Wayamba Journal of Animal Science – ISSN: 2012-578X; P975-P980, 2014
First Submitted July 29, 2014; Number 1395719429

INVESTIGATION OF THE TOXICITY LEVELS OF SUPPLEMENTAL DIETARY DL-METHIONINE FOR POULTRY IN A TROPICAL ENVIRONMENT

Annongu, A.A[1]; Karim, O.R[2]; Sola-Ojo, F.E[1]; Kayode, R.M.O[2] and Adeyemi, K.D[1]*
[1]Department of Animal Production, University of Ilorin, Ilorin Nigeria
[2]Department of Home-Economics and Food Science, University of Ilorin, Ilorin Nigeria

**Corresponding author:- Kazyadeyemi@gmail.com*

The study investigated the effect of supplementing dietary methionine on growth performance, serum biochemistry and liver histology in cockerels. A total of three hundred 1 day- old cockerel chicks were randomly assigned to diets containing 0.10, 0.25, 0.35, 0.45 or 0.55% of dietary methionine in a completely randomized design and fed for six weeks. Feed intake, weight gain, survival rate, serum cholesterol, transaminase activities and thiobarbituric acid reactive substance (TBARS) values and liver histology were determined. The result showed that feed intake and growth rate decreased with increasing level of dietary methionine although not significant ($p > 0.05$), while survival rate was 100% regardless of dietary treatments. Birds fed 0.10, 0.25 and 0.35% methionine had significantly higher ($p < 0.05$) weight gain, feed efficiency and HDL cholesterol but lower ($p<0.05$) LDL cholesterol and TBARS values than those fed 0.45 and 0.55% methionine. The activities of gamma glutamyl transaminase (GGT) and alanine transaminase (ALT) was significantly higher in birds fed 0.10 and 0.25 than 0.35, 0.45 and 0.55% methionine while aspartate aminotransferase (AST) activity decrease significantly ($p < 0.05$) with increasing levels of methionine. Normal hepatic architecture was observed in liver of birds fed 0.10 and 0.25% methionine while those fed higher levels had distorted hepatic architecture, the severity of which increased with increasing levels of methionine. Thus, inclusion of supplemented dietary methionine above 0.25% is likely to create health hazards in poultry in a tropical environment like Nigeria.

Key words: Methionine, cockerels, performance, serum cholesterol, liver histology.

It is necessary to possess adequate knowledge of the fact that food and feedstuffs contain organic and inorganic chemical substances essential or harmful to the health of man and animals. At times, even those substances considered beneficial become toxic to the body when taken in amounts above the tolerance level. Some chemical substances are potentially toxic while others can induce toxicity when included in excess. One of such nutrient is methionine (Kalbande et al., 2009). Methionine is one of the sulphur-containing essential amino acids which serve to build body proteins, repair worn out tissues and perform other functions in the body. Since essential amino acids cannot be synthesized from other compounds in the body and must be taken in diet, methionine per se constitutes the first limiting amino acids in diet of farm animals like poultry (Xie et al., 2007). When dietary supplementation is optimal, methionine improves growth and other performance indices including carcass quality (Xie et al., 2004). Excess dietary methionine however, has been shown to exert the most toxic side effect compared with the other amino acids (Carew et al., 1998; Acar et al., 2001). In most tropical countries, the level of methionine used is the normal 0.1-0.15% recommended by the National Research Council (NRC) of America in their nutrient requirements of poultry used in temperate regions. This level may be too low or high for optimum performance of poultry in tropical regions since it has been well established that ambient temperature play vital role in nutrient intake and subsequent nutrient utilization in poultry. For instance, under high ambient temperature, birds reduce feed intake to reduce heat production (Atteh, 2004). Besides, high ambient and relative humidity as experienced in Nigeria and other tropical countries may cause stress and induce a myriad of behavioral, physiological and biochemical responses that could pose challenges to growth performance, disease resistance, immunity and survivability (Htin et al., 2007). In such conditions, the birds feed needs to be nutritionally dense to meet the bird's nutritional requirement. In addition, in typical tropical environment like Nigeria, there is dearth of information on the optimum level of methionine for poultry. Thus, the objective of the current study was to determine the effect of supplementing graded levels of methionine above the normal 0.1-0.15% recommended by the National Research Council (NRC, 1994) on growth performance, some serum biochemical indices and liver histology in cockerels.

MATERIALS AND METHODS

Diets, animals, housing and feeding trial

Five iso-caloric and iso-nitrogenous diets were formulated. The reference diet (control) was made up of a corn-soybean meal as basic ingredients with the usual supplemental dietary methionine of 0.10% recommended by NRC (1994) while the other diets contained 0.25, 0.35, 0.45 and 0.55% supplemental methionine.

Table 1: Composition of the experimental diets on as fed basis.

	Levels of supplemented dietary methionine (%)				
Ingredients	0.10	0.25	0.35	0.45	0.55
Maize	60.00	60.50	61.50	64.00	65.50
Soybean meal	37.20	36.60	35.50	32.90	31.35
DL-methionine	0.10	0.25	0.35	0.45	0.55
Bone meal	1.50	1.50	1.50	1.50	1.50
Oyster shell	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100

Three hundred (300) day old Bankam breed of cockerel chicks from a commercial source were used for the experiment. They were housed in an electrically heated battery brooder cages. The housing temperature throughout the experimental period ranged from 31 to 33.5^{o}C. Sixty chicks were assigned to a dietary treatment partitioned into three replicates, each containing twenty chicks. Both feed and drinking water were supplied ad libitum. In the course of the trial, data was recorded on feed consumption, body weight gain, efficiency of feed utilization, growth and survival rates. Phenotypic observations were also made along with the performance characteristics. At the end of the trial, five birds per replicate were randomly taken and slaughtered for evisceration of liver samples for histopathology. Blood samples were collected from the slaughtered broiler cockerels for evaluation of serum biochemistry.

Plasma malonaldehyde/thiobarbituric acid reactive substances (MDA/TBARS), low and high density lipoproteins were analyzed. Serum enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transaminase (GGT) were determined by the colorimetric method using Sigma diagnostic (1985). Histopathological evaluation was conducted on liver samples.

Statistical analysis

The experiment followed a completely randomized design. Data obtained were subjected to GLM procedures of SAS. Significance was set at $p< 0.05$ and means were separated by Duncan multiple range test.

RESULTS

Consumption of supplemental dietary DL-methionine at graded levels and its effect on the performance characteristics is presented in Table 2. Feed intake and growth rate values were not significantly different ($p> 0.05$) among the treatments. However, the values of these parameters decreased with increasing levels of supplemented methionine. Ingestion of the graded levels of dietary methionine decreased ($p< 0.05$) body weight gain but seems to improve feed efficiency relative to the control diet ($p<0.05$).

Table 2: Effect of graded levels of dietary methionine on performance indices of cockerels

	Level of dietary DL-methionine (%)					
Indices	0.10	0.25	0. 35	0.45	0.55	SEM
Feed intake (g/b/d)	22.0	22.0	21.0	18.3	17.8	5.12
Weight gain (g/b/d)	6.2^{b}	6.7^{b}	6.75^{b}	5.9^{a}	5.7^{a}	0.45
Feed/gain	3.5	3.3	3.1	3.1	3.1	0.07
Growth rate (%)	42.0	41.3	41.0	41.0	39.0	6.40
Survival rate	100	100	100	100	100	14.09

a, b, c means having different superscripts along the same row are significantly different ($p< 0.05$).

Table 3 shows the effect of varying inclusion levels of methionine on TBARS/MDA, LDL and HDL cholesterol and serum enzymes in cockerels. TBARS/MDA and LDL significantly increased in response to increasing dietary levels of methionine while values of HDL decreased relative to the orthodox diet. There was no significant difference ($p> 0.05$) in the TBARS/MDA, LDL and HDL cholesterols among the 0.10, 0.25 and 0.35% methionine based diets but significant difference was observed on the 0.45 and 0.55% methionine diets. Activities of the marker enzymes, AST, ALT and GGT revealed that increasing dietary methionine levels caused reduction in the activities of the measured enzymes. There was no significant difference ($p> 0.05$) between the 0.10 and 0.25% in the values of GGT and ALT but differ significantly ($p< 0.05$) from other treatments. The control diet had a significantly ($p< 0.05$) higher AST activity than other treatments. There was no significant difference in the AST activity among the 0.25, 0.35 and 0.45 methionine supplements while 0.55% methionine had the lowest AST activity that was significantly different from the other treatments.

Micrographs 1-5 presented the influence of the different levels of methionine in diets on the histopathology of the liver samples of the experimental birds. The conventional diet with normal inclusion of methionine and the diet containing 0.25% methionine showed liver samples depicting normal hepatic architecture while the diet with 0.35% supplemental methionine elicited mild adverse effect on the liver of the group of cockerels receiving the diet. Diets containing 0.45 and 0.55% methionine showed liver samples with the highest adverse effects exemplified in hyperplasia (increase in cell size). The 0.55% methionine also caused some degree of lesion compared with the control diet

DISCUSSION

Body weight was gained more on diets containing 0.10, 0.25 and 0.35% gap methionine while weight gain was inferior in diets with high levels of methionine 0.45 and 0.55. Supplementing methionine at graded levels seems to improve feed efficiency but the apparent improvement failed to translate into body weight gain and growth rate.

Table 3: Effects of graded levels of methionine on serum cholesterol, TBARS and transaminase activities in cockerels

	Dietary levels of methionine (%)					
Indices	0.10	0.25	0.35	0.45	0.55	SEM
TBARS/MDA	0.87^{a}	0.86^{a}	0.93^{a}	1.50^{b}	1.54^{b}	0.23
LDL (mmol/L)	0.7^{a}	1.16^{a}	1.20^{a}	2.10^{b}	2.40^{b}	0.14
HDL (mmol/L)	2.86^{b}	2.70^{b}	2.00^{b}	1.36^{a}	1.00^{a}	0.36
GGT (IU/L)	6.86^{b}	6.56^{b}	5.86^{a}	5.50^{a}	5.40^{a}	1.23
ALT (IU/L)	7.00^{b}	7.50^{b}	5.50^{a}	5.00^{a}	4.50^{a}	1.40
AST (IU/L)	16.00^{c}	14.00^{b}	12.00^{b}	11.50^{b}	9.50^{a}	2.49

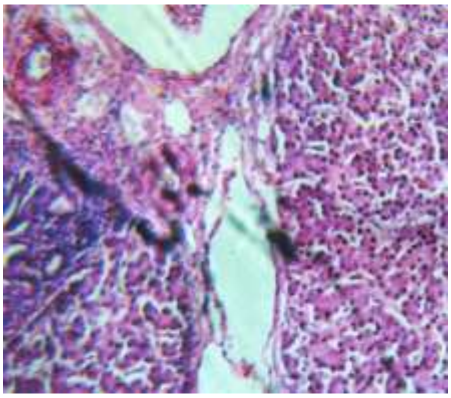
a, b, c means having different superscripts along the same row are significantly different ($p< 0.05$).

These findings agreed with past works (Acar et al, 2001; Xie et al., 2007) who observed depression in performance indices by feeding excessive supplemental amino acids beyond the requirement in diets. Hardwick et al. (1970) and Dibner et al. (2004) also reported that excessive dietary methionine was toxic to poultry and caused depression in body weight gain as well as reduced biochemical parameters, hematocrit and hemoglobin. Survival rate was nonetheless 100% in this study even though earlier reports (Katz and Baker, 1975; Han and Baker, 1993) confirmed that excessive feeding of individual amino acids could be toxic and might even cause increase mortality. The absence of mortality in this experiment suggests that the levels of methionine that could elicit lethal effect have not been reached. Increment in supplemental dietary levels of DL-methionine caused a concomitant increase in amounts of thiobarbituric acid reactive substances, MDA and low density lipoprotein. Toborek et al. (1996) observed similar effects concerning TBARS following increase in dietary methionine and reported that long term feeding of methionine-enriched diets brought about increase in the amount of LDL in response to the increase in the amount of reactive oxygen species. Increase in the amount of LDL in response to increase in level of DL-methionine in diets corroborates the suggestion of Munday (1989) that thio-compounds, methionine inclusive may be involved in production of free radicals that aid lipid peroxidation processes which probably is responsible for elevation of LDL following increase in levels of methionine in diets.

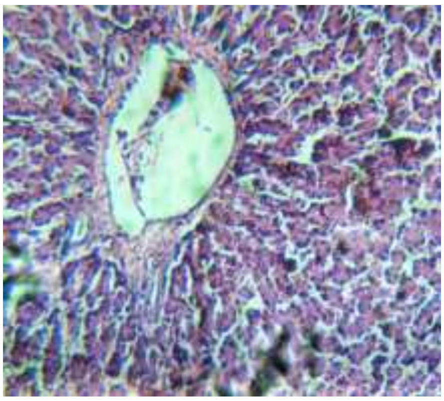
Increasing levels of supplemental methionine in diets led to a corresponding decrease in the activities of AST, GGT and ALT. Decrease in activities could be attributed to high methionine levels relative to the control since methionine-enriched diets have been shown to adversely influenced biochemical parameters including decrease in liver ATP and glycogen levels (Hardwick et al. 1970). Subsequent study (Finkestein and Bevenga, 1986) revealed that many methionine metabolites, S-adenosylmethionine, L-methionine-di-sulfoxide and methanethiol bind to cellular membranes and adversely affect or reduce enzyme activities.

Micrographs 1-5 present the histopathology of the liver samples of cockerels fed graded levels of methionine in diets. The 0.25% methionine diet had normal hepatic architecture similar to the reference diet while 0.35% inclusion of methionine showed mild adverse effects and highest deleterious effects were observed on 0.45 and 0.55% methionine. The adverse effects of high methionine diets are expected since excess dietary amino acids has been shown to release toxic ammonia through metabolism which temporarily builds up in the liver causing damage to the hepatic cells. The liver plays an important function in methionine metabolism making it vulnerable to the adverse effect of excessive methionine in diet. Especially for poultry, excess methionine is toxic and could significantly cause damage to body organs such as pancreatic acinar and induction of neurological changes (Katz and Baker, 1975).

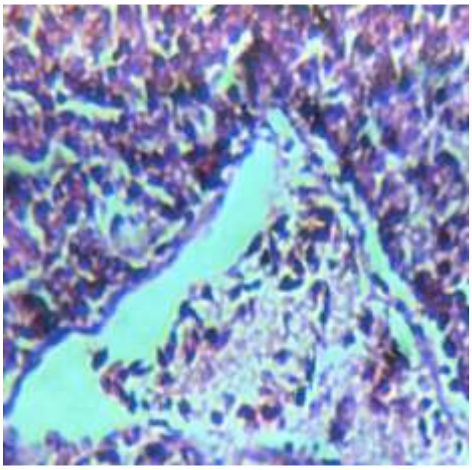
Micrographs showing liver of birds fed graded levels of methionine

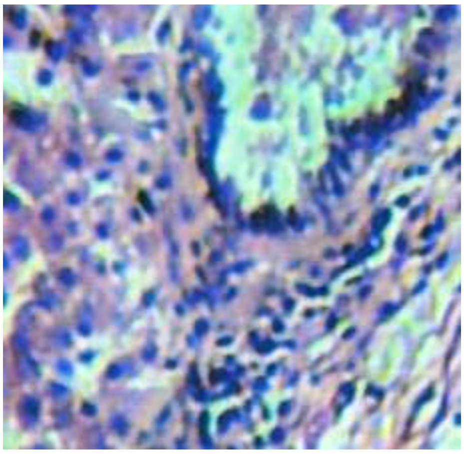


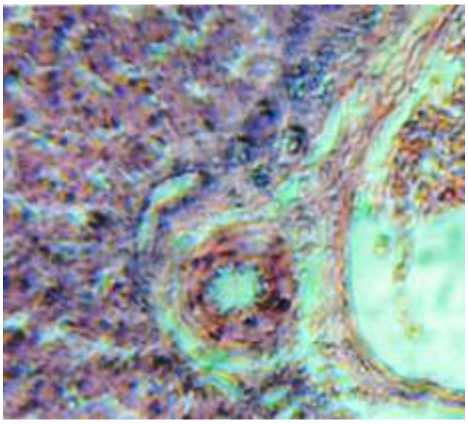
0.10 % Methionine (x400)



0.25 % methionine (x400)


0.35 % Methionine (x400)


0.45 % Methionine (x400)


0.55 % Methionine (x400)

CONCLUSION

Excess dietary supplementation of methionine negatively affects growth performance, decreases beneficial HDL cholesterol and transaminase enzymes, increases hazardous LDL particles and causes severe damages to the liver as observed in this study. Thus, it is recommended that poultry should not be fed dietary methionine above 0.25% in diet in a tropical environment like Nigeria.

REFERENCES

1. Abdollah A., Abolghasem G., Acar, N., Patterson, P.H and Barbato, G.F. (2001) Appetite suppressant activity of supplemental dietary amino acids and subsequent catch-up growth of broilers. Poult. Sci. 80: 1215-1222.
2. Atteh, J.O. (2004) Theory and Practice of Poultry Production. Adlek printers.
3. Benevenga, N.J and Steele, R.D. (1984) Adverse effects of excessive consumption of amino acids. Annual. Rev. Nutr., 4: 157-179.
4. Carew, L. B., Evarts, K. G and Alster. F. A. (1998) Growth, feed intake, and plasma thyroid hormone levels in chicks fed dietary excesses of essential amino acids. Poult. Sci. 77:295–298.
5. Dibner, J. J. (2003). Review of the metabolism of 2-hydroxy-4-(methylthio) butanoic acid. World's Poult. Sci. J. 59:99–110.
6. Finkelstein, A and Benevenga, N. J. (1986) The effect of methanethiol and methionine toxicity on the activities of cytochron C-oxidase and enzymes involved in protection from peroxidative damage. J. Nutr. 116, 204-209.
7. Han, Y., and Baker, D. H. (1993) Effects of excess methionine or lysine for broilers fed a corn-soybean meal diet. Poult. Sci. 72:1070–1074.
8. Hardwick, D.F., Applegarth, D.A., Cockrofit, D.M. (1970) Pathogenesis of methionine-induced toxicity. Metabolism, 19:381-390
9. Htin, N.N., Zulkifli, I., Alimon, A.R., Loh, T.C and Hair-Bejo, M. (2007) Effect of sources of dietary fat on broiler chickens exposed to transient high temperature. Arch.Geflugelk. 71 (2):74-80
10. Kalbande, V.H., Ravikanth, K., Maini, S and Rekhe, D.S. (2009) Methionine Supplementation Options in Poultry International Journal of Poult Sci. 8 (6): 588-591.
11. Katz, R. S., and D. H. Baker. (1975) Methionine toxicity in the chick: Nutritional and metabolic

implications. J. Nutr. 105:1168–1175.
12. Mezes, M., Virag, G.Y., Barta, M and Abouzed, A.D (1996) Effect of lipid peroxide loading in lipid peroxidation and on the glutathione and cytochrome systems in rabbits. Acta Vet. Hung; 44: 443-450.
13. Munday, R. (1989) Toxicity of thiols and disulphides: involvement of free radical species. Free radical Bio. Med. 7: 659-664
14. NRC (1994) Nutrient requirements of poultry, 9th revised Edition, National Academy Press Washington, D.C.
15. Sigma Diagnostic (1985) Transaminase ALP/GOT procedure No 505
16. Toborek, M; Kopleczna-Grazebieniak, C; Drozoz, M; Wicczorck, M. (1996). Increased lipid peroxidation as a mechanism of methionine-induced atherosclerosis in rabbits. Atherosclerosis, 115: 217-224.
17. Xie, M., Hou, S. S., Huang, W and Fan, H.P. (2007) Effect of Excess Methionine and Methionine
18. Hydroxy Analogue on Growth Performance and Plasma Homocysteine of Growing Pekin Ducks: Poult Sci. 86:1995–1999
19. Xie, M., Hou, S. S., Huang, W., Zhao, L., Yu, J. Y., Li, W. Y and Wu, Y. Y. (2004) Interrelationship between methionine and cystine of early Peking ducklings. Poult. Sci. 83:1703–1708.

