gain in goats (Yamia *et al.*, 2000). In this study, plasma glucose was similar to findings of Mutayoba *et al.* (2003), who reported a non significant effect of leucaena leaf meal on plasma glucose in pullets. The lower plasma protein with the increased leucaena seeds in the present study could have been due to reduced feed intake observed in these chicks. Similar findings reported by Mutayoba *et al.* (2003) who included leucaena leaf meal in pullets' diets. Plasma globulin was significantly ($p \leq 0.01$) influenced by the dietary treatments. Significantly ($p \leq 0.05$) the lowest value was observed for birds fed 12% leucaena seeds. In the current study, The reduced plasma minerals compared to control may be due to reduced feed intake and/or to the metal-chelation effect of mimosine (Sethi and Kulkarni, 1995) and mineral-chelation of phytate (Sell *et al.*, 2000). Birds in the current experiment apparently looked healthy and this agrees with the observation of Lee (1980). The results of haematological variables suggested that the experimental diets did not precipitate detrimental effect on the health status of chicks. However, sub clinical effects (growth depression and less feed intake) were observed. Further study of the composition of leucaena-based diets appears necessary to achieve most efficient utilization in broiler.

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