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# EVALUATION OF THE EFFICACY OF DIFFERENT FEED ADDITIVES TO ADSORBE T-2 TOXIN *IN VITRO*

ABSTRACT: In the trial, *in vitro* HPTLC — High Performance Thin Layer Chomatography was used to determine the amount of "free", i.e. unbound or non-decomposed T-2 toxin. Mean adsorption or degradation levels of T-2 toxin in examined feed aditives, in *in vitro* conditions, ranged from 26.06 to 34.84% and did not significantly differ among used adsorbents: inorganic (Minazel plus — Mz), organic (Mycosorb — Ms) and mixed (Micofyx — Mf). All these additives showed better adsorption ability in the acidic environment (pH 3).

KEY WORDS: T-2 toxin, glucomanane, modified clinoptilolite, mycofix, HPTLC

## INTRODUCTION

Trichotecenes are toxic products of secondary metabolism of several *Fusarium* fungi and over 170 are isolated and identified so far. For veterinary medicine the most important among them is T-2 toxin which is the most toxic (Humphreys, 1988). Its presence has been recorded all over the world implying that T-2 toxicoses are a very serious problem everywhere.

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 provide enzyme degradation of mycotoxins. Efficacy of alleviating harmful effects depends mostly on the 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) (Tomašević-Čanović et al., 2003). The feasibility of organic adsorbent utilization was also examined, particularly that of esterified glucomanane which is isolated from

the inner layer of yeast cell wall and possesses significant capability of mycotoxin adsorption (D e v e g o w d a et al., 1996). Recently, a new type of additive has been developed which contains microorganisms with the ability to inactivate mycotoxins by enzymatic modification of their structure (F u c h s et al., 2002).

## MATERIAL AND METHODS

In an *in vitro* trial, pure crystal T-2 toxin was used, *Fusarium* sp., concentration 99%, product of Biopur, Austria.

As mycotoxin binders were used:

a) modified clinoptilolite (*Min-a-zel Plus*, ITNMS Beograd) obtained by processing of clinoptilolite from Zlatokop zeolite.

b) esterified glucomanane (*Mycosorb*, Alltech USA) which is isolated from the inner layer of yeast cell wall.

c) mixed adsorbent, which contains inorganic binder, bacteria, enzymes, as well as phytogenic material extracted from plants (*Micofix Plus*, Biomin, Austria).

The *in vitro* investigation of the efficacy of different feed additives to adsorb or deactivate T-2 toxin was conducted according to standard procedure: addition of basic standard T-2 toxin solution (1 mg/ml in ethil-acetate) and 100 mg of tested additives. The contact between toxin and additives was reached in a magnetic mixer, in the buffer solution at pH3 and pH7, at 37°C during 1 h. Separation of unbound toxin remainder from the complex adsorbent-mycotoxin was done by filtration through quantitative filter paper Filtrak 391. Testing of wach feed additive was performed in 5 replications.

Determination of "free" i.e. unbound and non-degradated T-2 toxin was accomplished by TLC technique (HPTLC — High Performance Thin Layer Chromatography) according to the procedure of B a t a et al. (1983). The prepared samples were inflicted on the chromatographic panes Silikagel 60 F254 HPTLC Merck. Chromatogram development was done in the toluen-ethyl acetate-formic acid (50:40:10 v/v/v) system. Fluorescens was rised by panes splashing with 25% solution of sulfuric acid in methanol. Densitometric determining of T-2 toxin amount at 254 and 366 nm was performed with Camag TLC Scanner denzitometer with the software system Cats III.

Determination of toxin-adsorbent binding percentage was calculated from the difference between the total toxin amount that was added to the buffer solution and the amount of the unbound toxin remainder obtained by HPTLC.

## RESULTS

Results received in the *in vitro* conditions are shown in Table 1.

Tab. 1 — The amount and the percentage of T-2 toxin adsorbed by different feed additives

| Sample* (T-2 exp) | Amount of the unbound T-2, mg (T-2 unb), % | Amount of the bound T-2, mg, % | Average value of the bound T-2 toxin, %** |
|---|---|---|---|
| T-2 Mz, pH 7 | 35.24 | 14,56 | 29.24 |
| T-2 Mz, pH 3 | 34.21 | 15.59 | 31.30 |
| T-2 Ms, pH 7 | 34.98 | 14.82 | 29.76 |
| T-2 Ms, pH 3 | 32.45 | 17.35 | 34.84 |
| T-2 Mf, pH 7 | 36.82 | 12.98 | 26.06 |
| T-2 Mf, pH 3 | 34.26 | 15,54 | 31.20 |

* Cone. T2 empiric 50 mg/sample, experimentaly determined amount 49,8 μg (T-2 exp)

** $\frac{\text{(T-2 exp — T-2 unb)}}{\text{T-2 exp}} * 100\%$

As it could be seen from the table, amounts of the adsorbed T-2 toxin did not differ significantly between the samples tested in the solutions of the same acidity. Somewhat larger adsorbance percentage was noted in the solutions with lower acidity — pH 3.

The average value of adsorbed T-2 toxin by the innorganic binder Minazel Plus (Mz) was 29,24% at pH 7 and 31,3% at pH 3. The highest adsorbtion was achieved by organic adsorbent Mycosorb (Ms) — 29,76% at pH 7 and 34,84% at pH 3. On the other hand, the lowest adsorbtion was noted in the trials with the mixed binder Mycofix (Mf) — 26,06% at pH 7 and 31,20% at pH 3.

## DISCUSSION

Although an attempt was made to simulate the conditions in the gastrointestinal tract during the trial, it was impossible to reproduce the conditions existing in real system. During the experiment specific problem was the calculation of the adsorbed mycotoxin amount. Ledoux and Rottinghaus (1999) stated that in the *in vitro* trials only adsorbents with the ability to bind over 80% of mycotoxins should be taken in account.

In the performed experiments, in the solutions of the same acidity no significant differences in the adsorbed amounts of T-2 toxin were found. Higher level of adsorption was noted at the lower acidity (pH 3), when better contact between the adsorbent and toxin was reached. After the extraction of T-2 toxin by ethylacetate, layers which are easier and faster to split off were obtained and the segregated organic phase with T-2 toxin was plain giving clear spots without filth.

The obtained results indicated that T-2 toxin was highly adsorbed by the inorganic binder Minazel Plus (Mz) in *in vitro* conditions (29,24% at pH 7 and 31,3% at pH 3), as trichotecens do not possess polar functional group and natural zeolites are active only toward polar mycotoxins, especially aflatoxins. Results of Garsia et al. (2003) and Avantaggiato et al. (2005) showed that most of the tested adsorbents are not able to bind *Fusarium* mycotoxins. The reason for the relatively high level of T-2 adsorption was the

fact that adsorbent of the III generation (Minazel Plus), obtained from the processed natural zeolite with more than 80% of clinoptilolite and with balanced ratio of cations Ca/K/Na was used in the trials. By organic modification of its surface with surfactants (long-chain organic cations), i.e. changing of the surface polarity and hydrofobicity, new active centres have been created, as double layer of organic ligand which adsorbes not only polar, but also non-polar organic molecules, like T-2 toxin, while all basic characteristics of the material were preserved (Tomašević-Čanović et al., 2003).

Analysing the results regarding the adsorption of T-2 toxin by esterified glucomanane in *in vitro* conditions (29,76% at pH 7 and 34,84% at pH 3) similar conclusion was made to that reported by Raju and Devegowda (2002), Dawson (2001) and Devegowda et al. (2004) who claimed that, depending on the T-2 toxin dosage and duration of contact between the adsorbent and contaminated feed, percentage of adsorbed T-2 toxin by esterified glucomanane was 15—32%. Mycosorb exerts its protective effect through high adsorptive capacity of esterified glucomanane toward T-2 toxin and other mycotoxins, due to its large surface — aproximately 2,2 ha/kg EGM (Devegowda et al., 1996).

The obtained results showed somewhat higher level of adsorption or deactivation of T-2 toxin by Mycofix (26,06% at pH 7 and 31,20% at pH 3) as compared to *in vitro* inestigations done by Garcia et al. (2003) who noted 3,28% T-2 adsorption by Mycofix at pH 7. Biological detoxification of mycotoxins comes as a result of their degradation by enzymes or their biotransformation by interfering with whole microorganism cell or single enzyme system (Karlovsky, 1999). Besides enzyme or microbiological degradation of mycotoxins ("biotransformation") Mycofix contains adsorption component, innorganic binder, while the adsorption is based on the generation of hydratic junction between the mycotoxin and the binder.

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## ИСПИТИВАЊЕ ЕФИКАСНОСТИ АДСОРПЦИЈЕ Т-2 ТОКСИНА РАЗЛИЧИТИМ АДСОРБЕНТИМА У УСЛОВИМА *IN VITRO*

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Резиме

*In vitro* испитивањем одређивана је количина „слободног" тј. невезаног или неразграђеног Т-2 токсина техником танкослојне хроматографије (HPTLC — High Performance Thin Layer Chromatography). Просечне вредности адсорпције или деградације Т-2 токсина испитиваним адсорбентима у условима *in vitro* нису се значајније разликовале у зависности да ли је био умешан неорганski (Minazel plus, Mz), органски (Mycosorb, Ms) или мешовити адсорбент (Micofyx, Mf) и износиле су од 26,06 до 34,84%. Сви испитивани адсорбенти су показали већу способност адсорпције овог токсина у киселој средини (pH 3).