Reduction of ochratoxin A in broiler serum and tissues by *Trichosporon mycotoxinivorans*

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**Abstract**

The present study was planned to evaluate the possible transmission of ochratoxin A (OTA) in serum and targeted organs of broilers fed on two levels (500 and 1000 ppb) this toxin in the presence or absence of a toxin deactivator (containing a mycotoxin deactivating yeast *Trichosporon mycotoxinivorans*) at two inclusion levels (1 and 2 kg/ton of feed) to 270 day-old broiler chicks divided into nine groups (A–I) over a 42 days period. Serum samples were collected at 14, 28 and 42nd day of experiment, whereas, liver and kidney tissues were obtained from broilers slaughtered at 42nd day of experiment. The highest OTA levels were detected in serum, livers and kidneys of OTA treated groups without supplementation of toxin deactivator (groups D and G) at day 42 of experiment, while the residues were significantly (\(P < 0.01\)) lower in treatment groups (F and I) supplemented with toxin deactivator at 2 kg/ton of feed. The order of OTA level was serum > kidneys > liver.

**1. Introduction**

Ochratoxin A (OTA), a lactone containing secondary metabolite of toxigenic species of *Aspergillus* and *Penicillium* (Marquardt and Frohlich, 2000) is one of commonly occurring mycotoxins, produced during storage on food and animal feed. Contamination of poultry feeds with OTA is well documented (Binder et al., 2007; Hanif et al., 2008). Various field studies demonstrated that ochratoxins reduce growth rate, contribute to leg problems and malabsorption of nutrients, impair immune system and cause carcinogenicity (Hanif et al., 2008; Stoev, 2010).

In animal husbandry, various adsorbents such as clays and aluminosilicates are now included in feed for counteracting various mycotoxins. However, encouraging results have only been documented for aflatoxins and not for trichotheccenes and ochratoxins (Stanley et al., 1993). During the last decade, several studies focused on exploring a new approach for mycotoxin deactivation through microbial enzymatic action. A yeast strain named *Trichosporon mycotoxinivorans* isolated from hind gut of lower termite (*Mastotermitidae*) is reportedly effective for reducing dietary OTA and zearalenone (ZON) into their nontoxic metabolites (Politis et al., 2005; Schatzmayr et al., 2006). The present study was planned to assess the possible transmission of OTA into serum and tissues of broiler chickens fed two levels of OTA in the presence or absence of toxin deactivator (Mycofix Plus containing *T. mycotoxinivorans*) for a period of 42 days.

**2. Materials and methods**

A total of 270 day-old broiler chicks (StarBro) were divided into nine experimental and one control group (three replicates of 10 birds each) and treated with either or both OTA and toxin deactivator. Commercially prepared compound feed (protein 19.65% and ME 2840 kcal/kg) was artificially contaminated with two levels (500 and 1000 ppb) of OTA and a mycotoxin deactivator containing yeast *T. mycotoxinivorans* (6.0 \(\times\) 10^8 cells/g, Biomin GmbH, Austria). Guidelines of ethical use of animals (Dua, 2004) were followed. The extraction of OTA from serum and tissues (liver and kidney) was carried out as described by Curtui et al. (2001) and Guillaume et al. (2005), respectively. For statistical analysis, one-way ANOVA test of SPSS 10.0 was used. Duncan Multiple range test was used to compare differences between means with significance at the \(P < 0.01\) level.
3. Results and discussion

At day 14, no sera of any experimental group tested positive for OTA. Whereas, at day 28 and 42, sera of all experimental groups fed OTA without or with supplementation of toxin deactivator (groups D through I) tested positive for OTA residue. Serum OTA levels in group D and those fed same level with supplementation of toxin deactivator at 1 and 2 kg/ton of feed (groups E and F) fell significantly (P < 0.01). A similar trend in OTA reduction (P < 0.01) was observed in group H and I fed higher OTA level (1000 ppb) with supplementation of toxin deactivator at 1 and 2 kg/ton of feed (Table 1).

As can be seen that at day 14, no serum sample was positive for OTA. A short feeding period of 14 days may account for these negative results. In addition, OTA tends to concentrate in liver and kidneys for detoxification and elimination (Fuchs, 1988). It may be conjectured that OTA metabolism is faster during early chick life and this may account in part at least for these negative findings. Later on at day 28 and 42, successively increased levels were recorded in all experimental groups. Higher levels in serum as compared to kidney and liver may be attributed to strong binding between OTA and serum albumin (Bakker and Pieter, 2002). Ochratoxin A in serum is an indication of the systemic exposure to this mycotoxin and may be a useful diagnostic tool in detecting ochratoxin A in farm and research setting.

Livers and kidneys of broiler chickens in groups with no dietary addition of OTA (groups A, B and C) were bereft of this toxin at day 42. OTA residues in livers of birds fed a low dietary level (500 ppb) of OTA (group D; no toxin deactivator) and those fed the same OTA level with incorporation of toxin deactivator at 1 kg/ton (group E) and 2 kg/ton of feed (group F) decreased non-significantly. However, a significant reduction in kidney tissue was recorded. On the other hand, inclusion of OTA at a higher dietary level of 1000 ppb without toxin deactivator (group G) or with toxin deactivator at 1 kg/ton of feed (group H) and 2 kg/ton (group I) was associated with significantly (P < 0.01) higher in hepatic and nephritic residual levels of this toxin than the groups (D, E and F) fed 500 ppb OTA (Table 1). A significant (P < 0.01) ameliorating effect in terms of significant toxin reduction in a dose-dependent manner was observed in treatment group F followed by E, I and H, respectively.

The presence of OTA in liver and kidney in the present study is in line with the findings of some previous studies (Biro et al., 2002; Stoev et al., 2004). The accumulation of OTA residues in tissues especially liver and kidney is probably due to enterohpatic recirculation and hepatobiliary excretion of OTA. These metabolic routes provoke a direct toxic effect of this toxin in these organs. In view of low levels of OTA found in tissues and a short half life of OTA in chicken (4 h) (Fuchs, 1988), it can be assumed that the concentration of OTA in tissues decreases quickly during the period of deprivation of chickens before slaughter. However, considering that OTA is involved in the etiology of human renal diseases notably Balkan endemic nephropathy (Stoev et al., 1998), as well as the higher levels in tissues of chickens exposed to 1000 ppb than groups fed 500 ppb, the possible exposure of humans to this very hazardous and relatively heat stable toxin through chicken products should not be ignored. In as much as a significant accumulation of OTA in liver and kidney was also observed in the present study, discarding these organs at slaughter could be an effective preventive measure. Furthermre, by supplanting with OTA free feed for several days before slaughter, chances for carryover of OTA residues into food chain can be minimized (Stoev et al., 2004, 2010). The data of present study demonstrated that concentration of OTA residues in broiler chicken followed the pattern serum > kidney > liver. Similar trend was documented for pig by previous workers (Patterson and Roberts, 1979; Curtui et al., 2001).

The findings of present study revealed that supplementation of feed with a mycotoxin deactivator makes a desirable contribution to lowering OTA levels in serum, kidneys and liver. We previously documented that in general, histological changes in kidneys, liver, bursa and spleen were less pronounced in birds receiving OTA and toxin deactivator concomitantly (Hanif et al., 2008). These results provide a base for comprehensive future research to fully assess interaction of OTA with other mycotoxins, drugs and nutrients. One of the limitations of present study is that it did not attempt to determine OTA residues in tissues other than liver and kidneys. Secondly, in present study only OTA was analyzed but not the metabolites. Thirdly, potential effect of the yeast on pharmacokinetic bioavailability of OTA was not determined. These limitations should be addressed in future OTA residue studies. In addition, under field conditions broilers are not only fed feeds tainted with low to high OTA levels, but also receive drugs requiring elimination through kidney and liver. How do these drugs moderate or accentuate OTA residue levels remains to be investigated.

References


Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>OTA added in feed (ppb)</th>
<th>Toxin deactivator (kg/ton of feed)</th>
<th>OTA in serum samples (ng/ml)</th>
<th>OTA in tissue samples* (ng/g)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 14</td>
<td>Day 28</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>BD</td>
<td>BD</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>1</td>
<td>BD</td>
<td>BD</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>2</td>
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<td>BD</td>
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<td>D</td>
<td>500</td>
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<td>29.41 ± 2.12</td>
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<tr>
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<td>16.24 ± 1.52</td>
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<td>BD</td>
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<td>20.79 ± 2.32</td>
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<tr>
<td>I</td>
<td>2</td>
<td>2</td>
<td>BD</td>
<td>13.82 ± 0.58</td>
</tr>
</tbody>
</table>

BD = Below detection limit i.e. 1 ng/ml and 1 ng/g.

* Means carrying same superscripts within same column do not differ significantly (P > 0.01).

* Number of liver and kidney samples examined in each group for OTA concentration = 5.