Mycotoxins are fungal metabolites that may have deleterious effects in animals [1]. Mycotoxins are detected in cereal grains worldwide, with a prevalence of 88% on feed and raw feedstuffs [2].

Mycotoxins routinely occur in common feedstuffs such as corn, corn silage, small grains and small grain silage, especially when growing conditions are sub-optimal. It should be noted that mycotoxins may or may not be present in moldy feedstuffs. In addition, mycotoxins may be present in feeds that appear free of mold which makes mycotoxins a challenge for producers and nutritionists alike [3].

There is increasing evidence of animal feed contamination by several mycotoxins that is coming from the specialists in animal husbandry, animal feed production, veterinary medicine and mycotoxicology [4]. This can be explained by the ever growing database of toxic fungal metabolites, newly discovered facts about the synthesis of certain mycotoxins and their groups by different fungus types, the development of the system of permanent monitoring and the improvement of...
the methodology of research on mycotoxins contained in animal feed.

**Analysis of recent research.** According to Whitlow and Hagler, mycotoxins exert their effects through several means including 1) decreased feed intake, 2) reduced nutrient absorption and impaired metabolism, 3) altered function of the endocrine and exocrine systems, 4) suppressed immune function, 5) altered intestine microbial growth and 6) changes in white blood cell and neutrophil counts [5].

Müller et al. conducted an experimental investigation of weaner pigs and the influence of combined administration of ochratoxin A, fumonisins B1, deoxynivalenol and T2 toxin in quantities expected to be present in feeds of central European origin. They observed toxic effect of mycotoxins that slightly surpassed the toxico-biological effect after single administration of ochratoxin A [6]. The contamination of animal feed with several toxic compounds of micromycetes (i.e., microfungi) occurs quite frequently, since many kinds of Aspergillus, Penicillium and Fusarium produce more than one mycotoxin, and various fungi may contaminate grain components of animal compound feed. Thus, corn and wheat naturally contaminated with vomitoxin (i.e., deoxynivalenol, DON), 15-acetyl DON, Fusarium acid (FA) and zearalenone were included in the diet of start pigs.

Feeding pigs with purified mycotoxins fumonisin B1 and aflatoxin B1, together and separately influenced the immune system, biochemical, hematological and clinical parameters [7]. With the joint administration of mycotoxins, these indicators were more pronounced, and the toxic effects intensified and sometimes very significantly, especially liver disease syndrome [8].

The study demonstrated considerable decrease in growth rate, feed consumption and brain neurochemistry alterations [9]. The combined contamination of feedstuff with fungal toxins complicates the prevention of mycotoxicoses in animals, because mycotoxins have a wide range of physicochemical properties. Thus, application of only one method of detoxication and decontamination (e.g., the use of specific enterosorbet) is not always efficient [10].

Besides, it is well known that sorbents tend to bind (adsorb) and withdraw from the organism macro- and microelements, vitamins, and nutrients, which subsequently leads to the decrease in animal productivity and becomes the reason of the rejection of mycotoxin-binding pharmaceuticals.

Schell et al. used weaning and growing pigs to investigate the effect of sodium bentonite clay (1 %) on mineral metabolism in diets with or without aflatoxin. Feeding aflatoxin contaminated feed increased phosphorus (P), sodium (Na) and Zn absorption and retention suggesting a possible increased metabolic demand for these minerals when aflatoxin is present.

In addition, feeding sodium bentonite decreased Mg absorption regardless of the presence of aflatoxin. The addition of bentonite clay also decreased calcium (Ca) and Na absorption and retention aflatoxin contaminated diets and decreased Na absorption when the feed was free of aflatoxin. Similar to the effects of Hydrated sodium calcium aluminosilicate, Zn absorption and retention was decreased in diets supplemented with sodium bentonite. The effects of feeding sodium bentonite clay on iron (Fe) was confounded in this study by the increase in dietary Fe (446 ppm vs. 292 ppm) from the addition of the clay [11].

In addition to negatively affecting Zn, bentonite has also been shown to decrease copper (Cu) bioavailability in sheep [12] fed no supplemental trace minerals. Although the cation composition was not disclosed, bentonite was fed at 0.5% of the diet (as fed basis). In this study, bentonite decreased the ruminal solubility of Zn, Cu and Mg and led to significant decreases in Cu in both plasma (0.75 vs. 0.71 µg/ml) and liver (602 vs. 504 µg/g DM).

In conclusion, it appears utilizing silicate minerals as mycotoxin sequestering agents could lead to decreases in both Zn and Cu status. The interaction between complexed trace minerals and mycotoxin sequestering agents has not been researched. However, providing a portion of supplemental Zn and Cu as amino acid complexes may be warrant- ed to improve the likelihood of maintaining optimal trace mineral status when diets contain silicate-based mycotoxin sequestering agents [13].

Other mycotoxin sequestering agents include activated charcoal, cholestyramine, chlorophyllin and yeast cell wall-derived agents. Although these may be beneficial at reducing the impact of mycotoxins in humans, aquatic and other animal species, there is currently no data available on their interaction with mineral nutrition [14].

**The aim of the study** was to analyze the changes in vitamin and mineral metabolism in piglets under the influence of the feed additive Harufix+ in associated mycotoxicosis.

**Material and methods.** For the purposes of this study we formed 4 groups of weaner pigs, 10 piglets in each. The piglets in group 1 were administered with combined feed that contained Harufix+ in dosage of 1 kilo per ton of feed. The piglets in group 2 were fed with the feed that contained T2 toxin (0.1 mg/kg), fumonisins B1 (0.5 mg/kg), vomitoxin (deoxynivalenol, 0.1 mg/kg) and penicilllic acid (1 mg/kg). In order to produce T2 toxin we used Fusarium sporotrichiella, strain 2M.
which was produced at the State poultry research station of National Academy of Agrarian sciences of Ukraine). Other mycotoxins by micromycete isolates were incubated at the Department of microbiology and virology of Bila Tserkva National Agrarian University. The diet of group 3 piglets included a complex of mycotoxins and anti-toxic feed additive “Harufix+” (1 kg/ton). The feed of the animals of group 4 did not contain mycotoxins (control group). The experiment lasted 14 days. At the beginning and during the experimental period the animals were weighed. At the end of experimental period it was conducted biochemical laboratory analysis of piglets’ blood samples for evaluation the values of calcium, phosphorus, magnesium, zinc, manganese, ferum, copper, vitamins A and E. Blood for the study was taken from the piglets’ orbital venous sinus in vacuum tubes with gel and coagulation activator. The blood serum content of total calcium was determined in the reaction with calcium arsenase III, inorganic phosphorus – by UV-detection of phosphomolybdate complex, total magnesium – with the calmagite indicator, vitamin A – by the method of Bessey in the modification of VI Levchenko, vitamin E – in reaction with 2,2-dipyridyl. All these methods were carried out with reagents of research and production association "Philit-diagnostics" using a semi-automatic biochemical analyzer Stat Fax (USA). The serum content of ferum, copper, zinc and manganese was determined by atomic absorption spectrophotometry using an atomic absorption spectrophotometer Shimadzu (Japan).

Statistical processing of the results was performed using Statistica 10 (StatSoft Inc., USA, 2011).

Results. Based on animals’ weight tests, we come to conclusion that fodder additive “Harufix+” has positive impact on body weight growth. Thus, weight growth rate increase of the piglets in group 1 (i.e., those whose diet included the pharmaceutical, unlike the diet of the animals in control group) constituted 16 %, while their average weight growth rate was high and constituted 1.96 kg per day. This observation provides indirect evidence in support of the conclusion that this fodder additive does not bind and withdraws from the organism nutrients, vitamins, macro- and microelements.

We compared weight growth data of animals in group 2 (whose feed was contaminated with mycotoxins) and group 3 (whose diet was contaminated as well, but also included the feed additive). In comparison, we discovered the difference in 5 %. Average weight growth rate in group 2 was low and constituted 2.05 kg per day, which was the effect of the mycotoxins in the feed. The same parameter in group 3 constituted 2.15 kg per day (Figure 1). The difference in growth rates in these groups is apparent and strongly testifies to the protective capacity of Harufix+ in induced mycotoxicosis in weaner pigs. In the first group, the rate of weight gain was the highest and averaged 2.73 kg, which is due to the efficient use and absorption of feed nutrients against the background of additional use of feed additives.

The analysis of mineral and vitamins status in piglets did not reveal any disruption of their homeostasis. Furthermore, we observed the normalization of the ratio of certain elements of their mineral nutrition.

The content of total calcium in group 1 remained comparable to those in control group and...
it was $2.51\pm0.14$ mmol/L ($2.23–2.67$ mmol/L). It should be noted that in piglets of other experimental groups, the content of total calcium in the blood also did not have a significant difference compared with controls and animals of the first group (Table 1). The content of non-organic phosphorus in blood serum of piglets in group 1 was significantly higher ($p<0.001; +18.2\%$) relatively the corresponding parameter in group 4 ($2.75\pm0.055$) and it was in average $3.25\pm0.020$ mmol/L ($3.21–3.28$). Also it should be noted the significantly increasing in 1.2 times ($p<0.001$) the phosphorus content in animals of group 3, which received a complex of mycotoxins and anti-toxic additive “Harufix+” relatively the control group.

The changes in serum magnesium homeostasis were characterized by a probable decrease in its blood level in animals of the second group on average to $0.73\pm0.047$ mmol/L ($p<0.05$) compared with the control and 3rd groups, which may be due to impaired absorption of this macronutrient in the intestine or reduction of its reabsorption in the renal tubules due to the complex action of mycotoxins on these organs. In other experimental groups of piglets, the total magnesium serum content in the probably did not differ from the control (Table 1).

Ferum is a microelement that is in particular demand in the organisms of young, fast growing animals. Thus, Ferum was abundant in the blood of animals, whose diet included Harufix+, its content averaged $681.7\pm151.9$ μg/100 ml. The Ferum blood content of the piglets of groups 3 and 4 almost did not differ and averaged $505.8\pm182.1$ (207.0–835.5) and $529.0\pm268.0$ (212.0–1063.7) μg/100 ml respectively. Accordingly, in animals whose diet contained mycotoxins its content was on a considerably lower level and averaged $384.2\pm178.0$ μg/100 ml, which is 1.8 times less than in group 1 and 1.4 times less than in control group (Table 2).

Copper metabolism in the organism of those piglets that were administered this fodder additive did not undergo significant changes. The content of this micronutrient in animals of group 1 averaged $288.9\pm13.8$ (261.7–306.5) as opposed to $285.9\pm42.8$ μg/100 ml in those in control (Table 2). However, in the second experimental group, the blood copper content in piglets was $31.7\%$ lower compared to the control group, which indicates a negative effect of mycotoxins on the absorption of this trace element in the gastrointestinal tract of animals.

The serum Zinc content in group 1 averaged $38.7\pm2.31$ μg/100 ml, which is $34.8\%$ higher ($p<0.01$) than in control group ($28.7\pm1.85$). The highest level was in piglets of 3rd experimental group – $70.9\pm31.1$ μg/100 ml, which is 2.2 and 2.5 times higher than the same index in groups 2 and 4, respectively (Table 2). Probably, this is due to the

---

### Table 1 – The changes in macronutrient metabolism indexes in piglets

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca total, mmol/L</td>
<td></td>
<td>2.23–2.67</td>
<td>2.31–2.75</td>
<td>2.50–2.88</td>
<td>2.44–2.53</td>
</tr>
<tr>
<td>P non-organic, mmol/L</td>
<td></td>
<td>3.21–3.28</td>
<td>2.74–3.36</td>
<td>3.18–3.35</td>
<td>2.5–2.84</td>
</tr>
<tr>
<td>Mg total, mmol/L</td>
<td></td>
<td>0.95–1.14</td>
<td>0.64–0.80</td>
<td>1.05–1.2</td>
<td>0.74–1.33</td>
</tr>
<tr>
<td>Cu, μg/100 ml</td>
<td></td>
<td>397.9–917.7</td>
<td>203.8–740.2</td>
<td>207.0–835.5</td>
<td>212.0–1063.7</td>
</tr>
<tr>
<td>Zn, μg/100 ml</td>
<td></td>
<td>261.7–306.5</td>
<td>54.7–265.6</td>
<td>246.4–268.5</td>
<td>230.3–370.1</td>
</tr>
<tr>
<td>Mn, μg/100 ml</td>
<td></td>
<td>288.9±13.8</td>
<td>195.2±70.2</td>
<td>260.7±7.14</td>
<td>285.9±42.8</td>
</tr>
</tbody>
</table>

Note: * $p<0.05$; *** $p<0.001$ relatively control (group 4).

### Table 2 – The changes in micronutrient metabolism indexes in piglets

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe, μg/100 ml</td>
<td></td>
<td>681.7±151.9</td>
<td>384.2±178.0</td>
<td>505.8±182.1</td>
<td>529.0±268.0</td>
</tr>
<tr>
<td>Cu, μg/100 ml</td>
<td></td>
<td>261.7–306.5</td>
<td>54.7–265.6</td>
<td>246.4–268.5</td>
<td>230.3–370.1</td>
</tr>
<tr>
<td>Zn, μg/100 ml</td>
<td></td>
<td>34.2–41.8</td>
<td>28.2–36.9</td>
<td>37.5–133.1</td>
<td>26.7–32.4</td>
</tr>
<tr>
<td>Mn, μg/100 ml</td>
<td></td>
<td>19.6–34.0</td>
<td>13.0–46.8</td>
<td>16.0–39.2</td>
<td>15.2–16.6</td>
</tr>
</tbody>
</table>

Note: * $p<0.05$; ** $p<0.01$ relatively control (group 4).
positive effect of Harufix+ components on the zinc absorption against the background of mycotoxins damage.

Regarding changes in manganese levels, in piglets of all three experimental groups, its content in the blood averaged almost at the same level – 25.9–26.1 μg/100 ml, which is 1.6 times higher than the average value in animals of the control group (15.9±0.40 μg/100 ml). However, a probable increase in blood manganese levels was only in group 1 (p<0.05).

Similarly to the microelements values, our analysis of the vitamins A and E levels did not reveal their decrease that would result from the pharmaceutical use. Thus, the value of vitamin A in blood serum of group 1 was 14.3–25.8 and averaged 18.6±3.64 μg/100 ml, whereas the normal indicator for the piglets of this age is 20–50 μg/100 ml (Figure 2).

The highest average value of vitamin A was in the piglets blood of the control group – 29.1±4.6 μg/100 ml, while the lowest content was observed in animals of the 2nd experimental group, which was 1.9 times less than the control group (p<0.05). The analysis of blood serum for the value of vitamin A in group 3 was 38 % higher than in group 2 and 13.4 % – then in group 1 (Figure 2).

It should be noted that changes in the content of vitamin E in the blood of piglets under the complex influence of the association of mycotoxins without the use of enterosorbent were characterized by a significant decrease to 0.17±0.02 mg/100 ml (p<0.05) compared with animals of the first and third experimental groups. However, low level of vitamin E was observed in piglets of the control group – 0.19±0.03 mg/100 ml, which also did not take this feed additive (Figure 3).
The content of vitamin E in 67% of animals of groups 2 and 4 was less than the minimum level of 0.2 mg/100 ml. While in groups 1 and 3 we can observe a positive effect of the feed additive “Harufix+” components on the absorption and stability of tocopherol homeostasis, because the average value in the blood of piglets in these groups was in the range of 0.25–0.26 mg/100 ml.

Discussion. Minerals provide an important role in animals. Their importance is in maintaining a certain osmotic pressure of blood plasma, acid-base balance, permeability of various biological membranes, regulation of enzyme activity, preservation of the structures of biomolecules, maintenance of the motor and secretory functions of the digestive tract.

We found that in case of mycotoxicosis caused by toxins of Fusarium and Penicillium fungus, there were significant deviations in the content of total magnesium in the blood plasma of piglets, the level of which was 34, 2% less than in the control group. The use of the sorption additive “Harufix+” did not have a negative effect on the absorption of this macronutrient in the intestine, since its content in the blood of piglets of the 1st and 3rd experimental groups did not significantly differ from the control group.

The positive effect of feed additives on absorption and the absence of its sorption effect on phosphorus in the gastrointestinal tract of pigs was evidenced by a probable increase in its blood level in animals of groups 1 and 3 compared with controls (p<0,001). Phosphorus absorption in pigs is limited, depends on the sufficient amounts of vitamin D, and the presence of calcium in their nutrition [15, 16]. Apparently, such active transport of this macroelement is associated with the sorptive qualities of the Harufix+ components related to phosphorus accumulation and its transport into blood vessels. The significance of non-organic phosphates in the organism is determined not only by the fact that their great amounts are concentrated in bone tissue in the compound of calcium phosphate. Monosodium and disodium phosphates establish phosphorus buffer system in blood, which along with carbonate and protein buffers participates in acid-alkaline balance regulation. In parallel, phosphorus plays an important role in the processes of phosphorylation and dephosphorylation. This provides kidney absorption and excretion, as well as lipids and proteins transport [17]. This is demonstrated by the piglets’ body mass increase of group 1, i.e. in those that were administered fodder additive within their combined nutrition.

However, the blood total calcium content in piglets of all experimental groups remained at the level of control and was within physiological limits. That is, neither the use of feed additives nor the complex effect of the association of mycotoxins did not cause deviations in the homeostasis of this macronutrient. Probably, this can be explained by a rather severe complex and multicomponent endogenous system of ensuring the stability of calcium blood value, which involves mainly humoral factors and vitamin D also. Ensuring the calcium homeostasis stability and maintaining its physiological blood plasma level is carried out by various mechanisms, the main of which are changes in the degree of intestine absorption, the proximal renal tubules reabsorption and the calcium’s mobilization from the bone component [18].

The impact of Harufix+ on micronutrients absorption reveals itself in its positive influence on Zinc transport, which was reflected in a probable increase its blood level in piglets of the first group by 34.8% compared to control (p<0,01). The best result regarding Zinc homeostasis was obtained when using the sorbent in piglets with the associated influence of mycotoxins – 70.9±31.1 μg/100 ml, which is 2.5 times higher than the average value in the control group. Biochemical function of Zinc in the organism is related to the activity of ferment, which need Zinc as a necessary component or an activator. Up to date, Zinc was found present in more than 200 metalloenzymes, which take part in a range of metabolic processes, including synthesis and decomposition of carbohydrates, fats, proteins and nucleic acids [18].

It is important to sustain microelements homeostasis on sufficiently stable level since it determines their synergism in relation to metabolism stimulation in general. For instance, Ferum is highly needed for normal activity of dehydrogenases, catalase and peroxidase; Copper is needed for oxygenase, xanthine oxidase and urate oxidase; Manganese – for transferases; Magnesium – for phosphohydrolase and Zinc for carbonic anhydrase and carboxypeptidase [19, 20]. It is important to note that physiological anemia is the typical outcome of the insufficient Ferum in piglets organisms during the first weeks of their lives. Anemia causes the death of 20 to 30% suckling piglets during the first weeks of their lives. Our research has shown positive dynamics in the assimilation of Ferum in piglets that consumed a feed additive, both as part of common feed and against the background of its contamination by mycotoxins. In the second experimental group, the Ferum’s serum content was the lowest – 384.2±178.0 μg/100 ml.

The use of Harufix+ did not adversely affect the absorption of Copper and Manganese in the intestines of piglets, which was reflected in the constant levels of these micronutrients in the blood of ani-
mals of groups 1 and 3. Moreover, in the blood of piglets that consumed the feed additive, there was a probable increase in 1.6 times the content of serum Manganese compared to the control group (p<0.05).

The study content of vitamins A and E, the same as with mineral nutrients, has not been established decrease during treatment with study the feed additive. The obtained results testify to the active absorption in the gastrointestinal tract of the vitamin components within the fodder combined with fodder additive “Harufix+” and high biological accessibility of its transport forms. The efficiency of the pharmaceutical can be explained by its composition. Thus, the complex of mineral and organic components is formed by means of modification of organic cations of the mineral surface. As a result, not merely a mixture of organic and mineral components is formed, but a new organic complex. Acarbose that is a component of Harufix+ in combination the mineral components absorb mycotoxins and excrete them from the animal’s organism, as well as normalize the microflora of the intestines. Beta-glucan, which is also contained in this fodder additive, improves the function of the gastrointestinal tract, activating the enzymatic system of its mucous membrane.

Conclusions. 1. The study demonstrated antitoxic efficiency of fodder additive “Harufix+” in pigs with mycotoxicosis experimentally induced by a complex of mycotoxins, which was manifested by its efficient sorption properties characteristics relative to T-2 toxin, fumonisnin B1, vomitoxin and penicillic acid.

2. This fodder additive did not cause the disruption of beneficial components of nutrition digestion, it proved biologically harmless, and positively impacted body weight increase in the animals.

3. Monitoring of changes in the content of total calcium, inorganic phosphorus, magnesium, iron, zinc, copper, manganese and vitamins A and E in the piglets blood confirmed the stability of their homeostasis against the background of the sorbent.

Information on compliance with ethical standards. Experimental studies were conducted in compliance with the requirements of the Law of Ukraine No. 3447 – IV of 21.02.06 "On the animals protection from cruel treatment" and in accordance with the basic principles of the "European Convention for the protection of vertebrate animals used for experimental and scientific purposes" (Strasbourg, 1986), the Declaration on the Humane Treatment of Animals (Helsinki, 2000) and the National Congress on Bioethics “General Ethical Principles of Animal Experiments” (Kyiv, 2001).

Information about the interest conflict. The authors declare no interest conflict.

LIST OF LITERATURE
3. Висла́нко О.О., Зіно́в’єв С.Г., Гияр В.М. Ефективність використання нового сорбенту мікотоксінів у свиннях. Вісник Полтавської державної аграрної академії. 2010. № 2. С. 107–110.


REFERENCES


Моніторинг зміни показників мінерально-вітамінного метаболізму під впливом кормової добавки за експериментального асоційованого мікотоксину в поросяти

Андрійчук А.В., Мельник А.Ю., Вовкотруб Н.В.

Токсикобіологічна дослідження асоційованої мікотоксинної дії Penicillium и Fusarium (T-2 токсин у концентрації 0,1 мг/кг, фумонізин B1 – 0,5 мг/кг, вомітоксин (ДОН) – 0,1 мг/кг, пеницилова кілота – 1 мг/кг) супроводжувалася розвитком комплексного патологічного процесу в організмі відбулися поросят. У зв‘язку із цим було досліджено дії токсинозаційного та сербійну здатність комплексної кормової добавки “Харуфікс+” на основі маннозолігосахаридів. Вивчено вплив сорбенту на резорбтивну актівність мінеральних і вітамінних нутрієнтів корму за результатами біохімічних і лабораторних вимірювань.
Моніторинг змін показників мінерально-витамінного обміну при експериментальному ассоціаційному микотоксикозі поросят

Андрейчук А.В., Мельник А.Ю., Вовкотруб Н.В.

Токсикобіологічне дії ассоціації микотоксинів роду Penicillium і Fusarium (T-2 токсин в концентрації 0,1 мг/кг, фумонізин B1 – 0,5 мг/кг, вомітоксин (ДОН) – 0,1 мг/кг, пеницилловая кислота ‒ 1 мг/кг) супроводжувалося розвитком комплексного патологічного процеса в організмі отруєних поросят. Відносно цьому було вивчено деякі показники крові, а саме концентрацію елементів, що відображають стан елементного обміну. Отримані результати показали активне всмоктування в кишечнику витамінних компонентів у контексті застосування кормової добавки "Харуфикс+" та високу біологічну доступність його транспортних форм. Така ефективність добавки пояснюється, насамперед, її складом, а саме комплексом мінеральних і органічних компонентів, які формується шляхом модифікації органічними катіонами поверхні мінералу.

Ключові слова: микотоксини, микотоксикоз, макро- і мікроелементи, сорбент, вітамінний обмін, поросята.