EFFICIENCY OF MICOTOXIN SORBTION *IN VITRO* BY LIGNSORBENT

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Abstract

It was found that in an in vitro condition, Lignosorbent in an amount of 0.5 % showed a high sorbtion ability (70−100 %) for aflatoxin B1, patulin, zearalenone, sterigmatocystin and a lower sorbtion of T-2 toxin and deoxynivalenol (50 % and 65 %).

The doubling of the recommended amount of Lignosorbent did not provide a significant increase in the sorbtion of DON and T-2 toxins. Lignosorbent is able to sorb the mycotoxins of the trichothecen group (T-2 toxin, DON) by only 65−70 %. The maximum level of sorbtion of mycotoxins Lignosorbent is fixed at the 60th minute of exposure. The sorbtion capacity of Lignosorbent essentially depends on the polarity of the mycotoxins.

Key words: in vitro, Lignosorbent, mycotoxins, sorbtion ability

INTRODUCTION

The current legislation of Ukraine and the EU raises the requirements for the quality and safety of feeds, feed and food raw materials and food products, due to the contamination of feeds and feed raw materials by the mycotoxin. Most scientists came to the conclusion that the safe dose of mycotoxins does not exist, and to avoid contamination of feeds with toxic fungi is practically impossible [1].

Therefore, there is an urgent need for the implementation of veterinary and prophylactic measures, the development and introduction of new means and methods for the prevention and treatment of animal toxicosis, based on the use of natural sorbents with the affected food. Sorbents reduce the biological activity of mycotoxins, can bind, effectively hold and remove them from the gastrointestinal tract of animals [3]. The method of sorbtion is considered to be the most effective and safe in relation to animals [2, 11].

Today, in the domestic market of veterinary preparations of Ukraine there is a wide range of proposed sorbents, which can be conditionally divided into three groups: inorganic, organic and combined. Inorganic sorbents combine in their group zeolites, bentonites, various types of clays, sodium-calcium aluminosilicates, diatomaceous earth, etc. Neutralization of toxins by mineral sorbents is highly effective for polar aflatoxins and less effective for non-polar toxins. At the same time, mineral sorbents in the presence of oxides of somemetals in their composition can break the acid-alkaline balance and intestinal microbiocenosis [2]. It is possible to prevent such negative processes, by possibly using organ sorbents.

Among organic sorbents a special place is occupied by lignin [8], which is a complex polymer of phenolic nature with a cyclic structure, the basis of its structure being polycondensation aromatic rings. On the branched surface of lignin there is a large number of functional hydroxyl, carboxyl and other groups that are located in a certain ordered structure. According to the data of mercury porosity, the presence of a mesoporous with a radius of 3-10 and 100-150 nm in a hydrolyzed lignin and a macro pore with a radius of 500-5000 nm [5] has been established. Such a structure of the molecules of lignin provides it with a high enough sorbtion capacity. It is not by chance that it adsorbs well cholesterol, bile acids, vapors of organic solvents, phenol, slightly starch and poorly soluble sodium chloride, riboflavin, tyrosine, leucine, and others.

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The manuscript was received: 05.09.2018
Accepted for publication: 15.01.2019

Lignin and its products are widely used in medicine, veterinary medicine, the national economy and, mainly, in the feed industry in the production of premixes, where it is used as a filler [12].

Employees of the Laboratory of Food Sanitation of the Odessa Experimental Station of the NSC "IEKVM" on the basis of hydrolytic lignin developed a detoxifier of feeds – Lignosorbent [7].

In this connection, the purpose of our studies was to study in the simulation experiments *in vitro* the sorbtion properties of Lignosorbent in interaction with mycotoxins – patulin, aflatoxin B_1, sterigmatocystin, zearalenone, DON and T-2 toxin.

MATERIALS AND METHODS

To carry out the studies, the initial amount of sorbent studied was taken as the recommended amount of 500 mg/kg.

To prepare the test sample, we took a sample of Lignosorbent with a mass of 5 g, which was introduced into the flask with water, after which a solution of mycotoxin mixture was added with constant stirring. The solution contained a mixture of mycotoxins in accordance with the maximum allowable levels (MRL) of mycotoxins established in Ukraine in animal feeds: aflatoxin $B_1$of 0.1 mg/l, zearalenone – 2.0 mg/l, sterigmatocystin – 0.6 mg/l, patulin – 0.5 mg/l; deoxynivalenol – 1 mg/l and T-2 toxin respectively 0.2 mg/ l [10].

Experimental samples were held for 15, 30 and 60 minutes at 38±1°C and pH 6.0 in the incubation medium, after which it was centrifuged at 8000 rpm for 15 minutes and the supernatant was taken, which was used to determine mycotoxins with the use of TLC plates of ASK "Silufol" type UV-254 and "Sorbfil" [9].

The adsorbtion activity of the Lignosorbent relative to the mycotoxins was calculated from the mycotoxin concentration measure in the test sample at 15, 30 and 60 minutes after the sample was weighed using conventional formulas. Based on the results of two parallel studies, the mean value was determined.

A control sample was a solution of a mixture of mycotoxins with a corresponding mycotoxin content as in the test samples, but without Lignosorbent. The control sample was treated in the same way as the test sample.

RESULTS AND DISCUSSION

As a result of the studies, it was established (Table 1) that 0,5% Lignosorbent (I series of the experiment) showed sorbtion properties after 15 minutes of incubation with a mixture of mycotoxins.

Table 1 Sorbtion capacity of Lignosorbent,%

Exposuretime, minutes	Mycotoxins					
	Afla-toxin B_1	Patulin	Zearale-non	Sterigmo-cysteine	DON	T-2 toxin
I series of experience. Input of Lignosorbent in quantity 0,5 %						
15	60	45	65	35	25	20
30	100	100	100	65	40	35
60	100	100	100	70	65	50
II series of experience. Input of Lignosorbent in quantity 1 %						
15	80	60	70	50	40	35
30	100	100	100	75	65	55
60	100	100	100	80	70	65

Thus, during this time of exposure, the adsorbtion capacity of Lignosorbent with respect to aflatoxin B_1 averaged 60%, patulin - 45%, zearalenon – 65%, sterigmatocystin - 35%, deoxynivalenol - 25% and only T-2 toxin - 20%.

During the 30 minute exposure, Lignosorbent sorbedaflatoksin B_1, patulin and zearalenone by 100%, sterigmatocystin by 65%, and DON and T-2 toxin by 40% and 35%, respectively.

On the 60-th minute of the contact of Lignosorbent with mycotoxins in the incubation medium, the sorbtion of mycotoxins was registered: aflatoxin B_1, patulin and zearalenone – 100%, sterigmatocystin – by 70%, deoxynivalenol by 65% and T-2 toxin by 50% .

Our in vitro studies showed higher sorbtion by Lignosorbent (0,5%) of aflatoxin B_1, patulin, zearalenone, sterigmatocystin – 70-100% and lower T-2 toxin (50%) and deoxynivalenol (65%). In this regard, the recommended amount of Lignosorbent was decided to double – 1% (II series of experiments).

Introduced in the incubation medium Lignosorbent in an amount of 1% for the 15-th minute of interaction with mycotoxins sorbedaflatoxin B_1 by 80%, patulin – 60%, zearalenone – 70%, sterigmatocystin – 50%, deoxynivalenol – 40% and T-2 toxin by 35%.

At the 30-th minute Lignosorbent sorbedaflatoxin B_1, patulin and zearalenone 100% (complete sorbtion), sterigmatocystin by 75%, deoxynivalenol by 65% and T-2 toxin by 55%.

At the 60-th minute after the application of Lignosorbent, full sorbtion (100%) of aflatoxin B_1, patulin and zearalenone was recorded. Sterigmatocystine was sorbed by 80%, deoxynivalenolone 70% and T-2 toxin by 65%.

Thus, a doubling of the recommended amount of Lignosorbent did not provide a significant increase in the sorbtion of DON and T-2 toxins (70% and 65%).

To compare the sorbtion ability of the *in vitro* Lignosorbent with other drugs that are used in livestock in the south of Ukraine for the neutralization of fodder from mycotoxins, the following preparations were taken: Primix-Alfasorb –enterosorbent produced by NPP Arianda Ltd. (Odessa), MikofiksPlus 3. E (hereinafter Mikofiks) – sorbent mycotoxins produced by Vioin (Austria), Klinofid – sorbent of mycotoxins produced by Unipoint (Switzerland) and Amigo sorbent produced by AgroBaltTrade (Russia).

The results of studies on the sorbtion capacity of these preparations in vitro in an amount of 1% relative to aflatoxin B_1, patulin, zearalonone, sterigmatocystin, deoxynivalenol and T-2 toxin are shown in Table 2.

Table 2 Sorbtion capacity of sorbents after 60 min from the beginning of the experiment, %

Name sorbent	Mycotoxins					
	Afla-toxin B_1	Patulin	Zearale-non	Sterigmo-cysteine	DON	T-2 toxin
Alfasorb	100	100	100	80	72	90
Klinofid	100	100	70	75	90	80
Mycofix	100	100	100	90	90	90
Amigo	100	100	100	90	75	60
Lignosorbent	100	100	100	80	70	65

The materials in Table 2 indicate that none of the sorbents studied showed 100% sorbtion of the T-2 toxin. The higher sorbtion of T-2 toxin was noted in Klinofid, Alfasorb and Mycofix 80-90%, and somewhat lower in Lignosorbent and Amigo – 65% and 60%.

The somewhat low sorbtion ability of the sorbents studied DON and T-2 toxin in comparison with other mycotoxins is explained by their structural features - the presence of epoxide ring (12,13-epoxy-Δ9-trichothecene), which is the main target for the successful neutralization of mycotoxins. At the same time, it has been established [1, 6] that the epoxy ring of the trichothecenes is well protected against the action of various reagents, at the sacrifice of which they are capable of remaining for a long time without any changes.

It should be noted that the results of our research are consistent with other literature sources, which also note that not all sorbents are able to effectively neutralize fusarium toxins [1].

CONCLUSIONS

1. Studies have shown that under conditions of in vitro Lignosorbent in an amount of 0.5% showed a high sorbtion ability (70-100%) relative to aflatoxin B_1, patulin, zearalenone, sterigmatocystin and a lower sorbtion of deoxynivalenol and T-2 toxin, respectively, 65% and 50%.

2. The doubling of the recommended amount of Lignosorbent did not provide a significant increase in the sorbtion of DON and T-2 toxins. Lignosorbent is able to sorb the mycotoxins of the trichothecene group (T-2 toxin, DON) only by 65-70%, which indicates the dependence of the level of its sorbtion ability on the polarity of mycotoxins.

3. The received results of researches testify to the possibility of using Lignosorbent to prevent the development of mycotoxicoses in farm animals and poultry.

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