

**HEPATOPROTECTIVE, GROWTH PROMOTING AND HISTOPATHOLOGICAL
EFFECT OF NATURAL BETAININE IN BROILERS EXPERIMENTALLY INFECTED
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Article Received on 06/06/2019

Article Revised on 27/06/2019

Article Accepted on 17/07/2019

ABSTRACT

The objective of the present study was to evaluate the biochemical effect of betaine administration on bird performance after an experimental coccidial infection in broilers. Two hundred and fifty one day old Cobb broiler chicks were divided into 5 groups with different supplementations. Three groups are infected with 30000 *E. tenella* sporulated oocysts/chick. Blood samples were collected twice at age of 20 and 30 days of chicks for liver function evaluation then birds were sacrificed and livers were collected for histopathology. Results revealed that coccidiosis caused a significant increase in serum (AST, total cholesterol, triacylglycerols, VLDL, cortisol & CRP) and a significant decrease in serum TP, Albumin & LDL. Supplementation of betaine to infected group exhibited a significant decrease in serum level of (AST, ALP, total cholesterol, triacylglycerol, VLDL, cortisol & CRP) and a significant increase in serum TP, albumin and LDL activity after 3 days of the 1st and 2nd treatment doses when compared with untreated-infected group. In conclusion, Administration of betaine with coccidiosis caused significant improvement of all biochemical parameters, betaine has a protective effect against coccidiosis induced liver damage and oxidative stress in chicken so lead to increased body weight.

KEYWORDS: Natural betaine, Coccidia, Hepatoprotective, Growth Promoting, histopathological & *E. tenella*.**1. INTRODUCTION**

Chicken coccidiosis is considered a major parasitic problem in poultry farms, caused by intracellular protozoa of the family Eimeridae and causes severe economic losses. Seven distinct *Eimeria* species can infect chickens, develop and multiply in the intestinal epithelium causing extensive damage with destruction of intestinal villi leading to hemorrhage and death (Chapman, 2014), reducing the absorption and thus leading to weight loss, diarrhea, poor FCR and higher mortality of the affected flocks (McDougald and Reid 1997). Coccidiosis remains one of the most expensive and common diseases of poultry production in spite of advances in chemotherapy, management, nutrition, and genetics. It costs chickens producers worldwide at least 3 billion \$US annually (Dalloul and Lillehoj, 2006). Betaine is a powerful osmoprotectant synthesized from choline by choline oxidase in the liver. It plays a vital role in the integrity of cell membrane (Igwe *et al.*, 2015). Dietary supplementation of betaine to poultry diets improved weight gain and feed

efficiency by approximately 3 to 15% (Hassan *et al.*, 2005). Also, Betaine showed improvement in lipid metabolism and production performance of broilers. In intestinal cells subjected to osmotic disorder and dehydration; betaine is taken up and may have a stabilizing function to the cell membrane (Zeisel *et al.*, 2003). Osmotic-regulator role of betaine occurs at the cellular level where betaine acts as osmolyte and minimizes the loss of water from the cell; which can be of special importance in conditions of stress and dehydration caused by diseases or high temperature (Gudev *et al.*, 2011). Betaine acts as osmolyte able to help maintain cell volume (Craig, 2004). Betaine is found in many foods and it can be manufactured in the mitochondria. Betaine is extracted from sugar beet molasses or vinasses (Messadek, 2010). The aim of the present study was to evaluate the biochemical effect of betaine administration on bird performance after an experimental coccidiosis in broilers.

2. MATERIALS AND METHODS

2.1. Birds

Two hundred and fifty one day old Cobb chicks were obtained from a commercial broiler hatchery (El-Nile Company for poultry and rations). The birds were fed on balanced ration and water ad libitum and reared under hygienic condition. Ration was free from any anticoccidial drugs.

2.2. Eimeria strain

A field strain of sporulated *Eimeria tenella* oocysts were maintained at the Department of Parasitology, Faculty of Veterinary Medicine, and University of Sadat City by passages in chickens.

2.3. Betaine

Atcobeet® (40% natural betaine) oral solution was obtained from ATCO Pharma for pharmaceutical industries under license of Agrana-Austria for drinking water at dose of (ml/ L of drinking water).

2.4. Amprolium (20%)

Amprolium was obtained from Egyptian company for chemicals and pharmaceuticals (ADWIA), 10th of Ramadan city, Egypt in the form of 20% soluble powder and was used at dose (30g/50L) drinking water) for 5-7 days.

2.5. Experimental design

The chicks were allocated into 5 groups (50 birds for each group) as follows:- Group (1) was non-infected control negative group, Group (2) non infected and Betaine treated, Group (3) positive control group, Group (4) was infected and Amprolium treated and Group (5) was infected and Betaine –Amprolium treated group. At 2 weeks, groups 3, 4 and 5 were infected with 30.000 sporulated *Eimeria tenella* oocysts/birds orally. The treatment with betaine was started from the 17 days of age for three days then was repeated at the 27days of age for three days.

2.6. Examination of the birds

All groups were observed throughout the experiment to record the clinical symptoms and mortality rates%. Chicks in all groups were weighted every 10 days to determine the effect of betaine on body weight gain. Body weight gain was expressed in grams.

2.7. Serum collection

Blood samples were collected from all groups twice at 20 and 30 days of age in a tube without anticoagulant for serum collection. Serum was collected after centrifugation at 3000 r.p.m for 15 minute and stored at -20°C until use.

2.8. Estimation of serum biochemical parameters

Liver function tests were determined by a colorimetric methods as described using commercial kit as Aspartate amino-tranferase (Murray, 1984), Albumin (Doumas, 1971), Alkaline phosphates (Belfield and Goldberg,

1971) and Total protein (Burtis *et al.*, 1999).

Lipid profile test was determined calorimetrically that total cholesterol and triacylglycerols were determined according to (Schettler and Nussel, 1975), HDL-cholesterol (Gordon, 1977), LDL-cholesterol (Friedewald *et al.*, 1972) and VLDL- cholesterol (Bauer, 1982). Serum CRP concentration was determined according to the method described by (Burtis *et al.*, 1999).

2.9. Histopathology

Birds were sacrificed. Livers were rapidly removed, rinsed with isotonic saline, collected in 10% neutral buffered formalin and submitted to a pathology laboratory for histopathological examination. Formalin fixed samples were sectioned at 5 mm thickness and stained with Hematoxylin and Eosin (Bancroft and Gamble 2002).

2.10. Statistical analysis

The results were expressed as mean \pm SE using SPSS (13.0 software, 2009) program. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity. Values were considered statistically significant when $p < 0.05$.

3. RESULTS

3.1. Body weight

Table (1) revealed significant differences in mean of chick's weights between groups as well as between the control group and treatment groups throughout the experiment with P value < 0.05 .

The highest total body weight after 30 days was recorded in group 2 of (1.285 Kg) followed by group 1 of (1.250 Kg) and group 4 of (1.225 Kg), while *Eimeria* infected-Betaine-Amprolium recorded a significant lower total body weight in comparison with other groups (1.100 Kg).

3.2. Hepatoprotective effect of betaine on liver function of broilers experimentally infected with *E. tenella*.

The obtained data in tables (2 & 3) showed the effect of betaine on liver functions (AST, ALP,) and serum (TP, albumin, total cholesterol, triacylglycerols, VLDL, LDL, cortisol & CRP) in broiler chicks infected with *E. tenella*.

Supplementation of betaine to *E. tenella* infected group exhibited a significant decrease in serum level of (AST, ALP, total cholesterol, triacylglycerol, VLDL, cortisol & CRP) and a significant increase in serum TP, albumin and LDL activity after 3 days of the 1st and 2nd treatment doses when compared with untreated-infected group. The recorded results showed significant decrease in serum

liver enzymes activities. Also dosing of betaine with coccidiosis caused significant improvement of all previous parameters towards its normal ranges. These results suggested that betaine have a protective effect against coccidiosis induced liver damage and oxidative stress in chickens.

The obtained data presented in tables (2) and (3) revealed that coccidia infection caused a significant increase in serum AST, ALP, Cholesterol, TAG, VLDL, LDL, CRP and Cortisol activity but a significant decrease in serum TP, Alb and HDL compared with control normal group. Supplementation of betaine and/or amprolium to coccidian-infected group exhibited a significant decrease in AST, ALP, Cholesterol, TAG, VLDL, LDL, CRP, and Cortisol after 3rd day of the 1st and 2nd treatment doses but a significant increase in serum TP, Albumin, and HDL.

3.3. Histopathological protective action of betaine in intestinal cell of broilers experimentally infected with *E. tenella*.

Histological section of the negative control group showed normal structure of the liver with central veins and hepatic cords (Fig.1). Liver of positive control group showed congestion of hepatic vein and hyperplasia of bile duct (Fig.2). Also focal area of lymphocytic aggregations within hepatic parenchyma could be seen (Fig.3). Liver of both betaine–amprolium treated group showed normal appearance of hepatocytes with absence of lymphocytic aggregations (Fig.4 and 5). Mild congestion of central vein could be seen in betaine treated group (Fig.4) while severe congestion was seen in amprolium treated group (Fig.5).

Table (1): Growth promoting and body weight performance of betaine in broilers experimentally infected with *E. tenella*, (n=100).

Animal Groups	Body weight (g/chicken)			
	Zero day	After 10 days	After 20 days	After 30 days
1 st Group	197.50 ± 9.29 ^b	580.25 ± 29.06 ^b	860.75 ± 33.33 ^b	1250.45 ± 14.91 ^{ab}
2 nd group	185.45 ± 7.64 ^b	655.65 ± 22.91 ^a	875.35 ± 20.07 ^b	1285.20 ± 16.99 ^a
3 rd group	207.35 ± 8.97 ^b	560.85 ± 16.32 ^b	815.70 ± 21.42 ^c	1100.60 ± 14.43 ^d
4 th group	190.65 ± 6.66 ^b	680.30 ± 13.33 ^a	920.25 ± 11.05 ^a	1225.40 ± 22.42 ^c
5 th group	222.85 ± 5.28 ^a	685.45 ± 16.75 ^a	895.65 ± 16.33 ^b	1215.10 ± 10.67 ^b

☞ Mean values with different superscript letters in the same column are significantly different at (P<0.05).

Table (2): The Hepatoprotective effects of betaine on some blood parameters after 3 days of 1st Durations of treatment in broilers experimentally infected with *E. tenella*.

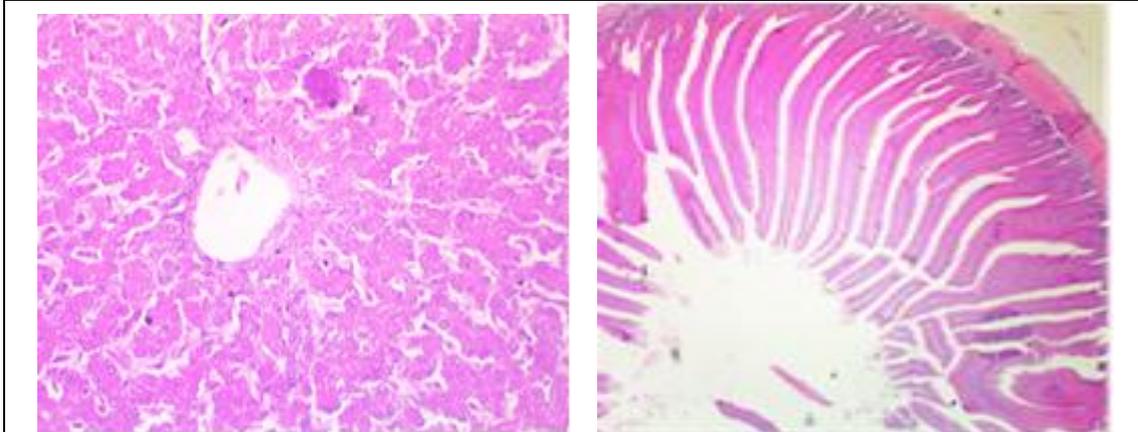
Parameter	1 st Group	2 nd Group	3 rd Group	4 th Group	5 th Group
AST (U/l)	86.45 ± 3.54 ^d	93.33 ± 2.09 ^d	307.10 ± 7.97 ^a	195.81 ± 3.30 ^b	152.25 ± 3.50 ^c
ALP (U/l)	26.33 ± 2.72 ^c	32.66 ± 2.90 ^c	110.80 ± 0.62 ^a	62.43 ± 2.29 ^b	75.94 ± 2.65 ^b
Total protein (g./dl)	7.32 ± 0.29 ^a	7.78 ± 0.22 ^a	5.96 ± 0.26 ^c	6.34 ± 0.23 ^b	6.50 ± 0.31 ^b
Albumin (g./dl)	3.61 ± 0.15 ^a	3.74 ± 0.15 ^a	2.39 ± 0.09 ^c	3.06 ± 0.19 ^b	2.99 ± 0.06 ^c
Cholesterol (mg/dl)	61.74 ± 2.90 ^d	52.67 ± 2.37 ^d	150.06 ± 3.07 ^a	93.74 ± 4.13 ^c	105.66 ± 3.25 ^b
Triacylglycerols (mg/dl)	58.85 ± 1.29 ^c	62.04 ± 1.69 ^c	183.11 ± 2.99 ^a	104.66 ± 1.38 ^b	112.33 ± 1.05 ^b
VLDL (mg/dl)	11.76 ± 0.86 ^c	12.60 ± 0.59 ^c	36.62 ± 1.03 ^a	21.93 ± 0.65 ^b	25.06 ± 0.68 ^b
LDL (mg/dl)	21.10 ± 1.77 ^c	26.71 ± 1.02 ^c	123.04 ± 1.83 ^a	73.59 ± 1.25 ^b	80.29 ± 1.40 ^b
HDL (mg/dl)	23.26 ± 1.31 ^a	20.94 ± 1.56 ^a	8.40 ± 0.68 ^c	18.22 ± 1.13 ^b	17.47 ± 1.40 ^b
Cortisol (mIU/dl)	7.26 ± 0.48 ^c	6.49 ± 0.50 ^c	57.15 ± 1.17 ^a	28.96 ± 2.09 ^b	31.55 ± 0.84 ^b
CRP (mg/dl)S	4.18 ± 0.36 ^c	3.59 ± 0.49 ^c	50.10 ± 2.69 ^a	20.47 ± 1.52 ^b	25.74 ± 1.42 ^b

Table (3): The Hepatoprotective effects of betaine on some blood parameters after 3 days of 2nd durations of treatment in broilers experimentally infected with *E. tenella*.

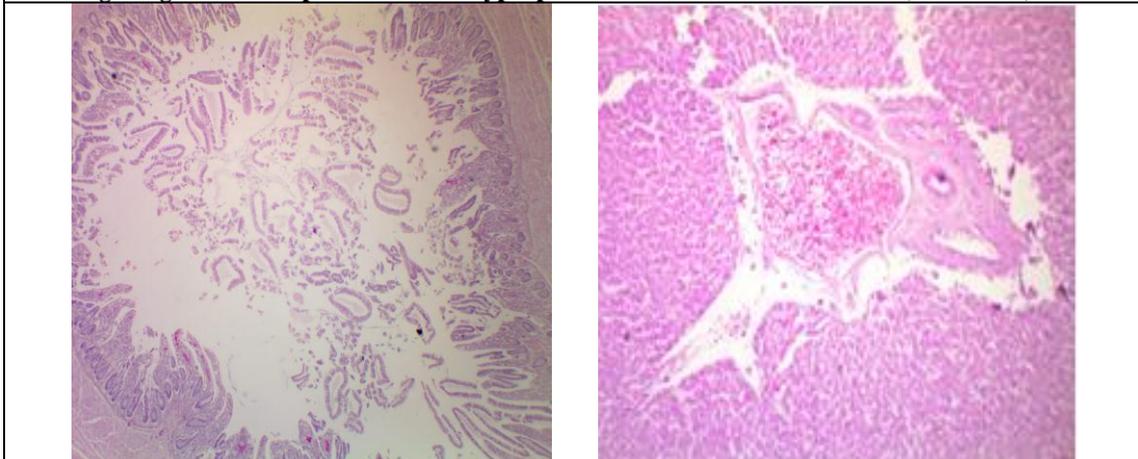
Parameter	1 st Group	2 nd Group	3 rd Group	4 th Group	5 th Group
AST (U/l)	91.13 ± 2.87 ^d	98.10 ± 1.62 ^d	349.47 ± 7.24 ^a	173.35 ± 2.78 ^c	127.18 ± 2.08 ^c
ALP (U/l)	28.13 ± 2.44 ^c	29.03 ± 1.38 ^c	124.13 ± 0.62 ^a	56.59 ± 2.18 ^b	80.08 ± 2.86 ^b
Total protein (g./dl)	7.67 ± 0.13 ^a	8.08 ± 0.57 ^a	4.26 ± 0.26 ^c	6.12 ± 0.34 ^b	6.32 ± 0.21 ^b
Albumin (g./dl)	3.69 ± 0.27 ^a	3.81 ± 0.15 ^a	2.06 ± 0.08 ^c	3.23 ± 0.07 ^b	3.18 ± 0.09 ^c
Cholesterol (mg/dl)	69.07 ± 2.44 ^d	58.63 ± 2.65 ^d	163.14 ± 3.07 ^a	118.29 ± 3.40 ^b	112.56 ± 3.09 ^c
Triacylglycerols (mg/dl)	64.16 ± 1.39 ^c	69.66 ± 1.12 ^c	201.11 ± 3.08 ^a	116.96 ± 1.40 ^b	96.22 ± 1.28 ^b
VLDL (mg/dl)	12.99 ± 0.77 ^c	13.93 ± 0.69 ^c	42.62 ± 1.12 ^a	24.39 ± 0.87 ^b	20.24 ± 0.65 ^b

LDL (mg/dl)	23.10 ± 1.33 ^c	24.66 ± 1.08 ^c	141.54 ± 1.97 ^a	61.56 ± 1.34 ^b	68.35 ± 1.27 ^b
HDL (mg/dl)	21.88 ± 1.84 ^a	19.03 ± 1.53 ^a	7.38 ± 0.55 ^c	16.55 ± 1.71 ^b	15.22 ± 1.46 ^b
Cortisol (mIU/dl)	7.91 ± 0.75 ^c	7.47 ± 0.54 ^c	66.24 ± 1.73 ^a	20.26 ± 1.19 ^b	22.21 ± 1.92 ^b
CRP (mg/dl)	4.75 ± 0.54 ^c	3.94 ± 0.24 ^c	63.27 ± 2.81 ^a	24.53 ± 1.25 ^b	31.40 ± 1.90 ^b

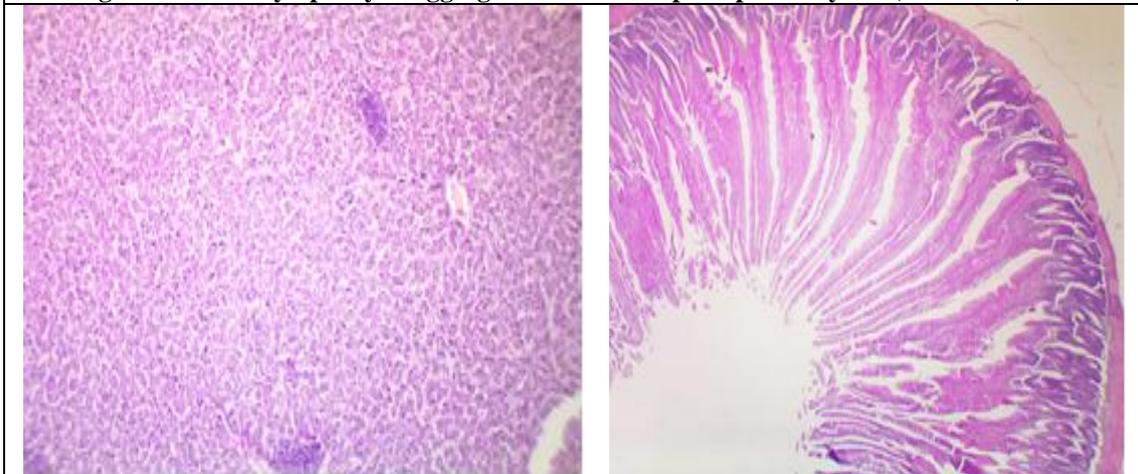
- **Fig.1: Histological section of chicken duodenum from negative control group (1st group) showing normal hepatic architecture. Notice normal central vein and hepatic cords. (H&E X40).**



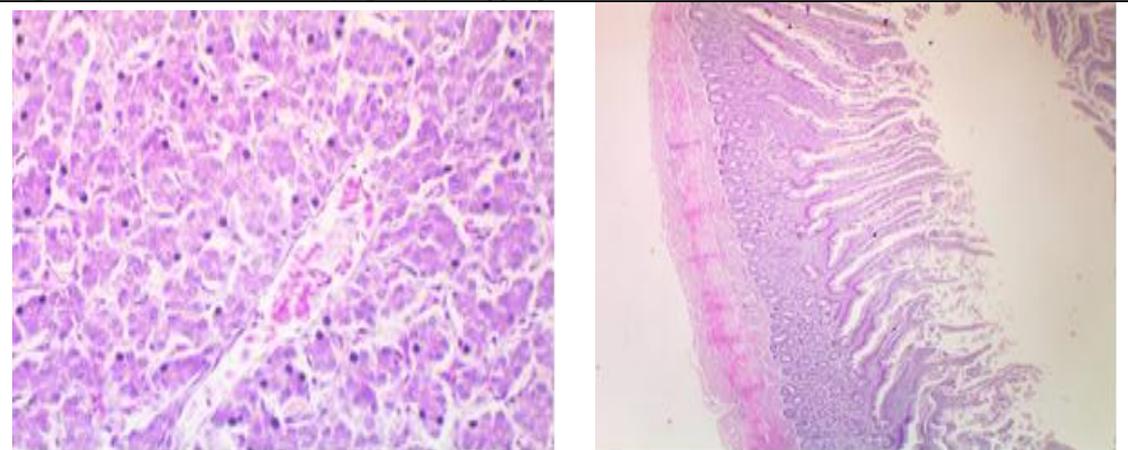
- **Fig.2 Histological section of chicken liver from coccidia Infected- untreated (3rd group) showing congestion of hepatic vein and hyperplasia of bile duct. H & E stain. (H&E X10).**



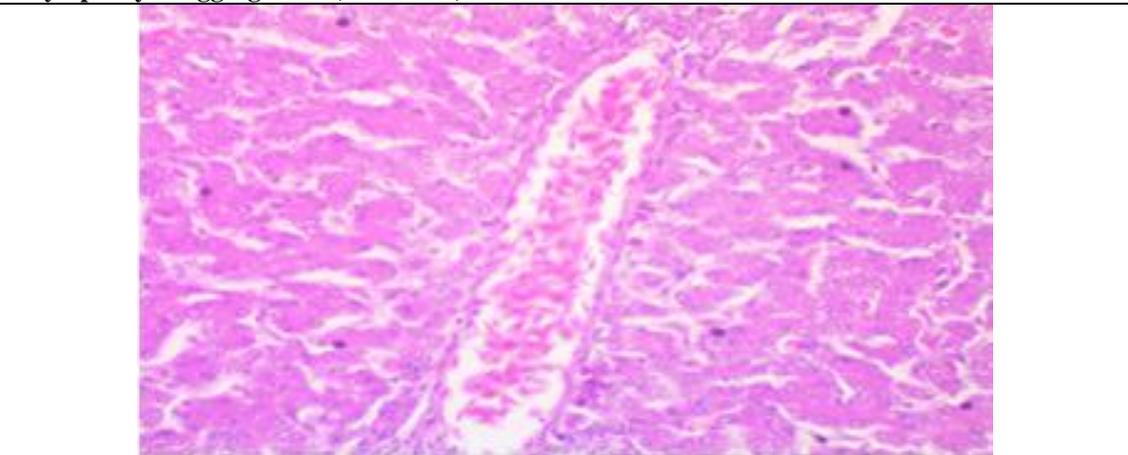
- **Fig.3 Histological section of chicken liver from coccidia Infected- untreated (3rd group) showing focal area of lymphocytic aggregations within hepatic parenchyma. (H&E X10).**



- **Fig.4** Histological section of chicken liver from 4th group (Coccidia infected-Betaine-Amprolium treated) group showing mild congestion of central vein. Notice normal appearance of hepatocytes and absence of lymphocytic aggregation. (X40).



- **Fig. 5:** Histological section of chicken liver from 5th group (Coccidia-Amprolium treated) showing severe congestion of central vein. Notice normal appearance of hepatocytes and absence of lymphocytic aggregation. (H&E X40).



4. DISCUSSION

The present study recorded that Coccidiosis in broiler reported a significant increase in serum AST, ALP, Cholesterol, TAG, VLDL, LDL, CRP and cortisol activity all over the periods of the experiment when compared with normal control group; that cortisol can be used as a biomarker of stress. Treatment with betaine to *E. tenella* infected group exhibited a significant decrease in AST, ALP, Cholesterol, TAG, VLDL, LDL, CRP and cortisol after 3 days of the 1st and 2nd treatment, but a significant increase in serum TP, Alb and HDL. These results are nearly similar to those reported by (Olthof *et al.*, 2005b) who demonstrated that high doses of betaine increased plasma LDL cholesterol which might offset the health benefit from homocysteine reduction, but the significance of the lipid effects has been questioned (Zeisel 2006). Supplemental dietary betaine improved weight gain and feed conversion in some poultry studies (Hassan *et al.*, 2005) and also the regulation of the lipid metabolism in laying hen (Zou *et al.*, 2002). Whereas, other studies showed minimal or no effect of betaine on animal performance (Feng *et al.*,

2006). These indications were partly confirmed by (Klasing *et al.* 2002) who reported improved intestinal cell functions in coccidiosis challenged broiler chickens. This was indicated by increased betaine levels in intestinal epithelium, a less severe shortening of duodenal villa and in more leukocytes in the epithelium and in the lamina propria; hypothesized that the latter could be associated with a more effective clearance of sporozoites. Intracellular betaine serves as an osmolyte that regulates cell volume and thereby tissue integrity (Lang., 2007). Betaine stimulates cell proliferation in the intestinal tissue so; the enlarged gut wall epithelium would provide an increased surface for nutrient absorption (Eklund *et al.*, 2005). In addition, Betaine has osmoprotective properties that aid in protecting intestinal cells, thus counteracting performance losses in coccidiosis, so betaine has a diverse range of beneficial effects on cellular metabolism (Ratriyanto *et al.*, 2009).

5. CONCLUSION

The obtained results showed that betaine impact beneficially at several critical points in the progression of

E. tenella induced tissue damage. These include amelioration of damage to gut and liver tissue also significantly decrease serum liver enzymes activities. These results suggested that betaine have a protective effect against coccidiosis induced liver damage and oxidative stress in chicken. Betaine increased body weight and improved feed conversion ratio.

6. REFERENCES

1. Bancroft JD, Gamble M. Theory and practice of Histological Techniques, 5th ed. Churchill Livingstone, London, New York and Philadelphia, 2002.
2. Bauer JD. "Clinical laboratory methods" 9th Ed, the C.V. Company Waistline Industrial Missouri 63116 Chapter, 1982; 33: 555.
3. Belfield A, Goldberg DM. Revised assay for serum phenyl phosphatase activity using 4 aminoantipyrine. *Enzyme*, 1971; 12: 561-573.
4. Burtis A, Carl A, Edward R, Ashwood MD. Tietz Textbook of Clinical Chemistry 3rd Edition, 1999.
5. Chapman HD. Milestones in avian coccidiosis research: A review. *Poultry Science*, 2014; 93(3): 501-511.
6. Craig, S.A. 2004. Betaine in human nutrition. *Am J Clin Nutr*. 80(3): 539-549.
7. Dumas B. Colorimetric determination of albumin. *Clinical Chemical Acta*, 1971.
8. Dalloul RA, Lillehoj HS. Poultry coccidiosis: recent advancements in control measures and vaccine development. *Expert Rev. Vaccines*, 2006; 5: 143-63.
9. Eklund M, Bauer E, Wamatu J, Mosenthin R. Potential nutritional and physiological functions of Betaine in livestock. *Nut. Res. Rev.*, 2005; 18: 31-48.
10. Friedewald W, Levy R, Fredrickson D. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*, 1972; 18: 499-502.
11. Feng J, Liu X, Wang YZ, Xu ZR. Effects of betaine on performance, carcass characteristics and hepatic betainehomocysteine methyltransferase activity in finishing barrows. *Asian-Aust. J. Anim. Sci.*, 2006; 19: 402-405.
12. Gordon T. Colorimetric determination of serum HDL cholesterol. *Amer. J. Med.*, 1977; 62: 707.
13. Gudev D, Popova-Ralcheva S, Ianchev I, Moneva P. Effect of betaine and air ammonia concentration on broiler performance, plasma corticosterone level, lymphoid organ weights and some haematological indices. *Biotechnology in Animal Husbandry*, 2011; 27: 687-703. DOI: 10.2298/BAH1103687G.
14. Igwe IR, Okonkwo CJ, Uzoukwu UG, Onyenegecha CO. The effect of choline chloride on the performance of broiler chickens. *Annu Res Rev Biol.*, 2015; 8(3): 1-8.
15. Klasing K, Adler K, Remus J, Calvert C. Dietary betaine increases intraepithelial lymphocytes in the duodenum of coccidia infected chicks and increases functional properties of phagocytes. *J. Nutr.*, 2002; 132: 2274-2282.
16. Hassan RA, Attia YA, El-Ganzory EH. Growth, carcass quality and serum constituents of slow growing chicks as affected by betaine addition to diets containing different levels of choline. *Int. J. Poultr. Sci.*, 2005; 4: 840-850.
17. Lang F. Mechanisms and significance of cell volume regulation. *J Am Coll Nutr*, 2007; 26: 613S-623. [SPubMedGoogle Scholar](http://pubmed.ncbi.nlm.nih.gov/).
18. McDougald LR, Reid WM. Coccidiosis. In: Calnek BW, Barnes HJ, Beard, CW, McDougald LR., Saif MY, editors. *Diseases of poultry*. Iowa State University Press, Ames, IA, 1997; 865-883.
19. Messadek, J. Modulation of nitric oxide synthesis by betaines. US Patent Application. <http://www.freepatentsonline.com/y2010/0305206.html>, 2010.
20. Murray. Report on the symposium "drug effect in clinical chemistry methods for Emtimation of AST. *Eur J Clin Chem Clin Biochem.*, 1984; 34: 1112-1116.
21. Olthof MR, van Vliet T, Verhoef P, Zock PL, Katan MB. Effect of homocysteine-lowering nutrients on blood lipids: results from four randomised, placebo-controlled studies in healthy humans. *PLoS Med.*, 2005; 2: 446-456.
22. Ratriyanto A, Mosenthin R, Bauer E, Eklund M. Metabolic osmoregulatory and nutritional functions of betaine in monogastric animals. *Asian-Aust. Anim. Sci. J.*, 2009; 22(10): 1461-1476.
23. Schettler, G, Nüssel E. Colorimetric determination of Triglycerides and cholesterol. *Arb. Med. Soz. Med. Präv. Med.*, 1975; 10: 25.
24. Zeisel, SH. Betaine supplementation and blood lipids: fact or artifact? *Nutr. Rev.*, 2006; 64(2): 77-79.
25. Zeisel, SH, Mar MH, Howe JC, Holden JM. Concentrations of choline containing compounds and betaine in common foods. *J. Nutr.*, 2003; 133: 1302-1307.
26. Zou, XT, Lu JJ. Effect of betaine on the regulation of the lipid metabolism in laying hen. *Agricultural science in China*, 2002; 1: 1043 - 1049.