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Effect of Choline Chloride Supplementation on Broiler Chicks Fed *Leucaena leucocephala* Seeds

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Abstract: This experiment was carried out to assess the effect of feeding leucaena seeds supplemented with choline on broiler chicks' performance. Two levels of choline chloride (0, 0.1%) were added to three levels of leucaena seeds (0, 6 and 12%) and incorporated in broiler starter and finisher diets. All performance parameters were depressed with the inclusion of leucaena seed diets. PCV% of birds fed any level of leucaena seed diets was not significantly different compared to control. Plasma GOT, ALP, Na, Pi, K and globulin were not affected by the dietary treatments. Plasma cholesterol of birds fed leucaena seed diets was significantly lower than that of control. Leg score of birds fed leucaena seed diets was significantly ($p \leq 0.05$) poorer compared to the control except for birds fed 6% choline supplemented leucaena seeds.

Key words: Leucaena, broiler, choline chloride, performance, leg score

INTRODUCTION

Leucaena seeds are moderate in lysine and methionine (Ter Meulen *et al*., 1979). Moreover, antnutritional factors interfere with the normal digestive function and reduce availability of nutrients such as amino acids (Wiryawan, 1997). Annison (1996) clearly demonstrated that choline is considered as a source of labile methyl group. It is now considered as a common dietary supplement for animal. Fisher *et al*. (2002) stated that choline functions as a methyl donor in the synthesis of methionine from homo cysteine. Most of those authors agree that where diets are low in crude protein and/or marginal in total sulphur amino acids, responses to both methionine and choline supplementation occurs. INRA (1997) has examined the response to dietary choline chloride supplementation (0, 400, 800 and 1600 mg/kg) in broiler diet. Feed conversion was maximized at 800 ppm. Consequently, this experiment was carried out to elucidate the implications of feeding leucaena seeds supplemented with choline on broiler chicks' performance.

MATERIALS AND METHODS

Ninety one-day-old unsexed commercial Hubbard broiler chicks were purchased from Arab Poultry Breeders Company-Egypt after being vaccinated against Marek's disease. The chicks were randomly assigned to five dietary treatments; each group was replicated three times.

Five isocaloric and isonitrogenous starter and finisher diets were formulated according to nutrient specifications recommended by National Research Council (NRC, 1994), as follows: Diet (A) was the control with 0% of *Leucaena leucocephala* seeds, diet (B) 6% of untreated leucaena, diet (C) 6% of leucaena supplemented with 0.1% choline chloride, diet (D) 12% of untreated leucaena and diet (E) 12% of leucaena supplemented with 0.1% choline chloride. For the first 3 weeks, the chicks were fed starter diets and then they were allocated to finisher experimental diets. The composition of starter and finisher diets were shown in Table 1 and 2, respectively. 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 conditions were visually examined 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 end of the experiment, blood samples were taken from jugular vein during slaughtering of two birds per pen. The blood was received in 10 ml test tube that contained ETDA.

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 was used to detect significant differences between treatment means.

RESULTS AND DISCUSSION

Mean values on the overall performance as affected by the inclusion of two levels of leucaena seeds with choline chloride are shown in Table 3. Overall

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

	Levels of *Leucaena leucocephala* seeds (%)				
Added choline (%)					
Ingredients (%)	0 (A) 0.00	6 (B) 0.00	6 (C) 0.10	12 (D) 0.00	12 (E) 0.10
Sorghum	59.00	58.00	57.90	56.00	55.90
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)	3202.82	3186.66	3183.22	3184.07	3180.63
CP %	23.59	23.10	23.09	22.85	22.84
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 %	25.02	24.48	24.29	24.14	24.50
Crude fiber %	4.70	4.91	4.88	5.11	5.17
EE %	4.85	4.97	4.84	4.76	4.73
Ash %	7.66	8.84	7.93	8.15	8.47
NFE %	25.02	24.48	24.29	24.14	24.50
Ca %	1.15	1.17	1.15	1.14	1.14
Total phosphorous %	1.62	1.58	1.59	1.59	1.59

*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 B3 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

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

	Levels of *Leucaena leucocephala* seeds (%)				
Added choline (%)					
Ingredients (%)	0 (A) 0.00	6 (B) 0.00	6 (C) 0.10	12 (D) 0.00	12 (E) 0.10
Sorghum	67.70	64.80	64.70	66.10	66.00
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)	3195.54	3198.60	3195.17	3213.25	3209.81
CP %	17.98	19.15	19.13	19.67	19.65
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 %	19.11	20.74	21.28	21.37	20.87
Crude fiber %	4.5	4.9	4.88	4.85	4.85
EE %	3.62	2.85	3.82	3.95	3.9
Ash %	6.95	8.22	7.03	8.11	8.10
NFE %	57.56	55.06	55.73	54.73	54.91
Ca %	0.90	0.87	0.88	0.90	0.91
Total phosphorous %	1.37	1.33	1.33	1.30	1.29

*As shown in Table 1

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

Added choline (%)	Dietary levels of *Leucaena leucocephala* seeds (%)					
Parameters	0 (A) 0.00	6 (B) 0.00	6 (C) 0.10	12 (D) 0.00	12 (E) 0.10	±SEM
Feed intake (g/bird)	3254.39^{a}±200.29	2414.02^{b}±87.73	2528.28^{b}±215.50	1425.59^{c}±57.81	1570.23^{c}±101.84	84.84
Body weight gain (g/bird)	1741.22^{a}±89.93	1152.11^{b}±102.8	1176.28^{b}±58.44	583.78^{c}±18.95	639.12^{c}±73.35	43.06
FCR (g feed/g Bwt gain)	1.87^{c}±0.03	2.10^{b}±0.12	2.15^{b}±0.09	2.44^{a}±0.08	2.47^{a}±0.15	0.06
PER (Bwt gain/protein consumed)	2.77^{a}±0.05	2.37^{b}±0.14	2.31^{b}±0.10	1.99^{c}±0.07	1.99^{c}±0.12	0.06
Leg score[1]	0.00^{c}±0.00	0.93^{ab}±0.12	0.57^{bc}±0.51	1.50^{a}±0.50	1.20^{ab}±0.17	0.19

Values are means of 4 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]0 for normal, 1 for slightly crippled, 2 for moderately crippled and 3 for completely recumbent and unable to walk

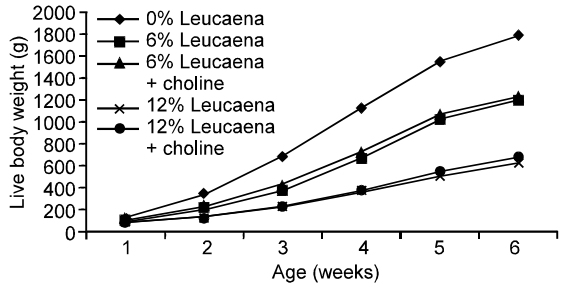

Fig. 1: Growth curve of broiler chicks fed dietary levels of *leucaena leucocephala* seeds supplemented with choline

performance parameters except for mortality were significantly ($p<0.01$) influenced by the dietary treatments. Birds fed the control diet significantly ($p<0.05$) showed best feed intake, body weight gain, FCR and PER values, whereas those that received 12% leucaena seeds diets significantly ($p<0.05$) showed the lowest values. The depression in feed intake associated with inclusion of leucaena seeds might have been due to different factors. Mimosine reduced feed intake through its appetite inhibitory effect (El-Harith *et al*., 1979 and Sethi and Kulkarni 1995). Figure 1 shows the growth curve of broiler chicks as affected by dietary leucaena and choline supplementation. It was revealed that the live body weight of birds fed the control and 6% leucaena seed diets was higher than that for birds received 12% leucaena seed diets. The depressed weight gain of broiler chicks as a result of feeding leucaena seeds diets is coinciding with the findings of Sethi and Kulkarni (1995). This depression may be due to insufficient intake of digestible nutrients (D'Mello *et al*., 1987). Supplementation of leucaena seeds with choline did not affect any of the overall performance parameters. Observations on overall performance indicated that choline chloride supplementation, did not significantly improved feed intake and body weight gain implying the low (1 g/kg diet) choline chloride supplement, hence the low methyl group supply. Mimosine and other antinutritional factors might have been responsible for adverse effect on the performance. For instance, mimosine inhibited protein biosynthesis in living body resulting in growth retardation (Sethi and Kulkarni, 1995). Those authors claimed that mimosine also inhibits some biological reaction through disturbing the action of metal containing enzymes.

Results of some haematological parameters (PCV%, Hb, RBC, MCV, MCH and MCHC%) as affected by inclusion of leucaena seeds with choline chloride supplementation are presented in Table 4. There was no significant ($p \geq 0.05$) effect of dietary treatments on RBC, MCV and MCH values. However, PCV% was significantly ($p \leq 0.05$) affected by the dietary treatments. The results of haematological indices indicated that the dietary treatments had no detrimental effect on chicks' health.

Plasma constituents of broiler chicks as affected by the inclusion of leucaena seeds supplemented with choline chloride are given in Table 5. Plasma GOT, ALP, Na, K, P and globulin were not significantly ($p \geq 0.05$) influenced by the dietary treatments while the other parameters were significantly affected. Plasma Ca of birds fed leucaena seeds diets was not significantly different, and significantly lower than that of control. The lower plasma Ca of birds fed leucaena seed diets may be due to low mineral intake or metal chelation effect of mimosine (Sethi and Kulkarni, 1995) and/or mineral chelation effect of phytate (Sell *et al*., 2000). These low levels of plasma Ca may be responsible for deteriorated leg score of birds fed on leucaena seed diets. Low plasma cholesterol of birds fed leucaena seed diets may be due to the effect of non starch polysaccharides. Similarly, Smits and Annison (1996) stated that viscous materials appear to act as a molecular filter because the severity of their effect varies with the molecular size of each nutrient. Hence the absorption of fats, fat soluble vitamins and cholesterol will be most affected as the bile salt micelles, used for transporting these substances in the gut, are large molecules compared with the size of sugars, amino acids, vitamins and minerals (Scott *et al*., 1982). Non starch polysaccharides may increase the viscosity of the digesta in the lumen of the gut, resulting in a reduction in the rate of hydrolysis of starch, protein and fat and a reduction in the transport and uptake of the products of starch, protein and fat digestion from the gut (Wiryawan, 1997). Plasma total protein of birds supplemented with leucaena seeds diets were not different from the control, whereas that of birds fed 6% choline supplemented leucaena seeds, was higher. This indicated that choline supplementation to the

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

	Dietary levels of *Leucaena leucocephala* seeds (%)					
Added choline (%)						
Parameters	0 (A) 0.00	6 (B) 0.00	6 (C) 0.10	12 (D) 0.00	12 (E) 0.10	±SEM
PCV %	24.67^{abc}±0.58	26.33^{a}±0.58	26.00^{ab}±0.00	23.67^{c}±1.15	24.33^{bc}±1.53	0.54
Hb g/dL	12.29^{a}±0.25	11.86^{ab}±0.65	11.14^{bc}±0.43	10.57^{c}±0.49	11.79^{ab}±0.21	0.25
RBC (x10^{6}/mm^{3})	2.94±0.55	2.77±0.64	2.88±0.26	2.27±0.28	2.38±0.13	0.24
MCV (fl)[1]	85.88±15.76	99.10±25.35	90.80±8.66	105.23±14.28	102.30±5.14	8.93
MCH (pg)[2]	42.79±8.01	44.75±12.49	38.83±2.38	46.79±3.59	49.59±1.81	4.02
MCHC %[3]	49.81^{a}±0.16	45.08^{abc}±3.40	42.86^{c}±1.65	44.77^{bc}±3.65	48.54^{ab}±2.56	1.51

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 choline chloride supplementation on plasma constituents and leg score of broiler chicks

	Dietary levels of *Leucaena leucocephala* seeds (%)					
Added choline (%)						
Parameters	0 (A) 0.00	6 (B) 0.00	6 (C) 0.10	12 (D) 0.00	12 (E) 0.10	±SEM
GOT U/L	28.33±2.08	30.67±2.08	31.33±4.51	37.33±2.08	31.33±4.16	1.84
ALP U/L	85.67±2.08	86.00±2.00	84.33±2.08	84.67±0.58	86.00±2.00	1.06
Ca mg/dL	8.27^{a}±0.40	7.20^{b}±0.10	7.20^{b}±0.20	7.13^{b}±0.15	7.30^{b}±0.10	0.13
Na mEq/L	136.67±3.06	146.67±7.64	143.67±5.69	144.00±4.00	148.67±8.50	3.54
K mEq/L	4.40±0.10	4.40±0.10	4.50±0.10	4.40±0.10	4.40±0.10	0.06
Pi mg/100ml	3.60±0.10	3.70±0.10	3.70±0.10	3.67±0.15	3.60±0.10	0.06
Total protein g/dL	3.79^{b}±0.04	3.86^{b}±0.16	4.17^{a}±0.13	3.91^{b}±0.14	3.69^{b}±0.19	0.08
Albumin g/dL	2.01^{abc}±0.07	1.93^{bc}±0.11	2.16^{a}±0.06	2.06^{ab}±0.04	1.84^{c}±0.15	0.06
Globulin g/dL	1.79±0.06	1.93±0.08	2.01±0.19	1.86±0.17	1.85±0.04	0.07
Cholesterol mg/dL	198.00^{a}±6.08	179.00^{b}±9.00	186.00^{b}±4.00	176.67^{b}±3.06	177.67^{b}±5.13	3.36
Uric acid	1.83^{b}±0.15	2.03^{ab}±0.15	2.23^{a}±0.21	1.77^{b}±0.15	2.00^{ab}±0.10	0.09
Glucose mg/dL	146.53^{c}±3.18	162.50^{b}±5.51	145.83^{c}±4.17	176.39^{a}±6.36	165.63^{ab}±9.38	3.52

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

leucaena seeds at 6% improved the quality of this diet. This could have been due to the role of choline as methyl donor (Scott *et al*., 1982). Plasma albumin of birds fed different leucaena seed diets was similar to the control; however, choline supplementation increased plasma albumin of birds fed 6% leucaena seeds.

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