Evaluation of biochemical characters of broiler chickens during dietary aflatoxin and clinoptilolite exposure

H. Oguz¹, Fıruze Kurtoglu², Varol Kurtoglu³*, Yavuz O. Birdane⁴

¹Department of Pharmacology and Toxicology, Biochemistry; ²Animal Nutrition and Nutritional Diseases; ³Faculty of Veterinary Medicine, University of Selçuk, Konya, Turkey, Department of Pharmacology and Toxicology; ⁴Faculty of Veterinary Medicine, University of Afyon Kocatepe, Afyon, Turkey

SUMMARY

Aflatoxin (AF) and clinoptilolite (CLI, a natural zeolite) were added to broiler food and some biochemical values and enzyme activities were evaluated. The experimental design consisted of six dietary treatments. (1) Control: basal diet; (2) CLI: basal diet plus 15 g CLI/kg diet; (3) 50 ppb AF: basal diet plus 50 ppb total aflatoxin; (4) 50 ppb AF + CLI: basal diet plus 50 ppb AF plus 15 g CLI/kg diet; (5) 100 ppb AF: basal diet plus 100 ppb AF; (6) 100 ppb AF + CLI: basal diet plus 100 ppb AF plus 15 g CLI/kg diet. A commercially available CLI was provided from the west region of Turkey and its chemical formula is “KNa₂Ca₂(Si₂₉Al₇)O₇₂·3₂H₂O”. For this a total of 576 day-old Ross broiler chicks were housed in six treatment groups from days 1 to 42. AF treatment significantly increased the serum Na levels and the aspartate-amino-transferase (ASAT) and alanine-amino-transferase (ALAT) enzyme activities, while total protein, albumin, total cholesterol uric acid, and K levels were not significantly different between groups. These results suggest that these low AF levels in food did not change the serum biochemistry but significantly affected the enzyme activities in broilers.

Key Words: aflatoxin, clinoptilolite, broiler, blood parameters

INTRODUCTION

Aflatoxins (AFs) are secondary toxic metabolites produced by certain fungi belonging to the genus Aspergillus and can occur as natural contaminants of poultry food (Leeson et al 1995, Oguz 1997). Aflatoxicosis in poultry can cause disease and increased mortality (Ibrahim et al 2000, Kubena et al 1998, Oguz and Kurtoglu 2000). Determination of biochemical toxic effects of AFs is important for diagnosis of toxicosis in broilers (Rosa et al 2001). AF toxicity in broilers may be manifested by decreased serum concentrations of total protein, albumin, total cholesterol (Kubena et al 1998, Oguz et al 2000a), uric acid

† E-mail: vkurtoglu@selcuk.edu.tr
(Kececi et al 1998), and increased hepatic enzyme activities such as ASAT and ALAT (Amer et al 1998, Santurio et al 1999). Since the beginning of the 1990s, adsorbent-based studies have been performed for removing AFs from contaminated food and minimising their toxicity in poultry. The natural and synthetic zeolites (Kececi et al 1998, Oguz et al 2000b), bentonites (Ibrahim et al 2000, Rosa et al 2001, Santurio et al 1999) and clinoptilolite (Oguz et al 2000a,b, Ortatatlı and Oguz 2001) were preferred because of their high binding capacities against AFs and their reducing effect on AF absorption from the gastrointestinal tract.

Therefore, the purpose of the present study was to evaluate the serum biochemistry and enzyme activity at low levels (50 and 100 ppb) and long-term AF exposure, and to determine the possible preventive role of dietary CLI on investigated values.

MATERIAL AND METHODS
Five hundred and seventy six day-old Ross broiler chicks of both sexes were obtained from a commercial hatchery, individually weighed and divided at random into six groups. The chicks were housed in heated batteries under fluorescent lighting and were fed a commercial food starter (maize and soybean base, 23% crude protein, 13.80 MJ/kg ME) up to 21 days and a grower diet (21.5% crude protein, 13.60 MJ/kg ME) up to 42 days.

Chickens consumed the diets and water ad libitum. The AF was produced from Aspergillus parasiticus NRRL 2999 culture (USDA, Agricultural Research Service, Peoria, IL, USA) via fermentation of rice by the method of Shotwell et al (1966) with minor modifications by Oguz (1997). Successfully fermented rice was then steamed to kill the fungus, dried and ground to a fine powder. The AF content in rice powder was analysed by the method of Shotwell et al (1966) and measured on a thin layer chromatography (TLC) - fluorometric densitometer (Camag-III, Basel, Switzerland) on the TLC spots. The AF within the rice powder consisted of 72.51% AFB1, 14.05% AFB2, 9.78% AFG1, and 3.66% AFG2 based on total AF in the ground rice powder. The rice powder was incorporated into the basal diet to provide the required amount of 50 and 100 ppb.

When the chicks reached 42 days of age, the feeding trial was terminated and 10 broilers from each treatment group were selected at random and bled by cardiac puncture. Serum concentrations of total protein, albumin, total cholesterol, uric acid, and the activities of ASAT and ALAT were determined on a spectrophotometer (Shimadzu, UV-2100, Japan) with commercial test kits (Bio-Clinica; Biobak Lab. Suppl, Istanbul, Turkey). The serum Na and K levels were determined by Flame Photometry (Jenway, PFP-7, USA). The data for serum biochemical and enzyme activity values were grouped and expressed as mean ± pooled standard errors of means. The results obtained were statistically
analysed using Duncan’s multiple range test (SPSS 1988). Statements of statistical significance are based on P<0.05.

RESULTS AND DISCUSSION

Serum total protein, albumin, total cholesterol, uric acid, and K levels were not significantly different between groups (Table 1). However, a numerical decrease was seen in total protein and uric acid levels in 100 ppb AF-fed chicks, compared to controls. The 100 ppb AF treatment also significantly increased the ASAT and ALAT enzyme activities and serum Na values. Serum ASAT activity was lower in chicks fed 50 ppb AF while serum Na levels were higher. The addition of CLI to the AF-containing diet showed no significant improvements in the parameters investigated.

Determination of serum biochemistry and enzyme activity can help in the diagnosis of aflatoxicosis cases before major clinical symptoms appear (Oguz et al 2000a). Previous studies performed with high levels of AF (2.5–5 mg/kg diet) showed significant decreases in serum total protein, albumin, total cholesterol and uric acid levels (Kubena et al 1998, Oguz et al 2000a, Rosa et al 2001), while serum Na and K levels were not affected (Santurio et al 1999). In the present study, the use of low AF levels (50 and 100 ppb) did not cause any significant changes in the total protein, albumin, total cholesterol, uric acid, and K levels, but a significant increase in Na values (P<0.05), compared to controls.

The differences between previous studies and our current findings can be related to the AF doses in the diet. The AF levels in the present study are 20-30 times less. Furthermore, no significant changes were reported in serum biochemistry for the lower dietary AF, such as 50 ppb (Abdelhamid et al 1994) and 200 ppb (Johri et al 1990), in broiler food. However, when AF levels increased in food up to 300 ppb and more (Jindal et al 1994, Raju and Devegowda 2000) the serum biochemistry was significantly affected and total protein, albumin and cholesterol levels were decreased. Serum ASAT and ALAT activities are considered sensitive indicators of hepatocellular damage/dysfunction, indicating liver inflammation, lesions or obstruction of the biliary tract (Kubena et al 1998). In the present study, serum ASAT and ALAT activities were significantly increased by feeding 100 ppb AF (P<0.05). These results agree with the reports of earlier studies (Jindal et al 1994, Amer et al 1998) which used, respectively, 500–750 ppb dietary AF in broilers. The use of agents that act as antidotes to the toxic effects of AF have therapeutic and economic importance (Ortatath and Oguz 2001) and another objective of the present study was to examine the preventive efficacy of the inert zeolite, CLI. In our previous studies (Oguz and Kurtoglu 2000, Oguz et al 2000a, Ortatatath and Oguz 2001) CLI was found to be a safe and effective preventive agent, which binds the AF molecules in the gastrointestinal tract. Its effectiveness was shown by the evaluations of performance parameters, biochemical–haematological changes and gross histopathological investigations of broilers given high AF
levels (2.5 ppm) over a period of 21 days. The preventive efficacy was not seen in the present study, probably because of the relatively low level of AF.

In support of this, Oguz et al (2000b) found that in using low levels of AF (50 and 100 ppb), the preventive efficacy of CLI could not be demonstrated. These results clearly demonstrate that low levels of AF (50 and 100 ppb) caused no significant difference in serum biochemical parameters, while ASAT and ALAT activities were affected by AF.

Table 1. Effects of aflatoxin (AF) and clinoptilolite (CLI) on biochemical values and enzyme activities for broiler chicks fed at 1-42 days of age

<table>
<thead>
<tr>
<th>Groups/Parameters</th>
<th>Group 1 (Control)</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF -CLI - 15 g/kg</td>
<td>AF 50 ppb CLI - 15 g/kg</td>
<td>AF 50 ppb CLI 15 g/kg</td>
<td>AF 100 ppb CLI - 15 g/kg</td>
<td>AF 100 ppb CLI 15 g/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total prot. g/dl</td>
<td>3.04 ± 0.11</td>
<td>3.16 ± 0.23</td>
<td>3.42 ± 0.34</td>
<td>3.13 ± 0.14</td>
<td>2.96 ± 0.09</td>
<td>2.79 ± 0.11</td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td>1.79 ± 0.06</td>
<td>2.05 ± 0.12</td>
<td>1.96 ± 0.09</td>
<td>2.09 ± 0.14</td>
<td>1.95 ± 0.08</td>
<td>2.11 ± 0.05</td>
</tr>
<tr>
<td>Total chol., mg/dl</td>
<td>100.63 ±</td>
<td>106.94 ±</td>
<td>110.66 ±</td>
<td>116.19 ±</td>
<td>107.77 ±</td>
<td>90.04 ±</td>
</tr>
<tr>
<td>Uric acid, mg/dl</td>
<td>6.64 ± 0.79</td>
<td>5.92 ± 0.60</td>
<td>3.89 ± 0.50</td>
<td>4.97 ± 0.66</td>
<td>4.67 ± 0.67</td>
<td>4.66 ± 0.55</td>
</tr>
<tr>
<td>Na, mmol/l</td>
<td>307.20 ±</td>
<td>381.20 ±</td>
<td>367.70 ±</td>
<td>358.30 ±</td>
<td>355.75 ±</td>
<td>342.60 ±</td>
</tr>
<tr>
<td>K, mmol/l</td>
<td>19.26 ±</td>
<td>22.16 ±</td>
<td>23.47 ±</td>
<td>23.84 ±</td>
<td>19.00 ±</td>
<td>20.87 ±</td>
</tr>
<tr>
<td>ASAT, IU/l</td>
<td>32.50 ±</td>
<td>26.03 ±</td>
<td>28.01 ±</td>
<td>21.13 ±</td>
<td>36.84 ±</td>
<td>40.49 ±</td>
</tr>
<tr>
<td>ALAT, IU/l</td>
<td>17.89 ±</td>
<td>23.39 ±</td>
<td>20.81 ±</td>
<td>39.02 ±</td>
<td>35.74 ±</td>
<td>40.65 ±</td>
</tr>
</tbody>
</table>

a – e Values within columns with no common superscripts are significantly different (P < 0.05), according to Duncan’s multiple range tests. Values represent the mean (SEM) of six groups of 10 broiler chicks each per treatment

CONCLUSIONS
The results suggest that the low AF levels in food did not change the serum biochemistry but significantly affected the enzyme activities in broilers.

REFERENCES


Raju, M. V. L. N. & Devegowda, G. (2000) Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry, and haematology in broilers exposed to individual and combined myco-