Ameliorating aflatoxicosis in commercial broiler chickens by dietary Mycosorb: Heamato-Biochemical studies

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ABSTRACT
A study was conducted to find out the ameliorating effect of dietary Mycosorb (esterified glucomannan, EGM) in experimental aflatoxicosis of broilers. For this purpose 270 number of day-old Anak 2000 chicks were procured and divided into 6 equal treatment groups. A control ration (T0) was prepared with conventional feedstuffs and an experimental diet (Tx) was prepared from this ration after incorporating aflatoxin (AF) @ 300 ppb produced on rice using Aspergillus flavus: NRRL 2999 strain through fermentation technology. From T1, three rations were prepared by mixing Mycosorb @0.025 per cent of feed (T1 M0.025), 0.50 per cent of feed (T1 M0.50) or 0.10 per cent of feed (T1 M0.10). A positive control diet (T2 M0.50) was prepared by mixing Mycosorb @ 0.5 per cent in the control ration. All 6 diets were fed to the respective groups for a period 42 days. Significantly (P<0.05) reduced level of serum cholesterol and serum protein levels were observed in aflatoxin-alone fed group; whereas increase surge of glucose, triglyceride and activities of serum enzymes (P<0.05) reduced level of serum cholesterol and serum protein levels were observed in aflatoxin-alone fed group; whereas increase surge of glucose, triglyceride and activities of serum enzymes viz., serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase were recorded in the aflatoxin-alone fed group. However, the increased parameters were corrected to normalcy when Mycosorb was added in the contaminated diet, particularly at 0.1 per cent level. The study showed an ameliorating effect of dietary Mycosorb during experimental aflatoxicosis.

Key words: Aflatoxin, Broiler chicken, Heamato-biochemical parameter, Mycosorb.

INTRODUCTION
Aflatoxins are metabolites of the fungi Aspergillus flavus and A. parasiticus, are highly hepatotoxic, neurotoxic, teratogenic and carcinoogenic compound and frequently contaminate poultry feeds even at low levels (Sapcota et al., 2006a). They impart various deleterious effects on vital organs such as liver and kidney resulting in reduction of body growth, poor feed utilization and lowering immunogenesis leading to mortality in broiler chickens (Girish and Devegowda, 2006). Significant changes in serum biochemical and haematological parameters are seen in aflatoxicated broiler chickens (Huff et al., 1986). Aflatoxicosis in broilers may be manifested by decreased serum concentrations of total protein, albumin, total cholesterol (Rosa et al., 2001). The Aflatoxin B1 decreased contents of Hb, immune complex rosette rate (ICR), increased number of RBC, and the erythrocyte functions were impaired (Tingting et al., 2015). Aflatoxicosis also leads to decreased serum alkaline phosphatase, RBC, PCV and Hb levels concomitant increase in liver and kidney weights and SGOT, SGPT, glucose levels (Sapcota et al. 2006b). Mycosorb (Esterified glucomannan, EGM) is a cell wall of yeast; Saccharomyces cerevisiae has received much attention in the recent times in minimizing adverse effects of aflatoxicosis in poultry (Arvind et al., 2003; Maldhure et al., 2015). EGM showed considerably high binding ability (80–97%) with AF (Diaz et al., 2002). The EGM partially and/or completely reversed the adverse effect of AF on performance, biochemistry haematology and immune responses of birds (Basmacoglu et al., 2005; Yildirim et al., 2011 and Maldhure et al., 2015). The main objective of this study was to evaluate the ameliorating effect of EGM so as to ascertain a practical method for AF detoxification.

MATERIALS AND METHODS
Aflatoxin B1 was produced in the laboratory through solid substrate fermentation in broken rice using pure culture of Aspergillus parasiticus, NRRL 2999 strain (Shotwell et al., 1966). The fermented rice was autoclaved (15 lbs pressure for 15 min) to kill the fungi, dried, (55-60°C), ground to powder form and its AF content was measured by the method described by Romer (1975) using TLC. A standard basal diet was prepared with toxin-free conventional feedstuffs (Table 1) and the rice powder grown with known amount of AFB1 was incorporated into it so as to provide the desired level of 300 ppb toxin per kg of diet. Mycosorb (Esterified glucomannan) required for the experiment was procured by M/s. Alltech Biotechnology Pvt. Ltd, Bengaluru and used in the experiment as per following schedule:

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Table 1: Ingredient and nutrient composition of the experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Starter (0-3 weeks)</th>
<th>Finisher (4-6 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>50.0</td>
<td>52.0</td>
</tr>
<tr>
<td>Rice Polish</td>
<td>6.0</td>
<td>12.5</td>
</tr>
<tr>
<td>Groundnut Cake</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>20.75</td>
<td>12.0</td>
</tr>
<tr>
<td>Fish meal</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Mineral Mixture¹ and vitamin*</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Nutrients**

| ME (Kcal/Kg)* | 2797.63 | 2842.48 |
| Crude protein (%) | 22.83 | 19.68 |
| Lysine (%)* | 1.0903 | 0.9328 |
| Methionine (%)* | 0.42005 | 0.4205 |

*Calculated value

¹Poultry min of M/s. Aries Agro-vet Industries Pvt. Ltd.

Day-old broiler straight-run chicks (n= 270) were procured from a private hatchery and were randomly distributed in to six treatment groups. Each treatment group was further divided in to three replicates with fifteen chicks. The chicks were fed different dietary treatments as shown in above tabular form. The feed was provided as per treatment and water was provided ad libitum to all the treatment groups throughout the experimental period. The broiler starter and finisher feed were provided up to third and fourth to six weeks of age, respectively (Table 1). The chicks were vaccinated against Ranikhet and Gumboro disease vaccines at 7th and 14th days of age, respectively. Uniform and standard managemental practices were provided throughout the experimental. The broiler birds in all the treatment groups were subjected on full feeding programme. At the end of the experiment five birds were selected at random from each group and about 5 ml of blood was collected aseptically from each of the birds. Then the blood samples were processed for separation of serum. The parameters were analyzed by using standard methods such as Serum cholesterol (CHOD/ PAP), Serum protein (Biuret Method), Serum glucose (DPEC - GOD/POD Ranbaxy), SGOT and SGPT (Reitman and Frankel’s method), Alkaline phosphates (Modified Kind and King’s method) and Triglyceride (Colorimetric enzymatic test using GPO of DiaSys). The data obtained on various parameters during this experimental trial were subjected to statistical analysis as described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

**Cholesterol:** In comparison to normal value of control group the cholesterol level in toxin-alone fed group decreased to the extent of 30.79% (Table 2). Interference with protein and fat mobilization metabolism and its utilization in the body of broilers by aflatoxin might have suppressed the serum cholesterol level (Mani et al., 2000). Lower cholesterol level due to aflatoxicosis was also recorded by Sapcota et al. (2006c), Khatke et al. (2013), Chibanga et al. (2014) and Lakkhawar et al. (2016). However, this condition was improved when Mycosorb was added to the diets of aflatoxin fed groups by which the serum cholesterol level was significantly (P<0.05) increased on dose dependent manner. This result is in agreement with the work of Ghabri et al. (2010), who reported that the EGM supplementation increased the serum cholesterol concentrations compared to the aflatoxin-fed group. The fact that S. cerevisiae influences for robust growth when supplemented in the diet of chickens, indicates that the lipid uptake is efficient (Diaz and Smith, 2005). In the present study, the increase in cholesterolemia in the EGM group may be due to the S. cerevisiae effect on the lipid uptake (Yildirim et al., 2011)

**Serum protein:** The serum protein levels found to be decreased significantly (P<0.05) in aflatoxin-alone fed group to the extent of 18.87 per cent as compared to control counterpart (Table 2). Reductions in serum total protein and albumin concentrations in chickens fed AFBI contaminated diets is said to be a result of inhibition of amino acid transport and mRNA transcription that leads to inhibition of DNA and

Table 2: Influence of dietary supplementation of Mycororb on haemato-biochemical constituents of broilers at 6 wks of age fed with aflatoxin

<table>
<thead>
<tr>
<th>Group</th>
<th>Haematological profile</th>
<th>To</th>
<th>Tx</th>
<th>TₓM₁</th>
<th>TₓM₂</th>
<th>TₓM₃</th>
<th>TₓM₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg%)</td>
<td>138.27±1.07</td>
<td>95.69±1.23</td>
<td>108.95±0.89</td>
<td>121.09±1.11</td>
<td>128.49±1.26</td>
<td>137.35±0.71</td>
<td></td>
</tr>
<tr>
<td>Protein (mg%)</td>
<td>3.18±0.12</td>
<td>2.58±0.11</td>
<td>2.71±0.10</td>
<td>2.90±0.40</td>
<td>3.00±0.45</td>
<td>3.27±0.45</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg%)</td>
<td>277.80±0.92</td>
<td>300.00±1.69</td>
<td>295.25±1.64</td>
<td>286.12±1.97</td>
<td>282.29±0.69</td>
<td>278.12±0.81</td>
<td></td>
</tr>
<tr>
<td>SGOT (IU/ml)</td>
<td>45.45±0.72</td>
<td>69.63±0.54</td>
<td>64.76±0.46</td>
<td>57.59±0.51</td>
<td>55.24±0.65</td>
<td>44.26±0.66</td>
<td></td>
</tr>
<tr>
<td>SGPT (IU/ml)</td>
<td>19.88±0.63</td>
<td>29.84±1.28</td>
<td>28.72±0.33</td>
<td>25.75±0.75</td>
<td>24.76±0.41</td>
<td>19.10±0.64</td>
<td></td>
</tr>
<tr>
<td>ALP (KA unit)</td>
<td>83.71±0.90</td>
<td>105.63±0.73</td>
<td>100.62±0.79</td>
<td>97.52±0.76</td>
<td>90.60±0.66</td>
<td>82.78±1.13</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>45.82±0.83</td>
<td>64.81±0.61</td>
<td>58.71±0.65</td>
<td>56.79±0.46</td>
<td>52.00±0.80</td>
<td>44.67±0.52</td>
<td></td>
</tr>
</tbody>
</table>

Means with at least one common superscript in a row do not differ significantly (P<0.05).
protein synthesis (Thaxton et al., 1974). The decreased serum protein level due to aflatoxicosis was also observed by Sapcota et al. (2006b), Verma et al. (2012) and Kumar Dhanpal et al. (2014) and Lakkhawar et al. (2016). Mycosorb, at medium (0.05 per cent) and high dose (0.10 per cent) could significantly improve the serum protein level as compared to toxin-alone fed group. The higher dose of Mycosorb (0.10 per cent) among the toxin-fed groups gave comparable value to that of control group reflecting in total counteraction. Basmacoglu et al. (2005) have shown that the dietary EGM supplementation at the dose of 0.10 per cent succeeded in significantly increasing the proteinemia in broilers during aflatoxicosis. Wang et al. (2006) and Che et al. (2011) also reported such observations.  

**Serum glucose:** The serum glucose level in the aflatoxin-alone fed group was found to be statistically higher than the control group to the extent of 7.99 per cent (Table 2). Similar findings were reported by Suksombat et al. (2011) and Valchev et al. (2014). The increased level of serum glucose level during aflatoxicosis might be due to breakdown of glycogen in the liver cells and subsequent release to the blood stream (Rosa et al. 2001). However, medium (0.05 per cent) or high (0.10 per cent) dose of Mycosorb could reduce the glucose level in the toxin fed broilers. Basmacoglu et al. (2005) also observed such a change when mycosorb @1g/kg was added in aflatoxin contaminated diet of broiler chickens.  

**Serum glutamate oxalo acetate transaminase:** In aflatoxin-alone fed group, the SGOT level increased significantly to the extent of 53.20 per cent as compared to control group. Similar findings that the aflatoxicosis in broiler increased SGOT level were reported by Motawe et al. (2014) and Kumar et al. (2016). The increased level of serum enzyme might be due to hepatic degeneration and subsequent leakage of this enzyme to the blood stream (Oguz et al., 2002). However, the elevated SGOT level was brought towards normalcy on dose-dependent manner when the diets of aflatoxin-fed birds were mixed with Mycosorb. Nevertheless, the restorative effect was not complete since even with the highest dose of Mycosorb the SGOT level remained elevated as compared to control group (45.45 vs. 55.24 IU/ml). Similar finding were also recorded by Cao and Wang (2014) when supplemented with mycosorb in aflatoxin contaminated diet.  

**Serum glutamate pyruvate transaminase:** The SGPT level in toxin-alone fed group was found to be 50.10 per cent higher than that of control group. The higher level of this enzyme was also reported by Kana et al. (2014) and Kumar et al. (2016). The explanation for higher SGOT level in the blood stream of aflatoxicated broilers also holds good for high SGPT levels. After the use of Mycosorb in the toxin-fed broilers the SGOT level decreased considerably and the trend was found to be similar to that of SGOT. Similar finding observed by Yildirim et al. (2011).  

**Alkaline phosphates and triglyceride:** The Alkaline phosphates (ALP) and triglyceride levels in toxin-alone fed birds increased significantly to the extent of 26.19 per cent and 41.44 per cent, respectively. The increased levels of ALP in aflatoxicated broilers was also reported by Chibanga et al. (2011) and Lakkhawar et al. (2016); whereas, increased levels of triglyceride in such birds were reported by Suksombat et al. (2011); Valchev et al. (2014) and Lakkhawar et al. (2016). Increase triglyceride levels were due to the hepatotoxic effects of AF characterized by the inhibition of protein synthesis and impairment of carbohydrate and lipid metabolism (Rosa et al. 2001). In present study Mycosorb, when added in diets of aflatoxin fed birds could be able to restore the levels of respective enzymes partially and results were dose dependent. Similar trend was observed by Basmacoglu et al. (2005). Banlunara (2005) indicated that activities of ALP was increased by AFB1, whereas, were decreased by EGM. Che et al. (2011) reported improved ALP levels by the feeding of mycosorb (0.05g/kg) in aflatoxicated broiler chickens.  

**CONCLUSION**  

The chemical compound present in Mycosorb responsible for this ameliorative effect need to be further studied and evaluated. It could be concluded that Mycosorb (@ 0.10 per cent helps in counteracting the adverse effects of AF in broiler chickens.  

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