HEPATOPROTECTIVE, GROWTH PROMOTING AND HISTOPATHOLOGICAL EFFECT OF NATURAL BETAINE IN BROILERS EXPERIMENTALLY INFECTED WITH E. TENELLA.

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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 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 (McDougall 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 (Daloull and Lillehoj, 2006). Betaine is a powerful osmotic stress protectant 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 maintains the osmotic equilibrium; stabilization to cellular function 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-transcerease (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 ± 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 ampirolium 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–ampilorium 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 ampirolium treated group (Fig.5).

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

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Body weight (g/chicken)</th>
<th>Zero day</th>
<th>After 10 days</th>
<th>After 20 days</th>
<th>After 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Group</td>
<td></td>
<td>197.50 ± 9.29b</td>
<td>580.25 ± 29.06b</td>
<td>860.75 ± 38.33b</td>
<td>1250.45 ± 14.91ab</td>
</tr>
<tr>
<td>2nd group</td>
<td></td>
<td>185.45 ± 7.64b</td>
<td>655.65 ± 22.91a</td>
<td>875.35 ± 20.07b</td>
<td>1285.20 ± 16.99a</td>
</tr>
<tr>
<td>3rd group</td>
<td></td>
<td>207.35 ± 8.97a</td>
<td>560.85 ± 16.32a</td>
<td>815.70 ± 21.42a</td>
<td>1100.60 ± 14.43a</td>
</tr>
<tr>
<td>4th group</td>
<td></td>
<td>190.65 ± 6.66b</td>
<td>680.30 ± 13.33a</td>
<td>920.25 ± 11.05a</td>
<td>1225.40 ± 22.42</td>
</tr>
<tr>
<td>5th group</td>
<td></td>
<td>222.85 ± 5.28a</td>
<td>685.45 ± 16.75a</td>
<td>895.65 ± 16.33a</td>
<td>1215.10 ± 10.67</td>
</tr>
</tbody>
</table>

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*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st Group</th>
<th>2nd Group</th>
<th>3rd Group</th>
<th>4th Group</th>
<th>5th Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/l)</td>
<td>86.45 ± 3.54a</td>
<td>93.33 ± 2.09a</td>
<td>307.10 ± 7.97a</td>
<td>195.81 ± 3.30a</td>
<td>152.25 ± 3.50a</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>26.33 ± 2.72a</td>
<td>32.66 ± 2.90a</td>
<td>110.80 ± 0.62a</td>
<td>62.43 ± 2.29a</td>
<td>75.94 ± 2.65a</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>7.32 ± 0.29a</td>
<td>7.78 ± 0.22a</td>
<td>5.96 ± 0.26a</td>
<td>6.34 ± 0.23a</td>
<td>6.50 ± 0.31a</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.61 ± 0.15a</td>
<td>3.74 ± 0.15a</td>
<td>2.39 ± 0.09a</td>
<td>3.06 ± 0.19a</td>
<td>2.99 ± 0.06a</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>61.74 ± 2.90a</td>
<td>52.67 ± 2.37a</td>
<td>150.06 ± 3.07a</td>
<td>93.74 ± 4.13a</td>
<td>105.66 ± 3.25a</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dl)</td>
<td>58.85 ± 1.29a</td>
<td>62.04 ± 1.69a</td>
<td>183.11 ± 2.99a</td>
<td>104.66 ± 1.38a</td>
<td>112.33 ± 1.05a</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>11.76 ± 0.86a</td>
<td>12.60 ± 0.59a</td>
<td>36.62 ± 1.03a</td>
<td>21.93 ± 0.65a</td>
<td>25.06 ± 0.68a</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>21.10 ± 1.77a</td>
<td>26.71 ± 1.02a</td>
<td>123.04 ± 1.83a</td>
<td>73.59 ± 1.25a</td>
<td>80.29 ± 1.40a</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>42.36 ± 1.31a</td>
<td>20.94 ± 1.56a</td>
<td>8.40 ± 0.68a</td>
<td>18.22 ± 1.13a</td>
<td>17.47 ± 1.40a</td>
</tr>
<tr>
<td>Cortisol (mIU/dl)</td>
<td>7.26 ± 0.48a</td>
<td>6.49 ± 0.50a</td>
<td>57.15 ± 1.17a</td>
<td>28.96 ± 2.09a</td>
<td>31.55 ± 0.84a</td>
</tr>
<tr>
<td>CRP (mg/dl/S)</td>
<td>4.18 ± 0.36a</td>
<td>3.59 ± 0.49a</td>
<td>50.10 ± 2.69a</td>
<td>20.47 ± 1.52a</td>
<td>25.74 ± 1.42a</td>
</tr>
</tbody>
</table>

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*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st Group</th>
<th>2nd Group</th>
<th>3rd Group</th>
<th>4th Group</th>
<th>5th Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/l)</td>
<td>91.13 ± 2.87a</td>
<td>98.10 ± 1.62a</td>
<td>349.47 ± 7.24a</td>
<td>173.35 ± 2.78a</td>
<td>127.18 ± 2.08a</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>28.13 ± 2.44a</td>
<td>29.03 ± 1.38a</td>
<td>124.13 ± 0.62a</td>
<td>56.59 ± 2.18a</td>
<td>80.08 ± 2.86a</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>7.67 ± 0.13a</td>
<td>8.08 ± 0.57a</td>
<td>4.26 ± 0.26a</td>
<td>6.12 ± 0.34a</td>
<td>6.32 ± 0.21a</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.69 ± 0.27a</td>
<td>3.81 ± 0.15a</td>
<td>2.06 ± 0.03a</td>
<td>3.23 ± 0.07a</td>
<td>3.18 ± 0.09a</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>69.07 ± 2.44a</td>
<td>58.63 ± 2.65a</td>
<td>163.14 ± 3.07a</td>
<td>118.29 ± 3.40a</td>
<td>112.56 ± 3.09a</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dl)</td>
<td>64.16 ± 1.39a</td>
<td>69.66 ± 1.12a</td>
<td>201.11 ± 3.08a</td>
<td>116.96 ± 1.40a</td>
<td>96.22 ± 1.28a</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>12.99 ± 0.77a</td>
<td>13.93 ± 0.69a</td>
<td>42.62 ± 1.12a</td>
<td>24.39 ± 0.87a</td>
<td>20.24 ± 0.65a</td>
</tr>
<tr>
<td>Parameter</td>
<td>Group 1 (mg/dl)</td>
<td>Group 2 (mg/dl)</td>
<td>Group 3 (mg/dl)</td>
<td>Group 4 (mg/dl)</td>
<td></td>
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<td>-----------------</td>
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</tr>
<tr>
<td>LDL</td>
<td>23.10 ± 1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.66 ± 1.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>141.54 ± 1.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.56 ± 1.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>21.88 ± 1.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.03 ± 1.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.38 ± 0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.55 ± 1.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>7.91 ± 0.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.47 ± 0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66.24 ± 1.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.26 ± 1.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>4.75 ± 0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.94 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.27 ± 2.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.53 ± 1.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

- **Fig.1**: Histological section of chicken duodenum from negative control group (1<sup>st</sup> 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 (3<sup>rd</sup> 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 (3<sup>rd</sup> group) showing focal area of lymphocytic aggregations within hepatic parenchyma. (H&E X10).
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


