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Effect of EDTA Supplementation on Broiler Chicks Fed *Leucaena leucocephala* Seeds

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Abstract: In the present experiment, 5 diets were formulated, control diet, 2 levels of leucaena seeds (6 and 12%) were supplemented with Ethylene Diamine Tetra Acetic Acid (EDTA) (0, 0.2%). Poor performance was associated with the inclusion of 12% leucaena seeds diets. Haemaological indices were not significantly ($p>0.05$) affected by the dietary treatments except PCV% and MCHC%. Though PCV% for birds fed leucaena seeds diets was not significantly ($p>0.05$) different compared to the control. Plasma constituents were not significantly ($p>0.05$) influenced by the dietary treatments except total protein, albumin and globulin. Leg abnormality showed that supplementation of 12% leucaena seed with EDTA had no effect. EDTA supplementation did not significantly ($p>0.05$) affected feed intake, FCR and PER.

Key words: Leucaena, broiler, EDTA, performance

INTRODUCTION

In many parts of the tropics and subtropics, animal and plant protein sources for poultry are fluctuating. This calls for search of new non-conventional sources such as leucaena seeds. Utilization of leucaena in poultry feeding is hindered by the presence of many antinutritional factors. However, mimosine is an important one (Ter Meulen *et al*., 1979), because of its metal-chelating ability which could probably disturb the action of metal-containing enzymes, especially those containing iron cations and cause inhibition of some biological reactions (Sethi and Kulkarni, 1995). Moreover, phytate can chelate mineral ions such as zinc and reduce the digestibility of amino acids and carbohydrates, impairing the availability of these compounds for poultry (Sell *et al*., 2000). Accordingly, using of natural and synthetic chelating agents can markedly improve mineral availability of grains that contain high phytate content. The high stability content of ethylene diamine tetra acetic acid (EDTA) in chelating metal ions enhances the availability of zinc and other divalent cations to chicks (Scott *et al*., 1982). Consequently, this experiment was designed to assess the effect of EDTA supplemented leucaena seeds diets on broiler performance.

MATERIALS AND METHODS

Seventy five, one day old unsexed commercial Hubbard broiler chicks were purchased from Arab Poultry Breeders Company-Egypt after vaccinated against Marek's disease. The chicks were divided into five treatment groups of 15 birds each and randomly assigned to the dietary treatments. Each group was further divided into 3 replicates of 5 birds each. The experiment was lasted for 6 weeks.

Five isocaloric and isonitrogenous starter and finisher diets were formulated according to National Research Council (NRC, 1994), Table 1 and 2. Diet (A) was the control with 0% of *Leucaena leucocephala* seeds, diet (B) 6% of untreated leucaena seeds, diet (C) 6% of leucaena seeds supplemented with 0.2% EDTA, diet (D) 12% of untreated leucaena seeds and diet (E) 12% of leucaena seeds supplemented with 0.2% EDTA. The chicks were fed starter diets for the first 3 weeks and then they were placed on finisher experimental diets. Feed samples were analyzed for proximate composition according to the methods outlined in the AOAC methods of analysis (1990).

Feed intake and body weight were determined weekly. Mortality was recorded daily as it occurred. Leg

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Table 1: Composition of experimental broiler starter diets containing graded levels of *Leucaena leucocephala* supplemented with EDTA.

	Levels of *Leucaena leucocephala* seeds (%)				
Added EDTA (%)	0 (A) 0.00	6 (B) 0.00	6 (C) 0.20	12 (D) 0.00	12 (E) 0.20
Ingredients					
Sorghum	59.00	58.00	57.80	56.00	55.80
Ground nut meal	19.00	12.00	12.00	8.00	8.00
Sesame meal	13.00	15.00	15.00	14.50	14.50
Super concentrates*	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	1.40	1.40	1.40	1.40	1.40
Nacl	0.30	0.30	0.30	0.30	0.30
Methionine	0.10	0.10	0.10	0.20	0.20
Vegetable oil	2.20	2.20	2.20	2.60	2.60
Calculated analysis					
ME (kcal kg^{-1})	3202.82	3186.66	3179.79	3184.07	3177.20
CP%	23.59	23.10	23.08	22.85	22.83
Crude fiber%	4.52	4.77	4.77	5.09	5.08
Ca%	1.12	1.14	1.14	1.12	1.12
Available Phosphorous%	0.45	0.45	0.45	0.45	0.45
Lysine%	1.09	1.10	1.10	1.12	1.12
Methionine%	0.58	0.60	0.60	0.69	0.69
Methionine+Cystine%	0.86	0.88	0.88	0.96	0.96
Determined analysis					
CP%	24.70	24.12	23.77	23.71	23.55
Crude fiber%	4.62	4.83	4.89	5.12	5.15
EE%	4.82	4.84	4.86	4.77	4.75
Ash%	7.88	9.15	8.65	7.65	8.15
NFE%	50.43	50.50	51.08	52.76	50.81
Ca%	1.16	1.16	1.17	1.15	1.14
Total phosphorous%	1.57	1.61	1.60	1.58	1.59

*Cp 40%, ME 2000 kcal kg^{-1}, 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^{-1}, Vitamin D3 50000 IU kg^{-1}, Vitamin E 500Mg kg^{-1}, Vitamin K3 60 Mg kg^{-1}, Vitamin B1/ Thiamin 20 Mg kg^{-1}, Vitamin B2/ Riboflavin 100 Mg kg^{-1}, Niacin Vitamin PP 600 Mg kg^{-1}, Pantothenic acid/ Vitamin B3 160 Mg kg^{-1}, Vitamin B6/ Pyridoxine 40 Mg kg^{-1}, Vitamin B12 300 Mcg kg^{-1}, Biotin/ Vitamin H 2000 Mcg kg^{-1}, Choline 10000 Mg kg^{-1}, Vitamin C 4000 Mg kg^{-1}, Folic Acid 30 Mg kg^{-1}, Iron 800 Mg kg^{-1}, Manganese 1400 Mg kg^{-1}, Copper 120 Mg kg^{-1}, Zinc 1000 Mg kg^{-1}, Iodine 6 Mg kg^{-1}, Cobalt 12 Mg kg^{-1}, Selenium 3 Mg kg^{-1}

Table 2: Composition of experimental broiler finisher diets containing graded levels of *Leucaena leucocephala* supplemented with EDTA

	Levels of *Leucaena leucocephala* (%)				
Added EDTA (%)	0 (A) 0.00	6 (B) 0.00	6 (C) 0.20	12 (D) 0.00	12 (E) 0.20
Ingredients					
Sorghum	67.70	64.80	64.60	66.10	65.90
Ground nut meal	5.00	5.40	5.40	4.00	4.00
Sesame meal	7.00	7.40	7.40	7.40	7.40
Wheat bran	11.50	7.60	7.60	2.20	2.20
Super concentrates*	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00
Nacl	0.30	0.30	0.30	0.30	0.30
Vegetable oil	2.50	2.50	2.50	2.00	2.00
Calculated analysis					
ME (kcal kg^{-1})	3195.54	3198.60	3191.73	3213.25	3206.37
CP%	17.98	19.15	19.12	19.67	19.64
Crude fiber%	4.38	4.67	4.66	4.65	4.65
Ca%	0.85	0.87	0.87	0.87	0.87
Available Phosphorous	0.37	0.37	0.37	0.36	0.36
Lysine%	0.95	1.01	1.01	1.04	1.04
Methionine%	0.39	0.40	0.40	0.41	0.41
Methionine+Cystine%	0.60	0.62	0.62	0.63	0.63
Determined analysis					
CP%	19.67	20.18	20.11	19.98	20.74
Crude fiber%	4.51	4.82	4.85	4.80	4.87
EE%	3.61	3.75	3.74	3.92	3.95
Ash%	7.45	8.15	6.94	8.07	7.88
NFE%	56.28	55.27	57.91	55.42	55.60
Ca%	0.88	0.88	0.89	0.91	0.89
Total phosphorous%	1.38	1.30	1.31	1.26	1.25

* As shown in Table 1

conditions were examined visually at 6 weeks of age and scored as 0 for normal, 1 for slightly crippled, 2 for moderately crippled and 3 for completely recumbent and unable to walk.

At the termination of the experiment, blood samples were taken from jugular vein during slaughtering of 2 birds per pen. The blood was received in 10 mL test tube that contained ETDA.

The design for this experiment was a Completely Randomized Design (CRD). Statistical analysis of the data was carried out using one-way analysis of variance (Steel and Torrie, 1980) in SAS version 6.12 (1985). Duncan's Multiple Range Test was used to detect significant differences between treatment means.

RESULTS AND DISCUSSION

Overall performance in response to different dietary treatments is presented in Table 3. Feed intake was significantly ($p<0.01$) affected by the dietary treatments. Birds fed 12% leucaena seeds diets significantly consumed the lowest feed quantities as compared to those fed other diets. Meanwhile birds on the control diet consumed the highest amount of feed. The depression in feed intake might have been associated with mimosine, which may inhibit appetite. This is in line with Sethi and Kulkarni (1995). Moreover, the growth curve of broiler chicks (Fig. 1) shows that higher live body weight was recorded for birds fed the control and 6% leucaena seed diets and the lower live body weight was observed in birds that received 12% leucaena seed diets. Weight gain was significantly ($p<0.01$) affected by the dietary treatments. It was found to be significantly ($p<0.05$) highest for birds fed the control diet compared to the others. Body weight gain for birds fed 6% leucaena seeds and 6% EDTA supplemented leucaena seeds was significantly ($p<0.05$) higher than those fed 12% leucaena seeds and 12% EDTA supplemented leucaena seeds. Consequently, low feed intake resulted in inadequate protein and essential amino acids (Scott *et al*., 1982). Growth depression may be brought up from mimosine blocking effect of metabolic pathways of aromatic amino acids and tryptophan (Sethi and Kulkarni, 1995). The poorest overall feed intake, weight gain, FCR and PER were observed for birds that received 12% leucaena seeds diets compared to control. Furthermore, neither feed intake nor FCR and PER was significantly affected by EDTA supplementation. Similar findings were observed by Kabuage *et al*. (2002), who reported that fortification of amaranth grain diets with EDTA did not improve broiler performance. On the other hand, leg abnormality conditions were significantly ($p<0.01$) affected by dietary treatments. Birds that received the control diet and 6% leucaena seeds significantly ($p<0.05$) showed better values compared to the others.

Haematological parameters of broiler chicks as affected by leucaena seeds and EDTA supplementation are given in Table 4. The results revealed that dietary treatments did not affect these parameters, except PCV% and MCHC%. MCHC% for birds fed leucaena diets was lesser than that for control except that for 6% leucaena seeds with EDTA which was not different. The results of haematological parameters suggested that dietary treatments had no detrimental effect on chick's health. Plasma constituents and leg score of broiler chicks as affected by leucaena seeds and EDTA supplementation

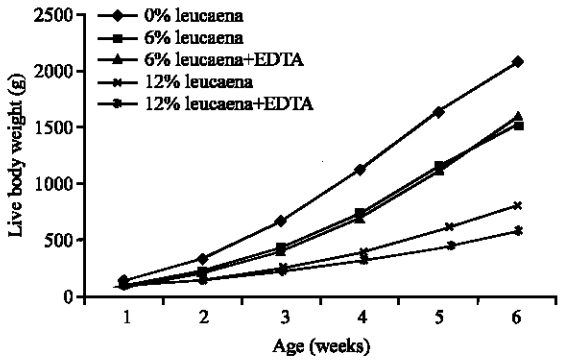

Fig. 1: Growth curve of broiler chicks fed dietary levels of Leucaena leucocephala seeds supplemented with EDTA

Table 3: Overall performance of broiler chicks as affected by *Leucaena leucocephala* seeds and EDTA supplementation

	Dietary levels of *Leucaena leucocephala* seeds (%)					
Added EDTA (%)	0 (A) 0.00	6 (B) 0.00	6 (C) 0.20	12 (D) 0.00	12 (E) 0.20	±SEM
Parameter						
Feed intake (g bird)	3580.25 ^{a}±486.55	2955.67^{ab}±270.29	2692.77 ^{b}±541.26	1382.32^{d}±279.24	1665.00^{d}±131.21	215.71
Body weight gain (g bird^{-1})	2042.23^{a}±250.77	1486.13^{b}±250.51	1541.42^{b}±202.43	543.76^{c}±198.34	767.40^{c}±141.42	122.73
FCR (g feed/g Bwt gain)	1.75 ^{b}±0.02	2.01 ^{b}±0.18	1.74 ^{b}±0.14	2.66 ^{a}±0.48	2.20 ^{ab}±0.23	0.15
PER (Bwt gain/protein consumed)	2.96 ^{a}±0.04	2.50 ^{ab}±0.22	2.91 ^{a}±0.24	1.88 ^{c}±0.37	2.25 ^{bc}±0.25	0.14
Leg score [1]	0.25 ^{b}±0.43	0.33 ^{b}±0.29	1.53 ^{a}±0.65	2.23 ^{a}±0.68	1.93 ^{a}±0.12	0.28

Values are means of 3 replicates per treatment, ^{abcd}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, [1]0 for normal, 1 for slightly crippled, 2 for moderately crippled and 3 for completely recumbent and unable to walk

Table 4: Some haematological parameters of broiler chicks as affected by *Leucaena leucocephala* seeds and EDTA supplementation

	Dietary levels of *Leucaena leucocephala* seeds (%)					
Added EDTA (%)	0 (A) 0.00	6 (B) 0.00	6 (C) 0.20	12 (D) 0.00	12 (E) 0.20	±SEM
Parameter						
PCV%	23.67^{abc}±0.58	26.00 ^{a}±0.00	21.67^{c}±0.58	22.67 ^{bc}±2.52	24.33^{ab}±1.53	0.79
Hb g dL^{-1}	14.81±0.51	13.48±0.26	13.04±0.26	10.81±3.56	10.52±1.56	1.02
RBC (×10^{6} mm^{-3})	2.47±0.30	2.41±0.26	2.31±0.04	2.10±0.63	2.13±0.15	0.19
MCV (fl) [1]	96.87±13.20	108.51±10.89	93.97±3.75	116.96±45.07	114.28±1.93	12.50
MCH (pg) [2]	60.41±5.58	56.19±4.64	56.53±1.34	56.17±26.47	49.29±5.32	7.23
MCHC% [3]	62.64 ^{a}±3.23	51.85 ^{bc}±0.99	60.19^{ab}±1.44	46.92^{c}±10.73	43.19 ^{c}±5.33	3.24

Values are means of 3 replicates per treatment, ^{abc}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, [1]Mean corpuscular volume, [2]Mean corpuscular haemglobin, [3]Mean corpuscular haemglobin concentration

Table 5: Effect of *Leucaena leucocephala* seeds and EDTA supplementation on plasma constituents and leg score of broiler chicks

	Dietary levels of *Leucaena leucocephala* seeds (%)					
Added EDTA (%)	0 (A) 0.00	6 (B) 0.00	6 (C) 0.20	12 (D) 0.00	12 (E) 0.20	±SEM
Parameter						
GOT U L^{-1}	34.67±6.66	33.67±4.04	38.33±8.96	40.67±3.06	33.67±4.04	3.33
ALP U L^{-1}	87.00±1.00	86.33±1.53	86.33±1.53	86.00±1.00	86.33±0.58	0.68
Ca mg dL^{-1}	7.87±0.06	7.73±0.55	7.87±0.12	7.87±0.15	8.13±0.67	0.23
Na mEq L^{-1}	140.67±4.16	141.33±3.21	139.00±1.00	140.00±2.00	140.33±1.53	1.53
K mEq L^{-1}	4.23±0.06	4.40±0.10	4.17±0.15	4.17±0.15	4.37±0.15	0.07
Pi mg 100 mL^{-1}	3.60±0.10	3.77±0.15	3.60±0.26	3.70±0.10	3.70±0.20	0.10
Total protein g dL^{-1}	5.03 ^{a}±0.82	4.98 ^{a}±0.67	4.04 ^{ab}±0.27	3.59 ^{b}±0.31	3.23 ^{b}±0.27	0.30
Albumin g dL^{-1}	2.39 ^{a}±0.34	2.39 ^{a}±0.17	2.10 ^{a}±0.20	1.97 ^{ab}±0.18	1.63 ^{b}±0.26	0.14
Globulin g dL^{-1}	2.64 ^{a}±0.49	2.59 ^{a}±0.50	1.94 ^{b}±0.19	1.62 ^{b}±0.13	1.60 ^{b}±0.02	0.19
Cholesterol mg dL^{-1}	194.33±5.03	199.67±6.81	200.33±9.07	191.67±5.51	195.00±5.57	3.79
Uric acid mg dL^{-1}	1.87±0.35	2.03±0.25	2.10±0.30	2.17±0.23	1.83±0.35	0.17
Glucose mg dL^{-1}	163.19±29.63	166.67±12.50	163.19±12.03	184.72±14.63	183.33±19.09	10.83

Values are means of 3 replicates per treatment, ^{abcd}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

(Table 5) showed that non of these parameters were affected by the dietary treatments except plasma total protein, albumin, globulin and leg score. The reduced total plasma protein and albumin associated with 12% leucaena seeds diets indicated the poor quality of these diets. Similarly, Tewe (1985) use plasma protein as an indirect measurement of dietary protein quality. This depression in plasma protein may be due to reduced feed intake of birds fed on 12% leucaena seed diets. Moreover, interference of mimosine with aromatic amino acids and tryptophan and that of trypsin inhibitors with protein digestion might have been also responsible for this depression. Better leg score was recorded for birds fed the control and 6% leucaena seeds compared to the others. The poor leg score might be due to low feed intake by these birds that resulted in low mineral intake or to metal chelation effect of mimosine (Sethi and Kulkarni, 1995) and/or mineral chelation effect of phytate (Sell *et al*, 2000).

CONCLUSION

Based on these results, it is suggested that, EDTA may provide appropriate findings if supplemented to leucaena seeds in different levels.

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