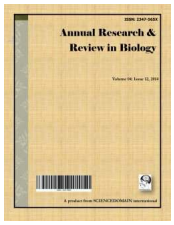

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The Effect of Choline Chloride on the Performance of Broiler Chickens

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Authors' contributions

This work was carried out in collaboration between all authors. Author IRI designed the study, wrote the protocol and interpreted the data. Author CJO carried out the haematology and the carcass analysis. Author UGU anchored the field study, gathered the initial data and performed preliminary data analysis; while author COO managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: A study was conducted to investigate the effect of graded levels of choline on the growth performance, dressed carcass yield, internal organs and haematological parameters of broiler chickens.
Materials and Methods: A total of three hundred day old mixed sex broiler chicks were shared into five treatment groups of sixty birds each and twenty birds for each of the three replicates. Birds in (T_1) belonged to the control. Those in T2 had 500 mg/kg choline chloride supplementation of their feed, those in T3 had 1,000 mg/kg choline chloride supplementation, and birds in T4 had 1,500 mg/kg choline chloride supplementation, while those in T5 had 2,000 mg/kg choline chloride supplementation. Data collected were the growth parameters, dressed carcass, internal organs and haematology.

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Results: There were significant differences ($p<0.05$) in the average final weight, average weight gain, daily weight gain, daily feed intake, and feed conversion ratio with improvement as the choline supplementation increased from 500 to 2,000 mg/Kg of feed. The dressed carcass and internal organs showed no significant differences ($p<0.05$). For the haematology, the red blood cell (RBC) count, white blood cell (WBC) count, lymphocyte count, monocyte count and basophil count showed significant differences ($p<0.05$).
Conclusion: It was concluded that choline should be included at 2,000 mg/kg of broiler's diet.

Keywords: Broiler; supplementation; choline chloride; performance; haematology.

1. INTRODUCTION

Nutrients are the nutritious components in foods that an organism utilizes to survive and grow. Macronutrients provide the bulk energy for an organism's metabolic system to function, while micronutrients provide the necessary cofactors for metabolism to be carried out. Both types of nutrients can be acquired from the environment [1]. Only part of birds' nutrient requirements is provided by the natural feedstuffs in their diets. Nutrient supplements must therefore be included in feed formulations.

Choline was first isolated from ox bile ("chole" in Greek). It was discovered by Adolph Strecker in 1864 and chemically synthesized in 1866 [2]. He isolated the platinum salt of a substance from pig and oxen gall and surmised it to be an ammonium base). Wurtz was the first to chemically synthesize choline by heating ethylene oxide with trimethylamine under acidic condition [3].

In poultry, choline's methyl group is available after the conversion to betaine in the liver. Choline has three essential metabolic roles, namely: - As a constituent of phospholipids; secondly, it helps to prevent fatty liver; and thirdly, as a precursor for acetylcholine synthesis. Choline also has non-essential metabolic functions: - As a labile methyl group, as well as prevention of perosis and fatty liver syndrome in broiler chicks [4]. Growing chickens can use betaine interchangeably with choline for the methylation function, but it cannot replace choline to prevent perosis.

Choline requirement of growing chicks decreases with age as it is generally not possible to produce a deficiency in birds over eight weeks of age. It was observed that methylation of aminoethanol to methylaminoethanol seems to be the rate-limiting step in choline biosynthesis for young birds. High levels of dietary methionine or other methyl donors, therefore, cannot completely spare the chick's requirement of dietary choline in contrast to observation in growing mammals such as the pig or the rat. Apparently, choline requirement of laying hens can be influenced by the level in the diet of the growing pullet [5]. Hens that received choline-free diets after eight weeks of age were able to synthesize all of the choline required for good egg production. Those that had received choline supplements in the growing diet required supplemental choline in the laying diet for maximum egg production. The deficiency signs noted in these hens were a reduction in egg production and an increase in fat content of the liver. With choline deficiency, however, choline content of the egg was not affected by low dietary choline.

Addition of 0.1% of supplemental methionine resulted in no response in laying hens to supplemental choline [6]. It appears that benefits from supplemental choline in layer diets occur mainly when supplemental methionine is just adequate to meet methionine requirements. It has been demonstrated that the addition of 0.11% choline plus 0.1% sulfate could essentially spare all supplemental methionine in broiler diets [7]. However, in turkey poult diets, responses to sulfate and choline addition were not equivalent to the addition of supplemental methionine [8]. Some scientists using young chicks, found that supplementation with methyl donors from either 0.23% choline or 0.23% betaine was equivalent to supplementation with 0.23% methionine in 21-day chick experiments, using basal diets containing 0.31% methionine and 0.43% cysteine [9]. Furthermore, some other researchers found that supplemental choline could replace up to two-thirds of the supplemental methionine required in broiler diets from 0 to 47 days in diets containing 0.30% methionine and 0.43% cystine in the starter phase, and 0.25% and 0.42% methionine and cystine, respectively, in the finisher phase [10].

Researches in humans have been done to know the importance of choline and it has been

discovered that choline is necessary for maintenance of pregnancy and lactation, memory development, gene expression, prevention of heart disease, cancer, fatty liver, cirrhosis, kidney necrosis and neural tube defects in neonates [11].

It was once believed that animals make enough choline in their bodies from other nutrients to meet their need for this important substance [12]. More recent research using choline-depleted diets has demonstrated that animals do require choline in feed to maintain body functions and growth [13].

Choline as a constituent of phospholipids, is essential in the building and maintenance of cell structure, as well as ensuring normal maturation of the cartilage matrix of bone, including the prevention of perosis in broiler [4]. Phosphatidylcholines (lecithins) are phosphoacylglycerols containing choline and they are the most abundant phospholipids of the cell membrane. Phosphatidylcholines represents a large proportion of the body's store of choline.

Choline is an essential nutrient in the production of phosphatidylcholine, one of the most important structural building blocks of a living cell. Its unique soap-like structure helps to keep the membrane fluid, yet mostly impermeable [14]. Phosphatidylcholine is a required component of VLDL particles. Without adequate phosphatidylcholine, fat and cholesterol accumulate in the liver [15].

Choline is a precursor for acetyl choline synthesis, the transmission agent for impulses along the sympathetic nervous system [4]. Choline is the backbone of a nervous system signal molecule or neurotransmitter called acetylcholine. Acetylcholine is indispensable for central and peripheral nervous system. The importance of acetylcholine cannot be overstated.

Choline exerts extremely important functions in maintaining the structure as well as the functional integrity of cells, organs and bodies. It is evident that dietary deficiency of choline will affect almost every organ in the body, particularly when animals are in the state of high metabolic activity and growth [16].

Lipid and lipotropes in the diet are closely related, since lipotropes are required for normal metabolism of lipids, and dietary lipid contents partially determine the lipotrope requirement [16].

It is evident that chicken exhibit fatty liver when the diet lacks sufficient choline. Choline aids fat metabolism in the liver, i.e. utilisation and outward transport of fat, so preventing abnormal accumulation of fat within hepatocytes - so-called "fatty liver" [4]. It has been shown that choline chloride supplementation significantly decrease the liver and abdominal fat deposition in the Japanese quail hence prevent fatty liver syndrome in the Japanese quail [17].

1.1 Purpose of this Work

The purpose of this work is to determine the choline requirement of broiler. Secondly, to find out the effect of choline supplementation on organ characteristics of broiler; and lastly, to determine the effect of choline supplementation on improving feed conversion rate.

2. MATERIALS AND METHODS

The experiment was conducted at the poultry unit of the Teaching and Research farm of College of Veterinary Medicine (CVM), Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. Umudike is located at latitude 5° 29' 33" north and longitude 7° 32' 56" east in rain forest zone of Nigeria. The climate of the region is characterized by a mean daily temperature of between 27℃ and 35℃ all through the year. Average rainfall of Umudike is about 2000 mm per annum with double maxima [18]. A total of three hundred day old mixed sex broiler chicks were used for the study. The chicks were weighed and brooded together for three weeks, after which they were shared into five treatment groups (T1, T2, T3, T4 and T5) of sixty birds each and twenty birds per replicate. Thus there were three replicates per treatment.

Birds in (T_1) belonged to the control. Those in T2 had 500 mg/kg choline chloride supplementation of their feed, those in T3 had 1,000 mg/kg choline chloride supplementation, and birds in T4 had 1,500 mg/kg choline chloride supplementation, while those in T5 had 2,000 mg/kg choline chloride supplementation. The birds were vaccinated against Newcastle disease and Infectious Bursal Disease. Feed and water were given ad libitum.

The basal experimental diet, as well as the proximate analysis of both starter and finisher diets are given in Tables 1, 2 and 3.

Table 1. Starter diet formulation

Feed ingredient	% of ration
Maize	55.93
Soya bean meal	39.45
Bone meal	3.00
Limestone	0.90
Salt	0.35
Broiler premix	0.25
Toxin binder	0.10
Enzyme	0.02
Total	100.00

**** Contains per 100 kg: Vitamin A (1200000 IU), Vitamin D3 (200000 IU), Vitamin E (3000 mg), Vitamin K3 (200 mg) Vitamin B1(200 mg), Vitamin B2 (500 mg), Vitamin B6 (400 mg), Vitamin B12 (2 mg), Pantothenic acid (1000 mg), Nicotinic acid (4000), Folic acid (100 mg), Biotin (15 mg), Iron (4000 mg) Manganese (8000 mg) Copper (1000 mg), Zinc (6000 mg), Iodine (100 mg), Cobalt (20 mg), Selenium (15 mg), Anti-oxidant (2500 mg)*

Table 2. Finisher diet formulation

Feed ingredient	% of ration
Maize	60.31
Soya bean meal	31.71
Wheat offal	3.88
Bone meal	2.22
Limestone	1.11
Salt	0.39
Broiler premix	0.28
Enzyme	0.11
Total	100.00

**** Contains per 100 kg: Vitamin A (1200000 IU), Vitamin D3 (200000 IU), Vitamin E (3000 mg), Vitamin K3 (200 mg) Vitamin B1 (200 mg), Vitamin B2 (500 mg), Vitamin B6 (400 mg), Vitamin B12 (2 mg), Pantothenic acid (1000 mg), Nicotinic acid (4000), Folic acid(100 mg), Biotin (15 mg), Iron (4000 mg) Manganese (8000 mg) Copper (1000 mg), Zinc (6000 mg), Iodine (100 mg), Cobalt (20 mg), Selenium (15 mg), Anti-oxidant (2500 mg)*

Table 3. Proximate Composition of Starter and Finisher diets

Parameters	% Composition starter	% Compostion finisher
Crude protein	23.94	20.28
Crude fibre	5.90	3.90
Crude fat	6.65	14.80
Moisture	10.9	11.65
Ash	13.15	11.95
NFE	42.16	37.42
Calcium	1.40	0.60
Phosphorus	0.16	0.14

The statistical model of the experiment is given as:

$$Y_{ijk} = m + C_i + Rj + €_{ijk}$$

Where,

Y_{ijk} = The dependent variable
m = Overall mean
ci = effect of choline
Rj = effect of replicate
€ijk = residual effect

2.1 Data Collection and Analysis

Data collected were live weight, feed intake, dressed carcass and internal organs. The values of feed intake and live weight were used to calculate feed conversion ratio. Blood samples were collected in a labelled sterile bottle with anti-coagulants (EDTA) through the jugular vein the birds. The blood samples were used to determine erythrocyte indices, leucocyte count and differential leucocyte count. The erythrocyte indices determined were packed cell volume (PCV), haemoglobin concentration and total erythrocyte count [19]; the white blood cell count including the differential count were also determined. Birds were killed, dressed and dressed carcass parts and internal organ parameters were collected [5,20,21]. The dressed carcass weight was expressed as percentage live weight; cut-parts weight as percentage dressed weight and weight of internal organs as percentage live weight. All percentage values were transformed using arc sine according to Preston [22]. Haematology data were log transformed before analysis. All data were subjected to the Analysis of Variance (ANOVA) and significant means separated using least significance difference (LSD). Significance was set at a value of $P < 0.05$.

3. RESULTS

From results shown in Table 4, there were significant differences ($p<0.05$) in the average final weight, average weight gain, and weight gain with T_5 (2,000 mg/kg of feed) showing the highest effect followed by T_4 (1,500 mg/kg of feed), then followed by T_3 (1,000 mg/kg of feed), then T_2 (500 mg/kg of feed) and finally T_1 (control). For the daily feed intake, there was significant difference ($p<0.05$) with T_1 (control) and T_3 (1,000 mg/kg of feed) having the highest feed intake respectively, followed by T_5 (2,000 mg/kg of feed), then T_4 (1,500 mg/kg of feed) and finally T_2 (500 mg/kg of feed). There was

significant difference ($p<0.05$) in the feed conversion ratio with T_2 (500 mg/kg of feed), T_5 (2,000 mg/kg of feed), and T_4 (1,500 mg/kg of feed) having the best feed to gain ratio respectively, then followed by T_3 (1,000 mg/kg of feed), and finally T_1 (control).

From results shown in Tables 5 and 6, there were no significant differences ($p<0.05$) in the carcase yield and the internal organs respectively.

Table 7 showed the results of the haematology. There were no significant differences ($p<0.05$) in the PCV, Hb, MCV, MCHC, MCH and eosinophil count. There were significant differences ($p<0.05$) in the RBC, WBC, lymphocyte, monocyte, and basophil counts.

4. DISCUSSION

There was a progression in weight gain as the dosage of choline is increased from 0 mg/Kg of feed (control) to 2,000 mg/Kg of feed (T5). This is in line with report by Emmert and Baker [13] who stated that using a choline-deficient basal diet showed an almost linear response to incremental addition of choline chloride up to 1115 mg/kg feed in chicks from 10-22 days of age; increasing choline chloride to 2000 mg/kg resulted in further weight gain improvements, but to a lesser extent; and that levels in excess of this had no benefit. Feed conversion ratio was maximized at T2 (500 mg/kg of feed), T4 (1,500 mg/kg of feed), and T5 (2,000 mg/Kg of feed); this is also in agreement with Emmert and Baker [13] who demonstrated that animals do require choline in feed to maintain body functions and growth.

The T1 (control) which had no choline supplementation had the highest feed conversion ratio which shows poor utilization of the feed.

The internal organs (liver, kidney, gizzard, spleen and heart) examined were not negatively affected by the various levels of choline used in this work. Though one of the benefits of choline in poultry production is the prevention of abnormal accumulation of fat in the hepatocytes or the development of fatty liver [23], the chickens used in this work did not show any fatty liver; thus the use of choline in this work was never therapeutic.

Like the internal organs, the carcass yield was also not significantly different ($p<0.05$) indicating that the various levels of choline used could not have interfered negatively with the muscle development.

Table 4. Values of growth parameters for each treatment at the end of 8 weeks

Parameters	T_1	T_2	T_3	T_4	T_5	SEM
Average final weight (g)	1150.00^{d}	1166.67^{d}	1216.67^{c}	1333.33^{b}	1433.33^{a}	19.55*
Average initial weight (g)	35.71	35.71	38.10	33.33	36.36	0.54^{ns}
Average weight gain	1114.29^{d}	1130.96^{d}	1178.57^{c}	1300.49^{b}	1396.97^{a}	19.64*
Feed intake (g/day/bird)	75.38^{a}	62.91^{d}	74.32^{a}	66.94^{c}	70.09^{b}	1.37*
Weight gain (g/day/bird)	16.94^{c}	18.66^{b}	18.74^{b}	20.06^{a}	20.73^{a}	0.35*
Feed conversion ratio	4.45^{a}	3.37^{c}	3.97^{b}	3.34^{c}	3.38^{c}	0.12*

a - d on the same row with different superscripts are significantly different ($p<0.05$)
**=significant, ns = not significant, T_1 =Control (0 mg/kg choline chloride), T_2 =500 mg/kg choline chloride, T_3=1000 mg/kg choline chloride, T_4 =1500 mg/kg choline chloride, T_5=2000 mg/kg choline chloride*
SEM=Standard error of mean

Table 5. Values of carcass yield for each treatment at the end of 8 weeks

Parameters	T_1	T_2	T_3	T_4	T_5	SEM
Live weight (g)	1150.00^{d}	1166.6^{d}	1216.67^{c}	1216.67^{c}	1333.3^{b}	19.55*
Dressed weight (%)	74.29	72.46	75.00	72.60	72.09	0.54^{ns}
Breast weight (%)	14.82	14.26	16.07	14.61	15.68	19.64^{ns}
Backcut (%)	10.52	10.30	10.25	10.47	9.86	1.37^{ns}
Thigh weight (%)	10.28	10.28	10.86	10.14	10.48	0.35^{ns}
Drumstick (%)	10.06	9.87	9.73	9.74	9.7	0.12^{ns}
Wing weight (%)	8.95	8.93	8.79	8.77	9.07	0.15^{ns}

a - d on the same row with different superscripts are significantly different ($p<0.05$)
**=significant, ns = not significant, T_1 =Control (0 mg/kg choline chloride), T_2 =500 mg/kg choline chloride, T_3=1000 mg/kg choline chloride, T_4 =1500 mg/kg choline chloride, T_5=2000 mg/kg choline chloride*
SEM=Standard error of mean

Table 6. Values of the weight of internal organs for each treatment at the end of 8 weeks

Parameters	T_1	T_2	T_3	T_4	T_5	SEM
Liver (%)	2.05	2.01	2.48	2.24	2.37	0.05^{ns}
Kidney (%)	0.77	0.73	0.75	0.80	0.80	0.03^{ns}
Spleen (%)	0.16	0.11	0.16	0.15	0.16	0.01^{ns}
Gizzard (%)	2.87	3.1	3.75	3.1	3.13	0.16^{ns}
Heart (%)	0.50	0.57	0.56	0.51	0.56	0.02^{ns}

a - d on the same row with different superscripts are significantly different (p<0.05)
**=significant, ns = not significant, T_1 =Control (0 mg/kg choline chloride), T_2 =500 mg/kg choline chloride, T_3=1000 mg/kg choline chloride, T_4 =1500 mg/kg choline chloride, T_5=2000 mg/kg choline chloride*
SEM=Standard error of mean

Table 7. Values of haematological parameters for each treatment at the end of 8 weeks

Parameters	T_1	T_2	T_3	T_4	T_5	SEM
Packed cell volume (%)	32.6	28.3	32.1	31.1	31.2	0.004^{ns}
Haemoglobin concentration (g/dl)	9.52	8.88	9.34	8.95	8.85	0.005^{ns}
Red blood cell count ($X10^6$/µl)	3.32^{a}	2.87^{b}	3.06^{ab}	3.11^{ab}	3.14^{ab}	0.005^{*}
Mean corpuscular volume (fl)	100.58	98.76	105.38	101.22	99.81	0.014^{ns}
Mean corpuscular haemoglobin concentration (g/dl)	29.24	31.53	29.28	28.87	28.41	0.248^{ns}
Mean corpuscular haemoglobin (picogram/cell)	29.58	31.11	30.64	29.26	28.32	0.005^{ns}
White blood cell COUNT ($X10^3$/µl)	7.58^{b}	8.58^{ab}	8.88^{a}	8.13^{ab}	9.2^{a}	0.004^{*}
Lymphocytes (%)	66.6^{ab}	70.0^{a}	62.2^{b}	67.4^{ab}	65.6^{ab}	0.004^{*}
Eosinophil (%)	3.2	3.0	3.2	2.8	2.8	0.005^{ns}
Monocytes (%)	3.6^{bd}	4.6^{a}	2.4^{c}	3.8^{ab}	3.0^{d}	0.014^{*}
Basophils (%)	0.2^{c}	0.2^{c}	0.2^{c}	0.6^{a}	0.4^{b}	0.027^{*}

a - d on the same row with different superscripts are significantly different (p<0.05)
**=significant, ns = not significant, T_1 =Control (0 mg/kg choline chloride), T_2 =500 mg/kg choline chloride, T_3=1000 mg/kg choline chloride, T_4 =1500 mg/kg choline chloride, T_5=2000 mg/kg choline chloride*
SEM=Standard error of mean

Blood represent a means of assessing clinical and nutritional health status of animals in feeding trials and the haematological variables most commonly in nutritional studies include PCV, RBC, Hb, Mean corpuscular haemoglobin count (MCHC), Mean corpuscular volume (MCV) and clotting time [24]. Increased WBC count may be as a sign of an inflammatory response [25], most commonly the result of infection, but may also occur following certain parasitic infections or bone tumors. It may also occur after strenuous exercise, convulsions such as epilepsy, emotional stress, pregnancy and labour, anesthesia, and epinephrine administration. In this study, though there were significant differences ($p<0.05$) in the RBC, WBC, lymphocyte, monocyte, and basophil counts; all the data collected were within the normal ranges. This may be interpreted as the absence or low levels of parasitaemia and nutritional diseases in the birds used for this research.

5. CONCLUSION

Based on the findings of this work, it is advised that choline be included at 2,000 mg/kg of broiler's diet. Choline can be obtained cheaply from most pharmaceutical stores. More work should be done with higher doses of choline to find out if they will have a better effect than the 2,000 mg/kg of the diet.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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