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STATE OF METABOLIC PROCESSES IN CATTLE UNDER THE INFLUENCE OF BIOTIC CONTAMINANTS OF FEED

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Summary. Control of feed contamination by micromycetes and bacteria at all stages of their preparation, storage, and feeding of farm animals is an acute issue of feed safety and one of the principal measures that create an opportunity to prevent their negative impact on animal health. Therefore, the study aimed to investigate the state of metabolic processes in cattle of different physiological groups under the influence of biotic feed contaminants. The material for the research was grain fodder and coarse grinding grain of local production, roughage used on the farm. Veterinary and sanitary condition of grain products was established based on organoleptic, toxico-biological and microbiological studies. To determine the indicators of the state of metabolic processes, 3 groups of cows (n = 5–7) with different physiological conditions were formed: group I — non-pregnant cows, group II — pregnant animals with normal pregnancy, group III — cows after miscarriage. Biochemical parameters (level of total protein, albumin, globulins, vitamins A and E) in blood serum samples were determined spectrophotometrically by conventional methods. The study of the content of inorganic elements in the aggregate samples of bovine sera was performed using an X-ray spectrometer 'Spectroscan MAX'. Laboratory studies have proven the presence of biotic contaminants in the feed base of the experimental farm. Exceedance of maximum permissible levels of feed contamination (max 16.50×10^4 CFU/g when MPL 5.0×10^4 CFU/g) by toxin-forming micromycetes (due to the genera *Fusarium*, *Aspergillus*, *Penicillium*, *Mucor*, and *Rhizopus*; a total of 24 isolates of microscopic fungi were isolated, which showed high toxicity in 11.3% and weak — in 20.1% of samples) and total bacterial contamination (max 18.7×10^5 CFU/g when MPL 5.0×10^5 CFU/g), in the structure of which coliform bacteria and *Salmonella enterica* were isolated. In cattle that consumed feed with an excess of biotic contaminants, disorders of the digestive tract (diarrhea) and reproductive capacity (abortions in the first half of pregnancy) were observed and metabolic disorders were found in cattle: increased Iron (on average 1.5 times) and Bromine (on average 1.6 times) levels, a decrease in the concentration of vitamin A (by 17.4–39.8%), and vitamin E (by 10.0–12.5%), most pronounced in cows after abortion and pregnant cows, respectively, Manganese (on average by 12.5%) and Selenium (by 30.7%)

Keywords: metabolic disorders, microbiological contamination, micromycetes, toxicity

Introduction. High productivity of animals is a genetically determined body's ability to effectively transform feed nutrients, due to the intensive metabolic processes in animals at all levels — from hydrolysis and transport of feed nutrients in the digestive tract and use of energy from their metabolism to biosynthesis of proteins, lipids and other organic substances.

Among them a special place is occupied by minerals — macro- and ultramicroelements. For the manifestation of genetically determined potential properties of animals to synthesize quality products, it is necessary to create ecological conditions for their feeding and maintenance, which provide the optimal course of metabolic processes in the body (Orobchenko, 2012; Doletskyi, 2015).

Feeding poor quality feed to animals with high levels of toxin-forming micromycetes and residues of toxic metabolites of lower fungi — mycotoxins, which are formed under conditions of growth on forage crops in the field, during harvesting and preservation of crops, can lead to weakening of the body's resistance, the emergence of diseases, reduced productivity and deterioration of livestock products (Yaroshenko, 2016; Yaroshenko, Kutsan and Orobchenko, 2018; Kutsan et al., 2020; Kemboi et al., 2020).

According to the Food and Agriculture Organization of the United Nations (FAO), due to the high prevalence of microscopic fungi in almost all biological habitats and their high adaptive properties, 25–40% of the world's food and feed crops are contaminated with mycotoxins annually. This causes annual economic losses of about 20 billion dollars. They produce mycotoxins, which, when entering the body of animals during feeding, can cause dangerous diseases — mycotoxicosis (Gadzalo, 2017; Harčárová, Čonková, Sihelská, 2018).

In the event of action of main mycotoxins on animals, the period of the disease clinical manifestation is determined by a few hours or days. However, this is typical only in acute or subacute mycotoxicosis. Under the action of small doses of mycotoxins a chronic form of toxicosis develops, which is too difficult to diagnose and can appear only after a long time from the beginning of use of the contaminated feed (Gonçalves, Corassin and Oliveira, 2015). Therefore, it is important and relevant to study the assessment of animal welfare, which underlies the functioning of efficient production, especially in the context of the Association Agreement between Ukraine and the European Union (EU) and the WHO strategy 'One Health'(Boqvist, Söderqvist and Vågsholm, 2018; Vandicke et al., 2021; Chiesa et al., 2021).

Due to the fact that the control of contamination of feeds by micromycetes and bacteria at all stages of their preparation, storage and feeding to farm animals is an important issue of feed safety and one of the main measures to prevent their negative impact on animal health (MAPFU, 2012), the study was **aimed** to investigate the state of metabolic processes in cattle of different physiological groups under the influence of biotic feed contaminants.

Materials and methods. The work was performed in the State Enterprise 'Experimental Base 'Dachna' of the Breeding and Genetic Institute — National Center of Seed Science and Variety Study' (SE 'Experimental Base 'Dachna') (Dachna, Odesa District, Odesa Region), laboratories of epizootology, parasitology, monitoring of animal diseases and provision of the Odesa Research Station of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Odesa), laboratories of clinical biochemistry and toxicological monitoring of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (NSC 'IECVM') (Kharkiv).

The material for the research was grain fodder and coarse grinding grain of local production, roughage used on the farm in 2020–2021 (21 samples). The veterinary and sanitary condition of grain products was established on the basis of organoleptic, toxicological and microbiological studies.

Organoleptic analysis was used to determine the appearance, color, odor, and visible signs of fungal infection (Obrazhei, Pohrebniak and Korzunenko, 1998). In order to determine the degree of contamination of feed with microorganisms, mycological and bacteriological studies were carried out by seeding in nutrient media, isolation into a pure culture, according to generally accepted methods (MVDMAUSSR, 1976; MAPFU, 2012).

The species affiliation of microorganism isolates was determined by cultural and morphological characteristics of the isolated mycobiota (features of culture growth on different media, their size, shape, width, construction of the edge and center of colonies, intensity of growth, surface characteristics, color of colonies) (Pidoplichko and Mil'ko, 1971). The main test for determining the toxicity of feed was a skin test on rabbits (Malinin, Khmel'nitskiy and Kutsan, 2002).

To determine the indicators of the state of metabolic processes, 3 groups of cows ($n = 5–7$) with different physiological conditions were formed: group I — non-pregnant cows, group II — pregnant animals with normal pregnancy, group III — cows after miscarriage.

The presence of possible metabolic disorders in the cattle body was determined by biochemical indicators in blood serum samples, which were examined spectrophotometrically according to generally accepted methods (Stegniy et al., 2007; Vlizlo, 2012) and compared them with the reference levels given in the monograph (Levchenko, 2010).

The determination of the content of inorganic elements in the aggregate blood serum samples of cattle was carried out using the X-ray spectrometer 'Spectroscan MAX' following the X-ray fluorescence methodology developed at the NSC 'IECVM' and approved by the State Committee of Veterinary Medicine of Ukraine (protocol No. 1 of 23–24 December 2009) (Kutsan, Orobchenko and Kocherhin, 2014).

Results. SE 'Experimental Base 'Dachna' is a multi-industry enterprise. Crop production, where the leading industry is seed production, occupies the largest specific weight in the structure of commercial products of the enterprise. However, dairy cattle breeding, which has more than 200 cows in breeding stock and 260 heifers and calves, is also of great significance in the structure. Conditions for feeding and keeping animals comply with sanitary and hygienic norms. Veterinary and sanitary measures are carried out in a timely manner at the farm. All animals are vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea, bovine parainfluenza-3, pasteurellosis and escherichioses.

However, in the autumn period, alimentary abortions were registered among cows in the first third of the pregnancy — the expulsion of a dead fetus (miscarriage) together with the membranes without visible previous clinical signs and disorders of the gastrointestinal tract (diarrhea), the cause of which could be insufficient feeding, protein starvation or, on the contrary, overfeeding with protein substances, mineral starvation (Phosphorus, Calcium, Iron, Potassium, *etc.*), vitamin deficiency (A, E, C, D), feeding with poor-quality feed.

In order to determine the causes of abortions, laboratory tests were conducted for the sanitary quality of the feed and the presence of biotic contaminants (microscopic fungi and coliform bacteria).

According to the results of the sanitary condition of 21 feed samples (roughage: silage, haylage, straw; grain-compound feed, corn, barley) it was established that 47.6% of the feed samples met the sanitary and hygienic requirements and were allowed for feeding, 52.4% did not meet the maximum permissible levels (MPL). Thus, changes in organoleptic indicators were detected in 11 samples of roughage (straw, silage), which had a dark gray color without a shine, a musty mold smell with mycelium and sporulation of mushrooms; in samples of hay we observed a musty smell and a change in color from dark brown to black with the formation of compressed lumps. Such feed is suspicious; a change in color, smell and other characteristics indicates the development of microorganisms.

As a result of research, it was established that the sanitary condition of grain fodder met the requirements of the MPL. High rates of micromycete contamination were found in rough forages, which amounted to 13.25×10^4 CFU/g in straw, 16.5×10^4 CFU/g in haylage, and 11.25×10^4 CFU/g of forage in silage, which, respectively, in 2.7, 3.3, and 2.3 times exceeded the MPL indicator (Table 1), and 24 isolates of microscopic fungi were isolated.

Table 1 — Results of microbiological studies of feeds

Name of the feed	Microscopic fungi, CFU/g	Total bacterial pollution, CFU/g	The titer of coliform bacteria
Barley	1.65×10⁴	3.5×10⁵	2
Wheat	not found	2.9×10⁵	1
Combined feed	not found	3.5×10⁵	2
Wheat bran	0.95×10⁴	3.0×10⁵	1
Wheat straw	13.25×10⁴	9.5×10⁵	3
Haylage	16.50×10⁴	6.3×10⁵	3
Silage	11.25×10⁴	18.7×10⁵	3
MPL	≤ 5.0×10⁴	≤ 5.0×10⁵	≤ 3

The maximum indicators of total bacterial contamination were also found in roughage, which 2–3 times exceeded the MPL, and the titer of coliform bacteria was 3.

Mycological studies of two straw samples revealed toxigenic micromycetes of the genus *Fusarium oxysporum*. Samples of peas, combined feed, bran, haylage, silage, and straw were affected by fungi of the genus *Aspergillus* spp., *Mucor* spp., *Penicillium* spp., and *Rhizopus nigrus*, which showed high toxicity in 4 (19.1%) and weak toxicity in 7 (33.3%) samples.

The analysis of research results showed that representatives of the genera *Aspergillus* — 16 isolates (66.7%), *Fusarium* — 1 isolate (4.1%), *Penicillium* — 3 isolates (12.5%), and Mucoraceae family — 4 (16.7%) were the main contaminants of fodder in SE 'Experimental Base 'Dachna' in 2021 (Fig. 1).

A skin test on rabbits revealed weak toxicity of 7 feed samples (33.3%) and toxicity of 4 feed samples (19.1%).

The etiological structure of isolates of microorganisms was represented by cultures of coliform bacteria (69.2%) and *Salmonella enterica* (30.8%).

After the detection of a significant amount of biotic contaminants in feeds, a study of the state of metabolic processes in the body of cattle of critical groups was carried out by means of biochemical studies of blood sera. The results of the research are given in Tables 2–3.

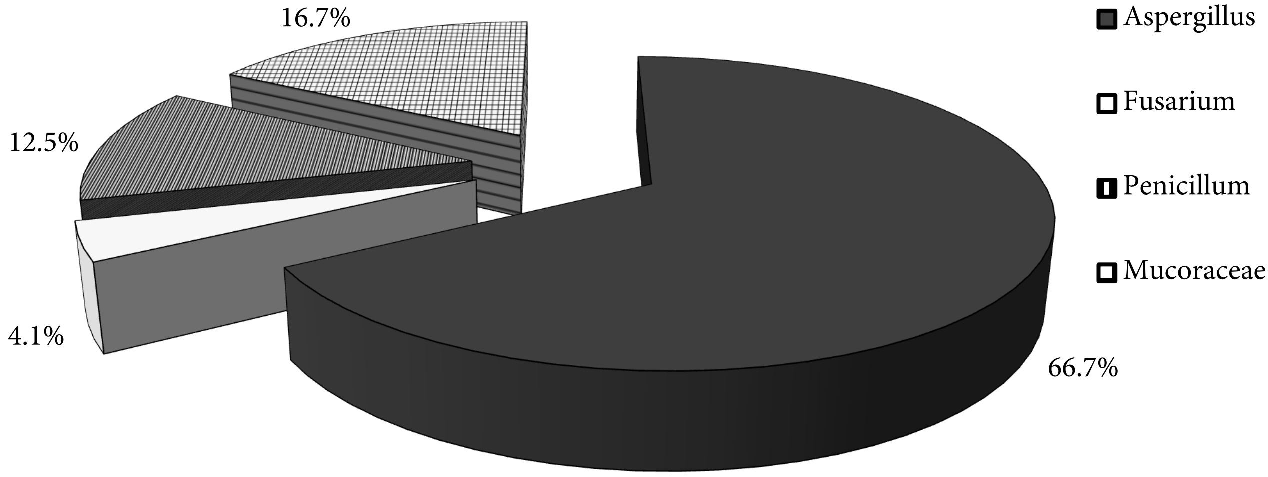

Figure 1. Taxonomic structure of mold fungi isolated from feeds for cattle

Table 2 — Results of biochemical studies of cows' blood sera (M ± m, n = 5–7)

Group	Indicators					
	Total proteins, g/l	Albumin, g/l	Globulins, g/l	Albumin/ globulin ratio	Vitamin A, μg%	Vitamin E, μg/ml
Group I (non-pregnant)	81.7 ± 0.6	36.5 ± 3.2	45.1 ± 2.3	0.80 ± 0.06	20.60 ± 0.30	3.6 ± 0.20
Group II (pregnant)	83.3 ± 2.5	35.2 ± 1.9	48.1 ± 3,7	0.76 ± 0.07	16.50 ± 1.30*	3.5 ± 0.15
Group III (after miscarriage)	80.3 ± 1.8	37.0 ± 1.2	43.3 ± 0.6	0.85 ± 0.01	15.05 ± 1.40**	4.0 ± 0.06
Reference level	72.0–86.0	27.5–39.4	28.9–48.6	0.80–1.00	not less 25.00	4.0–6.0

Notes: * — P ≤ 0.05, ** — P ≤ 0.001 relative to the indicators of the group I.

As evidenced by the data in the Table 2, the most pronounced deficiency of vitamin A was observed in cows — a decrease in concentration relative to the lower limit of the reference level was found in animals of all experimental groups: in non-pregnant animals the decrease was 17.4%, in pregnant animals — 34.0%, in cows after miscarriage — 39.8%. When determining the characteristics of the cow body supply in different physiological groups, it was established that the highest level of vitamin A supply was in non-pregnant animals. Compared to the indicator of the group I, the concentration of vitamin A was significantly lower by 20.9% (P ≤ 0.05) in pregnant cows, and by 27.1% (P ≤ 0.001) in cows after miscarriages.

In addition, a slight decrease in the supply of vitamin E in the cows' body was established — in the cows of the group I by an average of 10.0%, in the group II — by 12.5% compared to the lower limit of the reference indicators. In cows of group III, the indicator corresponded to the minimum level of reference values.

The indicators characterizing the state of protein metabolism generally corresponded to the reference values and did not differ significantly in the studied physiological groups of animals, with the exception of the globulin fraction content, which was close to the maximum values of the reference level. This determined the established tendency to decrease (by 5.0%) the albumin/globulin ratio in animals of the group II compared to the minimum reference values and indicators in the cows of the group I.

Studies of the content of inorganic elements in blood serum have established (Table 3) that in the body of animals of the group I (non-pregnant) and group III (after miscarriage) there is a deficiency of Manganese and an excess of Iron and Bromine, and in the group II (pregnant) there is a deficiency of Manganese and Selene and an excess of Iron and Bromine.

Table 3 — The content of inorganic elements in cows' blood sera (M ± m, n = 5–7)

Element	Group I (non-pregnant)	Group II (pregnant)	Group III (after miscarriage)	Reference level
Zinc, µg%	133.30 ± 1.67	133.50 ± 1.22	138.40 ± 1.84	100.00–150.00
Copper, µg%	101.60 ± 1.14	103.90 ± 1.29	110.00 ± 1.42*	80.00–120.00
Iron, µg%	271.40 ± 3.57	330.00 ± 3.76*	321.70 ± 3,84*	90.00–210.00
Manganese, µg%	3.60 ± 0.12	3.80 ± 0.17	3.40 ± 0.15	4.00–6.00
Selenium, µg%	7.90 ± 0.24	5.20 ± 0.13*	9.70 ± 0.27*	7.50–10.00
Lead, µg%	Not found	Not found	Not found	—
Nickel, mg%	5.10 ± 0.18	4.10 ± 0.16*	4.80 ± 0.11	2.80–5.40
Strontium, µg%	Not found	Not found	Not found	—
Cobalt, µg%	4.80 ± 0.14	4.10 ± 0.10*	4.30 ± 0.09*	3.00–5.00
Bromine, mg%	1.71 ± 0.06	2.47 ± 0.04*	2.19 ± 0.02*	0.70–1.30

Note. * — P ≤ 0.05 relative to the indicators of the group I.

Thus, in the group I Iron and Bromine concentrations exceeded the upper limit of the reference level by 1.3 times, respectively, while the concentration of Manganese was lower than the lower limit of the reference level by 10.0%.

In the group II concentrations of Iron and Bromine exceeded the upper limit of the reference level by 1.6 times and 1.9 times, respectively, while the concentrations of Manganese and Selenium were below the lower limit of the reference level by 10.0% and 30.7%, respectively.

In the group III concentrations of Iron and Bromine exceeded the upper limit of the reference level by 1.5 times and 1.7 times, respectively, while the concentration of Manganese was lower than the lower limit of the reference level by 15.0%. That is, the concentration of Iron in the blood serum of cattle of the experimental groups was higher than the upper limit of the reference level by an average of 1.5 times, Bromine — by an average of 1.6 times, while the concentration of Manganese decreased by an average of 12.5%, and Selenium — by 30.7%.

The analysis of the data in relation to the group I (conditionally control) indicates an increase (P ≤ 0.05) in the concentration of Iron in the blood serum of cattle of the group II by 17.9%, and Bromine — by 30.8%, while the concentration of Selenium, Nickel and Cobalt was lower (P ≤ 0.05) of the indicator of the group I by 51.9%, 24.4%, and 17.1%, respectively. In the blood serum of cattle of the experimental group III, the concentration of Iron exceeded the indicator of the conditional control by 15.8%, and Copper, Selenium, and Bromine — by 7.6%, 18.6%, and 21.9%, respectively, while the concentration of Cobalt was lower (P ≤ 0.05) than the indicator of the group I by 11.61%.

Discussion. Under the influence of fungi and bacteria, the physico-chemical properties of feeds change, various toxins and decomposition products of organic substances accumulate, which leads to animal poisoning. At the same time, the toxicity of feed is caused by the development of not one, but several types of toxic fungi on it. The course of animal poisoning may be in hidden and expressed forms, which depends on the generic and species composition of fungi, their toxicity, the degree of damage to the feed, and the sensitivity of animals. At the same time, the sensitivity of animals to viral and bacterial pathogens also increases, which causes the development of immunodepression, mycotoxicosis, symptoms of various diseases, the risk of ketosis (Marczuk et al., 2012), reduced productivity and reproductive function, abortions and death of animals (Volkov, 2005; Wakelin et al., 2016; Deveau et al., 2018).

Chronic toxicosis is characterized by a decrease in productivity, hemorrhages and necrotic changes in the digestive tract (especially in the large intestine), disorders of the function of hematopoietic organs due to damage to the liver and kidneys, changes in the cardiovascular system, biochemical indicators of blood, impaired reproductive function, abortions, the birth of a non-viable young animals (pregnant cows abort on the 3^{rd}–17^{th} days under the influence of toxic metabolites of fungi from the genus *Aspergillus*) (Malinin, Khmel'nitskiy and Kutsan, 2002; Adhikari et al., 2017).

According to our data, the above is confirmed both by clinical symptoms in cattle (in particular, such as diarrhea and abortions), and by disorders of metabolic processes in the body. A decrease in the level of vitamin A in the blood serum (A-hypovitaminosis) is observed in the event of a violation of its transformation into vitamin A in chronic inflammation of the intestinal mucosa, a lack of protein in the diet, easily soluble sugars, and the presence of antivitamins (nitrites, chlorides, *etc.*) in the feed. At the same time, the development of endogenous A-hypovitaminosis is caused by liver diseases (hepatodystrophy, purulent hepatitis), since they reduce the secretion of bile, the synthesis and release of bile acids, which participate in the emulsification of retinol. E-hypovitaminosis causes a violation of oxidative processes in organs and tissues, dystrophy and necrosis of hepatocytes, muscular dystrophy, decreased fertility, with a simultaneous lack of Selenium, white muscle disease develops. In addition, the intensive reproduction of mold fungi leads to significant losses of carbohydrates and a decrease in the quality of ensiled fodder due to partial consumption of lactic acid, when animals eat such fodder, A-vitaminosis of animals and fermentation processes in the digestive tract of cattle increase (Parakhyn et al., 2006). Which is reflected in the biochemical profile of the experimental groups of animals — a decrease in the concentration of vitamin A by (15.0-52.0%) and vitamin E in 70.0% of cows by (2.5–25.0%).

It should also be noted that oxidative stress is one of the most important toxic mechanisms of action of waste products of biotic feed contaminants (bacterial and mycotoxins). They are able to generate free radicals, including reactive oxygen species, which induce lipid peroxidation, leading to changes in membrane integrity, cellular redox signaling, and antioxidant status of cells, by excessive use of antioxidant system vitamins (A and E) (Wu et al. 2014). This may be one of the reasons for the established decrease in the body supply of them in the experimental cows.

As is known, the antioxidant system includes several links, one of which belongs to trace elements that are part of antioxidant protection enzymes. In particular, Selenium and Manganese play a leading role in these processes (Aguirre and Culotta, 2012; Fernández-Lázaro et al., 2020). It is possible that the decrease in the content of these elements in the blood serum of experimental cows is associated with the presence of endotoxicosis and excessive use of microelements.

Manganese deficiency can lead to delayed growth and development of animals, impaired reproductive function, and nervous system disorders. Due to a lack of Manganese, carbohydrate, mineral and vitamin exchanges are disturbed, acid capacity decreases, *etc.* Selenium in the body performs the function of an antioxidant, has an immunostimulating effect; affects reproductive function. Selenium deficiency is characterized by a violation of protein, carbohydrate, fat, mineral, vitamin (especially vitamins A and E) exchanges; liver necrosis; disorder of endocrine, cardiovascular, respiratory, digestive and nervous systems. Cow diseases such as metritis, ovarian cystitis, and udder edema are associated with Selenium deficiency (Kutsan, Orobchenko and Kocherhin, 2014).

Today, there is a debate in the world about Iron homeostasis under conditions of inflammation. Some authors indicate that inflammatory reactions in the body lead to anemia (Verma and Cherayil, 2017), while others, especially in the presence of chronic inflammation, claim an increase in the level of the element in the body (Wessling-Resnick, 2010). The last statement is consistent with our data: Iron concentration in cattle increased by an average of 1.5 times relative to the upper limit of the reference level.

Under conditions of excess reception, Iron accumulates in the organs and tissues of animals (especially in the liver) in the form of toxic hemosiderin; with an excess of Iron in the body, the assimilation of Calcium, Manganese, Zinc, vitamins A and E is disturbed, feed consumption and animal productivity decrease (Kutsan, Orobchenko and Kocherhin, 2014), which can also be the cause of a decrease in vitamin A and manganese in blood serum of cows.

Bromine reception in high doses in the body for 6 months causes disorders in carbohydrate and protein metabolism, fatty degeneration of the liver and degenerative-necrotic changes in the myocardium, leads to iodine deficiency, which causes growth retardation and pronounced changes in the endocrine system: a decrease in the content of thyroxine in the tissue of the thyroid gland, an increase in the amount of thyroid-stimulating and adrenocorticotropic hormones in the pituitary gland, a decrease in the content of thyroxine, testosterone and corticosterone in blood serum, an increase in follicle-stimulating hormone and insulin (Kutsan, Orobchenko and Kocherhin, 2014).

Regarding the content of vitamins and microelements in blood serum, their reduced or increased reception with feed and water should not be excluded either, but this is the topic of another work.

The animal's organism is placed in extremely harsh conditions of existence and requires constant monitoring of the state of health, maintenance of homeostasis of various systems and the organism as a whole. An important element of such control is biochemical monitoring, which makes it possible to detect the earliest deviations in health without allowing to go beyond physiological parameters. Changes in biogeocenoses, which occur constantly due to human economic activity and at the same time a natural deficiency of essential trace elements and the presence of a significant number of contaminants of biotic origin in feed, contribute to the emergence and spread of pathology of mineral metabolism in the body of farm animals, in particular in lactating cows (Doletskyi, 2015; Sachko et al., 2016).

Conclusions. 1. Based on the results of feed safety studies, it was established that 47.6% of the samples met the sanitary and hygienic requirements and were allowed for feeding, 52.4% of the feed did not meet the MPL, of which 33.3% of the samples were mildly toxic and 19.1% of the samples were toxic.

2. As a result of laboratory studies, high levels of biotic contaminants were found in the fodder base of the farm: the maximum total contamination with micromycetes was 16.50×10^4 CFU/g when the MPL was 5.0×10^4 CFU/g (due to toxin-producing representatives of the genera *Fusarium*, *Aspergillus*, *Penicillium*, *Mucor*, and *Rhizopus*, a total of 24 isolates of microscopic fungi were isolated) and the maximum total bacterial contamination was 18.7×10^5 CFU/g when the MPL was 5.0×10^5 CFU/g, in the structure of which coliform bacteria and *Salmonella enterica* were isolated (the titer of coliform bacteria was 3).

3. Clinically, an excess of biotic contaminants in feed was manifested in cattle by the occurrence of disorders of the digestive tract (diarrhea) and reproductive capacity (abortions in the first half of pregnancy).

4. Under the influence of biotic feed contaminants, metabolic disorders in the body of cattle were revealed: an increase in the level of Iron (on average by 1.5 times) and Bromine (on average by 1.6 times), a decrease in the concentration of vitamin A (by 17.4–39.8%), and vitamin E (by 10.0–12.5%), most pronounced in cows after miscarriage and pregnant, respectively, Manganese (by 12.5% on average) and Selenium (by 30.7%).

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