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EXAMINATION OF THE EFFICACY OF VARIOUS FEED ADDITIVES ON THE PATHOMORPHOLOGICAL CHANGES IN BROILERS TREATED WITH T-2 TOXIN

ABSTRACT: A 21-day-long experiment was performed on 160 one-day-old “Ross” broiler chicks. This research was done with the aim of investigating pathomorphological changes in broilers exposed to a relatively small amount of T-2 toxin (2 ppm) and the possibility of prevention and/or alleviating adverse effects of T-2 toxin using various feed additives. Pathohistological examination showed negative consequences of T-2 toxin in all examined organs as degenerative changes developed in small intestine mucosa, enterocytes and hepatocytes necroses, as well as lymphocytes depletion in bursa of Fabricius. Disparately from inorganic (Minazel-plus, Mz) and organic (Mycosorb, Ms) adsorbents, which did not provoke protective effects, in liver, small intestine and bursa of Fabricius of broilers who were given feed with T-2 toxin and mixed adsorbent (Mycofix-plus, Mf), mostly preserved structure of these organs could be noted.

KEY WORDS: T-2 toxin, broiler, pathomorphological changes, adsorbents

INTRODUCTION

Trichothecenes inhibit protein synthesis and from the pathological and immune point of view they are of extreme importance for poultry. When animals ingest relatively small amounts of T-2 toxin, clinical symptoms can miss and performances can remain unalterable during longer period. In this way toxin might be undiscovered although it causes subclinical changes that could be found at the pathomorphological examination.

A new approach to mycotoxin control is to alleviate and/or prevent harmful effects of mycotoxins in feed. Different feed additives are in use today, which either adsorb mycotoxins on their surface or they provide enzyme degradation of mycotoxins. The efficacy of alleviating harmful effects depends mostly on chemical structure of adsorbent, as well as on the type of mycotoxin.

Adsorbents are substances nonresorbable from the gut, which can physically bind some chemicals and thus block their resorption. Mineral adsorbents are commonly in use (active charcoal, hydrated sodium calcium aluminosilicate, sodium bentonit, dietary clay and zeolites) (T o m a š e v i ć - Č a n o v i ć et al., 2003). The feasibility of utilizing organic adsorbents is also examined, particularly esterified glucomanane which is isolated from the inner layer of yeast cell wall and which possesses significant capability of mycotoxin adsorption (D e v e g o w d a et al., 1996). Recently a new type of additive, containing microorganisms with the ability to inactivate mycotoxins by enzyme modification of their structure (F u c h s et al., 2002), has been developed.

The primary objective of this study was to overhaul the feasibility of alleviating and/or preventing pathohistological changes in broilers exposed to T-2 toxin by utilizing different feed additives.

MATERIAL AND METHODS

Pure T-2 toxin isolate, obtained under laboratory conditions by cultivation of *Fusarium sporotrichoides*, from 4 isolates: ITM-496, KF-38/1, M-1-1 i R-2301 (B o č a r o v - S t a n č i ć and R a d o š e v i ć, 1991) was used for feed contamination. To achieve proper homogenisation of T-2 toxin in the feed, ethil-acetate extract containing 2 ppm of T-2 toxin was 3 times sprayed at certain amount of feed that was afterwards mixed into the rest of the feed necessary for the experimental period (1—21. days).

After proper preparation of the feed samples, the determination of the present amount of T-2 toxin was done using ELISA method with monoclonal T-2 toxin antibodies (B a r n a - V e t r o i s a r., 1994).

Minazel-plus (Modified clinoptilolite, ITNMS Beograd), *Mycosorb* (Esterified glucomanane, Altech, USA) and *Mycofix-plus* (Biomim, Austria) were added to the feed in the amount of 0,2%.

In vivo trial 21 days long was performed on 160 one-day old “Ross” broiler chicks of both sexes. Animals were divided into 8 experimental groups, each containing 20 broilers: group 1: negative control, free from T-2 toxin and additives; group 2: positive control, 2 ppm T-2 toxin without binders; group 3: 2 ppm T-2 toxin + 0,2% *Minazel-plus*; group 4: 2 ppm T-2 toxin + 0,2% *Mycosorb*; group 5: 2 ppm T-2 toxin + 0,2% *Mycofix-plus*; group 6: 0,2% *Minazel-plus* without T-2 toxin; group 7: 0,2% *Mycosorb* without T-2 toxin; group 8: 0,2% *Mycofix-plus* without T-2 toxin.

Animal sacrifice was done in all experimental groups on the 21st day of the trial by method of cervical dislocation. Pathohistological examination was performed on liver, small intestine and bursa of Fabricius samples, embedded in paraffin, and sections of 3—5 m thickness were cut, stained using the standard hematoxylin eosin method (S c h e u e r et al., 1986) and observed under the optical microscope (Olympus BX-41).

RESULTS AND DISCUSSION

T-2 toxin affects almost all tissues and organs. Labile cells are mostly attacked by structural changes due to T-2 toxin cytotoxic effects. This mycotoxin causes pathomorphological alterations in liver, digestive system, bone marrow, skin, lungs, heart, reproductive organs, spleen, bursa of Fabricius and nervous tissue (Kubena et al., 1998, Bailey et al., 1998).

Pathohistological examination of liver from broilers fed with T-2 toxin contaminated feed without the adsorbents, revealed massive focal disseminated necrosis and numerous focal lymphocyte aggregates, which is in agreement with the findings of numerous other authors (Hoerr et al., 1981, Hoerr et al., 1982, Grizzle et al., 2004). Similar finding of periportal focal necrosis and hepatocyte degeneration was found in broilers that, besides T-2 toxin, also received inorganic and organic adsorbents Minazel-plus and Mycosorb with feed. Absence of protective effects in cases of T-2 toxicosis in broilers fed with diets containing zeolite or organic adsorbent, based on the pathohistological examinations, was also noted by Garsia et al. (2003) and Dvorska and Surai (2001).

In the liver of broilers which were given feed with T-2 toxin and mixed adsorbent Mycofix-plus, the liver moderate hepatocytes degeneration was observed, mostly in the form of the intracellular oedema and in fewer cases, lymphocyte infiltration in portal area was present. Preventive effect of Mycofix-plus was also noted in the research of Garsia et al. (2003) who observed toxic effects in liver at the bile ducts epithelium in the portal area. This beneficial effect is based on the enzymatic inactivation of the 12,13-epoxide ring of the trichothecenes and flavonoligands which are some of the components of Mycofix-plus and have the role to protect liver by blocking receptors in cell membrane of hepatocytes.

In the small intestine of broilers who were fed with T-2 toxin contaminated feed, on the 21st day, degeneration and descquamation of enterocytes, as well as limphocyte infiltration between intestine glandules was observed, which is in agreement with other investigations (Hoerr et al., 1981, Hoerr et al., 1982, Grizzle et al., 2004). Similar finding of degeneration and descquamation of enterocytes was noted in broilers that were, besides T-2 toxin, given adsorbents, inorganic and organic.

Contrary to these results, in the small intestine of broilers fed with feed contaminated with mixed adsorbent Mycofix-plus, protective effects were noticeable as in the case of pathohistological examination, only moderate enterocytes descquamation could be seen, but the lenght and the structure of small intestine villi remained normal.

Pathohistological inspection of bursa of Fabricius of broilers who were given T-2 toxin without adsorbents in feed showed atrophy of lymphoid tissue in the cortex and medulla, hereby observing vacuolisation in cortex lymphoepithelium, while lymphocyte depletion was expressed in medulla. This finding is compatible with observations of Hoerr et al. (1981), Hoerr et al. (1982), Grizzle et al. (2004), Fazekas et al. (2000). Similarly, protective failure was noted in tissue of bursa of Fabricius in broilers who were fed

with feed contaminated with added inorganic binder Minazel-plus, as well as organic adsorbent Mycosorb. This was in accordance with conclusions of Kubena et al. (1998) and Bailey et al. (1998).

Differing from organic (Mz) and inorganic (Ms) adsorbents, which application did not show protective effects in the tissue of bursa of Fabricius from broilers who were given feed with added toxin and mixed adsorbent Mycofix-plus preserved medullar and cortex structure, with dense lymphocyte population, especially expressed through diffuse proliferation of T-lymphocytes in cortex of follicles, was observed.

The protective effect of mixed adsorbent can be explained by the fact that enzymes, de-epoxidases, partially degraded the ingested T-2 toxin by selective destruction of its toxic 12,13-epoxy group (Fuchs et al., 2002). Besides the biological constituent, Mycofix-plus contains inorganic binder with the adsorption based on the production of hydrating connection between the mycotoxin and the adsorbent, as well as flavonoligands, terpenoid complexes and fycofite components which reduce inflammation, stimulate immune response and accelerate metabolic processes.

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ИСПИТИВАЊЕ УТИЦАЈА РАЗЛИЧИТИХ ДОДАТАКА ХРАНИ НА ИНТЕНЗИТЕТ ПАТОМОРФОЛОШКИХ ПРОМЕНА КОД БРОЈЛЕРА ТРЕТИРАНИХ Т-2 ТОКСИНОМ

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Резиме

Експеримент је изведен на 160 једнодневних бројлерских пилића провенијенције “Ross”, у трајању од 21 дан. Истраживања су била усмерена на омогућавање детаљнијег увида у патоморфолошке промене код бројлера изложених дејству релативно ниских доза Т-2 токсина (2 ppm), као и могућности превенције или ублажавања штетних ефеката коришћењем различитих адсорбената. Патохистолошким анализом исечака танког црева, јетре и Фабрицијеве бурзе, уочени су ефекти Т-2 токсина у свим испитиваним органима, у виду дегенеративних промена на слузници танког црева, некрозе ентероцита и хепатоцита, као и деплеције лимфоцита у Фабрицијевој бурзи. За разлику од неорганског (Minazel-plus,

Mz) и органског (Mycosorb, Ms) адсорбента чијом применом није дошло до заштитног ефекта, у ткивним исечцима јетре, црева и Фабрицијеве бурзе код бројлера који су путем хране добијали Т-2 токсин и мешовити адсорбент (Mycosifix, Mf), запажа се углавном очувана структура испитиваних органа.