EVALUATION OF THE EFFICACY OF CHOLINE CHLORIDE AS A FEED ADDITIVE IN BROILERS

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Abstract
The study evaluated the effect of choline chloride on growth, haematology, serum biochemistry, and relative organ weight of broilers. A total of 120-day-old chicks were randomly divided into four groups of 30 birds each, which was further sub-divided into 3 replicates of 10 birds each. Group A served as the un-supplemented control while the diets of groups B, C and D were supplemented with 0.5g/kg, 0.75g/kg and 1g/kg of choline Chloride (CC), respectively. The study lasted for six weeks. Result showed that dietary supplementation with CC did not enhance feed intake and weight gain. Group C had a significantly (P<0.05) higher feed efficiency (49.18%) than other choline-supplemented groups and control. There was no significant difference (P>0.05) in the mean values of AST, ALT, total protein and creatinine across all groups. However, the ALP and cholesterol values of group D were significantly (p<0.05) higher and its lymphocyte level significantly lower than all other groups. There was no significant (p>0.05) difference in the mean organ weights across all four groups, but the spleen weight (0.27g) of group D was significantly (p<0.05) higher than all other groups. Supplementation with CC at the level of 1g/ kg of feed seemingly had negative effect on serum cholesterol level and liver hepatocytes as evidenced in elevated values of these parameters in group D broilers.
Key words: Broiler, feed supplementation, choline, cholesterol, liver enzymes.

Introduction
The quest for cost-effective feeding and sustainable production of broilers have led farmers to compound feeds using relatively low-cost ingredients and supplemented with varied feed additives. Farmers have used antibiotics, hormones and many other feed additives to achieve this (Aidara-Kane, 2012). The potential threat to public health from inappropriate antibiotic use in food animals has led to a search for stable and healthier feed additives with positive effects.

Choline plays an essential role in several biological processes within the body such as building and maintaining cell structure as well as ensuring normal maturation of the cartilage matrix of bone, and the prevention of perosis in broilers (Workel, 2002). Very important to the poultry species, is the fact that choline provides the labile methyl groups necessary for the formation of methionine from homocysteine, by being oxidized to betaine (Zhang *et al*., 2013). However, Choline requirement of growing chicks decreases with age.

Conventional feed ingredients like maize and soybean contain choline chloride (CC) at levels lower than what is recommended for broilers (Fouladi *et al*., 2011). Broilers require about 1, 200 – 2,000 mg/kg choline in their feeds (depending on their growth rate). Yet, a typical diet based on corn and soybean (65% maize, 25% soybean, and 10% other) will contain about 1,040 choline from natural sources (Mavromichalis, 2015). Moreover, it is economical to add choline chloride directly to concentrate feed mixture (Erdman and Sharma, 1991).

This study was undertaken to evaluate the effect of CC on growth and health indices in broilers.

Experimental animals/design:

A total of 120 broilers (Ross breed) were procured from Agrited, Ibadan and randomly allocated into 4 groups (A – D) of 30 birds each. The groups were further divided into 3 replicates of 10 birds each. Experimental birds were acclimatized for one week before commencement of the study.

Composition of experimental diet

	Maize	Full-fat soya	Palm kernel meal	*Concentrate
Broiler starter:	60 %	30 %	0 %	10 %
Broiler Finisher:	50 %	30%	10 %	10 %

*Concentrate: Crude fibre 1.8%, Crude fat 4.13%, Sodium 1.41%, Lysine 2.45%, Methionine 32.53%, Methionine + cysteine 3.07%, calcium 5.80% and phosphorus 1.15%.
The compounded feed was divided into four parts; and CC was added at 0g, 0.5g, 0.75g and 1g/kg of feed, representing treatment groups A-D, respectively. Each group was fed their respective diet *ad lib* from day 1 of the experiment to day 42 (6 weeks)

Data collection
Feed intake was recorded daily and weight gain weekly using a sensitive weighing balance.
At the 6th week, 3 birds were randomly selected from each replicate and 5ml of blood collected from the jugular vein, 2 ml was discharged into prelabelled heparinized bottles and remaining 3 ml into clean sample bottles kept in slanted positions and allowed to clot. Serum was harvested after centrifuging the non heparinized blood samples at 3,000 revolution per minute for 3 – 5 minutes. Harvested sera were subjected to biochemical component assays while adhering to manufacturer's recommendations, and using commercial enzymatic kits. Alanine transaminase (ALT) and Aspartate transaminase (AST) activities were assayed by Reitman – Frankel colorimetric method and Alkaline phosphatase activities (ALP) by Phenolphthalein Monophosphate methods as described by Peters (1995). Serum cholesterol and triglyceride levels were assayed by enzymatic colorimetric method as described by Richmond (1973). Total protein and creatinine levels were assayed by Biuret method and modified Jaffe method respectively as described by Kohn and Allen (1995).
For relative organ weight, 12 birds were randomly selected (3 per group) and humanely sacrificed. The relative organ weights of the spleen, heart, liver, proventriculus, gizzard and abdominal fat were determined for each bird.

Statistical analysis
Data obtained were statistically analysed using Analysis of Variance (ANOVA). Significance was accepted at probability values ≤ 0.05. Means were compared using Duncan's new multiple Range Test (DVMRT) (Steel and Torre, 1960).

Results and Discussion
Results of the feed intake, weight gain and feed conversion ratio of broilers fed diets supplemented with varied level of choline chloride is presented in Table 1. The mean weight gain of group A was significantly ($p<0.05$) higher than group B and D but did not differ significantly from that of group C; of the supplemented groups, C and D had higher weight gain than group B. The mean feed intake of the birds across groups A, B and C did not vary significantly but were significantly higher than that of group D. Also, the mean feed efficiency of group C was significantly higher than that of groups A, D, and C.

The result of the serum biochemical parameters suggests that there was no significant difference ($P>0.05$) in the mean values among the four groups for AST, ALT activities and total protein and creatinine levels (Table 2). However, the mean value of ALP activities for group D was significantly ($P<0.05$) higher than group A, B and C. The mean cholesterol value of group D was significantly ($P<0.05$) higher than groups A and B and group C. While the mean triglyceride level of group A was significantly ($P<0.05$) lower than all other groups. Relative organ weight: There was no significant ($P>0.05$) difference in the mean of relative weights of the liver, heart, gizzard, abdominal fat and

proventriculus for the four groups (A, B C and D). However, the mean relative weight of the spleen in group D was significantly (P<0.05) higher than group A.

TABLE 1: Feed intake (kg/bird), weight gain (g/bird) and feed conversion ratio of broiler fed diets supplemented with choline chloride

Parameter	A (control)	B (choline 0.5mg/kg)	C (choline 0.75mg/kg)	D (choline 1mg/kg)
Initial weight(g)	44.03	43.50	44.00	43.60
Final weight(g)	2192.10±14.17^{c}	1668.47±13.17^{a}	2034.97±10.18^{bc}	1926.17±18.21^{b}
Mean weight gain(g)	2148.07±14.17^{c}	1624.97±13.04^{a}	1990.97±10.18^{bc}	1882.57±18.21^{b}
Mean feed intake(g)	4493.33±31.91^{a}	4790.00±41.63^{b}	4110.00±32.32^{a}	3876.67±83.33^{c}
Feed efficiency (%)	48.30±3.32^{b}	33.97±1.37^{c}	49.18± 5.81^{a}	48.61±1.41^{b}

Rows with different superscripts $^{a\ b\ c}$ indicates significant difference among groups (p<0.05). Values in the table represents means ± standard error of mean.

TABLE 2 Serum biochemical parameters of broilers fed diets supplemented with varied level of choline chloride at six weeks

Parameter	A (control)	B (0.5g choline)	C (0.75g choline)	D (1g choline)
Alkaline phosphatase (U/L)	11.5±16.59^{a}	21.2±36.80^{a}	18.4±18.40^{a}	44.2±1.24^{b}
Aspartate transaminase (U/L)	56.00±32.53^{a}	51.33±3.18^{a}	57.67±19.55^{a}	56.67±7.75^{a}
Alanine transaminase (U/L)	14.33±5.33^{a}	15.00±2.00^{a}	13.00±2.00^{a}	16.00±1.53^{a}
Total protein (g/dl)	2.43±0.16^{a}	2.57±0.13^{a}	2.29±0.25^{a}	2.86±0.24^{a}
Cholesterol (mg/dl)	106±10.02^{a}	101±9.72^{a}	104±7.35^{a}	168±13.25^{b}
Triglyceride (mg/dl)	40.28±5.01^{a}	51.39±9.12^{ab}	55.00±14.43^{ab}	51.04±27.74^{b}
Creatinine (mg/dl)	0.37±0.27^{a}	0.20±0.06^{a}	0.23±0.13^{a}	0.50±0.15^{a}

Rows with different superscripts $^{a\ b\ c}$ indicates significant difference among groups (p<0.05). Values in the table represents means ± standard error of mean.

TABLE 3 Relative organ weight of broilers fed diets supplemented with varied level of choline chloride at six weeks

Parameter	A (control)	B (o.5g choline)	C (0.75g choline)	D (1g choline)
Liver (g)	3.69±0.26^{a}	3.84±0.15^{a}	3.41±0.11^{a}	4.16±0.30^{a}
Spleen (g)	0.21±0.15^{a}	0.22±0.02^{ab}	0.23±0.01^{ab}	0.27±0.01^{b}
Heart (g)	0.67±0.05^{a}	0.60±0.05^{a}	0.71±0.07^{a}	0.68±0.02^{a}
Gizzard (g)	3.17±0.23^{a}	3.82±0.02a	3.41±0.28^{a}	3.43±0.40^{a}
Abdominal fat (g)	1.65±0.23^{a}	1.51±0.36^{a}	1.29±0.49^{a}	1.86±0.22^{a}
Proventriculus (g)	0.55±0.03^{a}	0.55±0.05^{a}	0.66±0.16^{a}	0.62±0.04^{a}

Rows with different superscripts $^{a\ b\ c}$ indicates significant difference among groups (p<0.05). Values in the table represents means ± standard error of mean.

Supplementation of broiler diets with the choline chloride used in this study did not improve production performance. However, at the inclusion level of 0.75g/kg feed, a slightly improved feed efficiency was observed but without a corresponding improvement in carcass yield. Elevated serum cholesterol levels are indicted with atherosclerosis (Glass and Witztum, 2001); elevated ALP levels are mostly seen in cases of liver damage (Senanayake *et al*., 2015). Dietary supplementation with CC

at the level of 1g/ kg of feed appears to be detrimental to liver hepatocytes and overall health of broilers and by extension could impact negatively on carcass quality of the meat.

Lack of significant difference between supplemented groups and the control was in contrast to McDevitt *et al*. (2000) report that CC could enhance production performance and overall carcass characteristics of broilers. Ratriyanto *et al*, (2009) reported lower activities of lipogenic enzymes and stabilization of protein and cell components against denaturing effect of high ionic strength; both, were not substantiated from our study.

It is recommended that Assay/analysis of 'industrial choline' should be carried out to estimate its actual choline content as well as its level of contamination before use.

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