EFFECT OF DIATOMACEOUS EARTH ON PERFORMANCE, INTERNAL ORGANS AND BIOCHEMICAL ALTERATIONS IN T-2 TOXICOSIS OF BROILER CHICKENS

SHIVASHANKAR, B. P.,1 NARAYANASWAMY, H. D.,1 SATYANARAYANA, M. L.,1 RAO, S.,1 RATHNAMMA, D.,2 MUNIYELLAPPA, H. K.3 AND SRIDHAR, N. B.4

1Department of Veterinary Pathology, 2Department of Veterinary Microbiology, 4Department of Veterinary Pharmacology and Toxicology, Veterinary College, Bangalore 560024. 3CU-ADMAS, Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore 560024; E-mail: shivashankarpatho@gmail.com

Received: April 15, 2015; Accepted: June 16, 2015

Abstract: The efficacy of Diatomaceous Earth (DE) was evaluated in broiler chickens by adding 400 and 800 mg/kg in the feed and T-2 toxin was fed at 0.5 ppm and 1 ppm in the diet for 35 days of age. The body weight, FCR, relative organ weights and serum biochemical alterations were studied. A total of three hundred and sixty newly hatched unsexed day-old healthy commercial broiler chicks were randomly divided into nine groups consisting of control and treatment groups. Feeding of T-2 toxin resulted in significantly (P<0.05) reduced body weight and higher feed conversion ratio at the end of the trial. T-2 toxin at 1 ppm showed significantly (P<0.05) increased relative weights of liver and kidney and decreased relative weights of thymus and bursa of Fabricius compared to control. Whereas, significantly (P<0.05) higher kidney weight and decreased weight of thymus was noticed at 0.5 ppm of T-2 toxin in the diet. The serum biochemical alterations in T-2 toxin fed groups included increased ALT, AST and creatinine values and decreased total protein, albumin, triglycerides and cholesterol levels. Birds with dietary supplementation of DE showed significantly (P<0.05) higher body weight and lower feed conversion ratio as compared to only T-2 toxin fed groups. However, DE supplemented birds did not differ significantly in relative organ weights of liver, kidney, thymus, spleen and bursa of Fabricius and serum biochemical values of ALT, AST, triglycerides, cholesterol and creatinine when compared to birds fed T-2 toxin alone. The study indicated that the addition of DE as an adsorbent in the diet was partially helpful in reducing the negative effects of T-2 toxin in broiler chickens.

Key words: T-2 toxin, Diatomaceous earth, Broiler Chicken

INTRODUCTION

Mycotoxins are naturally occurring fungal metabolites contaminating feeds and their ingredients which are used for livestock, poultry and human consumption. Majority of the common mycotoxins are known for their cytotoxic, genotoxic, immunotoxic, carcinogenic and teratogenic effects [1]. T-2 toxin is a member of trichotheccene group of mycotoxins produced mainly by Fusarium sporotrichioides and is widely prevalent in the environment contaminating livestock and poultry feed. Poultry feeds are prone to fungal growth resulting in elaboration of their metabolites during
different stages of the manufacturing process viz., production, processing, transport and storage [2].

The toxicity of T-2 toxin depends on various factors such as toxin dose, duration of exposure, age of birds and presence of other mycotoxins in the feed [3]. The toxic effects of T-2 toxin in poultry include inhibition of protein, DNA and RNA synthesis. Actively dividing cells (cells of the gastrointestinal tract, bone marrow, lymph nodes, spleen, and liver) are highly sensitive to T-2 toxin activity leading to alterations in relative organ weights and biochemical profile in poultry. Cytotoxicity results in lesions in feathers/skin and low performance in poultry production (decrease in weight gain, poor feed conversion, egg production, and hatchability) are main features of T-2 toxicity [4].

Decontamination of T-2 toxin from feeds and feedstuffs is a major problem in the poultry industry, as a large-scale, practical and cost effective method for detoxifying T-2 toxins is not still available. Among the current approaches, the use of non-nutritive adsorbing compounds such as Diatomites (Diatomaceous earth), Zeolites and Bentonite are preferred because of their binding capacity for T-2 toxin.

Diatomaceous earth (DE) is a naturally occurring siliceous sedimentary mineral formed from the microscopic skeletal remains of unicellular algae like plants called as diatoms. The typical composition of diatomaceous earth is 86% silica, 5% sodium, 3% magnesium and 2% iron. Diatomaceous earth with less than 7% composition of crystalline silica is generally recognized as a safe food additive in Canada and the United States [5]. Due to the high content of silicon dioxide, it has a large porosity and high adsorption capacity. Diatomaceous earth has shown the potential in vitro to bind aflatoxin, sterigmatocystin, T-2 toxin, zearalenone and ochratoxin [6]. Feeding of DE significantly improved feed conversion in broilers [7]. To our knowledge, there are no studies demonstrating protective role of diatomaceous earth in T-2 toxicosis of broilers in vivo. Therefore, the aim of the present study was to evaluate the effects of different concentrations of T-2 toxin in feed and efficacy of Diatomaceous Earth (DE) in alleviating the toxic effects of T-2 toxin in broiler chickens.

MATERIALS AND METHODS

The T-2 toxin was produced on whole wheat using *Fusarium sporotrichioides* MTCC 1894 [8] and quantified using thin layer chromatography (TLC) at animal feed analytical and quality assurance laboratory (AFAQAL), Veterinary College and Research Institute, Namakkal, Tamil Nadu, India.

Newly hatched three hundred and sixty unsexed day-old healthy broiler chicks were procured from a reputed commercial hatchery and reared in battery cage system with *ad libitum* supply of feed and water. Toxin free and toxin binder free starter and finisher broiler feed procured from Department of Poultry Science, Veterinary College, Hebbal, Bangalore, was used in the experiment. Diatomaceous earth was obtained from AGRIPOWER, Australia. All the chicks were vaccinated on days 7 and 14 of age using F1 strain of newcastle disease virus and infectious bursal disease (intermediate strain) respectively. The powdered wheat culture material containing known quantity of T-2 toxin was incorporated in the feed at the level of 0.5 ppm and 1 ppm and powdered diatomaceous earth binder was mixed in the feed to have final supplementation rate of 400 mg/kg and 800 mg/kg.

The experimental birds were randomly distributed into nine different groups containing 40 chicks each with following treatment diets for 35 days from the day of hatch.

The experimental trial was conducted as per the guidelines and approval obtained by the Institute Animal Ethical Committee (IAEC), Bangalore, India. At the end of 35 days, body weight and weekly feed consumption were recorded and feed conversion ratio was calculated. Six birds from each group were sacrificed and weights of liver, kidney, spleen, thymus and bursa were recorded and converted into relative organ weights (g %).

Samples of blood collected from six birds in each group on 35th day of trial were allowed to clot in test tubes and centrifuged at 1500 rpm for 20 minutes to separate the sera. The serum samples were analysed to estimate the concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins, albumin, triglycerides,
cholesterol and creatinine by using semi-automatic analyser (STATFAX 2000+, CPC Diagnostics Pvt. Ltd., India). The methodology and the reagents used in respect of each parameter were as per the recommendations of the manufacturer.

The results of the study were subjected to one-way analysis of variance (ANOVA) test. The means of different groups were compared by Duncan’s multiple range tests [9] using SPSS 16 software. The data were considered significant from one another at a P-value ≤ 0.05.

RESULTS

At the end of the experiment, birds in group I (control) and groups II and III (DE supplemented control) did not show significant difference in their body weight and feed conversion ratio (FCR). However, significantly (P<0.05) lowered body weight and higher FCR was recorded in birds fed 1 ppm of T-2 toxin (group V) and 0.5 ppm of T-2 toxin (group IV) as compared to control birds (group I) (Table 1). On the contrary, a significant increase in body weight and lowered FCR was seen in birds fed T-2 toxin and supplemented with diatomaceous earth in the diet (groups VI, VII, VIII and IX) as compared to only toxin fed groups IV and V.

The percent reduction in the body weight in birds fed with only T-2 toxin (Groups IV and V) at the end of the fifth week were 16.99 and 26.38 respectively as compared to untreated control (Group I). However, 9.72, 12.26, 12.23 and 16.01 per cent body weight gain was observed in birds supplemented with DE to toxin fed birds (Groups VI ,VII, VIII and IX respectively) at 35 days as compared to only T-2 toxin fed birds (groups IV and V).

Relative organ weights were evaluated as one of the parameters that could indicate changes in morphology and function of organs. The relative weights of various organs expressed as grams percent are presented in Table 1. Inclusion of 1ppm of T-2 toxin in the diet (group V) showed significant (P<0.05) increase in the relative weights of liver and kidney and decreased weight of thymus and bursa.

Table 1: Mean (±SE) Body Weight (g), Feed Conversion Ratio and Relative Organ Weights in broiler chicken fed with T-2 toxin, Diatomaceous earth (DE) and their combination in feed. Means in column with different superscripts differed significantly at (P<0.05)

<table>
<thead>
<tr>
<th>Group</th>
<th>T-2 Toxin (ppm)</th>
<th>DE (mg/kg)</th>
<th>Body Weight (g)</th>
<th>Feed Conversion Ratio</th>
<th>Relative Organ Weight (g%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>I</td>
<td>CONTROL</td>
<td></td>
<td>1356.17±19.55</td>
<td>1.84±0.04</td>
<td>2.84±0.14</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>400</td>
<td>1392.84±14.86</td>
<td>2.22±0.08</td>
<td>2.79±0.13</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>800</td>
<td>1424.14±16.69</td>
<td>1.81±0.06</td>
<td>2.76±0.09</td>
</tr>
<tr>
<td>IV</td>
<td>0.5</td>
<td>0</td>
<td>1125.65±27.48</td>
<td>2.25±0.04</td>
<td>3.02±0.20</td>
</tr>
<tr>
<td>V</td>
<td>1.0</td>
<td>0</td>
<td>998.33±22.18</td>
<td>2.82±0.06</td>
<td>3.48±0.26</td>
</tr>
<tr>
<td>VI</td>
<td>0.5</td>
<td>0</td>
<td>1235.17±35.66</td>
<td>2.11±0.02</td>
<td>2.95±0.28</td>
</tr>
<tr>
<td>VII</td>
<td>1.0</td>
<td>400</td>
<td>1120.83±15.23</td>
<td>2.56±0.01</td>
<td>3.31±0.13</td>
</tr>
<tr>
<td>VIII</td>
<td>0.5</td>
<td>800</td>
<td>1263.33±31.05</td>
<td>2.04±0.03</td>
<td>2.88±2.01</td>
</tr>
<tr>
<td>IX</td>
<td>1.0</td>
<td>800</td>
<td>1158.17±38.24</td>
<td>2.55±0.02</td>
<td>3.27±2.15</td>
</tr>
</tbody>
</table>

Table 2: Mean (±SE) values of serum ALT, AST, Total Protein, Albumin, Triglycerides, Cholesterol and Creatinine in broiler chicken fed with T-2 toxin, Diatomaceous earth (DE) and their combination in feed. Means in column with different superscripts differed significantly at (P<0.05)

<table>
<thead>
<tr>
<th>Group</th>
<th>T-2 Toxin (ppm)</th>
<th>DE (mg/kg)</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CONTROL</td>
<td></td>
<td>10.27±0.73</td>
<td>22.64±44.74</td>
<td>2.15±0.52</td>
<td>0.11±0.04</td>
<td>114.47±4.26</td>
<td>159.17±9.79</td>
<td>0.72±0.11</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>400</td>
<td>11.27±0.77</td>
<td>21.04±21.58</td>
<td>2.17±0.11</td>
<td>0.12±0.01</td>
<td>120.44±2.52</td>
<td>161.4±16.9</td>
<td>0.70±0.06</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>800</td>
<td>12.77±1.37</td>
<td>210.54±33.15</td>
<td>2.21±0.1</td>
<td>0.13±0.01</td>
<td>121.57±5.26</td>
<td>168.27±16.22</td>
<td>0.67±0.09</td>
</tr>
<tr>
<td>IV</td>
<td>0.5</td>
<td>0</td>
<td>12.74±0.53</td>
<td>211.37±79.77</td>
<td>1.87±0.49</td>
<td>1.36±0.27</td>
<td>81.04±4.92</td>
<td>102.47±17.76</td>
<td>0.95±0.08</td>
</tr>
<tr>
<td>V</td>
<td>1.0</td>
<td>0</td>
<td>31.87±0.47</td>
<td>3710±13.75</td>
<td>1.42±0.30</td>
<td>1.10±0.06</td>
<td>74.84±3.33</td>
<td>80.87±17.4</td>
<td>1.21±0.14</td>
</tr>
<tr>
<td>VI</td>
<td>0.5</td>
<td>400</td>
<td>12.15±3.25</td>
<td>213.07±61.64</td>
<td>2.52±1.0</td>
<td>1.82±0.06</td>
<td>85.44±5.46</td>
<td>108.34±16.5</td>
<td>0.91±0.06</td>
</tr>
<tr>
<td>VII</td>
<td>1.0</td>
<td>400</td>
<td>29.44±21.6</td>
<td>354.17±58.11</td>
<td>2.15±0.45</td>
<td>1.66±0.38</td>
<td>75.94±13.29</td>
<td>84.27±17.06</td>
<td>1.18±0.12</td>
</tr>
<tr>
<td>VIII</td>
<td>0.5</td>
<td>800</td>
<td>11.87±2.71</td>
<td>209.87±56.15</td>
<td>2.67±0.96</td>
<td>1.85±0.15</td>
<td>86.87±2.99</td>
<td>116.87±16.9</td>
<td>0.88±0.16</td>
</tr>
<tr>
<td>IX</td>
<td>1.0</td>
<td>800</td>
<td>27.77±0.07</td>
<td>348.24±39.5</td>
<td>2.23±0.86</td>
<td>1.73±0.06</td>
<td>77.97±3.8</td>
<td>88.9±13.69</td>
<td>1.15±0.04</td>
</tr>
</tbody>
</table>
compared to control (group I). In contrast, birds fed with 0.5ppm of T-2 (group IV) in the diet revealed significantly (P<0.05) higher kidney weight and decreased weights of thymus when compared to control group.

Only a marginal decrease in liver and kidney weights and marginal increase in thymus and bursal weights were noticed in birds supplemented with DE as compared to only T-2 toxin fed birds at the end of the experiment but did not differ significantly (P>0.05). However, the relative weights of spleen were neither increased nor decreased significantly in both toxin fed and DE supplemented birds as compared to control.

Chickens fed with 1 ppm of T-2 toxin (group V) had significantly (P<0.05) higher ALT, AST and creatinine levels and lowered total protein, albumin, triglyceride and cholesterol levels. Further, significantly (P<0.05) decreased total protein, albumin, triglyceride and cholesterol levels and increased creatinine levels were observed in birds fed with 0.5 ppm of T-2 toxin (group IV) than those of control group (Table 2).

The supplementation of DE in groups VI, VII, VIII and IX had improved the adverse effects caused by of T-2 toxin on the serum biochemical values by significantly increasing total protein and albumin levels as compared to only toxin fed groups (groups IV and V) while no significant changes were seen in the values of ALT, AST, triglyceride, cholesterol and creatinine in DE supplemented birds.

**DISCUSSION**

In the present study, significant (P<0.05) reduction in body weight and higher feed conversion ratio were noticed at the end of the fifth week in birds fed with 0.5 and 1ppm of T-2 toxin. These results are in agreement with earlier studies in which lowered body weight and poor feed efficiency were recorded when T-2 toxin was fed at 1 ppm and 0.5 ppm in the diet [10-12]. The Reduced weight gain observed in this study could be due to cytotoxic radiomimetic effects of T-2 toxin which causes impaired protein synthesis followed by inhibition of DNA and RNA synthesis [13]. Reduction in weight gain of T-2 toxin fed birds could further be explained by the fact that dividing cells such as cells of gastro-intestinal tract, liver, spleen and bone marrow are highly sensitive to T-2 toxin [14] which could interfere with the digestion and absorption of feed ingredients, contributing to poor feed efficiency.

The performance of the chicks was not affected by the addition of DE alone in the diet indicating the inert and non-toxic property of DE. The birds fed with T-2 toxin and supplemented with DE (group VI to IX) recorded significantly (P<0.05) increased body weight and reduced feed conversion ratio on fifth week as compared to only T-2 toxin fed birds (group IV and V). The increase in the body weight and reduced feed conversion ratio observed in this study could be attributed to adsorption of toxin during digestive process which rendered most of the toxin unavailable for absorption from the gastro intestinal tract.

There are no available data on in-vivo studies of ameliorating effects of DE in T-2 toxicosis in birds. However, in-vitro studies conducted by Natour and Yousef [6], Manafi et al.[15] and Bocarov-Stancic et al. [16] revealed in-vitro binding ability of DE for T-2 toxin with adsorption index ranging from 33.33 % to 74.28% at pH 3.0 to 6.9.

A few studies on evaluation of ameliorating effect of DE on aflatoxicosis, have shown significant increase in feed consumption and body weight [17] and improved FCR values [18,19] in birds fed with diet containing DE which supports the findings of this study. The basic mechanism of protective effects of DE against the toxicity of aflatoxin appear to involve sequestration of AFB1 in the gastro-intestinal tract and chemisorptions to the adsorbent (like other adsorbents such as hydrated sodium calcium aluminosilicate) which reduce the bioavailability [20,21]. In the present study, similar mechanism could be responsible for improved body weight gain in T-2 toxin fed birds supplemented with DE.

In addition, DE could help birds to maintain their body weight by way of increased feed intake. It may be possible that DE offers essential trace elements or may improve absorption of nutrients as DE consists of 86 to 94% silica, with the remainder containing alumina, calcium, phosphorus, sodium, potassium, magnesium, iron, sulfur, and other trace elements [22].

The relative weights of liver and kidney were higher and thymus and bursa of Fabricius' weights were
lower in T-2 fed birds compared to control. The increase in relative weights of liver and kidney could be attributed to degenerative and inflammatory changes incited by T-2 toxin and also accumulation of lipid in these organs as fat metabolism primarily occurs in the liver, while lipidemia with subsequent fat deposition might contribute for increased kidney weights [23]. Lymphoid necrosis and depletion of lymphoid cells observed in thymus and bursa of Fabricius in T-2 toxicoisis [24] could substantiate the reduced weights of these organs in the present study.

In DE supplemented birds (groups VI, VII, VIII and IX), the relative weights of lymphoid organs did not differ significantly with that of toxin fed birds. However, marginal increase in the relative weights of thymus and bursa of Fabricius was seen which indicate the beneficial role of DE in partially alleviating the destructive effects of T-2 toxin.

A significant reduction in total protein and albumin values were observed in T-2 fed birds as compared to control, which agreed with the findings of earlier workers [25,26]. The hypoproteinaemia and hypoalbuminaemia observed in T-2 toxin treated groups could be attributed to the reduction in feed consumption and hepatic damage, since liver is the major organ of protein synthesis especially albumin [27].

Feeding 1 ppm T-2 toxin in broilers resulted in significant increase of ALT and AST values at the end of the toxicity trial. These findings concurred with earlier reports [28-30]. The degeneration of hepatocytes and skeletal muscles and subsequent leakage of enzymes were the reasons for increase in the levels of ALT and AST in the toxin fed birds [31].

The levels of triglycerides and cholesterol were significantly (P<0.05) decreased when birds were fed with 0.5 ppm or 1ppm of T-2 toxin on 35 days of age. These results of decrease in triglycerides at 1 ppm of T-2 toxin were in consensus with the observations made by Balachandran et al. [32] and Ramesh [26]. Significant reduction in serum cholesterol level, observed in the present study, was in agreement with the earlier workers when broiler chickens were fed with 1 ppm of T-2 toxin from 0 to 28 days [29], 0.5 ppm of T-2 toxin from 0-4 weeks of age [33] and 4 ppm T-2 toxin from 0 to 42 days [34]. The decreased triglycerides and cholesterol values in toxin fed groups could be attributed to hepatic and enteric damage caused by T-2 toxin.

At 35 days of age, there was significant (P<0.05) rise in serum creatinine values in T-2 toxicated groups compared to control group. These results were in agreement with findings of Kubena et al. [20]. However, Yohannes et al. [35] reported no significant variation in serum creatinine values in broiler chicken fed with 2 ppm of T-2 toxin. The elevated levels of creatinine indicates impaired renal function consequent to kidney damage by T-2 toxin.

No significant (P>0.05) difference was observed in DE supplemented groups as compared to toxin fed groups for ALT, AST, triglyceride and cholesterol at the end of the study. However, significantly (P<0.05) increased serum total protein and albumin values were observed in DE supplemented birds compared to T-2 fed birds. The probable reason could be due to adsorption of T-2 toxin during digestive process which renders most of the toxin unavailable for absorption from GIT which might have reduced the liver damage and thereby, improved the total protein and albumin levels in DE supplemented birds.

**CONCLUSION**

The results of this study signify that the growth performances, relative organ weights and serum biochemical variables of broilers were negatively affected by T-2 toxin at 0.5 and 1 ppm in the diets. The addition of DE as an adsorbent at 400 and 800 mg/kg in the diet was beneficial in reducing the toxic effects of T-2 toxin. Further studies may be warranted to evaluate different dietary levels of DE to alleviate the detrimental effect of T-2 toxin in broilers.

**ACKNOWLEDGEMENTS**

We would like to acknowledge AGRIPOWER, Australia for providing DE and financial support and also ICAR-NAE project, Department of Veterinary Pathology, Veterinary College, Bangalore for providing the necessary facilities to conduct this research work.

**REFERENCES**
