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## CLINICAL AND PATHOMORPHOLOGICAL DIAGNOSTICS OF MYCOTOXICOSIS IN PARENT POULTRY FLOCK CAUSED BY T-2 TRYCHOTECENE

**ABSTRACT:** The most relevant mycotoxin from the trichotecene group, T-2, causes prominent cytotoxic effects. The toxin is a secondary product of fungi from the genus *Fusarium* that contaminates feed. Orally intaken, T-2 is absorbed fast in the upper digestive system and within only 3 to 4 hours later reaches liver, kidneys and muscle tissue. Clinical and pathological changes are sometimes not obvious.

The case of mycotoxicosis in a breeder flock of chickens, here presented, is aimed to underline the significance of clinical and pathological diagnosis supported with laboratory analysis that gave an objective causative diagnosis.

On the farm, the disease occurred suddenly and with total cessation of feed consumption. First cases were recorded in the flock at the age of 42 weeks. Grouping, intensive breathing and lying with overstretched legs and extended neck were symptoms observed in birds. Evident necrosis of beak tips and painful multi-focal necrosis in oral cavity were recorded during the clinical examination. On section, dark unclotted blood was first observed. Other *postmortem* findings included: filled gizzard with mucosal erosions and easy-removable cuticle, enlarged congested liver with multi-focal necrosis and subcapsular bleeding. The mortality rate increased by 4%, and the drop of laying rate was by about 18%. The fertility rate decreased by 22%. There was the increased number of rejected hatching eggs, 12%.

Culture of the complete diet resulted in approximately 150000 colonies per 1g of *Fusarium*. T-2 was detected by using ELISA in concentration of 480 µg/kg, which corresponded to the upper limit of maximum permitted concentrations for chickens, according to national legislations. This bylaw interpretation of “tolerable” concentrations of mycotoxins provokes controversy among experts and public.

**KEY WORDS:** clinical and pathological diagnostics, poultry, mycotoxins, T-2, legislative

### INTRODUCTION

Diseases caused by mycotoxins are non-contagious and usually feed related. Treatment with antibiotics or other drugs in general is not efficient. Due to their small molecular weight mycotoxins do not provoke immune response.

In practice, chronic intoxication occurs more frequently. Small concentration intake over a prolonged period and *vice versa*, higher concentration of mycotoxins for a short period of time have the same effects (R a f a i et al., 2000; N e š i ć et al., 2005).

Based on the target tissue, mycotoxins are classified as hepato-, nephro-, neuro- and cyto-toxins (S i n o v e c et al., 2000). Their biological effects are diverse: cancerogenic, mutagenic and theratogenic, immunomodulation and protein synthesis inhibition (S i n o v e c et al., 1993). The toxic effects and severity of the mycotoxin related disease depend primarily on the group and concentration of mycotoxins, relative duration of the exposure, the species, gender and age of poultry, general health and immune status, zoohygiene and zootechnolgy, nutrition and feeding regimen (Binder et al., 2007).

In the veterinary medicine, T-2 toxin that expresses prominent cytotoxic effect is one of the most relevant trichotecene toxins. It is produced by fungi from the genus *Fusarium* that contaminate crops in fields and feed during storage. Microorganisms grow under different environmental conditions and produce toxin at lower temperature range, from 4 to 8<sup>o</sup>C, but not at 32<sup>o</sup>C (Q u i n n et al., 1998; J a c o b s e n et al., 2013).

Orally intaken, the resorption of T-2 in the upper digestive tract is fast and after 3 to 4 hours reaches liver, kidneys and muscle tissue (S i n o v e c and J o v a n o v i ć, 1993). Residual concentrations of T-2 can be detected in eggs and meat. In comparison to chickens, turkeys and geese are more sensitive to T-2 (S i n o v e c et al., 2006).

In poultry, alimentary and nervous system disorders occur in cases of acute intoxication with trichotecenes. The letargy of birds, hyperpnoe and loss of balance are present. Haemorrhage is a regular finding in the alimentary tract and muscle tissue, as well as prominent focal necrosis and ulcerations in oral cavity and stomatitis. During the chronic course, decrease of feed consumption and body weight are recorded, and oral lesions and irregular moving are evident.

For several decades, mycotoxin related problems have represented a challenge for the researchers, veterinarians and farmers. Some confusion exists in relation to the clinical and pathomorphological diagnostics of the disease complex caused by mycotoxins (N e š i ć et al., 2011). In this paper, a case of mycotoxicosis in a parent poultry flock was presented in order to show how important it is to make diagnosis beforehand, clinically and pathologically. Finally, objective causative diagnosis was confirmed with laboratory findings.

## MATERIAL AND METHODS

The parent flock of heavy breed Ross 308 was relocated in winter to an exploitation farm with two separate houses, on the ground and the first floor, each containing 2200 birds. The complete diet produced for the heavy parent hybrid was purchased from one supplier.

The outbreak of the disease was observed simultaneously in both houses in 42 week old breeders which corresponded to the passed peak of production.

Based on the course, clinical observation and pathomorphological examination, the case of mycotoxicosis was suspected.

Feed was sampled and submitted for microbiological analysis. The samples were cultured on Sabouroud agar. After the incubation period, the grown colonies of fungi were purified and identified.

The content of T-2 was determined in feed using ELISA test, Ridascreen® (Art.No. R:3801, R-Biopharm, Germany), with detection limit 3.5 pp (R-biopharm, www.r-biopharm.de). The readings were processed in a software package Softv Rida®Soft Win (Art. No. Z9999, R-Biopharm, Germany) and the obtained results were interpreted according to the instructions provided by the manufacturer.

## RESULTS AND DISCUSSION

Weak activity and grouping along the side walls in the flock was first observed by the workers in the early morning. A veterinary clinical observation revealed the following:

- grouping of breeders in island-like formations close to the side walls leaving the central part of the house line feeders and drinkers almost empty,
- the feed consumption completely stopped,
- a minor part of the birds that were standing had difficulties to move, supporting themselves with extended wings (Fig.1),
- yellow to yellowish-green diarrhea and large quantity of feathers were observed on the litter,
- birds with retracted necks and poorly feathered were mostly lying with extended neck and overstretched legs, opened beak and accelerated breathing,
- beak necrosis and painful multi- focal necrosis in oral cavity were obvious (Fig. 2).

On necropsy, pathomorphological changes found were identical in all carcasses. They were all found in a characteristic position with extended neck, overstretched legs and with filthy feathers on abdomen and around the cloaca due to the yellowish to green diarrhea. Unclothed, dark colored blood was first observed at necropsy. Other *postmortem* findings included: gizzard filled with feed and mucosal erosions, and easy-removable cuticle (Fig.3), enlarged congested liver with multi-focal necrosis and subcapsular bleeding (Fig.4).

The dynamics of mortality and production parameters presented in Table 1 contain data for the age of 41, 42 and 43 weeks. The mortality rate increased by 4%, and the drop of laying rate was by about 18%. The fertility rate was decreased by 22%. There was an increased number of rejected hatching eggs of 12.2%. Clearly, all production parameters were violated. Although slow improvements in the flock started, the secondary loss was reflected in poor vitality of the one day old chickens that caused an increased number of afterclaims.

Tab. 1. – Mortality rate and production in breeders including one week before and after the outbreak.

Flock age (week)	Mortality rate (%)	Egg production (%)	Rejected eggs (%)	Fertilized eggs (%)	Hatched chickens (%)
41	0	72.5	0.8	92	83
42	4.25	54.6	13	70	61
43	Parent flock was excluded from the production				

The microbiological analysis – culture of complete diet, declared for nutrition of adult producing breeder chicken flock, was positive only to *Fusarium* fungi, in the calculated quantity of approximately 150000 colonies per gram. This result was at the upper limit of maximum permitted number of colonies for adult poultry (Službeni Glasnik, 2010). The T-2 concentration of 480 µg/kg was detected by ELISA, which was also at the upper limit of maximum permitted concentration for adult poultry (Službeni Glasnik, 2010).

Sudden clinical disease on the farm with high mortality and typical pathological changes, indicated that cumulative effects of T-2 were expressed. Numerous reports can be found on the death outcome in domestic animals due to consumption of the contaminated feed (P r o d a n o v et. al, 2011). In the literature, similar *postmortem* findings were described and it was noted that in comparison to chickens, species including turkey and goose were more susceptible to the toxin (S i n o v e c et al., 2006). Gross changes of the "X Disease" in turkeys, first described by W a n n o p (1961), were directed toward possible mycotoxicosis.

Until the laboratory confirmation was obtained, preliminary clinical and pathological diagnosis indicated that the following procedures had to be undertaken: First was to empty the feeding lines and replace the existing diet with the reliable safe one. The second action was supportive therapy with commercial product *Evitaselen* containing vitamins E and selenium. The late replacement of diet was unable to moderate the disease because of irreversible pathological changes. Early and beforehand mycotoxin detection and exclusion of the particular diet may help to mitigate the detrimental effects (N e š i ć et al., 2005). On farms with veterinary staff employed, it is possible to prevent, avoid or repair such losses. In this case further exploitation of the flock was found economically unjustified and it was excluded.

The laboratory findings of 150000 colonies per gram of *Fusarium* in the diet and the detected T-2 concentration of 480 µg/kg were high, nevertheless acceptable for consumption in adult poultry, according to the national regulations (Službeni Glasnik, 2010). Such interpretation provokes strong controversy among the experts. There can be no threshold concentration for the negative effects caused by mycotoxins and clearly, daily tolerable concentrations are impossible to determine (S i n o v e c et al., 2000; J a k i ć – D i m i ć et al., 2010; J a k š i ć et al., 2011). The regulations on the content of such and similar harmful substances in feed need to be corrected, particularly for more susceptible species and categories of poultry.



Fig. 1. – Flock grouping in island – like formation.



Fig. 2. – Necrotic process in oral cavity.



Fig. 3. – Gizzard: cuticle and mucosal erosions.

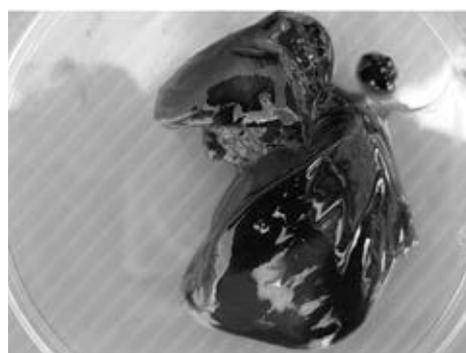


Fig. 4. – Liver: subcapsularly bleeding.

In spite of the fact that experienced veterinarians are able to recognize the symptoms without any difficulty and propose the therapy accordingly, further laboratory examinations are needed to confirm the diagnosis, including microbiological investigations and determination of mycotoxin content. In practice, cases of mycotoxicosis in poultry are frequently seen (K a p e t a n o v e t al., 2012).

## CONCLUSIONS

Clinical and pathological diagnostics is of great importance in the veterinary medicine due to efficient tools to note the disease, direct investigations toward specific laboratory tests and moderate the outcome. The results of numerous mycotoxin examinations indicate that, in many cases, the determined concentrations in feed do not exceed the levels regulated by the law. However, because of the cumulative effects and chronic exposure to mycotoxins, even in low doses, prompt expertise and action undertaken by the veterinarians are

inevitable, especially in more susceptible poultry species and categories. Additionally, in a number of feed plants and factories, components are obtained not in large quantities but rather capillary. In such circumstances a significant amount of complete mixtures may be delivered to poultry producers without the previous control, which indicates a need for more comprehensive feed monitoring.

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Running title:  
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### УЛОГА КЛИНИЧКЕ И ПАТОМОРФОЛОШКЕ ДИЈАГНОСТИКЕ МИКОТОКСИКОЗА ИЗАЗВАНЕ Т-2 ТОКСИНОМ КОД РОДИТЕЉСКОГ ЈАТА У ЕКСПЛОАТАЦИЈИ

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#### Резиме

Проблем присуства микотоксина у храни за животиње и последице које услед тога настају већ годинама представљају изазов за решавање за све који се са њима сусрећу. У том ланцу, који обухвата истраживања од превентиве до куративе, чест извор недоумица чини процес клиничког и патоморфолошког дијагностиковања обољења изазваних микотоксинима. За ветеринарску медицину најважнији микотоксин трихотеценске групе је Т-2 који испољава најизраженије цитотоксично дејство. Циљ рада је да се, кроз приказ случаја микотоксикозе живине, родитељског јата узраста 44 недеље у експлоатацији, истакне значај постављања клиничке и патоморфолошке дијагнозе уз лабораторијску анализу како би се поставила објективна етиолошка детерминација.

На фарми родитељског јата у експлоатацији приказана је нагла клиничка појава болести праћена потпуним престанком конзумирања хране. Већина груписаних јединки је лежала а при покретању отежано се кретала. Клиничком опсервацијом јата уочавала се некроза врха језика и ограничена некротична жаришта слузокоже усне дупље која су на додир јединки стварали бол. Након отварања мишићног желуца уочавао се садржај хране, ерозије зида желуца и кутикуле која се лако скидала. Јетра угинулих јединки карактерисали су: конгестија, заобљеност режњева, мултифокална некротична жаришта и супкапуларна крварења. Број угинулих јединки у току посматраног периода је био изнад технолошких норматива.

Натурални показатељи производње укључујући проценат снесених јаја, одабраних за инкубирање и лежења пилића били су далеко испод предвиђене технологије са тенденцијом опадања.

Добијене микробиолошке вредности потпуне смеше за исхрану родитељског јата у експлоатацији од 150 000 колонија плесни у 1 г рода *Fusarium* у чистој

култури и налаз трихотецена Т-2 од 450  $\mu\text{g}/\text{kg}$  на граници максимално дозвољене количине за пилиће како је регулисано постојећим законским актима изазива општу полемику у стручним круговима.

КЉУЧНЕ РЕЧИ: клиничка и патоморфолошка дијагностика, живина, микотоксини, Т-2 токсин, законска регулатива

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