

Growth rate, haematology and serum biochemistry of broilers fed diets supplemented with choline chloride

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Abstract

The study evaluated the effect of choline chloride (CC) supplementation on growth rate, haematology and serum biochemistry of broilers. 120-day-old broiler chicks were randomly divided into four groups of 30 birds each and these were further sub-divided into 3 replicates of 10 birds each. Group A served as the control while the diets of groups B, C and D were supplemented with 0.5 g/kg, 0.75 g/kg and 1 g/kg of CC respectively. 6 weeks post-supplementation, haematology, serum biochemistry, total weight gain, feed efficiency and carcass characteristics were determined. Group C (0.75 g/kg choline) 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 (4.42 U/L and 1.68 mg/dl respectively) were significantly ($p < 0.05$) higher than other groups. Lymphocyte counts of Group D was significantly ($p < 0.05$) lower than all other groups. The spleen weight (0.27 g) of group D was significantly ($p < 0.05$) higher than all other groups, but there was no significant difference ($p > 0.05$) in the relative weights of other organs of all four groups. The values of the breast weight/width, drumstick length/width, wing length and carcass length did not vary significantly across the supplemented-groups, but the breast-length, thigh weight/length/width, drumstick-weight, wing weight/width and carcass-weight of the control group were significantly higher than the supplemented-groups. Choline chloride supplementation at 0.75 g/kg may have contributed to improved feed efficiency but not with a corresponding excellent carcass yield.

Keywords: broilers; cholinechloride; growth; haematology; serum biochemistry

Introduction

The Nigerian poultry industry contributes tremendously to her national GDP and employment creation (Adebayo and Adeola, 2005), and serves as a significant source of good quality animal protein for its population. The industry, particularly broiler production, is especially attractive due to its short production cycle (6-7 weeks), ease of management, absence of religious or cultural restrictions on the meat and availability

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of market for the meat. However, with the ever-increasing human population of the country and globally, comes the increase in demand for high quality animal protein and hence the need for possible intensification and expansion of the industry if the sustainable development goals of zero hunger, responsible consumption and production are to be achieved.

One of the major problems associated with such intensification and expansion of the broiler industry is the high cost of poultry feed (Ayanrinde *et al.*, 2014). According to Afolayan and Afolayan (2008), in broiler production, over 70% of the overhead cost of production goes to feeding. Therefore, in a bid to achieve cost-effective feeding and sustainable production of broilers, farmers have taken to compounding local feeds using relatively cheap ingredients and supplementing with varying feed additives with or without scientific backing (Aidara-Kane, 2012). Enhancement of feed utilization in the birds using varying supplements and additives is a strategy that has been employed to reduce feed costs, improve production efficiency and profitability of the broiler production industry. Some of these additives, e.g., antibiotics, while promoting growth, improving feed efficiency and preventing/controlling diseases, may pose potential threat to public health from inappropriate use, and cause side effects detrimental to livestock and human health (EPC, 2005; Marshall and Levy, 2011). The restriction on the use of antibiotics as growth promoters in food animals as well as the need to achieve a profitable balance between the cost of feed, broiler performance and quality of end product, has prompted the search for more stable and healthier feed additives for use in broiler production.

Choline is an essential nutrient for all animals and in poultry is required as a dietary supplement (McDowell, 2000). In the current EU feed additive directive 70/524 and the new 1831/2003, it is listed as a vitamin (B4) and is used in poultry as a performance promoter in the form of choline chloride (Igwe *et al.*, 2015). Its functions include building and maintaining cell structure, prevention of abnormal fat accumulation in the liver (fatty liver) through fat metabolism, formation of acetylcholine, prevention of perosis/chondrodystrophy (anatomic deformation of leg bones in young birds), enhancing maximum egg production (Rajalekshmy, 2010) and a source of labile methyl group for methionine formation.

The molecule has been shown to result in improved carcass yield by sparing the dietary need for methionine in poultry, ruminant and pigs (Zang *et al.*, 2013), maximize broiler growth and improve breast meat yield, as well as improve feed conversion ratio, weight gain and carcass yield (Jadhv, 2008; Igwe *et al.*, 2015). Nevertheless, there have been reports of fraudulent choline chlorides on the market, with some producers adding chloride-containing compounds to the choline chloride to “enrich” the “chloride” content and maximize profit (Igwe *et al.*, 2015).

The study was therefore designed to evaluate the efficacy of choline chloride (CC) as a feed additive in broiler production with a look at the haematology and serum biochemistry of CC-supplemented broilers.

Materials and Methods

Study area, animals and sampling

The study was conducted at the Poultry Unit of the Department of Animal Health and Production, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

A total of 120 broiler chicks (Ross breed) with average initial body weight ranging from 43 g to 44 g were procured from Agrited® farm, Ibadan State, Nigeria. They were stocked into brooding pens, supplied with fresh water, fed twice a day and adapted to experimental conditions for one week prior to the start of the experiment. They were then randomly allocated into 4 groups (A – D) of 30 birds each and each group was further subdivided into three replicates of 10 birds each. All standard management procedures for broiler production were followed.

Experimental diet

Four iso-nitrogenous and iso-caloric diets were compounded (Table 1) for the groups. Diets were identical and differed only in the inclusion rates of the choline chloride (CC); the control diet (Group A) had no CC inclusion while the diets of the other three groups (Groups B – D) had 0.5, 0.75 and 1 g CC/kg of feed respectively.

Table 1. Composition of experimental diet

Feed ingredient	Broiler starter (kg)	Broiler finisher (kg)
Maize	60	50
Full fat soy cake	30	30
Palm kernel meal	-	10
Concentrate	10	10
Total	100	100

The Choline chloride, procured from a commercial source in Benin City, Nigeria was thoroughly mixed with the diets of the different groups of birds at the appropriate inclusion levels and all groups were fed their respective diets at a level of 3% body weight twice daily. Feed was manually administered and daily records of feed intake kept. The study lasted for 6 weeks.

Vaccination and health management

All birds were routinely vaccinated against Newcastle disease and Gumboro disease via the right routes and an Anti-stress preparation containing multivitamins (Maltaseryl®) administered for five days post vaccination. The birds were prophylactically treated with a coccidiostat (which contained sulphadimidine, diaveridine, vitamin A and vitamin K) against coccidiosis and secondary bacterial infections. Strict biosecurity measures were also employed in the course of raising the birds.

Growth

Body weight was measured at the beginning of the experiment and thereafter, weekly. Weekly weight gain and feed intake were measured and recorded.

*Haematology*Packed cell volume (PCV)

The packed cell volume was determined using the micro haematocrit method (Schalm *et al.*, 1975). Two third of capillary tubes were filled with anticoagulated blood samples and one end sealed with plasticine. The filled tubes were centrifuged at 10,000 revolutions per minute for five minutes using a micro haematocrit centrifuge (Hawksley, England). Then the PCV was read using the micro haematocrit reader.

Haemoglobin concentration

The haemoglobin concentration was determined by the cyanomethaemoglobin method (Kachmar, 1970). 0.02ml of blood sample was added to 3.98 ml of Drabkin's reagent in a clean test tube. The solution was mixed gently and kept at room temperature for 20 minutes to react. Then the absorbance of both samples and standard were read against a working reagent blank at a wavelength of 540nm using a digital colorimeter (Lab-Tech, India). The haemoglobin concentration of the blood sample was determined by multiplying the absorbance of the sample by the factor derived from the absorbance and concentration of the standard.

Erythrocyte count

The erythrocyte count was determined by the haemocytometer method (Schalm *et al.*, 1975). 0.02ml of the blood sample was added to 4 ml of the red blood cell diluting fluid, Turks solution, in a clean test tube, to

make 1:200 dilutions. Then a drop of the diluted blood was used to charge the Neuber counting chamber and allowed to stand for 2-3 minutes. Then it was placed on the stage of a microscope and viewed at $\times 40$ objective lens. Five groups of the sixteen squares of the counting chamber were counted. The number of erythrocyte counted was multiplied by 10,000 to obtain the erythrocyte count per microlitre of blood.

Total leukocyte count

The total leukocyte count was determined by the haemocytometer method (Schalm *et al.*, 1975). 0.02 ml of the blood sample was added to 4 ml of the white blood cell diluting fluid to make a 1:50 dilution of the blood sample using Formol citrate, in a clean test tube. A drop of the diluted blood was used to charge the Neuber counting chamber and allowed to stand for 2 minutes. Then it was placed on the stage of a microscope and viewed at $\times 10$ objective lens. Four corners of the squares of the counting chamber were counted to obtain the leukocyte count per microlitre of blood.

Differential leukocyte count

Blood smears of the samples collected were prepared on clean slides and stained with Leishmann's technique (Schalm *et al.*, 1975). The differential leukocyte count was enumerated by the meander counting method. The $\times 100$ objective lens of the light microscope was used for the count. The differential leukocyte cells were identified and recorded using the differential cell counter.

Serum biochemistry

On the 6th week, 3 birds were randomly selected from each group (one from each replicate) and 5ml of blood drawn from the jugular vein. The blood was allowed to clot and serum samples harvested for biochemical assay.

Alanine amino transaminase (**ALT**) and Aspartate amino transaminase (**AST**) activities were assayed by Reitman – Frankel colorimetric method while Alkaline phosphatase (**ALP**) activity was assayed by Phenolphthalein Monophosphate method as described by Peters *et al.* (1982).

Serum **cholesterol** and **triglyceride** levels were assayed by enzymatic colorimetric method as described by Richmond (1973), while total protein and creatinine levels were assayed by Biuret method and modified Jaffe method respectively as described by Kohn and Allen (1995).

Statistical analysis

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

Results and Discussion

From the results (Table 2), the mean weight gain (2148.07 g) of the control group (A) was significantly ($P < 0.05$) higher than the supplemented groups (B – 1624.97 g and D – 1882.57 g) but did not differ significantly from treatment group C. This implies that the CC used in the study did not significantly improve weight gain. This is in agreement with Swain and Johri (2000), Rafeeq *et al.* (2011) who reported that dietary choline at different inclusion levels had no significant effect on body weight in broilers. However, Hassan *et al.* (2005), Hossain *et al.* (2014), Igwe *et al.* (2015) and Giovani *et al.* (2017) all recorded increases in weight gain with dietary CC supplementation more than the control groups. This may be due to the type, source and level of possible contamination of the CC used in this study as well as the inclusion rate. Hossain *et al.* (2014) in their study recorded maximum weight gain at 0.2 g inclusion rate while Emmert and Baker (1997) observed

that increasing CC supplementation to 2000 mg/kg feed (2 g/kg feed) brought about reduced further weight gain and inclusion levels above this had no benefit. 0.75 g/kg CC inclusion levels used in the present study (Group C) appeared to offer maximum weight gain compared to other treatment groups.

Table 2. Feed intake (kg/bird), weight gain (g/bird) and feed conversion ratio (FCR) of broilers fed diets supplemented with varying levels of CC

Parameter	A (Control)	B (0.5 g cc/kg feed)	C (0.75 g cc/kg feed)	D (1 g cc/kg feed)
Initial weight (g)	44.03	43.50	44.01	43.60
Final weight (g)	2192.10± 54.17 ^c	1668.47± 53.17 ^a	2034.97± 10.18 ^{bc}	1926.17± 38.21 ^b
Mean weight gain (g)	2148.10± 54.17 ^c	1624.67± 53.04 ^a	1990.97± 10.18 ^{bc}	1882.17± 38.21 ^b
Mean feed intake (g)	4493.33± 32.91 ^a	4790.00± 41.63 ^b	4110.00± 32.32 ^a	3876.67± 33.33 ^c
Feed efficiency (%)	48.30±3.32 ^b	33.97±1.37 ^b	49.18±5.81 ^a	48.61±1.41 ^b

Different superscripts ^{a,ab,bc} indicate significant difference between the groups ($p < 0.05$).

The mean feed intake of the birds in the group with highest CC inclusion rate (D – 1 g/kg) was significantly ($p < 0.05$) lower (3876.67 g) than all other treatment groups and the control group. This finding is in agreement with studies by Hossain *et al.* (2014), who recorded reducing feed intake with increasing CC supplementation, such that feed intake was maximum at zero inclusion rate and minimum at 0.2 g of CC/kg feed. However, Swain and Johri (2000) observed no effect of CC on feed consumption. In the present study, treatment group C (0.75 g of cc/kg feed) recorded a feed efficiency of 49.18% which was significantly ($P < 0.05$) higher than that of the other supplemented groups and the control group A. This implies that at 0.75 g/kg the CC brought about efficient feed utilization such that 4110 g of feed was utilized to attain a final weight of 2034.97 g that did not differ significantly from the highest final weight of the control group (2192.10 g) – compared to the 4493.33 g of feed utilized by the control group to attain a final weight of 2192.10 g. This agrees with the study carried out by Rama Rao *et al.* (2001) where increasing feed efficiency was recorded with increasing dietary CC supplementation. It also agrees with studies by Hossain *et al.* (2014) and Igwe *et al.* (2015). The CC may have brought about improved feed efficiency through its ability to furnish labile methyl groups for the formation of methionine, the first limiting amino acid in poultry nutrition which when added to poultry diets provides a means for increasing the efficiency of protein utilization (Schutte and de Jong, 1999).

Blood analysis remains a means of assessing clinical and nutritional health status of animals in feeding trials and data in Table 3 show the effect of CC supplementation on haematological values across all groups.

Table 3. Haematological parameters of broilers fed diets supplemented with varied levels of CC

Parameter	A (Control)	B (0.5 g cc/kg feed)	C (0.75 g cc/kg feed)	D (1 g cc/kg feed)
PCV (%)	26.10±1.15 ^{ab}	24.00±1.73 ^a	29.33±1.76 ^b	31.00±1.53 ^b
Haemoglobin (g/dl)	9.33±0.67 ^{ab}	8.00±0.32 ^a	9.80±0.50 ^b	8.87±0.37 ^{ab}
RBC ($\times 10^6/\mu\text{L}$)	2.58±0.07 ^a	2.57±0.03 ^a	2.68±0.06 ^a	1.83±0.02 ^b
WBC ($\times 10^3/\mu\text{L}$)	39.05± 0.02 ^a	32.42± 0.04 ^a	49.88± 0.06 ^b	50.33± 0.01 ^b
Heterophils (%)	22.67±1.45 ^a	25.33± 2.40 ^a	41.00± 3.21 ^b	66.00± 3.06 ^c
Lymphocytes (%)	77.33±1.45 ^a	74.67±2.40 ^a	59.00±3.21 ^b	34.00±3.06 ^c

Different superscripts ^{a,ab,bc} indicate significant difference between the groups ($p < 0.05$).

The normal ranges of PCV and Haemoglobin (Hb) in chickens are 22-35% and 7-13 g/dl respectively (Bounous and Stedman, 2000); the PCV and Hb values recorded for all groups were within their normal ranges. However, the red blood cell (RBC) count of the group with the highest CC supplementation (Group D – 1 g CC/kg feed) was significantly ($P \leq 0.05$) lower than all the other supplemented groups, the control

group and the normal range of $2.5-3.5 \times 10^6 \mu\text{l}$ (Bounous and Stedman, 2000). The RBC values of all the other supplemented groups and the control group remained within their normal range.

Igwe *et al.* (2015) recorded a slightly reduced RBC with CC supplementation, however, the RBC values recorded were within the normal range of RBC for broilers. They attributed the reduction in RBC to possible low levels of parasitaemia and/or nutritional diseases in the birds.

Derilo and Balnave (1980) reported reduced RBC, reduced growth and an interference in the utilization of vitamin B6 as the clinical signs of increased levels of dietary CC supplementation in chicken. However, several studies have suggested that chickens very well tolerate high levels of CC supplementation and that CC in chicken diets at twice the recommended levels is quite safe (NRC, 1987; Leeson and Summers, 2001) especially as about 20,000 to 30,000 mg CC/kg of diet was needed to induce toxicity. According to Odle (1996), excess choline is oxidized to betaine which is beneficial to poultry, allowing animals to tolerate high levels without adverse effects. Kumar (2004); Kanduri *et al.* (2013) and Khose *et al.* (2018) however, recorded no significant effect of CC supplementation on RBC values of broilers While Ajit *et al.* (2014); Vivian *et al.* (2015) and Sakthir *et al.* (2017) reported a significant increase in the Hb, total RBC count and total WBC count of CC-supplemented broilers.

Such significantly reduced RBC value with CC supplementation as recorded in the present study may be due to possible contamination of the CC used with undesirable substances that have deleterious health effects on the red blood cells of birds. Workel (2014) reported a 0-50% contamination of CC samples from Chinese producers with toxic substances ranging from dioxin, lead, arsenic and mercury to sodium, ammonium, calcium, magnesium and potassium ions as well as a high trimethylamine (TMA) content. The TMA content of CC has been said to be one of the most important and limiting raw materials of CC, because high levels of this compound in the CC product, can result to toxicity in the birds which may manifest through adverse hematological effects. Chronic exposure to lead (and several other heavy metals) can inhibit the animal's ability to produce hemoglobin by interfering with enzymatic steps in the heme synthesis pathway thereby diminishing red blood cells (ATSDR, 2007).

While the white blood cell (WBC) count of Group D was significantly higher than that of the control group (A) and the group with the least CC supplementation (Group B), it did not differ statistically from that of Group C. This increase in the WBC counts of the supplemented groups, though in agreement with studies by Igwe *et al.* (2015) and Khose *et al.* (2018) that choline chloride improves immune status by increasing the WBC, did not however reflect in the lymphocyte count of the supplemented groups (as reported by Igwe *et al.*, 2015; Khose *et al.*, 2018) as all supplemented groups recorded declining lymphocyte counts with increasing levels of CC, such that the group with the highest CC supplementation had the lowest lymphocyte count ($34 \pm 3.06\%$), which was significantly lower than that of all the supplemented groups and the control group. The control group recorded the highest lymphocyte count ($77.33 \pm 1.45\%$), significantly higher than all the supplemented groups. This is contrary to findings by Igwe *et al.* (2015) and Khose *et al.* (2018) that CC significantly increased lymphocyte and total leucocyte counts by the 6th week of supplementation, suggesting a stimulation of the growth and functioning of the immune system.

The levels of serum biochemical constituents provide information on the health status of organs and tissues in the body as well as the metabolic state of the animal (Minafra *et al.*, 2010). The creatinine level of Group D (1g cc/kg feed) appeared higher than all groups, but the difference was not statistically significant. Furthermore, although there was no significant difference in the ALT values across all groups, the mean values of (ALP) and cholesterol of group D were significantly ($P \leq 0.05$) higher than all the other supplemented groups and the control group. Abnormal liver enzyme levels may signal liver damage or alteration in bile flow (Giannini *et al.*, 2005). ALP values generally increase when bile flow in the liver is obstructed (Schlaeger *et al.*, 1982; Moss, 1997; Giannini *et al.*, 2005) and since secretion into bile is the major route for cholesterol elimination (Boyer, 2013), it therefore follows that any condition/substance that would reduce bile flow through the liver would bring about a consequent increase in cholesterol levels as recorded in this study.

Triglyceride levels were also significantly higher in the CC supplemented groups than the control group and since the liver is actively involved in the oxidization of triglyceride to produce energy (Whitney, 2012; Bowen, 2020), it may be an indication of possible impairment of liver functions. Whitney (2012) also reported endotoxaemia as one of the conditions that could bring about hypertriglyceridemia. The increase in ALP, cholesterol and triglyceride levels recorded in the present study is in contrast to studies by Rahman (2005) who reported a decrease in serum cholesterol following supplementation of CC in broilers; Gangane *et al.* (2010) who observed hypocholesterolemic effect, decreased triglyceride levels and no significant changes in the gross pathological study architecture of liver after administration of synthetic/herbal CC to broiler chickens.

Table 4. Serum biochemical parameters of broilers fed diets supplemented with varied levels of CC

Serum parameters	A (Control)	B (0.5 g cc/kg feed)	C (0.75 g cc/kg feed)	D (1 g cc/kg feed)
Aspartate amino transaminase (AST) (U/L)	46.00±32.53 ^a	51.33±3.18 ^a	57.67±19.55 ^a	46.67±7.75 ^a
Alanine amino transaminase (ALT) (U/L)	14.33±5.33 ^a	15.00±2.00 ^a	13.00±2.00 ^a	16.00±1.53 ^a
Alkaline phosphatase (ALP) (U/L)	11.50±16.59 ^a	21.20±36.80 ^a	18.40±18.40 ^a	44.20±1.24 ^b
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)	106.00±10.02 ^a	101.00±9.72 ^a	104.00±7.35 ^a	168.00±13.25 ^b
Triglyceride (mg/dl)	40.28±5.01 ^a	51.39±9.12 ^{ab}	75.00±14.43 ^b	51.04±27.74 ^{ab}
Creatinine (mg/dl)	0.37±0.27 ^a	0.20±0.06 ^a	0.23±0.13 ^a	0.50±0.15 ^a

Different superscripts ^{a, ab, b, bc, c} indicate significant difference between the groups ($p < 0.05$).

The poor performance recorded in group D with the highest inclusion rate is inconsistent with previous studies reporting positive effects of CC on broiler performance. This may be due to associated residues or contaminants present in the synthetic CC used for the study. Studies have shown that increased TMA content of CC, beyond the permissible level of 200 ppm can result in reduced feed intake, low RBC counts (Leeson and Summers, 2001), inflammation, enlarged liver and spleen weights, increased liver enzymes activity and increased risk for cardiovascular diseases and arthrosclerosis (Wang *et al.*, 2011; Sun *et al.*, 2017; Chen *et al.*, 2017), some of which were observed in the treatment group D.

Conclusions

In conclusion, supplementation with the CC used in this study did not improve production performance and although at the inclusion level of 0.75 g/kg feed, a slightly improved feed efficiency was observed, this was without a corresponding improved carcass yield. While at the inclusion level of 1 g/kg diet, feed intake, growth, total RBC and lymphocyte counts were reduced and serum ALP, cholesterol and triglyceride were increased with enlarged liver and spleen.

We therefore recommend proper analysis or assay of all synthetic choline before use to ascertain their quality and degree of contamination with unwanted substances. The Argentometry method of CC analysis, although fast, reliable, cheap and commonly used, measures only the chloride content of the CC, giving room for fraud as any chloride-containing product can be added to enrich the CC; the method would not distinguish the source of the chloride, so that all chloride will be calculated as choline chloride (Igwe *et al.*, 2015). The Reinecke method of CC analysis, which is more specific for the choline content of the CC, is usually used to avoid this problem. Ion Chromatography method is another method for CC analysis that not only shows the actual choline content, but also the level of other ions, trimethylamine and other impurities present in the CC sample (Igwe *et al.*, 2015). Farmers are advised to choose reputable companies to buy synthetic CC from while bearing in mind that the lowest cost product may not offer high quality.

We also recommend the administration of proven high-quality CC at a decreasing dose as the broiler needs for the supplement decreases with age (NRC, 1994). Being unable to sufficiently synthesize choline, young broiler chicks need more CC and according to NRC (1994), broilers need 1300 mg CC /kg diet at 0-3 weeks, 1000 mg CC /kg diet at 3-6 weeks and 750 mg CC/kg diet at 6-8 weeks.

Authors' Contributions

C. Ezema: Conceptualization, Methodology, Project administration, Supervision, Funding, Review & editing. P. C. Ugwu: Writing – Original draft preparation Visualization, Funding, Review & editing. A. Morgan: Visualization, Funding, Review & editing. C. N. Uju: Supervision, Investigation, Validation, Funding. C. Aronu: Visualization, Funding, Review & editing. G. Sunday: Investigation, Data curation, Formal analysis, C. Ahamefula: Investigation, Data curation, Formal analysis. W. Idigoh: Investigation, Data curation, Formal analysis. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The use of the broilers for the study was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria Nsukka (**FVM-UNN-IACUC-2021-0685**), and the study was done in accordance with the regulations and guidelines of the committee.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Adebayo OO, Adeola RG (2005). Socio-economic factors affecting poultry farmers in Ejigbo local government area of Osun State. *Journal of Human Ecology* 18(1):39-41. <https://doi.org/10.1080/09709274.2005.11905804>
- Afolayan M, Olatunde A, Afolayan M (2008). Nigeria oriented poultry feed formulation software requirements. *Journal of Applied Sciences Research* 4:1596-1602.
- Agency for Toxic Substances and Disease Registry (ATSDR) (2007). Toxicological Profile for Lead. US Department of Health and Human Services Public Health Service.
- Aidara-Kane A (2012). Containment of antimicrobial resistance due to use of antimicrobial agents in animals intended for food: WHO perspective. *Revue Scientifique Technique* 31:277-287. <https://doi.org/10.20506/rst.31.1.2115>
- Ajit S, Doley P, Neeraj, Prasad J (2014). Effect of dietary supplementation of tulsi (*Ocimum sanctum*) leaf powder on haematology and serum biochemistry of broiler chicks. *International Journal of Biological and Pharmaceutical Research* 5(1):89-92.
- Ayanrinde OJ, Owosibo AO, Adeyemo AA (2014). Performance characteristics of broilers fed bread waste-based diets. *International Journal of Modern Plant and Animal Science* 2(1):1-11.

- Bounous D, Stedman N (2000). Normal avian hematology: chicken and turkey. In: Feldman BF, Zinkl JG, Jain NC (Eds). Schalm's Veterinary Haematology. Wiley, New York, pp 1147-1154.
- Bowen R (2020). Metabolic functions of the liver. Retrieved 2020 July 25th from: <http://www.vivo.colostate.edu/hbooks/pathophys/digestion/liver/metabolic.html>
- Boyer JL (2013). Bile formation and secretion. *Comprehensive Physiology* 3(3):1035-1078. <https://doi.org/10.1002/cphy.c120027>
- Chen K, Zheng X, Feng M, Li D, Zhang H (2017). Gut microbiota-dependent metabolite trimethylamine N-oxide contributes to cardiac dysfunction in Western diet-induced obese mice. *Frontiers in Physiology* 8:139. <https://doi.org/10.3389/fphys.2017.00139>
- Derilo YL, Balnave D (1980). The choline and sulphur amino acid requirements of broiler chickens fed on semi-purified diets. *British Poultry Science* 21:479. <https://doi.org/10.1080/00071668008416700>
- Emmert JL, Baker DH (1997). A chick bioassay approach for determining the bioavailable choline concentration in normal and overheated soybean meal, canola meal and peanut meal. *Journal of Nutrition* 127:745-752. <https://doi.org/10.1093/jn/127.5.745>
- EPC (2005). Ban on Antibiotics as Growth Promoters in Animal Feed Enters into Effect, European Commission – IP/05/1687. Available at: http://europa.eu/rapid/press-release_IP-05-1687_en.htm
- Farina G, Kessler ADM, Ebling PD, Marx FR, César R, Ribeiro AML (2017). Performance of broilers fed different dietary choline sources and levels. *Ciência Animal Brasileira* 18. <https://doi.org/10.1590/1089-6891v18e-37633>
- Gangane GR, Gaikwad NZ, Ravikanth K, Maini S (2010). The comparative effects of synthetic choline and herbal choline on hepatic lipid metabolism in broilers. *Veterinary World* 3(7):318-320.
- Giannini EG (2005). Liver enzyme alteration: a guide for clinicians. *Canadian Medical Association Journal* 172(3):367-379. <https://doi.org/10.1503/cmaj.1040752>
- Hassan RA, Artia YA, EL-Ganzory EH (2005). Growth, carcass quality and serum constituents of slow growing chicks as affected by betaine addition to diets containing different levels of choline. *International Journal of Poultry Science* 4:840-850. <https://doi.org/10.3923/ijps.2005.840.850>
- Hossain M, Das GB, Hassan M, Shaikat A (2014). The effect of choline chloride supplementation on performance parameters and carcass characteristics of broiler. *Iranian Journal of Applied Animal Science* 4(2):373-378.
- Igwe IR, Okonkwo CJ, Uzoukwu UG, Onyenegecha CO (2015). The effect of choline chloride on the performance of broiler chickens. *Annual Research and Review in Biology* 8(3):1-8. <https://doi.org/10.9734/ARRB/2015/19372>
- Jadhav NV, Nagbhushana V, Shivi M, Kartikesh SM (2008). An evaluation of comparative effects of feeding synthetic and herbal choline on broiler performance, nutrient balance and serum activities. *Veterinary World* 1(10):306-309.
- Kachmar JF (1970). Determination of blood haemoglobin by the cyanomethaemoglobin procedure. *Fundamentals of clinical chemistry*, WB Sanders company, Philadelphia, pp 268-269.
- Kanduri AB, Saxena MJ, Ravikanth K, Maini S, Dandale M, Kokane SS (2013). Performance assessment of broiler poultry birds fed on herbal and synthetic amino acids. *Advances in BioResearch* 4(3):1-8.
- Khose KK, Manwar SJ, Sedmake ED, Ingole RS, Rathod PR (2018). Supplementation herbal choline in diets on haematological indices and development of vital organ in broilers. *Journal of Entomology and Zoology Studies* 6(6):1054-1058.
- Kumar V (2004). Biocholine as a replacer of choline chloride in commercial broilers. MVSc. Thesis, University of Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan Mathura, Uttar Pradesh, India.
- Kohn RS, Allen MS (1995). Enrichment of proteolytic activity relative to nitrogen in preparation from the rumen for *in vitro* studies. *Animal Feed Science and Technology* 52(1-2):1-14. [https://doi.org/10.1016/0377-8401\(94\)00711-H](https://doi.org/10.1016/0377-8401(94)00711-H)
- Leeson S, Summers JD (2001). *Scott's Nutrition of the Chicken*, 4th Ed. University Books, Guelph.
- Marshall BM, Levy SB (2011). Food animals and antimicrobials: impacts on human health. *Clinical Microbiology Reviews* 24:718-733. <https://doi.org/10.1128/CMR.00002-11>
- McDowell LR (2000). *Vitamins in Animal and Human Nutrition*, 2nd Ed. Wiley-Blackwell: Hoboken, New Jersey, USA, pp 793.

- Minafra CS, Marques SFF, Stringhini JH, Ulhoa CJ, Rezende CSM, Santos JS, Moraes GHKD (2010). Biochemical serum profile of broilers fed diets supplemented with alfa-amylase from *Cryptococcus flavus* and *Aspergillus niger* HM2003. Revista Brasileira de Zootecnia 39:2691-2696. <https://doi.org/10.1590/S1516-35982010001200020>
- Moss DW (1997). Physiochemical and patho-physiological factors in the release of membrane-bound alkaline phosphatase from cells. Clinica Chimica Acta 257(1):133-40. [https://doi.org/10.1016/S0009-8981\(96\)06438-8](https://doi.org/10.1016/S0009-8981(96)06438-8)
- NRC (1987). Vitamin Tolerance of Animals. National Academy of Sciences-National Research Council, Washington, D.C.
- NRC (1994). Nutrient Requirements of Poultry, 9th Edit., Nutrient Requirements of Domestic Animals. National Academy of Sciences-National Research Council, Washington, D.C.
- Odle J (1996) Ingredients for pig feed: betaine and carnitine. Feed Management 47(1):25-27.
- Peters TT, Biamonte GT, Doumas BT (1982). Protein in serum, urine and cerebrospinal fluid and albumen serum. In: Tietz NW (Ed). Selected Methods of Clinical Chemistry. W.B. Saunders Company, Philadelphia, pp 884-887.
- Rafeeq M, Pasha TN, Rashid N, Hilal B, Shahzad I (2011). Effect of supplementation of methionine, betaine and choline on the performance of broiler chicken in early life fed methionine deficient ration. Journal of Animal and Plant Science 21(4):778-780.
- Rahman BMDA (2005). Effect of dietary choline and methionine on broiler chicks performance and some blood parameters. MSc. Thesis, University of Khartoum, Khartoum, Sudan.
- Rajalekshmy P (2010). Effects of dietary choline, folic acid and vitamin B12 on laying hen performance, egg components and egg phospholipid composition. ETD collection for University of Nebraska - Lincoln. AAI3428429. <https://digitalcommons.unl.edu/dissertations/AAI3428429>
- Rao SR, Sunder GS, Reddy MR, Praharaj NK, Raju MVLN, Panda AK (2001). Effect of supplementary choline on the performance of broiler breeders fed on different energy sources. British Poultry Science 42(3):362-367. <https://doi.org/10.1080/00071660120055340>
- Richmond W (1973). Cholesterol enzymatic colorimetric test chop-PAP method of estimation of total cholesterol in serum. Clinical Chemistry 191:1350-1356.
- Sakthi PM, Gopala K, Murthy TR, Vijayanand T (2017). Effect of blend herbal supplement on haematology and serum biochemistry in commercial layer chicken. Journal World's Poultry Research 7(2):48-56.
- Schalm OW, Jain NC, Carrol EG (1975). Veterinary haematology. Leo and Febiger, Philadelphia. pp 140-152.
- Schlaeger R, Haux D, Kattermann R (1982). Studies on the mechanism of the in-crease in serum alkaline phosphatase activity in cholestasis: significance of the hepatic bile acid concentration for the leakage of alkaline phosphatase from rat liver. Enzyme 28(1):3-13. <https://doi.org/10.1159/000459078>
- Schutte JB, de Jong J (1999). Ideal amino acid profile for poultry. Cahiers-Options-Mediterranees 37:259-263.
- Steel RGD, Torrie JH (1960). Principles and Procedures of Statistics. McGraw-Hill, New York.
- Sun G, Yin Z, Liu N, Bian X, Yu R, Su X, ... Wang Y (2017). Gut microbial metabolite TMAO contributes to renal dysfunction in a mouse model of diet-induced obesity. Biochemical and Biophysical Research Communications 493(2):964-970. <https://doi.org/10.1016/j.bbrc.2017.09.108>
- Swain BK, Johri TS (2000). Effect of supplemental methionine, choline and their combinations on the performance and immune response of broilers. British Poultry Science 41(1):83-88. <https://doi.org/10.1080/00071660086457>
- Vivian UOO, Ndofofor-Foleng HM, Olorunleke SO, Uguru JO (2015). Evaluation of growth performance, haematological and serum biochemical response of broiler chickens to aqueous extract of ginger and garlic. Journal of Agricultural Sciences 7(4):167-173. <https://doi.org/10.5539/jas.v7n4p167>
- Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, ... Hazen SL (2011). Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472(7341):57-63. <https://doi.org/10.1038/nature09922>
- Whitney SM (2012). Triglycerides. In: Wilson DA (Ed). Clinical Veterinary Advisor: The Horse. W. B. Saunders Company, Philadelphia.
- Zhang JJ, Wang LX, Ruan WK, An J (2013). Investigation into the prevalence of coccidiosis and maduramycin drug resistance in chickens in China. Veterinary Parasitology 191:29-34. <https://doi.org/10.1016/j.vetpar.2012.07.027>



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