Сучасне птахівництво

***UDC 636.5.087.7:615.246.2***

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# The influence of nutrients and surfactants on T-2 toxin adsorption

**Abstract.** ***The influence of nutrients and a surfactant on T-2 toxin adsorption and extraction from water solutions was investigated. The decrease of T-2 toxin adsorption was more pronounced in the presence of surfactant than of nutrients, which might be related to the intensive adsorption of surfactants and their ability to solubilize T-2 toxin. The influence of chyme surfactants (bile acids, feed emulsifiers) on mycotoxins adsorption might be an important reason for existence of discrepancies between in vitro and in vivo mycotoxin binders efficacy.***

**Keywords:** *mycotoxin binders, T-2 toxin, surfactants, bile*

Fusarium fungi colonize crops and produce a variety of metabolites, of which T-2 toxin is one of the most dangerous. The prevalence of crops contamination with T-2 toxin [4], along with comparatively higher susceptibility of poultry to this mycotoxin, than to such widespread ones as zearalenone, fumonisins or vomitoxin, create a need for methods of T-2 toxicosis prevention. However, diversity, persistence and irregular distribution of mycotoxins as well as of mycotoxin-producing fungi, make such methods as routine grain decontamination or use of fungicides impractical. Therefore, the use of anti-mycotoxin feed additives gained considerable popularity.

Physical adsorption of mycotoxins in the gastrointestinal tract (GIT) is believed to be an important mode of action of such additives, which is why they are commonly referred to as "mycotoxin binders". However, their efficacy is not constant, the discrepancies between their in vitro and in vivo efficacy, as well as between ability to counteract the effects of chronic and acute mycotoxicoses are known, and binders effective against aflatoxicosis might appear ineffective against T-2 toxicosis [9-13].

### 1. List of adsorbents used

| № | Type | Characteristic |
|---|---|---|
| 1 | Carbon based | Birch activated carbon |
| 2 | | Activated carbon |
| 3 | | Shungite |
| 4 | Clay based | Gaize |
| 5 | | Zeolite |
| 6 | | Silicon dioxide |
| 7 | | Bentonite based commercial mycotoxin binder |
| 8 | Plant polymer based | Lignin |
| 9 | | Yeast cell wall based commercial feed additive (1) |
| 10 | | Yeast cell wall based commercial feed additive (2) |
| 11 | Combined | Lignin and birch activated carbon mixture 2:1 |
| 12 | | Commercial mycotoxin binder |

Influence of GIT conditions on adsorption of mycotoxins is believed to be among the main reasons for the above-mentioned discrepancies, but only acidity level is commonly simulated during the in vitro testing of mycotoxin binders, in spite of the abundance of binders, which retain their activity towards mycotoxins under broad pH range [8].

Accordingly, investigation of the influence of GIT conditions (presence of amino acids, lipids, carbohydrates, bile acids, feed emulsifiers) on T-2 toxin adsorption is of interest.

**Methods.** T-2 toxin was produced by extraction from Fusarium sporotrichioides 2m-15-206 culture on grain, purified by column chromatography and crystallized from diethyl ether [3].

Adsorbents were chosen on the basis of their relatively high activity towards T-2 toxin and zearalenone shown in previous work [1] (table 1).

T-2 toxin adsorption was studied from the:

- water solution;
- surfactant solution, which contained tween 20 (polyoxyethylensorbitanmonotaulorate) 0.5% vol.;
- solution of nutrients, which contained:
  - » amino acids: DL-valine, L-isoleucine, DL-leucine, DL-lysine, DL-methionine, DL-threonine, DL-tryptophan and DL-phenylalanine, 0.5 g/l each;
  - » carbohydrates: 1.3 g/l D-glucose and 14 g/l sucrose;
  - » lipids: sunflower oil 40 ml/l;
  - » compound feed extract (obtained by extraction of chicken compound feed with water: 200 g/l, for 24 hours);
- combined solution of nutrients and surfactants.

The initial concentration of T-2 toxin in solutions amounted 10 mkg/ml, concentration of ethanol – 0.5% vol. The temperature of the solutions was 42°C, time of incubation – 3 hours. Solutions without adsorbents were used as controls.

T-2 toxin concentration in solutions upon centrifugation was determined by disc diffusion method [5]. The percent of adsorption was calculated as follows:

$$A=((C_{cont}-C_{exp})/(C_{cont}))\times 100,$$

where:

A – percent of adsorption;

$C_{exp}$ – concentration of adsorbate in test solution;

$C_{cont}$ – concentration of adsorbate in control solution.

If the concentration of T-2 toxin in control solution did not exceed that in experimental one, the adsorption was considered to be nil.

Chloroform extraction of T-2 toxin was performed from:

- water solution;
- 3% citric acid solution;
- 3% sodium bicarbonate solution;
- 0.5% tween 20 solution;
- amino acids and carbohydrates solution;
- compound feed water extract.

Tween adsorption was studied from its 0.5% solution. Concentration of tween was measured by an original method, based on the ability of surfactants to interfere with dye adsorption by activated carbon, which was performed as follows:

- addition of activated carbon (40 mg) to 4 ml of the solution to be analyzed with subsequent 3-hour incubation while stirring;
- centrifugation of the mixture obtained 3000 rpm for 3 min and separation of the sediment;
- addition to the sediment obtained 4 ml of 0.03% "brilliant green" dye water solution with subsequent 1-hour incubation at 25ºC while stirring;
- centrifugation of obtained mixture 3000 rpm for 3 min;
- dilution of obtained supernatant with water 7.5 fold;
- optical density measurement of diluted supernatant ("StatFax 2100" reader, wavelength 630/450 nm);
- the concentration of surfactant was calculated using a calibration graph.

The same method was used to study the influence of adsorbents on bile surfactant properties.

Extraction of tween was studied from its 0.5% solution. Chloroform, emulsified in the solution was removed by heating the solution to 60ºC and shaking.

Statistical analysis was performed, using the Students t-test.

Results and discussion. In total, three experiments had been conducted. The first one was dedicated to the comparison of the influence of nutrients and surfactants on T-2 toxin adsorption, the second – to the study of pH, nutrients and surfactants influence on T-2 toxin extraction, and the third – to the investigation of surfactant adsorption and extraction.

***Experiment 1. Comparison of the influence of nutrients and surfactants on T-2 toxin adsorption.*** Adsorption of T-2 toxin was the highest in water solution and the lowest – in the combined solution of nutrients and surfactants, in which statistically significant adsorption was exhibited only by activated carbon (№2). Despite that the amount of nutrients in the solutions was greater than that of surfactants, T-2 toxin adsorption by all the adsorbents used, except for the activated charcoal, was higher in the solution of nutrients (table 2).

So, in agreement with the results of previous study [2], the presence of nutrients and especially of surfactants can decrease the adsorption of T-2 toxin.

Such a strong influence of surfactants on the T-2 toxin adsorption might be explained, firstly: by competitive adsorption, caused by their ability to concentrate on phase borders, and secondly: by the increase of T-2 toxin solubility in the presence of surfactants, caused by its micellar solubilization. In order to test these speculations, adsorption of surfactants and their influence on T-2 toxin extraction were investigated.

***Experiment 2. Adsorption of surfactants.*** All adsorbents used, except for Shungite, actively sequestered tween from water solutions – in spite of its high initial concentration (0.5 g/l), the adsorption amounted 50 to ≥90%, thus reaching 1/10 of adsorbents mass, which might support the suggestion about intensive competitive adsorption of surfactants (fig. 1).

**2. T-2 toxin adsorption from the solutions of different composition (M±m; n=4, amount of adsorbents 25 g/l)**

| Type | № | Adsorption, % | | | |
|---|---|---|---|---|---|
| | | Solution | | | |
| | | Water | Nutrients | Surfactants | Nutrients and surfactants |
| Carbon based | 1 | ≥90 | 23±13 | 2±11 | 0 |
| | 2 | ≥90 | 52±5* | 77±1* | 27±5* |
| | 3 | ≥90 | 25±12 | 4±3 | 0 |
| Clay based | 4 | 61±10* | 20±9 | 0 | 5±12 |
| | 5 | 41±18* | 16±7 | 8±8 | 0 |
| | 6 | 59±12* | 17±14 | 13±13 | 0 |
| | 7 | 58±7* | 31±13* | 13±6* | 13±11 |
| Plant polymer based | 8 | 68±3* | 38±7* | 21±5* | 6±11 |
| | 9 | 32±12* | 33±10* | 19±12 | 9±10 |
| | 10 | 15±15 | 26±10 | 4±15 | 10±6 |
| Combined | 11 | 73±8* | 29±15* | 10±10 | 10±13 |
| | 12 | 78±4* | 29±11* | 12±17 | 12±10 |

*Note: * — differ from the control significantly P<0.05.*

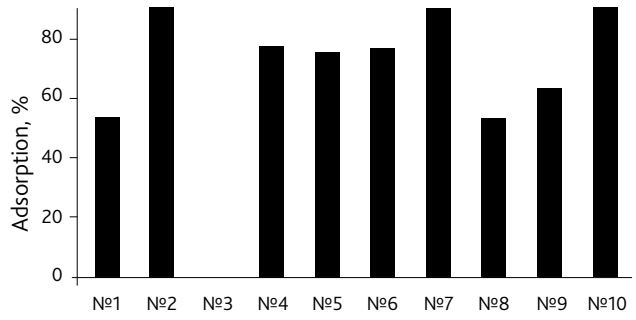


**Fig. 1.** Adsorption of tween 20 (amount of adsorbents 50 g/l)

*Note: the yeast-based adsorbents were excluded from the experiment because they contained unidentified surfactants.*

Incubation of the hen bile solution with the adsorbents impaired its ability to interfere with "brilliant green" dye adsorption by activated charcoal. In that respect, incubation with binders was analogous to manifold dilution of the bile solution (fig. 2). It can be speculated, that such effect was caused by the adsorption of bile components (predominantly surface active ones– bile acids and phospholipids).

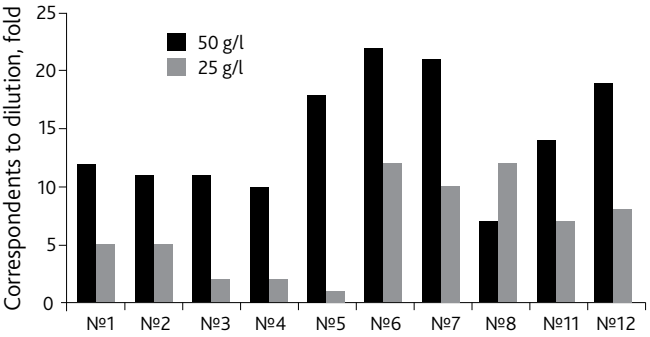


**Fig. 2.** Impairment of the surface activity of the bile solution (10% vol.), after its incubation with binders

***Experiment 3. The influence of pH, nutrients and surfactants on T-2 toxin chloroform extraction.*** T-2 toxin was efficiently extracted from water – the coefficient of its chloroform/water distribution amounted 41. In the presence of nutrients the extraction was significantly lower, but the lowest it was in the presence of the surfactant. The decrease of T-2 toxin extraction in the presence of sodium bicarbonate and citric acid was statistically insignificant (fig. 3).

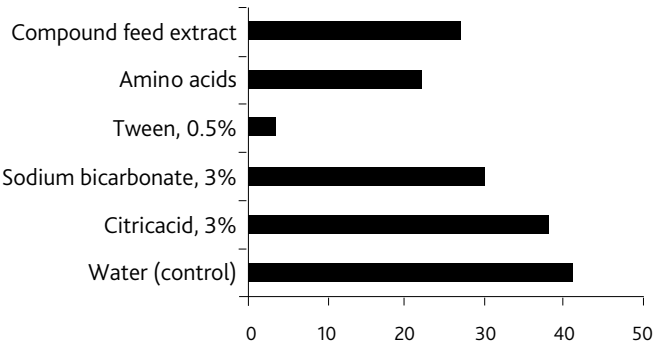


**Fig. 3.** The influence of the dissolved substances on the T-2 toxin chloroform/water distribution coefficients (chloroform 9%)

However, the ability of chloroform to extract tween appeared to be much lower than to extract T-2 toxin – the chloroform/water distribution coefficient of tween amounted about 3. Thence, an attractive explanation for the decrease of T-2 toxin extraction, might be its micellar solubilization, which can be supported by the high chloroform/water distribution coefficient of T-2 toxin (that supposed to facilitate its inclusion in the hydrophobic inner core of surfactant micelles), and also by the ability of surfactants to solubilize another fusariotoxin – zearalenone [7].

So, both the surfactant competitive adsorption and the T-2 toxin solubilization could influence the T-2 toxin adsorption. However, a possibility might exist not only of T-2 toxin micellar solubilization but also of its adsolubilization in adsorbed surfactant layers, similarly to other lipophilic compounds [6].

The existence of such surfactant-mediated mechanism of mycotoxins sequestration, because of its low specificity, could explain the discrepancies between mycotoxin binders efficacy against chronic and acute mycotoxicoses.

It is possible, that for unmediated mycotoxins adsorption from surfactant solution to occur, the energy of their adsorption should be no less that that of surfactant adsorption and of mycotoxin solubilization. Calculating the Gibbs energy of T-2 toxin adsorption and extraction from water solution with the equation [14]:

$$\Delta G = RT \times \ln K\_d,$$

where:
ΔG – Gibbs energy, J/mol;
R – universal gas constant (8,314 J/mol);
T – Kelvin temperature;

Kd – distribution coefficient (ratio between the amount of adsorbate, bound by the unit of the adsorbents mass and equilibrium concentration of adsorbate in solution), it can be seen, that for charcoal, clay, polymer based, combined adsorbents the ΔG amounts correspondingly <-15, -10, -8, -13, and for chloroform -10 kJ/mol, which is similar to that of zearalenone adsorption by talk and diatomite (from -13 to -17 kJ/mol [14]), but is considerably less than ΔG of aflatoxin adsorption by some aluminosilicates (up to -29 kJ/mol [12]), which could explain the high efficacy of aluminosilicates against chronic aflatoxicosis.

## Conclusions

The presence of surfactants might interfere with T-2 toxin adsorption and extraction, presumably because of their ability to concentrate on phase separation borders and solubilize T-2 toxin.

During in vitro mycotoxin binder testing, it is advisable to simulate the influence of surfactants.

The possibility of using the ratio between the energies of adsorption of mycotoxins and that of surfactants, as the criterion for the comparison of mycotoxin binders, requires additional attention. ■

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## Вплив нутрієнтів та поверхнево-активних речовин на адсорбцію Т-2 токсину

**Анотація.** ***Досліджено вплив нутрієнтів та ПАР на адсорбцію та екстракцію Т-2 токсину з водних розчинів. Зниження адсорбції Т-2 токсину було більш вираженим за присутності ПАР, ніж нутрієнтів, що може бути пов'язане з інтенсивною адсорбцією ПАР та їх здатністю солюбілізувати Т-2 токсин. Вплив ПАР хімусу (жовчних кислот, кормових емульгаторів) на адсорбцію Т-2 токсину може бути важливим фактором, що обумовлює розбіжності між in vitro та in vivo ефективністю адсорбентів мікотоксинів.***

**Ключові слова:** *адсорбенти мікотоксинів, Т-2 токсин, поверхнево-активні речовини, жовч*

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## Влияние нутриентов и поверхностно-активных веществ на адсорбцию Т-2 токсина

**Аннотация.** ***Изучено влияние нутриентов и ПАВ на адсорбцию и экстракцию Т-2 токсина из водных растворов. Снижение адсорбции Т-2 токсина было более выраженным в присутствии ПАВ, чем нутриентов, что может быть связано с интенсивной адсорбцией ПАВ, а также их способностью солюбилизировать Т-2 токсин. Влияние ПАВ химуса (желчных кислот, кормовых эмульгаторов) на адсорбцию Т-2 токсина может быть важным фактором, обуславливающим расхождения между in vitro и in vivo эффективностью адсорбентов микотоксинов.***

**Ключевые слова:** *адсорбенты микотоксинов, Т-2 токсин, поверхностно-активные вещества, желчь*

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