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PATHOHISTOLOGICAL CHANGES IN KIDNEY AND LDH ACTIVITY IN BROILER TREATED WITH DIFFERENT DOSES OF OCHRATOXIN A

ABSTRACT: The three-week long trial was performed on day-old Hybro broilers divided into four groups. After 14 days long preexperimental period, the experimental groups were offered feed contaminated with 0.5, 1.0 and 1.5 ppm OA during 7, respectively. At the end of the trial blood and kidney samples were taken for investigations.

In broilers feed with 1.5 ppm of OA histopathological examination of the kidney tissue revealed changes located in proximal tubules. Some cells were dim and swollen. These changes produced particular or total reduction in tubular lumen of kidney. Acute tubular necrosis existed in some of tubulocytes in form of small foci. Fragmentation of necrotic mass and presence of fresh red blood cells were also detected.

The LDH activity was significantly greater in broilers of experimental groups compared with control group.

All presented data indicated that intensity of pathohistological alterations and LDH activity depends upon dietary OTA level. Positive correlation between pathohistological changes and increased LDH activity caused by OTA was noticed. Thus, LDH activity measure could be used as early diagnostic tool in measuring changes caused by OTA.

KEY WORDS: ochratoxin, broiler, kidney, LDH

INTRODUCTION

Ochratoxins are highly toxic compounds commonly produced as secondary metabolites by two species of fungi: *Penicillium verrucosum* Dierckx and *Aspergillus ochraceus* Wilhelm (*alutaceus*) (Friskyud and Samson, 1991). In recent years, ochratoxin A (OA) has received considerable attention because it can not only seriously affect animal performance and health, but it may also have deleterious effects on humans. Of greatest concern in humans (Marquardt and Frohlich, 1992) is its implicated role in an irreversible and fatal kidney disease (Balkan endemic nephropathy).

Microscopic lesions in ochratoxicosis are most prominent in the kidney. On light microscopy, severe distension, enlargement and hypertrophy of the renal proximal convoluted tubules and thickening of the glomerular basement membrane are seen in kidney sections of broilers receiving 2—4 ppm dietary OA for 20 days. (Dwivedi and Burns, 1984). The same pathohistological changes were reported by Mraz and Kosutzky (1992), after feeding broilers with 0.85 ppm OA during 42 days. Pathohistological examination revealed epithelial dystrophy of proximal tubules, presence of eosinophilic granulocytes in tubular lumen, glomerular dystrophy and cell infiltrate of intertubular space.

The broilers' kidneys have high activity of LDH comparing to the other animals (Cubena, 1974). Increased serum LDH was reported only in broilers with kidney disorders. Kubena and Harvey (1994) described a significantly increased LDH activity in broilers treated with 2 ppm OTA for 21 days. Ayed (1991) also described an increased LDH activity in broilers treated with low doses of OTA (0.5 ppm/7 days).

The present study was, therefore, designed to assess the effect of short-term treatment with graded levels of dietary OA on the pathohistological changes in kidney tissue of broilers, as well as correlation between pathohistological changes and increased LDH activity caused by OTA.

MATERIAL AND METHODS

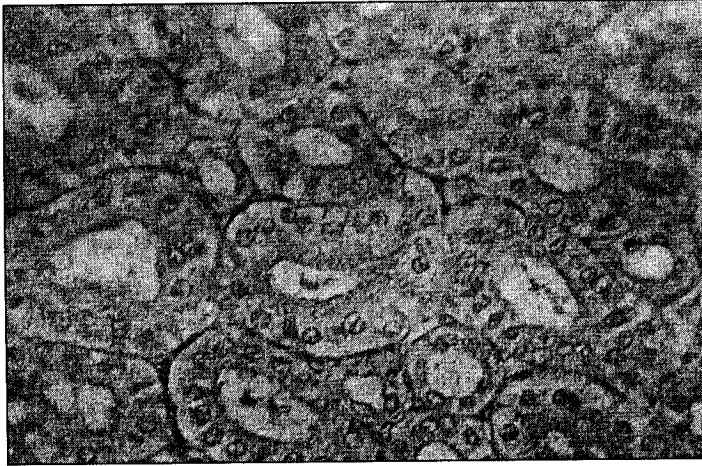
Experimental design. After 14 days long preexperimental period, a total of 48 broilers were submitted to the trial. Birds were divided into three experimental groups (A, B, C), and one control group (K). Experimental groups were fed with contaminated feed.

Diet. All groups of broilers were fed with commercial mash, which consisted of standard feedstuffs and contained enough nutrients to meet all requirements. In the mixture for A, B and C experimental groups the 99% pure ochratoxin A (Sigma, O — 1877), obtained from *Aspergillus ochraceus* culture (303-47-9), was added in an amount enough to provide 0.5, 1.0 and 1.5 mg OTA/kg of feed, respectively.

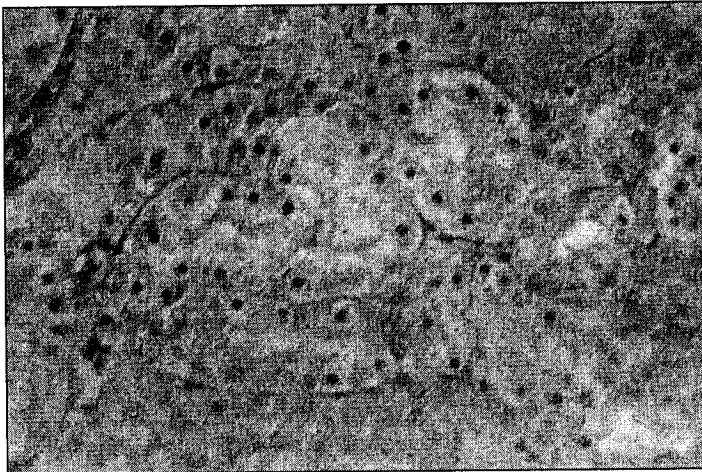
Sample collection. Kidney and blood samples were taken after the period of toxin administration (21st day). In the shortest possible period the samples of kidney were taken for histological investigation. Kidney samples were fixed in 10% neutral formalin and absolute alcohol and were formed in paraffin. Thickness of the cut was 5—8 μm and they were stained (Scheur and Chalk, 1986) using standard methods (HE). Also, using an automated, clinical-chemistry analyzer (SMAC Technicum 3000) the determination of the serum activities of LDH was done.

RESULTS

Histopathological changes in kidney were not detected in broilers of control group and broilers of experimental groups fed with 0.5 and 1.0 ppm of OA.

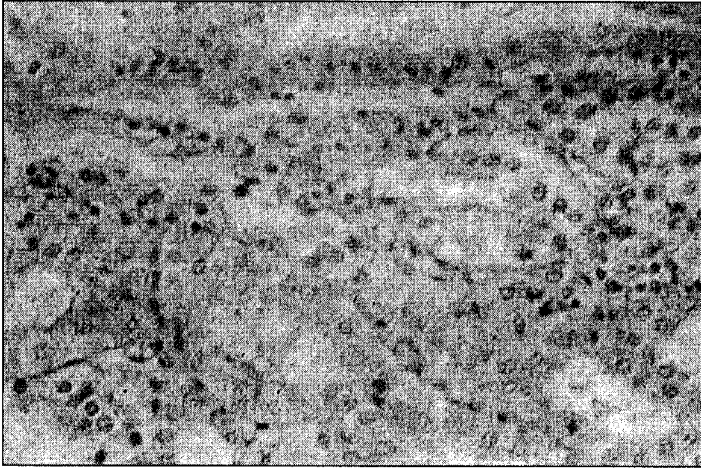


In broilers fed with high doses of OA (1.5 ppm) during 7 days period histopathological examination of the kidney tissue revealed changes located in proximal tubules. Some cells were dim and swollen. These changes produced particular or total reduction in tubular lumen of kidney.

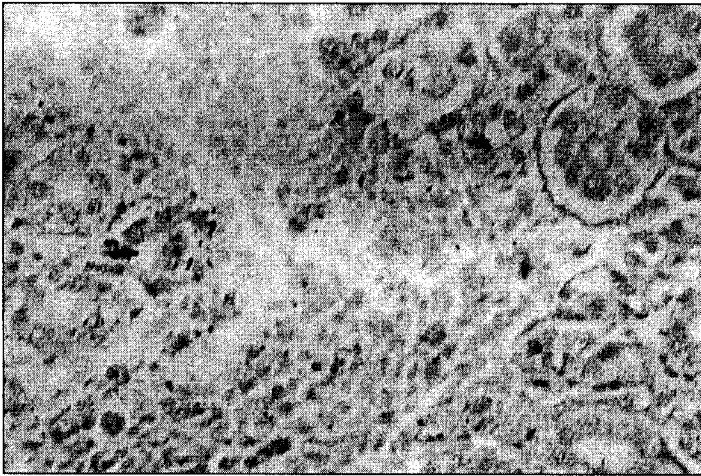


Basement membrane of epithelial cells was intact. Cytoplasm of tubulocytes was filled with fine granules and nucleus was masked. Also, reduction in volume and hyperchromatosis of nucleus in epithelial cells of proximal tubules was detected. In some of epithelial cells in changed tubules light vacuolization was expressed and transparent cytoplasm was detected.

Acute tubular necrosis existed in some of tubulocytes in form of small foci. Fragmentation of necrotic mass and presence of fresh red blood cells were also detected. In two of six sacrificed broilers intensive extravasation near necrotic center was found. The structure of glomeruli was better preserved than tubules; thus their normal formation is more easily observed.



The LDH activity was significantly greater in broilers of experimental groups compared with the control group.



DISCUSSION

In our trial pathohistological changes were detected in tissue samples of animals receiving 1.5 mg OA/kg feed during 7 days. According to our findings, K u b e n a et al. (1989) detected in broilers fed 2 mg OA/kg feed enlargement of kidney epithelial cells with dark nuclei, which indicate early degenerative changes in proximal tubules of intoxicated animals. Similar changes, although with almost double dose of toxin, were described by H a r v e y et al. (1987), who fed broilers with 3.5 mg OA/kg feed during 28 days. Pathohistological changes included dilatation and necrosis of tubules.

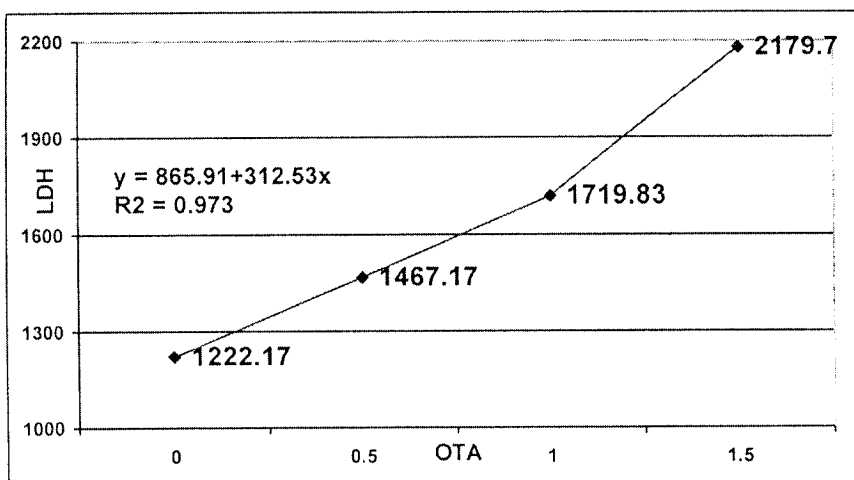


Figure 1. The enzyme LDH concentrations in broilers 21-days of experiments

Stojković et al. (1984) presented persistence of a protein fraction with small molecular weight in blood (20.000 Da) which binds OA more specifically than plasma albumins. The authors concluded that the binding of OA to this protein might be relevant to its predominant nephrotoxic effect, because such molecules can easily pass through the normal glomerular membrane, enabling the accumulation of OA in the kidney.

All described pathological changes induced after OA treatment had a primary localization in proximal kidney tubules and could be connected with toxin metabolism. Increasing LDH activity could indicate alteration of tubulocytes related with OTA effect.

All the presented data indicated that intensity of pathohistological alterations and LDH activity depends upon dietary OTA level. A positive correlation between pathohistological changes and increased LDH activity caused by OTA was noticed. Thus, LDH activity measure could be used as an early diagnostic tool in measuring changes caused by OTA.

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**ПАТОХИСТОЛОШКЕ ПРОМЕНЕ У БУБРЕЗИМА БРОЈЛЕРА И
АКТИВНОСТ ЛАКТАТ ДЕХИДРОГЕНАЗЕ ТРЕТИРАНИХ
РАЗЛИЧИТИМ КОЛИЧИНАМА ОХРАТОКСИНА А**

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Резиме

Оглед је изведен на Нубро-бројлерима подељеним у четири групе и трајао је 21 дан. Након четрнаестодневног припремног периода огледне групе су храњене храном контаминираном охратоксином А у количини од 0.5; 1.0 и 1.5 ppm током 7 дана. На крају огледа узети су узорци крви и ткива за испитивања.

Код животиња које су 7 дана добијале количину од 1.5 ppm ОА патохистолошким прегледом бубрега уочава се да су променама углавном захваћени проксимални бубрежни тубули. Поједине епителне ћелије су мутне и набубреле, што је довело до делимичног или потпуног сужења лумена бубрежних тубула. Код мањег броја бубрежних тубула запажа се акутна тубуларна некроза у виду ситних огњишта. Честа је појава фрагментације некротичне масе, као и налаз свежих еритроцита.

Активност ензима LDH на крају огледа била је сигнификантно виша код бројлера свих огледних група у односу на контролну групу.

Све наведене чињенице указују да интензитет патохистолошких промена у бубрегу зависи од концентрације охратоксино А у храни, при чему активност ензима LDH може да послужи као користан параметар за процењивање степена алтерација бубрега.