Deoxynivalenol and Ochratoxin A inactivation in broiler chickens’ feed

Received for publication, August 21, 2012
Accepted, December 17, 2012

HOREA SARANDAN1, MIHAI DECUN1, VIRGIL PAUNESCU2, VALENTIN ORDDODI3, FLORINA BOJIN2, IOAN HUTU1, CALIN POP1, SIMONA ZARCULA1, CAIUS BURIAN2, GABRIELA TANASIE2 and MIHAI SARANDAN1

1 Banat University of Agricultural Sciences and Veterinary Medicine Timisoara, Faculty of Veterinary Medicine
2 University of Medicine and Pharmacy “Victor Babes” Timisoara, Romania, Faculty of Medicine
3 University of Medicine and Pharmacy “Victor Babes” Timisoara, Romania, Faculty of Pharmacy
Address for correspondence: mihai.sarandan@gmail.com
Banat University of Agricultural Sciences and Veterinary Medicine Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului 119, Timisoara, Romania

Abstract

This work was designed to test the possibility of mycotoxin inactivation in broiler chicken feed contaminated with low doses of DON (1 mg/kg) and OTA (0.5 mg/kg) throughout a production cycle, evaluated in terms of growth parameters, histopathological lesions, haematological and immunological parameters.

There were not recorded mortalities during the experimental period, there were observed a few cases of diarrhea and feed regurgitation. The continuous exposure to DON and OTA for six weeks was reflected into histological lesions alteration of the blood biochemical and immunological parameters. The mycotoxins produced characteristic microscopic lesions in kidney, liver and crop samples; they increased the blood uric acid concentration, modified haemoglobin and red blood cell levels, but did not alter haematocrit, white blood cell counts or leukocyte formula. Both humoral and cellular immunological parameters were modified: serum lysozyme, properdin and phagocytic activity.

The inclusion of the mycotoxin inhibitor reduced the severity of histological lesions and had a significant effect on body weight, feed conversion ratio, serum lysozyme, properdin and phagocytic activity values, ensuring broilers’ protection against detrimental mycotoxin effects and thus providing a reliable feed reconditioning alternative.

Key words: histological lesions, lysozyme, phagocytic activity, properdin, uric acid.

Introduction

Mycotoxins are toxic substances produced mostly as secondary metabolites by filamentous fungi, mainly Aspergillus, Penicillium, and Fusarium species, which may be present in human foods or animal feeds [1].

Microbial spoilage is a problem because it can induce nutritional losses, off-flavors, formation of mycotoxins and potentially allergenic spores. Therefore, besides being an economic problem, unwanted fungal growth can cause some serious health hazards that have to be monitored carefully [2].

A wide spectrum of filamentous fungi is often found in various food commodities, where they can cause extensive damage. Although prevention of fungal growth and mycotoxin production on plants and in feedstuffs is usually considered as the best approach to impede the harmful effects on animal and human health, decontamination/detoxification of contaminated products is also of prime importance [3]. During a national research scale more than 100 toxinogenic moulds isolated from agricultural and food products [4].
Toxin-producing moulds may invade plant material in the field before harvest, during post-harvest handling and storage and during processing into food and feed products. Due to the multiple possible origins of fungal infection, any prevention strategy for fungal and mycotoxin contamination must be carried out at an integrative level all along the food production chain [5]. Although crop rotation [6] and prevention methods to avoid mycotoxin contamination on the field prior to harvest, during harvesting or storage have been developed, it is currently not possible to eliminate the risk of a mycotoxin contamination entirely [7]. Trichothecenes are closely-related sesquiterpenoids with an epoxy ring and a variable number of hydroxyl, acetoxy or other substituents, mostly produced by fungi of the *Fusarium* genus, commonly found world-wide on cereals. The trichothecenes causing most concern are T-2 toxin, which is the most acute toxic trichotheccene, HT-2 toxin, nivalenol and the most frequently occurring trichotheccene, deoxynivalenol (DON) [8]. Chronic exposure to DON at low doses causes growth retardation and immunotoxicity whereas much higher doses can interfere with reproduction and development [9].

Ochratoxin A (OTA) is a mycotoxin produced by several species of *Aspergillus*, and *Penicillium* fungi that structurally consists of a para-chlorophenolic group containing a dihydrioisocoumarin moiety that is amide-linked to L-phenylalanine. OTA is detected worldwide in various foods and feed sources and can have several toxicological effects such as nephrotoxic, hepatotoxic, neurotoxic, teratogenic and immunotoxic [10]. Upon ingestion, accumulation of ochratoxin A in the organism occurs predominantly in blood, liver and kidney [11].

The data on combined toxic effects of mycotoxins are generally limited, particularly with respect to trichothecenes, and it is known that the issue of combined toxicity is very complicated [12]. It has been reported that feeding mycotoxins in combinations could result in pronounced adverse effects in avians [13]. Considering the increasing food price indices [14] the inactivation of mycotoxins’ from contaminated feed becomes an important economic aspect to back up the use of new strategies for improving growth performance [15, 16].

The present study was designed to test the possible reconditioning of a dual mycotoxin contaminated feed, by inclusion of the mycotoxin deactivator Mycofix® Plus (MPL) from Biomin GmbH. The efficiency of the product was evaluated through the reduction of specific signs of mycotoxicosis reflected on the growth parameters, biochemical, hematologic and immunologic parameters, macroscopic and histological aspects of target organs (liver, kidney and crop) from one to 42 days of age. MPL action is achieved by adsorption, biotransformation and bioprotection. Adsorbents selectively bind and immobilize mycotoxins reducing toxin bioavailability while specific enzymes eliminate the toxicity of trichothecenes, zearalenone and OTA by altering their chemical structure. The integrated plant and algae extracts minimize toxin-related lesions by their hepatoprotective and immunomodulatory action expressed by increasing the secretion of anti-inflammatory cytokines, decreasing the secretion of pro-inflammatory cytokines and stimulation of the macrophages activity.

**Materials and methods**

**Experimental design**

The experiment was performed on 270 *Hybro G* broiler chickens, from one to 42 days of age. The broilers were randomly distributed into five even lots (T1- T5) and each lot was kept in six battery cages, with nine birds per cage, respecting the temperature, ventilation and lighting recommendations for this hybrid. Broilers were fed starter feed up to four weeks and grower feed from the fifth week until the end of the experiment, according to the *Hybro G* manual.
from Nutreco. The fodder had the same energy, protein, vitamin and mineral content for all lots. Pure mycotoxins (Biopure Referenzsubstanzen GmbH) premixed on fishmeal support and/or MPL (Biomin GmbH) were added into the feed in order to attain homogeneous concentrations. Lots T2, T3 and T4 were administered 1mg/kg feed DON and 0.5 mg/kg feed of OTA. The feed was supplemented with MPL in dose of 0.5 kg/ton for lots T3 and T5 and 1 kg/ton for lot T4. In order to verify uniform dispersion of the mycotoxins in feed, ten feed samples per lot were analyzed for OTA and DON both in contaminated and not contaminated feed. Mycotoxin levels were determined by High Performance Liquid Chromatography (HPLC) according to AOAC Official Methods (PVM 2:1997 for DON and method PVM 973.37 for OTA.

The broilers were weighed (Kern PEJ 4200) on the first experimental day and then weekly until the end of the experiment. All the birds were vaccinated at seven and 21 days against Newcastle disease and at nine days against Gumboro disease using water administered vaccines (Romvac Company).

At 42 days blood samples were taken from six birds from each lot for haematological, biochemical and immunological tests. The same birds were euthanized by exsanguination, subjected to necropsy examination. Samples of liver, kidneys and crop were collected for histological examination.

**Growth parameters**

All the birds were weighed weekly until the end of the experiment and average body gain was determined. The average feed intake and feed conversion ratio (FCR) were weekly determined for each cage.

**Necropsy and histopathology**

At slaughter, the liver and kidneys were examined, weighed and their weights were reported as percentage of body weight. For histopathological examination liver, kidney and crop samples were fixed in formalin-alcoholic solution (Merck), included in paraffin (Merck), sliced (Slee Mainz Cut 4062), stained using hematoxyline-eosine (H&E) (Merck), and examined by microscopy (Optika B-350) under 10, 20 and 40 magnification factors.

**Blood parameters**

Blood samples were taken using anticoagulant blood collecting tubes from the alar vein. Haemoglobin (Hb), haematocrit (Ht), erythrocytes (RBC) and leukocytes (WBC) numbers were determined by hemocytometry (MS 9-5 - Melet Schloesing). Red blood cell indices were calculated: mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC). The WBC formula was determined on peripheral blood smears using the May-Grunewald-Giemsas stain (Biometric Technology).

Serum uric acid levels were determined using an automated analyzer (Hospitex Eos Bravo Forte). Serum lysozyme concentration was determined by comparing the optical density (Specord 210) of a *Micrococcus lypseikticus* strain (ATCC 4698) after contact (30 minutes, room temperature) with the samples or the lysozyme standard (Sigma) [17]. Properdin was measured by the colorimetric assay [17] in the presence of Biuret reagent (Biometric Technology) after inulin-complexion (Sigma). Phagocytic activity (PA) was determined by the standard plate count method [17] using plate count agar (Oxoid,) and a reference *Staphylococcus* strain (ATCC 9144).

**Statistical analysis**

Data were analysed by ANOVA followed by a Tukey test (SPSS 15.0). Differences among means with P<0.05 were accepted as statistically significant. Values in tables are means and pooled standard errors of the mean (SEM).
Results and discussion

The HPLC analysis of the feed showed a homogeneous (coefficient of variation below 5%) distribution of the mycotoxins in the contaminated feed (lots T2, T3 and T4) while in the non-contaminated feed (lots T1 and T5) there were not detected either DON or OTA. Since mycotoxins’ effects depend on the dose and organism’s weight, the daily mycotoxin intake was calculated based on the OTA and DON concentration in feed, feed intake and body weight throughout the experiment (Table 1).

Table 1. Mycotoxin intake

<table>
<thead>
<tr>
<th>Lot</th>
<th>Experimental week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2</td>
<td>DON</td>
<td>144.04</td>
<td>155.87</td>
<td>146.06</td>
<td>124.93</td>
<td>95.62</td>
<td>76.42</td>
</tr>
<tr>
<td></td>
<td>OTA</td>
<td>72.02</td>
<td>77.94</td>
<td>73.03</td>
<td>62.46</td>
<td>47.81</td>
<td>38.21</td>
</tr>
<tr>
<td>T3</td>
<td>DON</td>
<td>143.21</td>
<td>146.84</td>
<td>137.74</td>
<td>117.43</td>
<td>96.62</td>
<td>78.81</td>
</tr>
<tr>
<td></td>
<td>OTA</td>
<td>71.61</td>
<td>73.42</td>
<td>68.87</td>
<td>58.71</td>
<td>48.31</td>
<td>39.41</td>
</tr>
<tr>
<td>T4</td>
<td>DON</td>
<td>122.70</td>
<td>149.84</td>
<td>136.52</td>
<td>109.89</td>
<td>88.01</td>
<td>77.93</td>
</tr>
<tr>
<td></td>
<td>OTA</td>
<td>61.35</td>
<td>74.92</td>
<td>68.26</td>
<td>54.95</td>
<td>44.01</td>
<td>38.97</td>
</tr>
</tbody>
</table>

Growth parameters

There were not recorded any mortalities during the experiment. On day one the average body weight of the broilers in all the lots was 37.54±1.56 g with a low variability (CV=0.44). Diarrhea and feed regurgitation were observed in a small number of cases in the first week in broilers from lots T2 and T3. Growth parameters for all the lots are shown in table 2.

Table 2. Growth parameters of the broilers in lots T1-T5

<table>
<thead>
<tr>
<th>Week</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>SEM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (g)</td>
<td>11.5±0.50</td>
<td>10.6±0.56</td>
<td>11.35±0.77</td>
<td>12.44±0.37</td>
<td>12.09±0.59</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Daily weight gain (g)</td>
<td>18.2±0.52</td>
<td>17.86±0.29</td>
<td>18.52±1.09</td>
<td>16.9±0.91</td>
<td>19.65±0.58</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Daily feed (g)</td>
<td>144.68±7.46</td>
<td>141.9±2.37</td>
<td>146.9±3.32</td>
<td>157.65±3.68</td>
<td>141.85±5.37</td>
<td>2.03</td>
</tr>
<tr>
<td></td>
<td>FCR (kg feed/kg gain)</td>
<td>1.59±0.04</td>
<td>1.71±0.10</td>
<td>1.64±0.06</td>
<td>1.36±0.07</td>
<td>1.64±0.06</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*a,b* significant difference (P<0.05)
There were observed statistically significant differences between the weights of the broilers in T4 and T2 (p=0.003) at 7 days of age, between lots T4 and T2 (p=0.005) and between T5 and T2 (p=0.002) at 14 days of age, between lots T4 and T2 (p=0.010) and between lots T5 and T2 (p=0.018) at 21 days of age, between lots T4 and T3 (p=0.048), between T4 and T2 (p=0.006) at 28 days of age, between T4 and T2 (p=0.005), between T4 and T3 (p=0.039) at 35 days of age, between T4 and T3 (p=0.012), and between T4 and T3 (p=0.018) at the end of the experimental period at 42 days of age. Statistically significant lower feed conversion ratios were recorded for lot T4 compared to T2 (p=0.012) in the first experimental week.

**Necropsy and histopathology**

Liver and kidney examination revealed no visible changes of colour or signs of renal or visceral uric gout. There were not recorded statistically significant differences between the weights of liver (p=0.118) and kidneys (p=0.707) in the experimental groups.

Histopathological examination showed hepatic, renal and crop lesions; the most severe were found in lots T2 followed by T3 and T4 (figure 1). No lesions were observed in lots T1 and T5 which were not exposed to mycotoxins.

![Figure 1. Histopathological aspects of kidney (K), liver (L) and crop (C) samples from lots T2, T3 and T4](image)

In lot T2 the proximal tubules were most affected showing swelling of the tubular epithelial cells and stenosis of the tubular lumen. There were also observed hypertrophy of the renal glomerules with disseminated focal necrosis and vascular lesions: ectasia, congestion and perivascular edema. In lot T3, less severe lesions were found: areas of disseminated focal necrosis with a partial disappearance of the tubular epithelium and granular degeneration of the cells in the tubular epithelium. In lot T4, renal lesions were still present, but less severe than in lot T3: the cytoplasm of the renal cells showed basophilic granulations of various sizes, with nuclei in different stages of necrobiosis, cortical hyper chromatosis, pycnosis and lysis of the nuclei.

Liver samples from lot T2 showed granular degeneration, fragmentation and dissociation of the liver cords, focal necrosis in the liver cords, granular-vacuolar degeneration, peri-ceniplobular cellular infiltrates, ectasia of the sinusoidal capillaries, and congestion of the interlobular vein with leuko diapedesis, erythro diapedesis and perilobular edema. In the samples from lot T3 the hepatic lobules generally maintained their architecture, with some areas of lipid infiltration of the hepatocytes and predominantly vascular lesions while in T4 the liver cords were maintained intact, with some focal granular perilobular and rare cytolysis. Vascular lesions were caused by active hyperaemia of the centrilobular vein. Crop lesions observed in samples from lot T2 consisted of cornification of the superficial epithelial layers, the presence of necrotic areas, ectasia of the vessels in the submucosa and inter-fascicle muscular edema with diffuse leukocytes infiltrate. In lot T3 there were observed: hypertrophy of the epithelium with keratinisation of the superficial layers, vascular...
congestions, perivascular edema and rare fibrin nets. In the samples from lot T4 the lesions were less severe: the germinative layer was intact (nuclei in different stages of mitosis) and there were some vascular changes in the submucosa, expressed as arterial congestions, leuko and erythro diapedesis, clots of fibrin in the submucosa and peri-fascicle edema in the muscular layer.

**Blood parameters**

The blood parameters measured at six weeks for all the lots (table 3) were statistically analyzed and compared to physiological reference ranges [18].

**Table 3. Biochemical and haematological parameters**

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>SEM</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht</td>
<td>0.31±0.01</td>
<td>0.31±0.01</td>
<td>0.31±0.01</td>
<td>0.30±0.02</td>
<td>0.30±0.01</td>
<td>0.005</td>
<td>0.940</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>8.78±0.27a</td>
<td>10.28±0.62</td>
<td>11.65±0.54a</td>
<td>10.55±1.05</td>
<td>9.28±0.47</td>
<td>0.326</td>
<td>0.036</td>
</tr>
<tr>
<td>RBC (10^6/mm³)</td>
<td>2.22±0.11</td>
<td>2.38±0.15</td>
<td>2.35±0.18</td>
<td>2.03±0.18</td>
<td>2.21±0.12</td>
<td>0.005</td>
<td>0.501</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>139.36±4.88</td>
<td>133.52±6.93</td>
<td>134.47±6.13</td>
<td>150.6±4.57</td>
<td>137.61±5.08</td>
<td>2.587</td>
<td>0.228</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>33.01±1.71</td>
<td>37.48±1.58</td>
<td>34.47±1.86</td>
<td>30.73±1.08</td>
<td>30.73±1.08</td>
<td>0.827</td>
<td>0.002</td>
</tr>
<tr>
<td>WBC (10^9/µL)</td>
<td>28.03±1.83</td>
<td>27±0.80</td>
<td>30.57±1.25</td>
<td>28.93±1.25</td>
<td>28.27±0.60</td>
<td>0.553</td>
<td>0.349</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.75±0.54a, 8.13±0.34ab</td>
<td>7.57±0.18a</td>
<td>6.14±0.44b</td>
<td>6.13±0.47b</td>
<td>0.244</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Lysozyme (µg/mL)</td>
<td>28.87±1.96a</td>
<td>16.03±1.18ab</td>
<td>18.82±0.76a</td>
<td>21.53±2.07</td>
<td>24.37±2.77b</td>
<td>1.137</td>
<td>0.001</td>
</tr>
<tr>
<td>Properdin (µg/mL)</td>
<td>26.73±2.01a</td>
<td>16.25±0.47ab</td>
<td>19.44±0.59abc</td>
<td>22.24±0.69b</td>
<td>24.76±1.83bc</td>
<td>0.878</td>
<td>0.0001</td>
</tr>
<tr>
<td>Phagocytic activity</td>
<td>0.30±0.021a,b</td>
<td>0.19±0.017ab</td>
<td>0.19±0.01b</td>
<td>0.27±0.01ab</td>
<td>0.37±0.02ab</td>
<td>0.0144</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*data were analysed by means of ANOVA followed by a Tukey test

a, b, c significant difference (P<0.05)

Hematocrit values were between normal limits and there were no statistical significant differences between the lots. Statistically significant (p = 0.032) higher Hb values were observed for T3 when compared to T1, and also for MCHC between T3 and T1 (p=0.002) and between T3 and T5 (p=0.022). WBC counts were between physiological limits and there were no significant differences between lots and also for the WBC formula.

The high levels of uric acid measured for lots T2 and T3, above the reference values (6.28±1.06 mg/100 ml) [17], are related to the renal lesions which hamper excretion. Uric acid concentration in the serum of the birds showed statistically significant differences between the lots. It was significantly higher in lots T2 (p=0.03) and T3 (p=0.034) compared to T1 and in lot T2 compared to T4 (p=0.017) and to T5 (p=0.016). There were no significant differences between lots T4 and T1.

There were recorded statistically significant differences for lysozyme values between lot T1 and T2 (p=0.001), T3 (p=0.009) and also between lot T2 and T5 (p=0.037). The recordings for properdin followed the same pattern as for lysozyme: there were differences between the control lot T1 and T2 (p=0.000), T3 (p=0.004), and between T2 and T4 (p=0.02), and T5 (p=0.001), and between T3 and T5 (p=0.046).

The phagocytic activity values recorded in the lot fed mycotoxins without MPL, T2, were significantly lower compared to T1 (p=0.001), T4 (p=0.022) and T5 (p=0.000). Lot T3 had also a statistically significant lower phagocytic activity compared to T1 (p=0.001), T4 (p=0.035), and T5 (p=0.000).
Although both DON and OTA were present at low concentration, they did influence the health status and production characteristics of the broilers. The symptoms of mycotoxicosis manifested by the broilers in the experimental lots consisted of alteration of growth parameters, histopathological lesions in the kidneys, livers and crops, changes of the blood and immunological parameters.

Based on the histopathological lesions, which are mostly characteristic for OTA intoxication and since the ingested dose was closer to the LD50 for OTA compared to DON, it may be assumed that it was the mainly responsible for these changes. Nephritis signs were present in the kidneys of the broilers in lots T2, T3 and T4, similar to previously described lesions and also histological studies of mycotoxins’ effects on broilers’ livers report mononuclear cellular infiltration when fed 0.8 ppm OTA up to four weeks of age [19]. Similar lesions were reported when administering 2 ppm of OTA in broiler feed up to six weeks of age [20]. Compared to these studies, the lesions observed in this experiment may be attributed to the mixed mycotoxin exposure, longer exposure time or both. Previous research also indicate the increase of relative weight of gizzard, erosions of the proventriculus’ mucosa, as a consequence of DON irritation of the upper gastrointestinal tract, while other studies report no lesions due to DON [21] or that OTA and DON interact additively [22]. In this experiment there were observed only histological lesions of the proventriculus, probably due to the low exposure, but the irritation of the mucosa was clinically confirmed by a few cases of feed regurgitation.

The LD50 for one day old chickens is 2.14 µg/g and 3.6 µg/g for three weeks old birds [22]. For DON, the LD50 consecutive to oral administration was determined to be 140 mg/kg bw [23]. The minimal dietary growth inhibitory OTA dose for chickens is 2 µg/g [22], while for DON studies report that dietary concentrations below 15 mg/kg had no adverse effect on body weight gain, feed consumption, or feed efficiency of broilers and report an increase in feed efficiency in broilers fed a diet with 16 ppm DON making it impossible to establish a simple dose-response relationship between growth depression and dietary concentrations of DON for broilers [24].

In the present experiment the maximum OTA level was 77.94 µg/kg and for DON it was 155.87 µg/kg for lot T2 in the second week. The low mycotoxin exposure did not affect broiler’s body weight and there were no significant differences between the control lot T1 and lots fed mycotoxins. No significant weight differences were found between T5, T4 and T1 suggesting that MPL does not have a significant effect as a growth promoter. Still, the combined OTA and DON effects became visible in the second and third experimental week when lot T2 was compared to lot T5. The low age of the broilers, higher mycotoxin daily intake per body weight, especially in the second week, for T2, opposed to the addition of MPL administered to T5 led to a statistically significant difference. This difference became clear when comparing T4 to T2 where were recorded significant differences throughout the experimental period. The significant differences which appeared from the fourth experimental week to the end of the experiment between T4 and T3 are due to the higher mycotoxins’ ratio per kg of body weight for T3 from the fourth week, and to the higher MPL content of the feed administered to T4. Although low doses of mixed DON and OTA did not have a detrimental effect on broiler’s body weight (no differences between T1 and T2) the inclusion of MPL has a significant effect on broiler’s body weight, and from the fourth week forth an increased dose of MPL should be used.

The lower feed conversion ratios recorded for lot T4 compared to T2 may be explained as a reduced metabolic effort to counteract mycotoxin effects in chickens, especially in the first week of life, when broilers are most vulnerable.
The higher uric acid concentrations observed for lot T2 compared to T1, T4 and T5 shows that the combined effects of DON and OTA, even at low doses modify the excretory activity, and impede especially the clearance of uric acid. The difference between T3 and T1 but not between T4 and T1 suggests, as observed for body weight, that a higher inclusion of MPL ensures a better protection. The statistical analysis of uric acid serum concentration confirms the extent of renal lesions observed during the histological examination. Previous studies report a significant increase of the uric acid levels in birds fed OTA contaminated diets at similar [19] or four times the mycotoxin levels used herein [25]. In chicken, OTA’ intestinal absorption is at about 40% [26] and the elimination half-life, of 4.1 hours following oral administration [27], is longer in blood than in tissues [26] which confirms the differences observed between the lots for the haematological examination. The statistical analysis of uric acid serum concentration confirms the extent of renal lesions observed during the histological examination. The haematological and immunological parameters measured expressed the most visible effects of the intoxication, with the most significant differences between the lots. This may be explained by the persistence of mycotoxins in the blood stream, bound to serum proteins [26] and to DON and OTA induced changes in the hematopoietic system of [28].

Haemoglobin levels were within the reference values for all the samples measured. Previous research shows haematocrit modification in birds fed Fusarium mycotoxins only after the eighth week and no modification of the haemoglobin or MCHC [29]. Other research shows the mould-contaminated (OTA, aflatoxin and T-2 toxin) diet significantly increased the levels of WBC, Hb and Ht, and significantly decreased RBC level of broilers up to 43 days of age [30].

The lower lysozyme values recorded in lot T2 compared to T1 and T5 signify an impairment of humoral immunity due to the mycotoxin mix. The difference between lot T3 and T1 suggests once again a higher dose of MPL should be used. The function of natural cellular defence, expressed by the phagocytic activity, was also affected by OTA and/or DON contamination of feed. As previously described, OTA decreases phagocytosis and locomotion of heterophils and monocytes [31]. There was also reported severe lymphocytolysis and depletion with cellular sparsity in the lymphoid organs of OTA treated groups [32]. Under field conditions, there may be chances of increased secondary infections in those birds exposed to mycotoxin contaminated diets in addition to other stressors [13]. For the phagocytic activity there were recorded more statistically significant differences between the lots, probably to the more subtle factors involved in cellular immunity. Differences between T2 and T1 and between T3 and T1 reflect the effect of the mycotoxins, whereas when compared to T4 and T5 the stimulation of the macrophages activity of the components in MPL may also be implied. Similar studies report significantly decreased phagocytic activities and locomotion of heterophils in broilers fed 4 and 8 μg OTA/g for 21 days, and a reduction in bactericidal activities of heterophils in groups fed 8 μg OTA/g diet [13]. Compared to their results, the reduction of the phagocytic activity tested herein, at a much lower exposure to OTA may be the result of the mixed DON and OTA effects.

The reduced cellular defence functions correlates to the parameters of the humoral immunity. The difference between lots T4 and T5 (p=0.003) but not between them and the control lot T1, suggests a slight immune stimulatory activity of some of the components in MPL together with a residual cellular toxicity of the feed from T4. The inclusion of MPL at 0.5 kg/ton in T3 was not high enough to cover negative effects of combination of DON and OTA on phagocytic activity. Biotransformation of OTA has not been elucidated in detail and the data regarding OTA metabolism are controversial. The contribution of metabolites in OTA toxicity is still unclear [26]. The lower phagocytic activity of lot T3 compared to T2 may be due to incomplete degradation of mycotoxins to secondary metabolites which may interfere the immunological mechanisms.

Overall, these data show an impairment of both humoral and cellular immunity due to low doses DON and OTA, which is compensated by the inclusion of 1kg/ton MPL.
Conclusions

The inclusion of 0.5 mg/kg OTA and 1 mg/kg DON in broilers’ feed from one to 42 days of age causes detrimental effects on birds’ health, expressed as histological lesions and alteration of the blood parameters and innate humoral and cellular immunity. Histological lesions observed in observed kidney (also emphasized by high uric acid levels), liver and crop samples were mainly characteristic for OTA intoxication, but their presence at a lower dose compared to other studies investigating OTA intoxication alone, suggests the added effect of DON. Reduced innate humoral and cellular parameters are also a consequence of the combined effect of DON and OTA. The growth parameters were not significantly influenced by this mycotoxin combination, and there were not recorded mortalities. Still, in farm conditions, the influence of decreased immunity may be reflected as higher incidence of secondary infections leading to economical loss. The addition of MPL diminished the severity of histological lesions, significantly improved biochemical and immunological parameters and also had a significant effect on body broilers’ weight. Our study suggests that from the fourth week forth the feed should be supplemented with 1% MPL in order to attain a reliable feed reconditioning alternative.

Acknowledgements

This work was published during the project “POSTDOCTORAL SCHOOL OF AGRICULTURE AND VETERINARY MEDICINE”, POSDRU/89/1.5/S/62371 and “BIOTEHNOLOGII CELULARE SI MOLECULARE CU APLICATII IN MEDICINA” - POSDRU/89/1.5/S/60746, co-financed by the European Social Fund through the Sectorial Operational Programme for the Human Resources Development 2007-2013.

References

Deoxynivalenol and Ochratoxin A inactivation in broiler chickens’ feed