Проведено дослідження впливу важких металів, а саме Кадмію і Плюмбуму на організм молодняка свиней та ефективності засобу з попередження інтоксикації важкими металами. Важкі метали згодовувались окремо і сумісно, у дозах, що перевищують гранично допустимі концентрації у кормах у 10 та 20 разів. Тим самим, змодельовано процес інтоксикації біологічного об'єкту (свині) важкими металами, що може статись внаслідок забруднення довкілля. Дослідження проводилися з метою визначення напряму і ступеню впливу важких металів на живу масу, масу внутрішніх органів тварин, ступінь накопичення у продукції свинарства, а також визначення ефективності антитоксичної добавки в якості детоксиканту важких металів. Статистична обробка отриманих даних дозволила побудувати математичні моделі і встановити кореляційний зв'язок між вивчаємими факторами.

Встановлено, що важкі метали мають суттєвий негативний вплив на інтенсивність росту тварин і сила впливу зростає із дозою. Це підтверджує і високий кореляційний зв'язок між цими ознаками (коефіцієнт кореляції (r) дорівнює 0,854. Так, жива маса свиней наприкінці досліду як в I-ій так і у II-ій серії за дії хемотоксикантів зменшувалася на 5,5–14,8 % порівняно з контролем. Сила впливу залежала і від самого токсину. Так, найбільший негативний ефект спостерігався за дії самого Кадмію та Кадмію і Плюмбуму разом. Тварини, які на фоні інтоксикації Плюмбумом і Кадмієм отримували антитоксичну кормову добавку зберігали інтенсивність росту на рівні контролю, а за результатами I-ої серії перевищували його показники. Важкі метали в більшій мірі накопичувалися в печінці і нирках, найменше - в легенях, серці і м'язах. Побудовані рівняння регресії показали, що головний вплив на збільшення вмісту Кадмію і Плюмбуму у органах і м'ясі відіграє зростання дози відповідного елементу у кормах. Вміст Плюмбуму у кормах впливав на вміст Кадмію у м'ясі – із збільшенням його концентрації, вміст Кадмію у м'ясі зменшувався.

Отримані в результаті досліджень дані дають уяву про напрям і ступінь дії важких металів на біологічні об'єкти. Математичні моделі можуть використовуватись для прогнозування вмісту токсинів у продукції свинарства

Ключові слова: екоцидний вплив, міграція ксенобіотиків, антитоксична добавка, безпечна продукція свинарства

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DEVELOPMENT OF TECHNIQUES TO PREDICT AND PREVENT BOTH THE EFFECT OF XENOBIOTICS AND THEIR MIGRATION INTO PIG-DERIVED PRODUCTS

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1. Introduction

Studies in recent years have testified to the critical state of the environment that is caused by an increase in the number of different pollutants not inherent to the environment before [1]. Among these pollutants, special group is the heavy metals. Conditionally named "heavy metals" are understood to represent a group of metals that have a density above 6 g/cm^3 and a relative atomic weight exceeding 50 a. u. m. [2], most of which are toxic (zinc, cadmium, mercury, plumbum, and others) [3]. These xenobiotics quickly migrate and accumulate in the components of the biosphere (air, water, soil – plants – animals), thereby hindering the production of environmentally safe agricultural products [4].

It is known that human health directly depends on the quality and environmental safety of products that are consumed; therefore, this issue requires special attention [5].

Studying the mechanism of influence, the intensity of migration throughout food chains and searching for ways to reduce the content of heavy metals in products of animal husbandry is of great practical importance. This is relevant for the production of pork as well.

This is essential in those regions where it is impossible to prevent the pollution of agrobiogeocenoses as a result of strong anthropogenic influence and that leads to high levels of accumulation of chemotoxicants in the fodder and products of animal husbandry. Under these conditions, it is important to know the mechanism of action of toxicants, the intensity of migration throughout trophic chains, as well as effective techniques for predicting and preventing the negative effect of heavy metals.

2. Literature review and problem statement

One of the major environmental problems of modern humanity is the problem of the continued growth of the content of compounds of heavy metals in the soil, water and atmosphere of the industrially developed countries and cities. Of all the pollutants of soils, the heavy metals account for about 60 % [6]. Moreover, in many cases the content of toxicants that exceed MPC is observed not only around the sources of industrial emissions of metals, but also in areas remote from these places [7].

The anthropogenic sources of the presence of heavy metals in ecosystems are quite varied: emissions from industrial enterprises, transport, combustion of fossil fuel, sewage, solid waste landfills, mineral fertilizers [8].

The main danger of heavy metals is that they are capable to gradually accumulate in the links of the food chain, with the greatest concentration to be at its final link [9]. Thus, the cattle that consume contaminated fodder accumulate metals in specific organs and a person who takes meat and dairy products from them gets a larger concentration of these elements [10]. In addition, certain heavy metals are capable of biotransformation, the result of which are metabolites that have even greater toxicity than the original compounds [11].

Among the main heavy metals, the most toxic ones to the organism of animals are Cd and Pb. These elements demonstrate significantly more pronounced toxicological properties, even at the lowest concentrations [12]. Every year. biogeochemical cycles receive $3\cdot 10^5$ t Pb, $2\cdot 10^3$ t Cd. Ukraine outperforms the United States and developed European countries by 3.0–6.5 times in terms of the anthropogenic chemical load [1].

Both toxicants are very toxic, they block the work of enzymes that are important to the life activity of the body, disrupt the performance of internal organs [13], reduce the body's resistance to disease [14], they exert negative impact on heredity and destroy the red blood cells [15].

When excess amounts of cadmium and plumbum enter the body, it disturbs the exchange of some essential elements (ferrum, cuprum, sulfur, iodine) [16], increases the excretion of calcium and phosphorus from the body and decreases the strength of bones [17].

Heavy metals negatively affect the productivity of animals [18], change the chemical composition, indicators of biological value and sanitary quality of animal husbandry products [19]. Such products negatively affect health of people.

Most studies into the influence of heavy metals on the body of animals are limited to studying the accumulation of heavy metals in organs [20] and the physiological status of animals at a slight excess in the content of heavy metals [21], or even at the level of the maximally permissible concentrations in fodder [22]. The impact of excessive doses of heavy metals is often explored at laboratory animals, which do not have any industrial value and provide for the understanding the mechanism of action of toxins on biological objects [23]. Most research is directed at investigating a single toxin [22] or a certain mix [24], which does not give any clear pattern about the nature of interaction between heavy metals.

The development and implementation of means to prevent the negative effect of heavy metals on the body of animals is the pressing issue at present. There are quite a lot of different preparations with such an effect [25]. However, these are mostly the one-component preparations [26], which are not capable to solve the problem because heavy metals have a combined effect on the body. Natural adsorbents, which are also widely used as detoxicants, along with heavy metals actively remove essential elements (cuprum, zinc, ferrum), required by animals for normal life activity [25]. Proven to have been effective are such synthetic preparations as unitiol, calcium thetacine [10], they, however, may cause various side effects that make their long-term and preventive application rather problematic [27]. Promising in this direction is the development and application of comprehensive additives with natural components (herbs, vitamins, microelements, organic compounds of microelements) [10].

Studying the impact of toxic doses of heavy metals when they are jointly or separately applied makes it possible, by constructing an imitation model of the ecocidal efffect, to define the mechanism of action, the extent and character of the accumulation of toxins in organs and meat. Statistical processing of the acquired data enables establishing a correlation relationship between these attributes and to construct mathematical models of dependence of the content of heavy metals in fodder on the concentration in meat and internal organs. These mathematical models would make it possible, based on the indicators of heavy metals content in one of the links (fodder), to reliably predict the amount in the next one (livestock products). Given this, there is a need to control the content of heavy metals in trophic chains, specifically "fodder–animal–animal products (meat)" for receiving more environmentally safe products for the consumer.

It is also interesting to build mathematical models of the content of heavy metals in trophic chains, which make it possible, based on the indicators of heavy metals content in one of the links (soil, fodder), to reliably predict their quantity in the next one (fodder, livestock products).

3. The aim and objectives of the study

The aim of this work is to explore the direction and the extent of influence of xenobiotics, as well as techniques for predicting and preventing their migration to the body of young pigs.

To accomplish the aim, the following tasks have been set:

– to determine the extent of impact of the examined heavy metals on the live weight and the weight of internal organs of animals during experiment;

– to determine the degree of accumulation of cadmium and plumbum in the internal organs and meat of young pigs during fattening at different doses of these heavy metals in fodder and the effect of an experimental fodder additive as a detoxicant;

– to construct mathematical models of dependence of the live weight of animals, the weight of separate organs, and the concentration of toxicants in meat and internal organs on the dose of the consumed heavy metals;

– to perform a cluster analysis of research results in order to categorize data and identify their specific structure.

4. Materials and methods of research

We studied the process of biogenic migration of heavy metals (cadmium and plumbum) within the system "fodder – body of pig – products (meat)" under conditions of artificial ecocidal impact, as well as the efficiency of application of the anti–toxic fodder additive, at a farm in Poltava Oblast (Ukraine). The scientific-industrial experiment was performed on the castrate boars of large white breed at the age of 3.5 months. The starting live weight was 30 kg. Based on the principle of pairs–analogs, we formed 5 groups per 10 heads each. The first group was taken to be control. Following a 15-day comparative period, the diet of pigs from the examined groups was introduced with salts of heavy metals, such as cadmium and lead, in the dosage that exceeds the maximally permissible concentration for the mixed fodder for pigs by 10 times, thus creating a model of the ecocidal impact that reflects the level of pollution at anthropogenic areas. The animals from group V were additionally fed with the antitoxic fodder additive in a dose of 30 g per head per day. Composition of the additive included such components as dry extract of oak, grass flour of herbs, metionats of cuprum, manganese, zinc, and cobalt, vitamins A, C, D_3 and E. The same pattern was applied for the second series of our study, with a dose of heavy metals exceeding the maximally permissible concentration for mixed feed for pigs by 20 times (Table 1).

Table 1

Experiment procedure

Group	Quantity of heads	Conditions of experiment
Preparatory period, 15 days (in experimental series I and II)		
I–V	10	BD (basic diet) (fodder content of Pb<5 mg/kg, Cd<0.4 mg/kg)
Main period, 138 days (in experimental series I and II)		
Series I		
I (control)	10	BD (fodder content of Pb<5 mg/kg, Cd<0.4 mg/kg)
II (experiment)		BD+Pb (10MPC)
III (experiment)		BD+Cd (10MPC)
IV (experiment)		BD+Pb (10MPC)+Cd (10MPC)
V (experiment)		BD+Pb (10×MPC)+Cd (10MPC)+ +anti-toxic fodder additive
Series II		
I (control)	10	BD (fodder content of Pb<5 mg/kg, Cd<0.4 mg/kg)
II (experiment)		BD+Pb (20MPC)
III (experiment)		BD+Cd (20MPC)
IV (experiment)		BD+Pb (20MPC)+Cd (20MPC)
V (experiment)		BD+Pb (20MPC)+Cd (20MPC)+ +anti-toxic fodder additive

Note: BD – basic diet

We fed experimental animals in group, two times. The diet in terms of energy supply and nutrient substances corresponded to acting norms. The content of cadmium and plumbum in the mixed feed did not exceed MPC. The basic diet was daily supplemented with acetic acid salts of plumbum and cadmium in doses that matched the procedure of our research, taking into consideration the content of these heavy metals in mixed feed (Table 2). In the mixed feed, the content of plumbum and cadmium was less than the maximally permissible concentration: plumbum – 3.7 mg/kg (MPC is 5 mg/kg), cadmium – 0.109 mg/kg (MPC is 0.4 mg/kg).

In order to investigate the intensity of accumulation of heavy metals in the internal organs and tissues, we slaughtered, at the end of the experiment, three animals from each group. The concentration of plumbum and cadmium in fodders and biological material was determined at the atom–absorbing spectrophotometer AAS–30 (Germany).

Table 2

Arrival of plumbum and cadmium to the body of the examined young pigs

Indicator	Series in experiment									
	I					II				
	Group									
	I	II	III	IV	V	I	II	III	IV	V
Background content in fodder: cadmium, mg/kg	0.109					0.109				
plumbum, mg/kg	3.7					3.7				
Added per 1 kg of fodder: pure cadmium, mg	–	–	3.89			–	–	7.89		
pure plumbum, mg		46.3		46.3			96.3		96.3	
1 kg of fodder after addition of toxicants contained: pure cadmium, mg	0.109	0.109	4	4	4	0.109	0.109	8	8	8
pure plumbum, mg	3.7	50	3.7	50	50	3.7	100	3.7	100	100

Materials of the research were processed using mathematical-statistical methods employing the software packages Excel-2010 (Microsoft Corp.) and Statistica-10 (Soft Stat Inc.) [32, 33].

We constructed the mathematical model of dependence of live weight of piglets Y or the weight of their separate organs on a dose of the consumed heavy metals X applying the package "Data Analysis" from the software MS Excel-2010. We used the Regression tool and a method of selection of the most informative variables (the largest value for determination factor R, the smallest value for significance level by Fisher p_F, under condition for the significance of all its coefficients by Student at the level p_{St} not greater than 0.05).

5. Results of studying the effect of heavy metals on the bodies of pigs and pig-derived products

5. 1. Degree and character of the effect of heavy metals on the live weight and the weight of internal organs

When setting animals for fattening, the piglets both in series I and II in our experiment did not differ in the live weight with no reliable difference between the series registered. At the end of the experiment, the difference in live weight between control group and the examined group was reliable in two series of the experiment. Thus, the live weight of animals from the examined groups II, III and IV in series I of the research decreased in comparison to control, respectively, by 2.8 % ($P\geq0.95$), 5.5 % ($P\geq0.99$) and 4.4 % ($P\geq0.99$). The live weight of animals from the examined group V, on the contrary, increased compared to control by 4.0 % ($P\geq0.99$), indicating the positive effect of the anti–toxic plant additive.

In series II of the experiment, the live weight of animals in the examined groups decreased even more that in control, and a decrease in this indicator was observed also in the animals from group V. Thus, the live weight of animals from the examined groups II, III, IV, and V was less, in contrast to control group, respectively, by 5.7 % ($P\geq0.999$), 9.0 % ($P\geq0.999$), 10.8 % ($P\geq0.999$), 1.2 % ($P\geq0.90$) (Fig. 1).

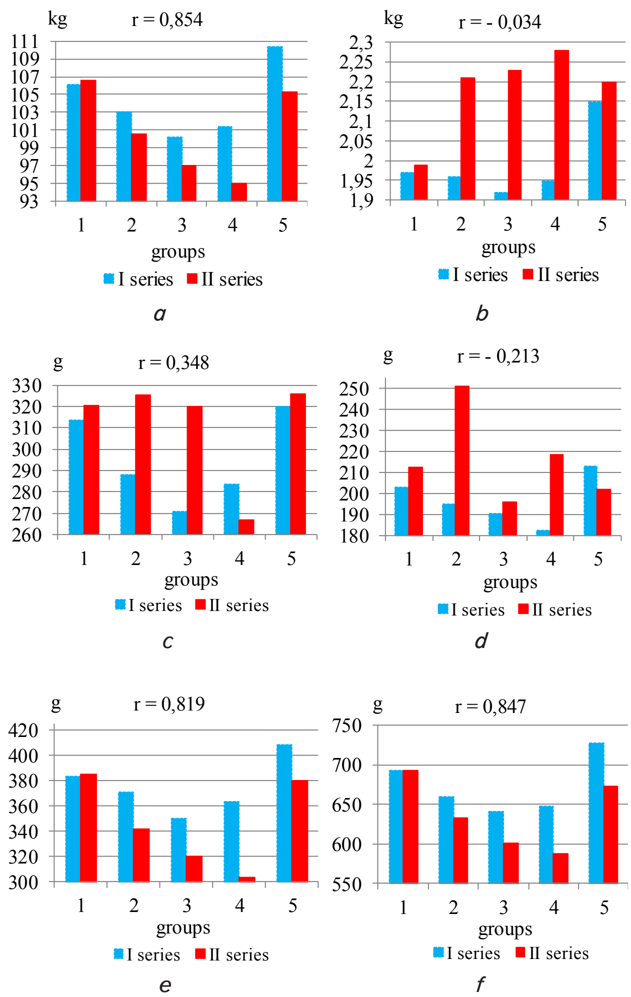

Fig. 1. Changes in weight: *a* – live; *b* – liver; *c* – kidney; *d* – spleen; *e* – heart; *f* – lungs – animals in the examined groups (Table 1), and a degree of relation between indicators and an increase in the dose of heavy metals (between the two series of experiments)

A correlation analysis of the acquired data using the tool "Correlation" revealed that between the live weight of animals and the applied doses (indicators in series I, II of our experiments) of heavy metals there is a high correlation relationship that is indicated by the value for correlation coefficient r=0.854.

Regarding the impact of various doses of heavy metals on the weight of the internal organs of animals, it was found that an increase in the dose of toxicants leads to a decrease in the weight of the heart (Fig. 1, *e*) and lungs (Fig. 1, *f*). As regards the weight of the liver (Fig. 1, *b*), kidney (Fig. 1, *c*) and spleen (Fig. 1, *d*), the same dependence is observed only when a dose of the toxicants exceeds MPC by 10 times. With a further increase in the dose of heavy metals (up to 20 MPC), the weight of these organs, on the contrary, increases. However, in all cases we observe a strong compensating effect from the developed anti–toxic fodder additive.

A correlation analysis of this data indicates that between these two varying attributes (weight of the organ and a dose) there are established relations of varying strength and direction. Thus, the degree of correlation connection was the highest between a dose of heavy metals and the weight of heart and lungs (r>0.8) (Fig. 1, *e*, *f*), a weak and a very weak correlation was observed between a dose of toxicants and the weight of kidney (r>0.4) (Fig. 1, *c*), a very weak negative correlation for the weight of liver (r>0.03) and spleen (r>0.2) (Fig. 1, *b*, *d*).

When constructing a mathematical model of dependence of the live weight of piglets or the weight of their specific organs (demoted Y) on a dose of the consumed heavy metals, the content of pure cadmium in fodder (that is, dosage) is denoted X_1, and the content of pure plumbum, respectively, X_2. We constructed the model using the software MS Excel-2010 applying the tool "Regression" from the package "Data Analysis". We employed the method of selection of the most informative variables (the highest values for determination factor R^2 of the entire regression equation and the lowest value for significance level of this equation by Fisher P_F, provided the significance level of all its coefficients by Student is at level not larger than P<0.05). The regression equation was derived in the following general form:

$$Y = a_0 + a_{11}X_1 + a_{21}X_2 + a_{12}X_1^2 + a_{22}X_2^2,$$

that is, in the form of a second-degree polynomial based on indicators X_1 and X_2. When constructing the regression equations, we discarded data on group *V* acquired from experimental series I and II, because along with the heavy metals the rations for these groups of piglets include the antitoxic vegetable additive. Results for the two series of experiments are given in Table 3.

Table 3

Dependence models of the live weight of piglets and the weight of their organs on a dose of the consumed heavy metals

Regression equations	R^2	$>p_F$
Live weight, kg		
$Y = 105{,}618 - 0{,}829X_1 - 0{,}032X_2$	0.92	0.002
Liver, kg		
$Y = 1{,}943 + 0{,}00223X_1^2 + 0{,}0000136X_2^2$	0.78	0.02
Kidney, g		
$Y = 2377{,}595X_1 - 493{,}322X_1^2$ $(0{,}13 < X_1 < 4{,}7;\ 4{,}4 < X_2 < 58{,}8)$ $Y = 2519{,}021X_1 - 264{,}662X_1^2$ $(4{,}7 < X_1 < 9{,}4;\ 58{,}8 < X_2 < 117{,}6)$	0.999 0.996	0.025 0.044
Spleen, g		
$Y = 194{,}709 + 0{,}00263X_2^2$	0.54	0.038
Heart, g		
$Y = 384{,}447 - 5{,}731X_1 - 0{,}244X_2$	0.88	0.005
Lungs, g		
$Y = 686{,}447 - 7{,}513X_1 - 0{,}322X_2$	0.89	0.004

Data from Table 3 indicate that the main influence on the change in the live weight of piglets and the weight of their individual organs (except for spleen) is exerted by the concentration of pure cadmium X_1 (because for indicator X_1 the regression coefficients are by more than an order of magnitude higher than that of X_2). For liver, an in increase in X_1 leads to an increase in weight, and in all other cases – to a decrease. For spleen, the main factor of the increase in its weight is the increase in the concentration of plumbum X_2. Application of the two regression equations to determine the weight of the kidneys over different ranges of change in the doses of heavy metals is predetermined by the complex nature of this dependence (Fig. 1, *b*), with the influence of X_2 on the change in weight being negligible.

All the calculated regression equations are credible and meaningful since the probability of the null hypothesis is low p_F<0.05. The determination factor testifies to the share of influence of the independent variable on the dependent one, and in all equations this indicator exceeds 70 %. That is, by applying the proposed regression equations, it is possible, with a high degree of reliable probability, to determine the live weight of animals and the weight of internal organs when altering the doses of heavy metals in fodder.

5. 2. Degree of accumulation of cadmium and plumbum in meat and internal organs of animals

Examining the internal organs for the content of plumbum and cadmium showed that most of these metals accumulated in kidneys and liver, and less (in sequence) in spleen, lungs, heart, and muscles (Fig. 2, 3, *a–e*).

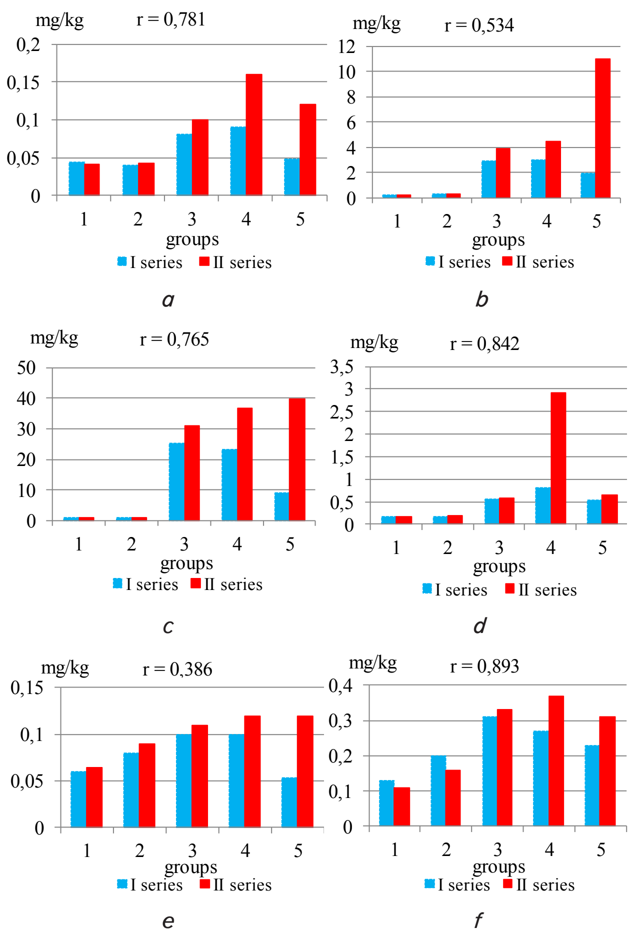

Fig. 2. Content of cadmium in: *a* – meat; *b* – liver; *c* – kidney; *d* – spleen; *e* – heart; *f* – lungs of animals in the examined groups (Table 1), and the degree of relation between indicators and an increase in the dose of heavy metals (between the two series of experiments)

Regarding cadmium, its largest concentration was observed in the kidneys and liver of animals from the examined groups III and IV (Fig. 1, *b, c*), the lowest in muscles, both in series I and II of experiments (Fig. 2, *a*). Also significant was the accumulation of cadmium in the spleen of the examined animals, especially animals from group IV, which were fed cadmium and plumbum together (Fig. 2, *g*).

It should be noted that under the joint action of toxicants in series I of experiments, the accumulation of cadmium in the kidneys, liver, lungs was smaller in comparison with indicators for group III (which were affected by cadmium only), which can be attributed to the antagonistic action of plumbum on the accumulation and redistribution of cadmium in the body (Fig. 2, *a–f*). The increasing dose (series II) led to the largest accumulation of cadmium in the organs of pigs from group IV compared to animals from other experimental groups for both series I and II (Fig. 2, *a–f*).

The content of cadmium in animals from group V, where the animals were fed, along with heavy metals, the experimental fodder additive, decreased in the organs and tissue compared to animals from examined group IV. However, in series II of experiments we observed a sharp growth of this heavy metal in the liver and kidneys, even compared to piglets from group IV, which can indicate the activation of the detoxification function in the body and the intense discharge of heavy metals (Fig. 2, *b, c*).

A correlation analysis of the above data revealed a high degree of relation between a dose of heavy metals and the content of cadmium in meat (r=0.781), kidney (r=0.765), spleen (r=0.842) and lungs (r=0.893). The medium and weak statistical relationship was observed between a dose of toxicants and the content of cadmium in the liver (r=0.534) and heart (r=0.386).

The accumulation and distribution of plumbum in the internal organs and tissues of animals from the examined groups were uneven. Thus, its largest content was observed in the liver, kidney, and spleen of animals from experimental groups II, IV and V, which can be explained by the intensive work of these organs when the body was exposed to the heavy metal intoxication. An increase in the dose led to an increase in the degree of accumulation of plumbum in the internal organs. Feeding the experimental fodder additive stimulated the discharge of plumbum from the body, resulting in that its content in the internal organs and tissues decreased (Fig. 3, *a–f*). In this case, we observed a very high correlation relationship between a dose of heavy metals and plumbum content in the spleen of the examined animals (r=0.989). A high statistical relationship was observed between an indicator for a dose of heavy metals and plumbum content in meat (r=0.741), liver (r=0.861), kidney (r=0.766), and lungs (r=0.745).

5. 3. Mathematical models of dependence of the toxicant concentration in meat and internal organs on a dose of the consumed heavy metals

The mathematical models of dependence of the content of cadmium and plumbum in the meat and organs of pigs (denoted Y) from experimental groups on a dose in the fodder (X_1 is the content of pure cadmium in fodder, X_2 is the content of pure plumbum) (Tables 4, 5) were calculated in the manner similar to that used to construct the mathematical model of dependence of the live weight of piglets or the weight or their separate organs on a dose of the consumed heavy metals (chapter 5.1).

Results of calculating the content of cadmium for two series of experiments are summarized in Table 4.

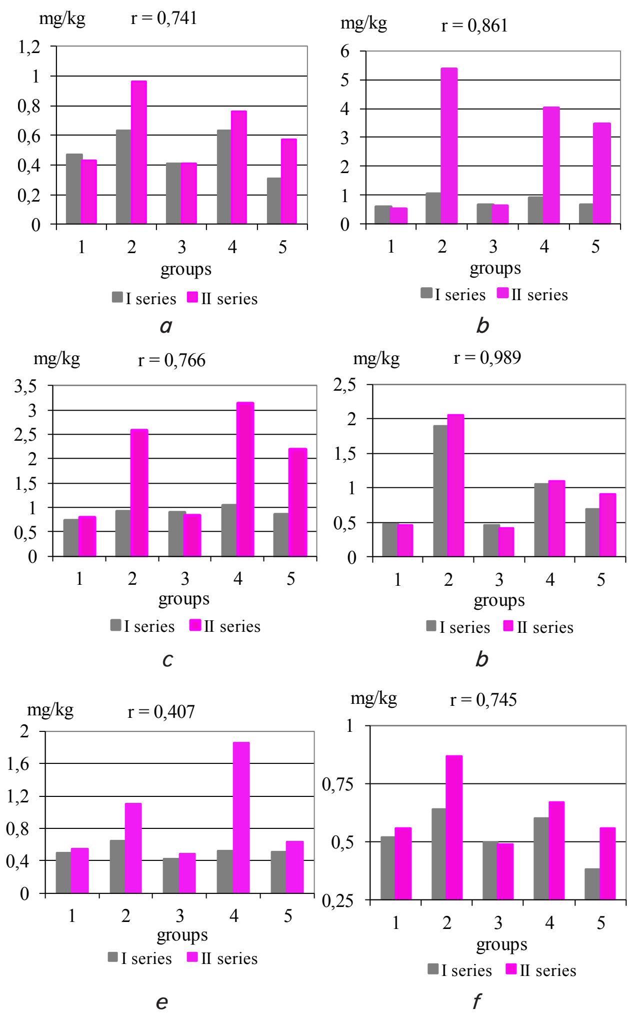

Fig. 3. The content of plumbum in: *a* – meat; *b* – liver; *c* – kidney; *d* – spleen; *e* – heart; *f* – lungs of animals in the examined groups (Table 1) and the degree of indicators' relation to an increase in a doses of heavy metals (between the two series of experiments)

Table 4

Dependence models of the content of cadmium in the tissues of piglets on a dose of heavy metals in fodder

Organs and tissues	Regression equations	R^2	$>p_F$
Meat	$Y=0{,}0119X_1-0{,}000437X_2$	0.93	0.0009
Liver	$Y=0{,}481X_1$	0.97	0.000004
Kidney	$Y=3{,}896X_1$	0.97	0.000006
Spleen	$Y=0{,}178X_1$	0.70	0.0066
Heart	$Y=0{,}0142X_1$	0.65	0.011
Lungs	$Y=0{,}0425X_1$	0.78	0.0024

Data from Table 4 show that all regression equations are practically reduced to the pair-wise linear regression equations (except for the first equation for a linear multiple regression for indicators X_1 and X_2). In this case, the main effect on the increase in the content of cadmium in the tissues of piglets is exerted by an increase in the dose of this element in fodder (also shown in Fig. 2, *a–f*). According to the first equation of regression, the content of cadmium in meat is affected by the concentration of plumbum in fodder, an increase in its concentration X_2 leads to a decrease in the content of cadmium in meat (that is, plumbum replaces it).

Regression equations for the relationship between the content of plumbum in fodder and the degree of its accumulation in the organs and tissues of examined animals are given in Table 5.

Table 5

Dependence model of the content of plumbum in the tissues of piglets on a dose of the consumed heavy metals

Organs and tissues	Regression equations	R^2	$>p_F$
Meat	$Y=0{,}00819X_2$	0.77	0.003
Liver	$Y=0{,}0354X_2$	0.90	0.0002
Kidney	$Y=0{,}0235X_2$	0.88	0.0004
Spleen	$Y=0{,}0159X_2$	0.80	0.0018
Heart	$Y=0{,}0123X_2$	0.83	0.0012
Lungs	$Y=0{,}00759X_2$	0.66	0.011

All the regression equations shown in Table 5, which are practically reduced to the pair-wise linear regression equations, indicate that the main influence on the increase in the content of plumbum in the tissues of piglets is exerted by an increase in the concentration of this toxicant in fodder, which is confirmed also in Fig. 3, *a–f*.

All equations are statistically significant by Fisher (p_F<0.05) with the high values for determination factors, which is why they can be reliably applied to predict the content of cadmium and plumbum in meat and internal organs.

5. 4. Cluster analysis of results of studying the dependence of live weight of piglets and the weight of their organs on a dose of the consumed heavy metals

To solve the task on data classification and to identify certain patterns in their structure, we employed a cluster analysis to process the results of studying the dependence of live weight of piglets and the weight of their organs on a dose of the consumed heavy metals. To this end, all 10 experimental groups for both series of our study were combined into a single large sample. Clustering was based on the Ward's method, which makes it possible to distinguish between clusters even of small size, using the software Statistica-10 (Soft Stat Inc.). The results are shown in Fig. 4 in the form of a dendrogram.

A vertical axis of the dendrogram contains numbers of groups (from 1 to 5 for experimental series I; numbers from 6 to 10 match groups from 1 to 5 for experimental series II). The horizontal axis denotes the so–called linkage distance – the smaller it is the stronger the link between clusters. The Figure shows that the strongest link exists between control groups 1 and 6, followed by 2 and 4 – in which we fed separately plumbum (the dose is 10 MPC) and cadmium and plumbum together (the dose is 10 MPC); they are then joined by group III in which we fed only cadmium (the dose is 10 MPC), that is, cluster (2; 4; 3) is composed of the experimental groups exposed both to the joint and separate effect of the toxicants in series I.

Next, one cluster is formed by groups 5 and 10 whose diet was supplemented with the developed antitoxic plant additive. They have formed, together with control groups, cluster

(1; 6; 5; 10) in which the concentration of heavy metals is minimal and compensated. Next, the cluster was formed by groups 8 and 9 from series II, in which we fed only cadmium, and cadmium and plumbum jointly, in a dose of 20 MPC, this cluster was joined by group 7 where the animals were fed only plumbum in a dose of 20 MPC.

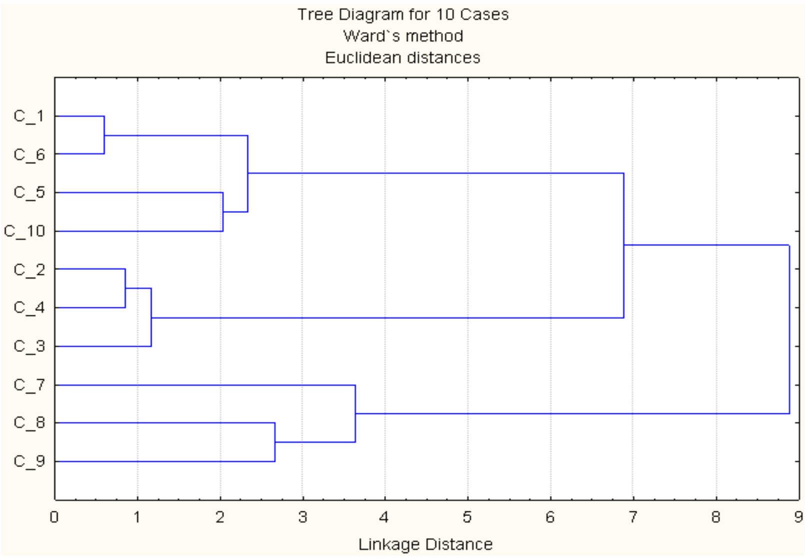

Fig. 4. Dendrogram of cluster analysis (10 groups of animals)

Thus, one cluster was formed in the absence of excessive action of heavy metals and the compensating action of the plant additive against the background of heavy metal intoxication; two clusters – under the action of cadmium and plumbum, both jointly and separately, in doses of 10 and 20 MPC.

6. Discussion of results of studying the effect of heavy metals on the organism of pigs and pig-derived products

Feeding the animals with plumbum and cadmium, both jointly and separately, in doses of 10 MPC and 20 MPC, had an impact on the indicators of live weight and the weight of internal organs from the examined animals. The resulting live weight of the fattening pigs both in series I and II of experiments, under the action of heavy metals, decreased by 5.5–14.8 % in comparison with control, while the largest negative effect was observed in the animals from experimental group 3 in series I, and experimental group IV in series II of experiments. Animals that were treated, against the background of plumbum and cadmium intoxication, with the antitoxic plant additive demonstrated live weight at the level of control, and even surpassed it in series I of experiments.

Thus, the data obtained indicate that an increase in a dose of heavy metals enhances their negative effect on the live weight of animals. This is also confirmed by the high correlation link between these attributes (correlation coefficient (r) is equal to 0.854 (Fig. 1, *a*).

An analysis of influence of cadmium and plumbum on the weight of internal organs has revealed a clear-cut relationship between a dose of the examined toxicants and the weight of heart and lungs (increasing the dose reduced the weight of these organs), which is confirmed by the high degree of correlation (r>0.8).

A complicated dependence emerged between the dose of heavy metals and the weight of kidney, liver, and spleen, because an increase in the dose led in some cases to a decrease, and in some cases to an increase, in the weight of these organs. The relationship between these attributes was of a weaker and the smallest degree. That is, it is not correct to confidently argue on that increasing the dose of cadmium and plumbum in fodder for animals leads to a decrease in all internal organs. This statement may only relate to the weight of the lungs and heart. Decrease in the live weight and the weight of internal organs from animals exposed to the action of heavy metals can be explained by the mechanism of action of toxicants, specifically a decrease in the activity of enzymes, including the gastrointestinal tract, the influence of the digestibility and metabolism of nutrients. The growth of the weight of liver, kidney, spleen in series II of experiments, along with a decrease in these indicators in series I, can be explained by the intensification of work of these organs-detoxicants in the body with an increase in dose. This is also confirmed by the character of distribution of heavy metals in internal organs.

Thus, it was established that cadmium was mostly accumulated in kidneys (Fig. 2, *c*), and plumbum in liver and spleen (Fig. 3, *b*, *d*). The least accumulation of these xenobiotics was observed in lungs, skeleton and heart muscles. A statistical analysis of the acquired data has revealed that the level of the content of heavy metals in fodder significantly affects the content of cadmium in meat, kidneys, spleen, and lungs, which is confirmed by correlation coefficients, respectively r=0.781, r=0.765, r=0.842, r=0.893. The medium and weak correlation relationship was observed between a dose of toxicants and the content of cadmium in liver (r=0.534) and heart (r=0.386). The largest plumbum content in fodder affected its content in the spleen of examined animals (r=0.989), in meat (r=0.741), liver (r=0.861), kidney (r=0.766) and lungs (r=0.745).

The analysis confirms that cadmium and plumbum mainly affect kidneys, liver, and spleen. In this case, changes occurred both in their weight and the level of their accumulation of toxicants. All this points to the fact that these organs are the targets for the action of these heavy metals.

We observed a positive effect of the anti-toxic plant additive on reduction of the cumulation and improvement of the elimination of plumbum and cadmium, both in series I and II of experiments. In addition, animals that were treated with the plant additive had a larger weight of the internal organs compared to control, due to the larger live weight and better development of these animals. That is, the pharmacological properties of the additive's components and their combination ensure binding the toxins in the body and their quick removal both in the digestive tract and in the metabolic process, the activation of immune and antioxidant systems, normalization of metabolism, hemopoiesis, and strengthening of the organism in general.

The goal of this research was to construct mathematical models of dependence of the examined indicators (live weight, the weight of internal organs, the concentration of heavy metals in internal organs and meat) on a dose of the consumed heavy metals and various types of their effect (separate and combined); the following results were obtained in the course of research: the principal influence on a change in the live weight of piglets and the weight of their separate organs (except for spleen) is exerted by the concentration of pure cadmium. For spleen, the main factor of its increase in weight is the increase in the concentration of plumbum. All computed regression equations are reliable and meaningful (p_F<0.05, R^2>0.70), and thus could be used in order to predict live weight of animals and weight of their internal organs at different content of heavy metals in fodder.

The regression equations that we constructed, reflecting a dependence of the content of toxicants in meat and internal organs on their dose in fodder, demonstrated that the main influence on the increase in the content of cadmium and plumbum in organs and meat is exerted by an increase in the dose of the respective element in fodder. It was established that the content of plumbum in fodder affects the content of cadmium in meat – an increase in its concentration led to a decrease in the content of cadmium in meat (that is, plumbum replaced it).

The constructed equations are reliable (p_F<0.05) and could be used to predict the content of cadmium and plumbum in meat and internal organs.

In order to determine the homogeneity and to systematize the examined groups according to the degree of influence of the investigated factor (a dose of heavy metals on the live weight and the weight of internal organs), we have applied the method of cluster analysis.

The result was the identified 3 main clusters: cluster 1 combined control groups (the influence of heavy metals is lacking) and the groups in which we added the antitoxic plant additive; clusters 2 and 3 were composed of the groups exposed to the excessive effect of cadmium and plumbum, both jointly and separately, in doses of 10 and 20 MPC.

The research results obtained elucidate the character of the impact of heavy metals on the body of animal, a technique to reduce the negative effect of toxins, and methods to forecast the transition of heavy metals along the links of a trophic chain. The advantage of this study, compared to analogs, is that we carried it out under industrial conditions, engaging animals that are valuable in production, and under the action of excessive doses of different combinations of heavy metals. The research results could be employed for improving a technology for obtaining high-quality environmentally safe products of animal husbandry (pig production) under conditions of fodder contamination with heavy metals. The mathematical models derived could be applied to predict the direction of effect and the degree of accumulation of chemotoxicants in the body of animals.

The study is limited by that it involved animals of the same sex–age group, specifically fattening pigs. It would be interesting to investigate the impact of heavy metals over the entire cycle of growing animals and under the action of different combinations of doses of cadmium and plumbum. The issue of interaction between cadmium and plumbum at different ratios of doses remains to be investigated. However, such a research is characterized by a large-scale character and is rather complex when undertaking it under industrial conditions.

7. Conclusions

1. It was established that under the action of heavy metals in doses of 10 and 20 MPC there was a decrease in the live weight of animals at the end of the experiment, by 5.5–14.8 %, and the degree of influence increased in proportion to the dose (r=0.854) while depending on the metal and the type of action. The action of heavy metals and an increase in dose led to a decrease in the weight of heart and lungs (r>0.8); the weight of liver, kidneys, and spleen, on the contrary, increased. We used the experimental additive that decelerated the negative effect of heavy metals on the body with the best effect observed in series I of our experiments.

2. It was established that heavy metals mostly accumulated in liver (within 1.04–5.36 mg/kg) and kidney (0.77–39.59 mg/kg), with the lowest content in heart (0.08–1.86 mg/kg) and muscles (0.04–0.96 mg/kg). The increasing dose of toxicants led to an increase in their concentration in meat, kidneys, spleen, lung, liver, which was confirmed by strong correlation relationship (r>0.7). The medium and weak correlation relationship was observed between a dose of toxicants and the content of cadmium in liver (r=0.534) and heart (r=0.386). We observed, in the groups in which animals were fed with heavy metals jointly, a change in the pattern of accumulation of toxins, due to the antagonistic mutual effect of cadmium and plumbum.

3. We have constructed mathematical models of dependence of live weight, the weight of internal organs, and the degree of accumulation of toxins in the body of animals, depending on a dose of cadmium and plumbum in fodder and the type of action. The equations derived are reliable (p_F<0.05) with the high values for determination factor, thus they could be reasonably applied in order to predict the effect of chemotoxicants on the organism of animals and the accumulation in pig-derived products.

4. We have performed a cluster analysis of the acquired data using the Ward's method. This method made it possible to organize the experimental groups according to the degree of influence of heavy metals on live weight and the weight of internal organs; 3 homogeneous clusters were identified. Control group formed cluster 1 with the groups where the antitoxic plant additive was introduced, while clusters 2 and 3 were composed of the groups exposed to excessive effect of cadmium and plumbum, both jointly and separately, in doses of 10 and 20 MPC.

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