EFFECT OF FUMONISIN B1 ON LYMPHATIC ORGANS IN BROILER CHICKENS - PATHOMORPHOLOGY

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Abstract

The purpose of this study was to establish the pathomorphological alterations in the lymphatic organs of broiler chickens fed forages containing fumonisin B1 (FB1), known to cause immunodeficiency. The birds were treated with mycotoxin isolated from corn contaminated with F. verticilloides (moniliforme). Apoptotic and necrotic changes were recorded under light and electron microscopes. Significant morphological alterations such as focal necrosis and an increased number of apoptotic lymphocytes and epithelial cells were found mainly in the thymus and spleen, while the bursa Fabricii was less affected. It can be concluded that FB1 causes in vivo suppression of development, differentiation, and function of the lymphoid cells and the stromal epithelial reticular cells, and activates the processes leading to cell death in organs of the immune system of chickens.

Key words: broiler chickens, lymphatic organs, fumonisin B1, pathomorphology.

The presence of fumonisins in fodder poses a dangerous threat for the poultry industry. These mycotoxins are produced by fungi of the genus Fusarium and are considered to be immunotoxic (6, 11, 19). They can cause various health disorders of great economic importance because of the compromised immune system and inadequate postvaccinal immune response. That is why the study of the feed-born mycotoxicoses and particularly fumonisins, with their diverse effects is very important.

A number of pathological effects of fumonisins were reported in birds: haemorrhages, leukocyte infiltrations, fatty infiltration, necrotic lesions, fibrosis in the liver, kidneys, lungs, heart, intestines, gizzard, bursa of Fabricius, and pancreas, oedema and haemorrhages in the brain (7), thymic cortical atrophy (20), multifocal hepatic necrosis, and biliary hyperplasia (9). Moreover, a reduction in the size of the spleen along with a depletion of white pulp, thinning of cardiomyocytes, lymphoid cell depletion from bursal follicles, and renal tubular nephrosis were also observed (4). Electron microscopy (EM) studies revealed nuclear disintegration in the chicken peritoneal macrophages (1), cytoplasmic and nuclear enlargement in cells of the liver, lungs, kidneys, heart, and pancreas, thickening of the membranes of the smooth endoplasmic reticulum and dilation of the rough endoplasmic reticulum with loss of ribosomes together with vacuolated and deformed mitochondria (7). However, EM investigations on the ultrastructure of the immune system organs in FB1-treated chickens are quite insufficient.

The purpose of this study was to establish the pathomorphological alterations in the thymus, spleen, and bursa Fabricii in chickens experimentally fed forages containing fumonisin B1, known to cause immunodeficiency.

Material and Methods

Corn with 40% humidity was contaminated with a culture of F. verticilloides and cultivated for 14 d at 20-22°C. Fungal cultures were dehydrated at 105°C to 13% humidity and the content of fumonisin B1 (FB1) was determined by ELISA (Ridascreen, R-Biopharm AG, Germany). No other mycotoxins were detected. The fodder for the chickens was prepared with a concentration of the toxin amounting to 10 mg/kg of corn, which is the most frequent pollution of crops in Bulgaria.

Fifty broiler chickens (hybrid Cobb) were allotted into experimental and control groups. The experimental group consisted of 40 chickens, which were fed FB1 contaminated fodder from the 7th d post
hatching to the 45th d of their life. The control group of 10 chickens was fed mycotoxin-free fodder for the same period. All the procedures performed on animals were approved by the local Animal Care and Use Committee. The chickens were euthanized on day 45 and autopsies were performed. The lymphatic organs were observed for their position, size, colour, and texture. Samples from the thymus, spleen, and bursa of Fabricius were routinely processed for histopathological examination and stained with haematoxylin and eosin. The observation was carried under light microscope (Leika DM 500B, Wetzlar, Germany) and images were recorded using a connected digital camera. Lymphocytes, lymphoblasts, and epithelial reticular cells were counted and the numbers of normal, apoptotic, necrotic, and mitotic cells were evaluated. Results were expressed as mean ± S.E.M. The statistical significance was evaluated by one-way ANOVA, followed by Bonferroni’s post hoc test using GraphPAD InStat (Software, USA). Values of P<0.05, P<0.01, and P<0.001 were considered significant.

For EM observation, tissue samples from the immune system organs were fixed in 4% glutaraldehyde for 24 h and postfixed in osmium tetroxide for 2 h. After dehydration in absolute ethanol and acetone, the samples were processed in propylene oxide and embedded in Durcupan. Ultrathin sections were contrasted with uranyl acetate and lead citrate and observed under a JEOL 1200 EX transmission electron microscope.

Results

Gross pathology examination revealed no changes in the size, colour, and texture of the examined organs. In rare cases only single haemorrhages in the thymus and in the bursa Fabricii were detected.

Microscopic examination showed that mainly the cortex and, to a smaller extent, the medulla of the thymus, and the white pulp and marginal zone of the spleen were affected in the experimental animals. The following alterations, characteristic for the process of cell death were observed in the lymphocytes, lymphoblasts, and epithelial cells: brightened, vacuolated or shrunk cytoplasm, polymorphic, fragmented or pyknotic nuclei, and chromatin margination. Necrotic epithelial reticular cells with blurred extensions were detected in the medulla and in the perivascular spaces of the thymus and white pulp of the spleen. The bursa Fabricii was slightly affected and only the number of the necrotic epithelial cells was significantly elevated (P<0.01) in the experimental group in comparison with the control one (Table 2). The number of apoptotic and necrotic epithelial cells and the number of apoptotic lymphocytes in the thymus and spleen, as well as the number of necrotic lymphocytes in the thymus were significantly elevated (P<0.001 and P<0.05, respectively) in the treated birds, compared to the controls (Tables 1 and 2). A difference in the number of the cells with morphological signs of mitosis was not noted in both groups (Tables 1 and 2). Only single macrophages were observed in the examined organs.

The observed changes are presented in the following figures:
### Table 1
FB1 effects on the lymphocytes in the organs of the immune system of chickens

<table>
<thead>
<tr>
<th></th>
<th>lym bursa c</th>
<th>lym bursa ex</th>
<th>lym spl c</th>
<th>lym spl ex</th>
<th>lym thy c</th>
<th>lym thy ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>23.81± 3.98</td>
<td>32.44±2.29</td>
<td>14.19±2.74</td>
<td>39.56±3.96***</td>
<td>21.50±3.18</td>
<td>51.56±2.46***</td>
</tr>
<tr>
<td>nec</td>
<td>5.93±1.18</td>
<td>8.06±1.19</td>
<td>1.31±0.44</td>
<td>4.87±1.38</td>
<td>3.43±0.63</td>
<td>8.93±1.96*</td>
</tr>
<tr>
<td>mit</td>
<td>1.87±0.6</td>
<td>0.31±0.19</td>
<td>2.0±0.42</td>
<td>0.75±0.32</td>
<td>1.8±0.49</td>
<td>0.87±0.38</td>
</tr>
</tbody>
</table>

Mean ± S.E.M.; apop-apoptosis; nec-necrosis; mit-mitosis; lym-lymphocytes; bursa-*bursa Fabricii*; spl-spleen; thy-thymus; c-control; ex-experimental group; *P<0.05, **P<0.01, and ***P<0.001.

### Table 2
FB1 effects on the epithelial cells in the organs of the immune system of chickens

<table>
<thead>
<tr>
<th></th>
<th>ep bursa c</th>
<th>ep bursa ex</th>
<th>ep spl c</th>
<th>ep spl ex</th>
<th>ep thy c</th>
<th>ep thy ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>1.43±0.27</td>
<td>2.81±0.35</td>
<td>1.62±0.29</td>
<td>8.81±1.05***</td>
<td>4.06±0.51</td>
<td>11.81±0.86***</td>
</tr>
<tr>
<td>nec</td>
<td>1.12±0.29</td>
<td>2.81±0.35**</td>
<td>0.81±0.19</td>
<td>3.75±0.39**</td>
<td>1.69±0.23</td>
<td>4.93±0.29***</td>
</tr>
<tr>
<td>mit</td>
<td>0.25±0.09</td>
<td>0.18±0.09</td>
<td>0.5±0.16</td>
<td>0.18±0.09</td>
<td>0.43±0.14</td>
<td>0.18±0.09</td>
</tr>
</tbody>
</table>

Mean ± S.E.M.; apop-apoptosis; nec-necrosis; mit-mitosis; ep-epithelial reticular cells; bursa-*bursa Fabricii*; spl-spleen; thy-thymus; c-control; ex-experimental group; *P<0.05, **P<0.01 and ***P<0.001.

**Fig. 4.** Chicken on FB1 diet; thymus cortex: A) nucleus (N) of a lymphocyte with an enlarged perinuclear space (arrow) and an atrophic nuclear membrane; B) elliptic formation in the site of the RER cisternae (GER).

The electron microscopic study revealed morphological alterations characteristic for apoptosis in the lymphocytes in the thymus cortex and white pulp of the spleen of FB1-treated birds. The shape of nuclei was altered and an enhanced perinuclear halo and margination of the heterochromatin were observed (Fig. 4-A). The double layer structure of the nuclear membranes was difficult to distinguish. The ratio of the heterochromatin and euchromatin remained unaltered, but the heterochromatin was highly compacted. The RNP structures - the perichromatin and interchromatin granules were difficult to observe in the euchromatin space. The dense fibrillar component and the granular components in the nucleoli were hardly discerned. Free ribosomes and groups of polysomes were found in the cytoplasm. Well shaped cisternae of ER were not visible in the lymphocytes. The RER in the T-cells represented elliptic compact structures (Fig. 4-B). The mitochondria,
in most cases, had lost their characteristic pattern. Empty vacuoles surrounded by residues from double-layer membranes and a complete lysis of the mitochondrial cristae were found. The structures of Golgi complex were not detected in the thymus and spleen samples. Erythrocytes with stratified nuclear membranes, increased perinuclear spaces, and leakage of nuclear content were observed in the spleen of FB1-treated birds (Fig. 5).

Unlike in thymus and spleen, the pathological alterations in the lymphocytes from the bursa Fabricii were not so pronounced. The hetero-euchromatin ratio in the nucleus was preserved. The RNP structures and the nucleolar subcomponents were clearly distinct. In rare cases alterations in the mitochondria with lysis of the cristae were found. The ER and Golgi complex structures were not easily discernible.

**Discussion**

The presented data showed that birds treated with fumonisin B1 developed significant pathomorphological alterations mainly in the thymus and spleen and to a lesser extent in the bursa of Fabricius. In this study apoptosis in the cortex of the thymus cannot be explained solely by the natural turnover of the T-lymphocyte population, since the number of apoptotic lymphocytes was significantly higher compared to the controls, as a main consequence of the FB1 treatment. Our results are similar to those described by Sharma et al. (16, 17) who reported that FB1 induces additional cytotoxicity due to a local activation of cytokines produced by T-cells, leading to an increased cell death. Similar alterations in the RER, observed in this study, were reported by Deshmukh et al. (3) and Javed et al. (7). It is probable that the RER alterations refer to the phases of organelle destruction in the process of cellular apoptosis following the FB1 impact. This could account for the widespread destruction of the mitochondrial structures and vacuolisation of the cytoplasm. The effect of FB1 on the epithelial cells can influence the endocrine function of the thymus and thymosin secretion, which regulates the proliferation and differentiation of T-lymphocytes and, in consequence, their function could be detrimentally impaired.

The extensive apoptotic and necrotic changes in the spleen of chickens fed FB1-polluted crops disturb the performance, proliferation, and activation of T and B lymphocytes and their antigenic response, resulting in the absence of plasma cells and antibody production (lack of ER structures). Decreased spleen cell viability and mitogenic response caused by FB1 were reported by Keck and Bodine (8). The impaired erythrocytes in the spleen of the FB1-treated chickens could lead to a poor oxygen supplementation and disruptions in the protein exchange, which would eventually result in dystrophic changes. Abnormal erythrocytes in broilers fed forages containing FB1 and FB2 were described by Dombrink-Kurtzman et al. (5). In our experiment, the bursa Fabricii remained relatively devoid of toxic damage, although an elevated number of apoptotic B-lymphocytes were recorded, even though, the difference was insignificant when compared to controls. However, the apoptotic processes could compromise parts of B-lymphocyte proliferation and could lead to a suppression of humoral immunity. In turkeys, which receive feed contaminated with *Fusarium equiseti* and *Fusarium moniliforme*, a suppression of humoral immunity was reported (2, 10). In addition, a significant reduction of antibody titres in mycotoxin-treated birds has been shown (18). The diminution of macrophages content and their activity in the organs of the avian immune system, together with significantly affected tissues could be attributed to the effect of FB1. Chatterjee et al. (1) reported that dietary intake of FB1 may damage the macrophages in consumers rendering the latter susceptible to infection. Studies in chickens have shown that FB1 caused dose-dependent death of peritoneal macrophages (15). Moreover, it has been reported that *Fusarium proliferatum* culture material caused a 34% lower phagocytic activity of macrophages and a significant suppression in the total Ig and IgG levels in chickens (14). It is evident that the altered T-lymphocytes cannot accomplish an effective cooperation with the antigen-presenting cells, which are also affected by the toxin. Several in vitro studies indicate that fumonisin-induced changes in the key enzymes involved in the cell cycle regulation, differentiation, and apoptosis are initial or secondary, and trigger the process of cell death (12), which could result in pleiotropic effects such as modulation of the transcription and activation of enzymes involved in signal transduction (13).

It can be concluded that FB1 in vivo strongly suppresses the development, differentiation, and function of the lymphoid cells and stromal epithelial reticular cells and activates the processes leading to cell death in the organs of the chicken immune system. This will inevitably cause disruption in cellular and humoral
immune competence and also may affect certain endocrine functions of the thymus.

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References