Nigerian J. Anim. Sci. 2021, 23 (1): 28-34

Performance and serum biochemistry of broiler chicken fed dietary supplemental methionine, choline and inorganic sulphate

***Adejumo R.O., Abiola O.A. and Longe O.G.**

Department of Animal Science, University of Ibadan, Ibadan, Nigeria

****Corresponding Author:** tundeadejumo@yahoo.co.uk +2347031819553*

***Target Audience:** Researchers, Animal scientists, Medical practitioners*

Abstract

An experiment was conducted to examine the sparing effect of choline and/or sulphate on methionine in serum biochemical indices of broiler chickens in a 21-days feeding trial. Two hundred (200) unsexed one-day old Arbor Acre broiler birds were randomly distributed into five dietary treatments consisting 5 replicates of 8 birds per replicate in a completely randomized design. Diet 1 was the control, a basal diet without any of the supplements (methionine, choline or sulphate, diet 2 had the basal diet with methionine (0.30%) only, diet 3 was the basal diet with 0.30% methionine + 0.10% choline, diet 4 was the basal diet with 0.10% choline+ 0.25% sulphate and diet 5 was the basal diet with 0.30% methionine+ 0.10%choline+ 0.25% sulphate. On day 21, two birds per replicate were bled at the jugular vein into heparin bottles for serum biochemical indices. Results showed that feed intake of birds fed methionine, methionine+choline and methionine+choline+sulphate supplemented diets were similar but significantly higher than those fed diets supplemented with choline+sulphate and the control diet. Similar trend was observed in the final body weight gain of birds on experimental diets. Improved feed conversion ratio was recorded for birds fed methionine, methionine+choline and methionine+choline+sulphate supplemented diets. Diets had significant (P<0.05) effect on glucose, blood urea nitrogen, creatinine, triglyceride, cholesterol, albumin and globulin but liver enzymes were not significant. Supplementation of methionine with the combination of choline and sulphate resulted in overall improvement of broiler biochemical parameters when compared with control and birds fed combination of choline and sulphate alone. Supplementing methionine with choline and sulphate also spares methionine in improving the health status of the broiler birds by regulating the serum lipids.

***Keyword:** Broiler chicken, Methionine, Choline, Sulphur, Serum biochemistry.*

Description of Problem

The significance of sulphur amino acids in poultry nutrition, especially methionine cannot be overemphasized. Methionine is an essential amino acid that provide methyl group which are needed for several metabolic reactions such as the synthesis of carnitine and creatinine (6), its sparing effect on choline has also been reported (2). Besides the sparing effect on choline, methionine is also the primary source of sulphur which is required for the synthesis of many sulphur containing compounds e.g. mucopolysaccharides (chondroitin sulphate) and cerebroside sulphate. Methionine improves energy utilization in broilers and also a lipotropic agent in preventing fatty liver (3). The discovery that methionine and choline are essential in the nutrition of chicks and turkey poults has played an important role in the production of highly efficient feed for poultry. However, the study of their interrelationship as labile methyl donors is still a subject of research and discussion among several nutritionists. Although sulphate has not been considered dietary essential, there are reports suggesting its possible sparing action in

sulphur amino acid (23). The interrelationship between choline and methionine and the interrelation between sulphur containing amino acids and sulphate have been studied (11 and 12).

Methionine can furnish methyl groups for choline synthesis in most species of poultry. Choline, however, is effective only in sparing methionine which otherwise would be used to make up for choline shortage when it is deficit. Methionine is not used up for choline synthesis if there is an adequate level of dietary choline. In formulating typical poultry diets, methionine is frequently one of the most limiting amino acids. Therefore, it would be impractical for marginal quantities of methionine to be wasted for synthesis of the vitamin when supplemental choline can be provided more economically. Meanwhile, in supplementing poultry diets with methionine and (or) choline, a third nutrient, sulphur, must also be considered. Sulphur is present in a number of body metabolites (e.g., mucopolysaccharides) and if not adequately supplied in the diet, sulphur amino acids would likely be degraded. It was reported that sulphate must be present for choline to spare a maximum amount of methionine (12). The practical implication is that sulphate and choline need to be adequately provided in diets so that the more expensive and often marginally deficient nutrient methionine is not used to provide either of these nutrients. Therefore, the objective of this study was to examine the sparing effect of choline and/ or sulphate on methionine on the serum biochemistry of broiler chickens.

Materials and Methods

The experiment was carried out at the Teaching and Research Farm of the University of Ibadan, Ibadan. Two hundred (200) unsexed one-day old Arbor Acre broiler chicks obtained from a reputable hatchery in Ibadan, Nigeria were used for this study. The birds were randomly distributed into five dietary treatments consisting of five replicates in a treatment with eight birds per replicate in a completely randomized design. The study lasted for 21days. Diet 1 was the control, a basal diet without any of the supplements (methionine, choline or sulphate, Diet 2 had the basal diet with 0.30% methionine only. Diet 3, was basal diet with 0.30% methionine+ 0.10% choline. Diet 4 was basal diet with 0.10% choline+ 0.25% sulphate while diet 5 had the basal diet with 0.30% methionine+ 0.10% choline+ 0.25% sulphate (Table 1). Methionine was supplied in the form of DL-Methionine, while choline was from choline chloride and sulphate as potassium sulphate. Feed and cool clean water were provided *ad libitum* during the period of the experiment.

Performance indices

Feed was presented to the birds unrestricted and feed intake was calculated as the difference between amount given and the left over. The birds were weighed at the start and end of the study and the values were used to calculate body weight gain and the feed conversion ratio

Blood collection

At day 21, two birds per replicate were bled through the jugular vein and about 3mls of blood were collected into heparinized bottles and allowed to coagulate. Coagulated blood samples were centrifuged (2000rpm for 15min) after which it was stored at -20^{o}C for further analysis of serum biochemical parameters.

Serum biochemical analyses

Glucose was determined by spectrophotometric method of (21) and (20). Aspartate aminotransferase and alanine aminotransaminase were determined manually by spectrophotometric method as described by (19). Urea was determined by urease method

and creatinine by Folin-Wu filtrate method as described by (18). Total serum protein was determined using biuret method as described by (16) while albumin was determined using the BGG (Bromocresol green) method as described by (14). The serum globulin was calculated by subtracting serum albumin from total serum protein. Cholesterol was determined by enzymatic colorimetric reaction according to method of (20).

Proximate analysis of diets

Chemical compositions of the experimental diets were analyzed to calculate the dry matter, crude protein, crude fibre, total fat and ash using the method of (1).

Statistical analysis

Data obtained were subjected to statistical analysis of variance (ANOVA) (17) using the software and treatment means were separated using Duncan multiple range test (5).

Table 1: Gross composition (g/100gDM) of experimental broiler starter diets

INGREDIENT %	Control diet	Methionine	Methionine+Choline	Choline+Sulphate	Methionine+Choline+Sulphate
Maize	51.00	51.00	51.00	51.00	51.00
SBM	25.50	25.50	25.50	25.50	25.50
Full fat soya	20.00	20.00	20.00	20.00	20.00
Wheat bran	2.95	2.65	2.55	2.60	2.30
Limestone	1.00	1.00	1.00	1.00	1.00
DCP	2.00	2.00	2.00	2.00	2.00
Methionine	0.00	0.30	0.30	0.00	0.30
Choline chloride	0.00	0.00	0.10	0.10	0.10
Potassium sulphate	0.00	0.00	0.00	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30
Vit/min premix	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Crude protein	23.09	23.05	22.98	23.01	22.95
ME (kcal/kg)	3057.34	3048.64	3048.64	3039.94	3028.34
Calcium	0.92	0.92	0.92	1.00	0.96
Avail.phosphorus	0.48	0.48	0.48	0.48	0.48
Methionine	0.24	0.54	0.54	0.24	0.54
Lysine	1.28	1.28	1.28	1.28	1.28
Choline	0.12	0.12	0.16	0.16	0.16
Sulphur (%)	0.02	0.02	0.02	0.08	0.08

Composition of Vitamins/kg diet:A-12,000i.u D3-3000,i.u E-30mg K3-2.5mg B1-2mg B2-2.5mg Niacin-40mg Calpan-10mg B6-3.5mg B12-0.02mg Folic Acid-1mg Biotin-0.08mg Antioxidant-125mg.

Composition of Mineral/kg diet: Sodium chloride-10mg Potassium chloride-62.5mg Sodium bicarbonate-37.5mg Sodium Acid Phosphate-10mg Sodium Citrate-81.25mg Calcium Lactate-21.25mg Lactose-312.5mg

SBM: Soyabean meal **DCP:** Di-Calcium phosphate **ME:** Metabolisable Energy

Table 2: Growth performance of broiler chicks fed supplemental methionine, choline and/or sulphur

Parameter	Control diet	Methionine	Methionine+ Choline	Choline+ Sulphate	Methionine+ Choline+Sulphate	SEM
Initial body weight	44.00	43.40	43.65	43.5	43.10	0.49
Final body weight	424.13^{b}	536.11^{a}	547.15^{a}	425.13^{b}	541.89^{a}	11.69
Feed intake	853.90^{b}	1001.68^{a}	967.70^{a}	893.90^{b}	980.78^{a}	21.46
Weight gain	380.13^{b}	492.71^{a}	503.50^{a}	381.63^{b}	498.79^{a}	36.14
Feed conversion ratio	2.25^{a}	2.03^{b}	1.92^{b}	2.35^{a}	1.97^{b}	0.05

^{ab}:means along the row with similar superscript are not significantly different (P>0.05)

Table 3: Serum biochemical indices of broiler chickens fed supplemental methionine, choline and/or sulphur diets

Parameter	Control diet	Methionine	Methionine+ Choline	Choline+S ulphate	Methionine+C holine +Sulphate	SEM
Glucose (mg/dl)	205.66^{a}	206.50^{a}	209.07^{a}	207.50^{a}	180.08^{b}	5.64
Blood Urea Nitrogen (mg/dl)	3.31^{c}	4.84^{b}	4.53^{b}	7.17^{a}	8.27^{a}	0.43
Creatinine (mg/dl)	0.72^{b}	0.97^{a}	0.98^{a}	1.05^{a}	1.06^{a}	0.03
Triglycerides (mg/dl)	63.69^{a}	65.25^{a}	34.09^{b}	72.79^{a}	35.49^{b}	6.78
Cholesterol (mg/dl)	131.50^{c}	197.88^{a}	204.48^{a}	173.86^{b}	124.01^{c}	7.84
Total protein (g/dl)	2.27	2.53	2.52	2.32	2.56	0.11
Albumin (g/dl)	1.43^{b}	1.68^{a}	1.62^{ab}	1.43^{b}	1.63^{ab}	0.22
Globulin (g/dl)	0.83	0.85	0.89	0.89	0.93	0.12
Aspartate amino transferase (i.u/l)	257.11^{a}	209.31^{b}	207.83^{b}	224.59^{b}	211.40^{b}	6.41
Alanine amino transferase (i.u/l)	6.24^{b}	6.80^{b}	8.10^{ab}	9.36^{a}	8.21^{ab}	0.65

^{abc}: means along the row with similar superscript are not significantly different(P>0.05)

Results and Discussion

The growth performance of experimental birds fed dietary supplemental methionine, choline and/or sulphate as shown in Table 2. Significantly different values were observed among treatment means for total feed intake, final body weight, body weight gain and feed conversion ratio of birds fed dietary treatments. Feed intake of birds fed methionine, methionine+choline and methionine+ choline+sulphate supplemented diets were similar but significantly higher than those fed control and supplemental choline+sulphate diets. Supplemental methionine had sparing effect on choline (2) and sulphur. Similar trend was observed in the final weight and body weight gain of birds on experimental diets. Improved feed conversion ratio was recorded for birds fed methionine, methionine+choline and methionine+choline+ sulphate supplemented diets. It could that the absence of methionine in the control diet and supplemental choline+sulphate diet resulted in poor utilization when compared with the fed

methionine supplemented diets. Methionine supplies the body need of sulphur and could be used to produce choline during choline shortage but supplemental choline+sulphate will only spare and cannot totally replace methionine. According to (3), supplementing methionine in broilers resulted in lowered feed conversion ratio but with elevated abdominal fat.

The serum biochemical parameters of broilers fed dietary supplementation of methionine with choline and/or sulphate is shown in Table 3. The value (180.08mg/dl) obtained for glucose for Methionine+Choline+ Sulphate was significantly different and lower than values obtained for other diets across the treatment. Brody (3) reported that the influence of methionine in broiler diets serves to improve energy utilization in the body, therefore lower value obtained for methionine+ choline+ sulphate could suggest better availability of methionine in the presence of choline combined with sulphate in the diet compared with other treatments that lacked the dietary supplementation of either or combination of methionine, choline and sulphur. Also, sulphur is a component of insulin, an important hormone that regulates the uptake of glucose by cells for use as energy, a lack of sulphur can result in low insulin production or cell resistance to insulin, insulin stimulates glucose oxidation and storage. Sulphur is also needed for thiamine and biotin production which are needed for normal carbohydrate metabolism. Lower circulating plasma glucose (180.08mg/dl) recorded in this study when methionine was supplemented with choline and sulphur suggests enhanced efficiency of insulin in the presence of sulphur. However, (8) reported that circulating circus of glucose in chickens is 190 to 220 mg/dl but (13) reported values of 152 to 182 mg/dl.

The values obtained for urea nitrogen in this study were not consistent with the values (0.90 to 1.85 mg/dl) reported by (13). However, values obtained for choline+sulphate and methionine+choline+sulphate were significantly higher than values obtained for methionine and methionine+ choline and could infer possible influence of choline combined with sulphur in the two diets. The presence of choline combined with sulphate could have a sparing effect on the sulphur amino acids present in both choline+ sulphate and methionine+ choline+ sulphate to increase the protein digested in the intestine, hence increased metabolism of amino acid resulting in higher serum urea nitrogen. Urea is synthesized by hepatocytes from ammonia generated by catabolism of amino acids derived either from digestion of proteins in the intestine or from endogenous tissue proteins. Values obtained for creatinine for birds on the experimental diets were significantly higher than the control diet and similar to the values reported by (13). This may be connected to the breakdown of creatine phosphate in the muscle to release the by-product creatinine. Creatinine is a measure of muscle activity. A reduced muscle activity of birds fed the un-supplemented diet could be linked to the reduced weight gain observed for such birds, since muscle activity is higher with greater muscle deposition with the presence of amino acids (Table 3).

Values obtained for triglycerides in this study were significantly lower in diets supplemented with the combination of methionine and choline chloride with or without sulphur. This possibly suggests that supplementation of methionine choline and/or sulphate had regulatory effect on the triglyceride level of growing broiler birds. Methionine and choline are known to interfere with fat metabolism (15). It is well known that requirement of choline is critical for regulating lipid metabolism, (10). The mean values obtained for cholesterol in this study for diets supplemented with methione and

methionine+choline compared to the control revealed that supplementation of methionine with or without choline in the absence of sulphur could possibly increase serum cholesterol at the early stage of growth of broiler chickens. Though a significantly lower value was recorded for cholesterol in methionine+choline+sulphate yet the final body weight and the feed intake of the birds on this diet were not significantly different from diets fed supplements of methionine and methionine+choline (Table 3). It has become clear that fat accretion is closely related to the rate of gain and nutritional management practices.

Albumin is a blood transport protein that binds many molecules and drugs including hormones, lipoproteins and amino acids (2). The addition of any of the supplements had no significant effect on the total protein. Values obtained for globulin were also consistent with that reported by (13). Hyperproteinemia in most birds is indicated by plasma total protein concentrations of greater than 4.5g/dl (22). The mean values obtained for aspartate aminotransferase (AST) in this experimental study shows a significantly lower value when compared to the control diet which suggests a better liver performance and better utilization of the supplements. The enzyme levels are especially useful in assessing subtle and early changes in biliary obstruction and active cirrhosis, (4). Dietary supplementation of choline with or without methionine reduces liver tumour incidence (6&7). The mechanisms by which choline deficiency is thought to be carcinogenic include enhanced cell proliferation, altered methylation status and altered signal reduction (7).

Conclusion and Applications

1. Based on the findings of this study, the supplemental choline combined with sulphate would spare methionine to improve the health status of broiler birds.
2. Supplementation of methionine with choline and sulphate will result in overall improvement of broiler performance and reduce the risk of excessive fat deposition.

References

1. A.O.A.C. (2019). Association of Official Analytical Chemist. *Method of Analysis, 21st edition, Washington DC, USA.*
2. Mohammad M, Arash A, and K. Heshmatollar, (2018). Partial Replacement of Dietary Methionine with Betaine and Choline in Heat Stressed Broiler Chickens. *The Journal of Poultry Science Vol. 55(1) (2018): 28-37*
3. Brody T., (1994). Nutritional Biochemistry Academic Press Inc. *USA, 658p.*
4. Das H, Ali M.A, Devi L.I, Kirthika P., GaliJ. M. R and P. Behera (2017). Studies on some important enzymes of non-descript sheep of Assam. *Journal of Livestock Science 8: 59-62*
5. Duncan, D.B., (1955). Multiple Range and F-Tests. *Biometrics, 11:1-42*
6. Tripodi, F., Badone, B., Vescovi, M., Milanesi, R., Nonnis, S., Maffioli, E., Bonanomi, M., Gaglio, D., Tedeschi, G., & Coccetti, P. (2020). Methionine Supplementation Affects Metabolism and Reduces Tumor Aggressiveness in Liver Cancer Cells. *Cells, 9(11), 2491.*
7. Mehedint, M. G., & Zeisel, S. H. (2013). Choline's role in maintaining liver function: new evidence for epigenetic mechanisms. *Current opinion in clinical nutrition and metabolic care*, *16*(3), 339–345.
8. Hazelwood R.L, (1986). Carbohydrate metabolism. In: *Avian Physiology (Ed. P.D Shirkie) pp303-325 Spring verlag, New York.*
9. Hernawan E., Wahyuni S. and H. Suprapti, (2012).The Levels of Blood Glucose,

Triglyceride, Final Body Weight and Abdominal Fat Percentage of Broiler Under Sex-Separated and Straight Run Rearing System. *Lucrari Stiintifice-Universitatea de Stiinte Agricole si Medicina Veterinara, Seria Zootehnie 2012 Vol 57 pp28-33 ref. 17*

10. Jadhav N., Maini S. and K. Ravikanth, (2008). Comparative efficacy studies of herbal and synthetic choline supplements on broiler growth and performance. *The Internet Journal of Veterinary Medicine*vol. 5; 2
11. Waldroup, Park &Motl, M & Yan, Fenglong& Fritts, C. (2006). Effects of Betaine and Choline on Response to Methionine Supplementation to Broiler Diets Formulated to Industry Standards1. *Journal of Applied Poultry Research. 15. Pages 58-71*.
12. John T. Brosnan, Margaret E. Brosnan (2006). The Sulfur-Containing Amino Acids: An Overview. *The Journal of Nutrition, Vol 136 (6) Pp1636S-1640S*
13. Mitruka B.M and H.M Rawnsley, (1981). *Clinical biochemical and haematological reference values in normal experimental animals, New York Mass Publishing*.
14. Peters T., Biamonte G.T. and B.T. Doumas (1982). Protein (total protein) in serum, urine and cerebrospinal fluid; albumin in serum. *Selected method of clinical chemistry. Vol. 9. W.R*
15. Pooja J., K. Astrid, K. Harald, M. Michaela, L. Carolin, H. Gerald, C. Thierry and Michael T. (2014). Role of Adipose Tissue in Methionine-Choline-Deficient Model of Non-Alcoholic Steatohepatitis (NASH). Biochim Biophys Acta. (2014) Jul; (1842)(7): 959-970
16. Reinhold G.J., (1953). *Standard methods of clinical chemistry; Vol; pp 88: Reiner M. (Edn); Academic press, New York.*
17. SAS Institute, (2010). SAS/STAT User's Guide. Release 6.08 editions. *SAS Institute Inc., Cary, North Carolina.*
18. Schirmeister, J., Willmann H. and H. Kiefer. (1964). Plasma Creatine as Indicator of Renal Function. *Dtsch. Med. Wochenschr. 89: 1018 -23*
19. Schmidt, E., and F.W. Schmidt, (1967). Release of enzyme from the liver. *Enzyme. Biology Clinical Vol.3:1- 8*
20. Siedel, J., Schlumberger H, Klose S., Ziegenhorn J. and A.W. Wahlefeld, (1981). Improved reagent for theenzymatic determination of serum cholesterol .*Journal of Clinical Chemistry and Clinical Biochemistry Vol* 19:838-842
21. Teuscher A. and P. Richterich, (1971). Schweiz. Med. Wschr pp101, 345 and 390
22. Thrall, M.A, 2004. Veterinary hematology and clinical chemistry. *Lippincott Williams and Wilkins, Philadelphia, pp:518*
23. Ernest R., B.L Damron and R. H Harms, (1972). The Requirement for Inorganic Sulphate in the Diet of Chicks for Optimum Growth and Feed Efficiency. *Poultry Science 51: 1606-1612.*