EFFECT OF ENTEROCOCCUS FAECIUM DSM 7134 PROBIOTIC UPON SOME HEMATO BIOCHEMICAL VALUES IN ACUTE OCHRATOXICOSES IN BROILERS

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ABSTRACT

The effect of a single strain probiotic Enterococcus faecium DSM 7134, upon some biochemical and haematological parameters in broilers, was examined after a single dose application of ochratoxin A (OTA). For that purpose, a total of 160 Ross broiler chicks were randomly distributed into four different treatments with two replicates of 20 chicks each: 1) control; 2) probiotic 3.300 x 10⁹ CFU, tested at 0.2 g/l in drinking water; 3) OTA 50 mg/l, in drinking water; and 4) probiotic plus OTA, in same amounts as in groups 2 and 3. Blood samples were collected by cardiac puncture 6 h and 12 h after ochratoxin A application. Evaluated parameters in chicks’ blood were: total protein, albumin, urea, creatinine, haematocrite and haemoglobin.

Data for all parameters at 12 hours after OTA application, revealed significant (p<0.01) differences in group receiving only mycotoxin (OTA) when compared with data from the chicks in group receiving probiotic and ochratoxin (P+OTA). Hematological analyses showed significant (p<0.01) decreased hemoglobin value in chicks from group OTA at 12 hours post exposure to mycotoxin, compared to those at 6 hours post exposure. Chicks from the group receiving only probiotic, at 12 hours showed significant (p<0.01) decrease in creatinine value compared to that of the control group. It can be concluded, that administration of probiotic Enterococcus Faecium DSM 7134 in drinking water together with ochratoxin, may well be a contribution to a healthy broiler production, especially if probiotic is used as an integral part of a larger concept including management factors.

Key words: Enterococcus faecium DSM 7134, ochratoxin A, broilers, biochemical profile, haematological values

INTRODUCTION

Ochratoxin A (OTA), a secondary metabolite mainly produced by Aspergillus ochraceus and Penicillium viridicatum, has been shown as a potent nephrotoxin to animals and human, even though animals may vary in their susceptibility to the toxin effects (1).

Ochratoxin is the main cause for the Balkan Endemic Nephropathy (2), and according to the International Agency for Research on Cancer (IARC) (3), OTA is classified as on of the possible carcinogens in group 2B. Besides being nephrotoxic, ochratoxin is considered to cause a broad range of toxicological effects such as hepatotoxic, neurotoxic, immunotoxic, genotoxic and teratogenic effects in both human and animals (4). Ochratoxin can adversely affects animal production, especially in swine and poultry industries. Particularly in poultry production, ochratoxicoses can cause significant losses due to its effects on performance and health, by reducing the growth rate and feed consumption, reducing immune response, increasing mortality and poor feed conversion (5-8). Furthermore, OTA affects the biochemical profile in poultry, by altering the amounts of total blood protein, albumin, globulin, urea, triglycerides, uric acid and creatinine.
Diets contaminated with ochratoxin fed to broilers, could lead to leucopenia and lymphopenia (11), and decreased number of red blood cells and hemoglobin concentration could be observed, as well (12).

In the past decades, research was focused on finding solutions for mycotoxin detoxification, by binding it to different adsorbents, adding antioxidants to the diet and other feed supplements (13-16). Results have showed that not all binders have the same capacity to protect animals against the detrimental effects of mycotoxins. Some experiments (17) showed that, most of the adsorbents, by being beneficial to the host organism by binding the mycotoxins from the feed, in the same time they may impair the use of the essential nutrients from the feed, as well. For that reason, it is very important that the methods used for mycotoxin decontamination should not influence or contribute to losses of nutritional feed value or palatability.

Recently more attention is given to the use of specific bacterial strains which are able to biotransform mycotoxins (18). Until now, several bacterial strains have been proven for in vitro and in vivo biotransformation of different mycotoxins such as: aflatoxins, ochratoxin, trichotecenes and zearalenone (19-26). These beneficial bacteria are so called probiotics. They are defined as nonpathogenic live microbial food supplements, which beneficially influence human (27) and animals health by reducing pathogen colonization and therefore contribute to human and animal welfare (28-30).

Based on the data on field outbreaks of ochratoxicosis resulting in poor growth rate and poor feed efficiency (31), particularly being quite pronounced in young broiler chicks (9), this research was conducted in order to study the effect of acute experimental OTA toxicity upon hematological and biochemical profile in young broiler chicks.

**MATERIAL AND METHODS**

**Experimental birds and management**

This study was carried out on 160 day-old Ross broiler chicks, received from a commercial hatchery, from a maternal flock appropriately vaccinated according to the established immunoprophylaxis program. During the experiment and afterwards, animals were treated according to the “Guide for the care and use of laboratory animals” approved by the National Research Council (ILAR)(32) in USA.

**Experimental design**

One hundred and sixty 1-day-old Ross broiler chicks, after weighing were equally assigned in two groups of 80 chicks: 1) control (C); 2) probiotic (P), receiving 0.2 g/l in drinking water, with concentration of 3.300 x 10^9 cfu. Both groups were kept on a floor system under standard hygienic and ambiental conditions, and were supplied feed and water ad libitum, throughout the experimental period of 14 days. During this period chicks were fed commercial starter ration (23.5 % crude protein and 3025 kcal metabolizable energy (ME)/kg). At 14 days of age, chicks were randomly distributed into four different treatments with two replicates of 20 chicks each: 1) control (C); 2) probiotic 3.300 x 10^12 cfu, tested at 0.2 g/l in drinking water (P); 3) OTA 50 mg/l, in drinking water (OTA); and 4) probiotic plus OTA, (P+OTA), in same amounts as in groups 2 and 3. Calculated OTA intake was 5.85 mg per chick in a single dose, while calculated probiotic intake concentration was 0.023 g with concentration of 3.300 x 10^9 cfu/chick/day. Detailed layout of the experiment is presented in Table 1.

Blood samples were collected by cardiac puncture at 6 and 12 hours after toxin application. After blood was taken, small aliquot was immediately transferred into a 500 ml tubes with anticoagulant, and the rest was transferred into serum tubes. Evaluated parameters in chicks’ blood were: total protein, albumin, urea, creatinin, haematocrite and haemoglobin.
Table 1: Chick’s grouping and treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diet and treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - control</td>
<td>Basal diet</td>
</tr>
<tr>
<td>OTA (Ochratoxin A)</td>
<td>Basal diet + OTA, 50 mg/l, in drinking water</td>
</tr>
<tr>
<td>P - probiotic</td>
<td>Basal diet + probiotic, 0.2 g/l in drinking water, with concentration of 3.300 x 10^9 cfu</td>
</tr>
<tr>
<td>P+OTA Probiotic and ochratoxin A</td>
<td>Basal diet + probiotic, 0.2 g/l in drinking water, with concentration of 3.300 x 10^9 cfu and OTA, 50 mg/l, in drinking water</td>
</tr>
</tbody>
</table>

**Hematological parameters**

For hematological analyses blood samples with anticoagulant (K₃EDTA) were taken from fifteen randomly selected birds from each group, 6 and 12 hours after OTA application. Hemoglobin and hematocrit concentration were determined by automated cell counter hematology analyser, Celly (Hy-cell Diagnostics, France).

**Biochemical parameters**

Blood was collected from 15 chicks without anticoagulant and serum was extracted by centrifugation at 3000rpm for 10min, followed by serum storage at −20 °C till analyses were done. Commercially available colorimetric kits from Human (Germany) were used for the determination of total protein, albumin, urea and creatinine, according to the manufacturer’s instruction. All analyses were performed on spectrophotometer Cecil 2000 series, (Cecil Instrument 2000 England), according to the manufacturer’s protocol.

**Statistical analysis**

Data obtained from all parameters in this study were subjected to descriptive statistics, where mean and standard deviation were calculated. For comparison within the groups, t-dependent test was used, and differences between groups were analysed with Tukey, ANOVA by using SPSS software. For all parameters, differences with p<0.01 were considered statistically significant.

**RESULTS AND DISCUSSION**

**Effect on hematological parameters**

Data from the analyses of hemoglobin and hematocrit are presented in Table 2. Data presented in Figure 1 show significant decrease in hemoglobin concentration 12 hours after toxin application, in group fed with ochratoxin compared with the results at 6 hours in the same group. Lower values for hemoglobin were detected in group P+OTA compared with the group that received only probiotic, but were not significant. However, values from group P+OTA compared to those from group OTA were statistically (p<0.01) higher at 12 hours after toxin application (Figure 2).

Values for hematocrit concentration showed in Table 2, were decreased in group OTA, when compared to control and P+OTA, after 6, while 12 h after toxin application, the decrease of the hematocrit concentration in this group was statistically significant (p<0.01). Also in group P+OTA, decreased values for hematocrit were noticed, compared with those in group fed with only probiotic, both 6 and 12 h, after toxin application. Gradually with time, changes in values of hematocrit concentration within all groups were not detected, except for the group fed with probiotic, where value at 12 h was increased compared to the same at 6h. This was not a significant increase. Decreased values for hemoglobin and hematocrit in broilers fed ochratoxin A, are in correlation with findings carried out by (33-34, 12). Reason for decreased Hb values could be impaired protein synthesis by ochratoxin, which is confirmed in this study by lower values for serum albumin in OTA group (33,35).
Effect on biochemical parameters

Mean values with standard deviation of serum total protein (TP) and albumin (ALB) concentrations are presented in Table 2. Significant reduction (p ≤ 0.01) was observed for both parameters in group OTA at 12 hours after application, when compared to P+OTA group (Figure 2). Total protein and albumin values in group P+OTA, 12 hours after toxin application are higher than 6h after application. On the contrary from this results, TP and ALB values in OTA group, 12h after mycotoxin application are lower than those measured at 6h. These are not significant results.

Our results are in accordance with similar findings reported by several authors (9,10,34). Decreased values of total protein and albumin could be due to liver histopathological changes observed in this experiment, but not presented in this paper. Reduction of protein and albumin values is a result of an impaired protein synthesis by the toxicological effect of ochratoxin A, as well. Higher values for TP and ALB in P+OTA group, compared to OTA group, confirm the protective role of probiotic, as far as protein synthesis is concerned.

Table 2. Values (M±SD)¹ of hemoglobin (Hb), hematocrit (Hct), total proteins (TP), albumins (ALB), urea (UREA) and Creatinin (Creatinin) in four different groups (Control; OTA; Probiotic; P+OTA) measured after 6 and 12 hours from the application of ochratoxin A in the OTA and P+OTA groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>OTA</th>
<th>Probiotic</th>
<th>P+OTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>6h</td>
<td>12h</td>
<td>6h</td>
<td>12h</td>
</tr>
<tr>
<td>Hb (%)</td>
<td>9,20±0,15</td>
<td>9,27±0,14</td>
<td>7,31 a±0,19</td>
<td>6,99 a±0,14</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>26,07±1,16</td>
<td>26,73±0,98</td>
<td>20,37±1,68</td>
<td>20,17±1,33</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>3,25 ±0,26</td>
<td>3,39±0,25</td>
<td>2,34±0,15</td>
<td>2,29±0,14</td>
</tr>
<tr>
<td>ALB (g/dl)</td>
<td>1,93±0,17</td>
<td>1,97±0,15</td>
<td>1,38±0,13</td>
<td>1,33±0,15</td>
</tr>
<tr>
<td>UREA (mg/dl)</td>
<td>1,09±0,28</td>
<td>1,08±0,11</td>
<td>1,73±0,54</td>
<td>1,91±0,22</td>
</tr>
<tr>
<td>Creatinin (mg/dl)</td>
<td>0,26±0,02</td>
<td>0,27±0,04</td>
<td>0,38±0,02</td>
<td>0,45±0,06</td>
</tr>
</tbody>
</table>

¹Data are means ± standard deviation, a-i Values for each criteria in a row with same superscript are significantly different at p<0,01

Figure. 1. Changes (p<0.01) on hemoglobin (Hb), creatinin and urea concentration in groups with mycotoxin (OTA and P+OTA) after 6 and 12 hours after the application of the mycotoxin.
Mean values with standard deviation of serum urea and creatinine, 6 and 12 h after ochratoxin A application are showed in Table 2. We found that, creatinine values in group OTA, 12 hours after toxin application, are significantly higher (p<0.01) compared to those obtained at 6 hours in same group (Figure 1). However, urea concentration in group P+OTA, was found to be statistically lower at 12 hours after toxin application, compared to the values obtained at 6 hours (Figure 1). Concerning the concentration of both urea and creatinine, higher values are noticed in OTA group compared to control and P+OTA group at 6 and 12h after toxin application, but were not significant, except for the values at 12 hours compared to P+OTA group (p<0.01). These findings are in agreement with those by (10, 34, 35). Decreased creatinine and urea values in group fed with probiotic compared to control group, are a confirmation of the ameliorating effect of the probiotic in counteracting the toxic effect in experimental ochratoxicoses.

Increased serum creatinine and urea concentrations in ochratoxin group, are an indicator of an inflammatory or degenerative changes in the kidney (36), and it is assumed that ochratoxin induced nephrotoxicity could be due to interference with transport function in collecting tubule cells together with diffused impairment of the proximal tubules function.

Still, further research need to be done in order to better understand the dose and time dependent toxicological effect of OTA and to confirm the probiotic effect upon broiler biochemical profile and performances, by using different probiotic bacteria as single strains or in a mixture.

**CONCLUSIONS**

The objective of the present study was to evaluate the acute toxic effect of a single dose application of OTA in broiler chicks, upon certain blood parameters. Obtained results confirm that OTA is an important mycotoxin in broiler production, due to the induced changes in hematobiochemical analyses.

Based on the reviewed literature, as well as on data obtained in this study, we can conclude that single dose application of ochratoxin A in concentration of 50 mg/l in drinking water, affects broil-
er’s hematological and biochemical parameters. It resulted in significant reduction in hemoglobin and hematocrit percentage, decrease in total serum proteins and albumin, but increase in concentration of urea and creatinine.

We think that administration of probiotic Enterooccus Faecium DSM 7134 may well be a contribution to a healthy broiler production at least if they are used as integral part of a larger concept including management factors.

REFERENCES


