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Application efficiency of complex pharmacotherapy schemes at combined mycotoxicosis in young bulls

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Abstract. The article presents data on the study of the effectiveness of tetra-p feed additive and toxin adsorbents Minazel plus and Sorbitox introduced into feed, naturally contaminated with mycotoxins. The experiment was conducted on young bulls of 9 months age, which for 3 months got the preparations in diets in the following concentrations: the first group – tetra-p – 5.0 kg/t of feed, Minazel plus – 1.5 kg/t of feed, the second group – tetra-p – 5.0 kg/t of feed, Sorbitox (1.5 kg/t of feed). The results of the experiment determined that the use of tetra-p in combination with preparations with sorption activity had a pronounced therapeutic effect on the body of young bulls. An improvement in the clinical condition of animals was observed from 14-16 days of the experimental period and was manifested by an increase in appetite, normalization of the gastrointestinal tract, as well as by an increase in body weight gain by 11.7 % and 10.3 %. Blood biochemical parameters were characterized by an increase in the level of total protein by 12.4–15.8 % ($p \leq 0.01$), albumin by 18.0–21.3 %, urea by a factor of 1.53–1.44 ($p \geq 0.01$), cholesterol by 1.4–1.56 times and triglycerides by 1.6 times. The activity of liver enzymes decreased by 18.3–21.4 % (ALT) and by 25.7–27.1 % (AST). It has been proved that the developed scheme improves the effectiveness of treatment of mycotoxicosis in animals by the optimizing effect on metabolism, restoration of impaired homeostasis, activation of reparative processes of the liver tissue and increased deposition, activation and utilization of pollutants, produced by microscopic fungi.

1 Introduction

The problem of mycoses and mycotoxicoses remains one of the main problems in animal husbandry. They are specific diseases that arise as a result of eating feed affected by toxic metabolites of molds [1]. The greatest danger to farm animals is represented by microscopic saprophytes fungi that infect the feed substrate during storage: *Aspergillus, Penicillium, Fusarium, Alternaria, Mucor, Rizophormus*, etc. Today more than 350 species of microscopic fungi are known that produce a huge amount of mycotoxins, among which the most dangerous are aflatoxins, ochratoxin A, zearalenone, trichocetins, etc. [2]. Existing classifications of mycotoxins are based on their mechanism of action or organotropic effects. From the point of view of chemistry, they are heterogeneous, fairly stable organic compounds with the very different molecular characteristics. Mycotoxins are formed on all feed crops, without exception, both on growing plants and in the process of grain maturation and storage [3].

Mycotoxins are not a random occurrence in wildlife, but are an adaptive-protective mechanism that microscopic fungi have developed in the course of evolution for their self-defence in order to prevent birds, animals and humans from eating their habitats. That is why they are universal toxic metabolites that can cause serious disturbances in the physiological state of living organism, regardless of the type of grain consumer. Moreover, mycotoxins have the property to accumulate in the body in much higher concentrations than they can be found in the feed, showing cumulative and synergistic effects, in which their combined influence on the body is greatly enhanced.

Many mycotoxins have carcinogenic, teratogenic, mutagenic, allergenic, immunosuppressive and embryotoxic properties [4]. The direction of their action on the body and the severity of the pathological process depend on many factors, which include dosage, the duration of the intake of toxins in the body, the type of animal, its sex and age, as well as biological manifestations, etc. However, in all cases there is a failure of the vital organs and systems of the animal's body. Moreover, the action of mycotoxins is often initially hidden, and the clinical manifestations of mycotoxicosis occur when the damaging effect of toxic metabolites has already become irreversible, which poses a serious danger to the health of animals and humans [5, 6].

An increase in the export and import of grain between countries, a gradual climate change in the world contributes to a significant increase in the widespread prevalence of feed crops with various mycotoxins, which can lead to uncontrolled contamination of feed with toxic products of mushroom metabolites. That is why the problem of mycotoxicosis extends beyond the borders of

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individual countries and is so important that it undoubtedly requires the development of justifications for the strategy for the prevention and elimination of toxins along the entire chain – from the field to the person [7].

Their extremely widespread prevalence, the increased frequency of cases of feed poisoning occurring acutely or latently in many livestock farms with a certain frequency, as well as indisputable evidence of their danger to human and animal health, forces specialists to develop a methodology for an integrated approach to fighting this group of diseases. It is achieved through the use of effective tools allowing not only to reduce the contamination of toxins in feed raw materials, but also to use a variety of physiological and biochemical mechanisms of the living organism due to various preparations and feed additives, specially created for its solutions.

In this connection, a promising direction in the system of prophylaxis and therapy of combined mycotoxicoses is the creation of complex schemes with a combination of adsorbents and feed additives that protect the functions of organ systems, act on the principle of nonspecific antidotes, and ensure the elimination of mycotoxins from the body [8, 9].

For the prevention and treatment of chronic combined mycotoxicosis in cattle, the Krasnodar Research Centre for Animal Husbandry and Veterinary Medicine has developed a tetra-p feed additive with the exchange-stabilizing, probiotic and hepatoprotective properties. As active substances, tetra-p contains diacetophenonyl selenide, beta-carotene, vitamin E, vitamin C and plant phospholipids, dissolved in vegetable oil, from the microbial mass of living bacteria *Bacilis subtilis* 945 (B-5225), as well as wheat bran and grist obtained when processing oilseeds.

The mechanism of action of tetra-p is provided by beta-carotene, which is involved in the synthesis of fatty acids, enhances the rate of glycolysis in muscles, kidneys and liver, has radioprotective and immunomodulating properties, and organic selenium with hepatoprotective and antioxidant properties. Fodder grist of microbiological carotene is a source of essential and bioavailable amino acids and vitamins C and E. Probiotic *Bacilis subtilis* enhances non-specific and specific immunity, stimulating the growth of normal intestinal microflora [10].

Two sorbents, Minazel Plus (MINAZEL PLUS) and Sorbitox, were used as preparations that enhance the action of tetra-p, which is a multicomponent toxin neutralizer.

Minazel Plus is a sorbent of mycotoxins, an organic complex obtained by the interaction of positively charged ions of organic and mineral components. Minazel Plus contains natural zeolite with a minimum clinoptilolite concentration of at least 85 %.

Sorbitox is a combined sorbent of mycotoxins and represents the inner shell of the yeast cells of *Saccharomyces cerevisiae* (at least 80 %), as well as calcium aluminosilicate (not more than 20 %). Its sorbing effect is provided by adsorption of a wide range of mycotoxins in the gastrointestinal tract of animals due to structural features and a large porous structure.

Both sorbents, when introduced into feed, actively adsorb mycotoxins on their surface, preventing them from entering the blood and body tissues, thereby preventing their negative effects on animals, increasing productivity and reducing the risk of developing diseases [11].

2 Materials and Methods

A study of the effectiveness of the developed tetra-p feed additive and toxin adsorbents in the integrated therapeutic regimen at combined mycotoxicosis was carried out on 60 young bulls of 9 months age, selected according to the principle of paired analogues with a body weight of 220 ± 3.5 kg, formed in three groups (n = 20).

Young bulls of the control group received feed of the basic ration (BR), naturally contaminated with mycotoxins. Since the concentration of mycotoxins in feed exceeded the maximum permissible level (MPL) for these pollutants, which under the conditions of a long experiment could lead not only to development of clinically pronounced mycotoxicosis, but also to the death of animals, fodder benzonite was introduced into the rations of this group in a dose of 1 % of concentrated feed mass.

The bulls of the experimental groups were fed with tetra-p feed additive at the dose of 5.0 kg per ton of feed and adsorbents: the 1st experimental group received Minazel Plus (1.5 kg/t of feed), the 2nd experimental group received Sorbitox (1.5 kg/t of feed), which were pre-mixed with feed in special mixers (Table 1).

Table 1. Scheme of therapeutic measures

Group (n=20)	Experiment duration, days	Experiment structure
control	90	BR, naturally contaminated with mycotoxins + bentonite
1 experimental	90	BR, naturally contaminated with mycotoxins + tetra-p + Minazel Plus
2 experimental	90	BR, naturally contaminated with mycotoxins + tetra-p + Sorbitox

The conditions and the ration of all the bulls participating in the experiment were identical and corresponded to the keeping technology on the farm — a year-round stall system with a tethered method for keeping the bulls. The duration of the experimental period was 90 days.

The influence of the additives introduced into the ration on the metabolic state and the functional state of the liver of animals was controlled in dynamics through clinical and biochemical studies, reflecting the development of possible pathological processes in the body, the dynamics of body weight and the level of safety and morbidity of calves.

Weighing of animals was carried out four times – at the beginning of the experiment, after 30 and 60 days and at the end of the experiment (on the 90th day). Blood for laboratory studies was taken on the 30th, 60th and 90th days of the experimental period.

Blood biochemical parameters were determined on an automatic chemical analyzer – Vitalab Selectra Junior with software version 1.0 (manufactured by Vital Scientific N. V, Netherlands) using reagents from ELITech Clinical Systems (France) and Analyticon biotechnologies AG (Germany).

Mycotoxicological studies of feed were carried out by the method based on measuring the content of mycotoxins in samples using indirect solid-state competitive enzyme-linked immunosorbent assay (ELISA), according to GOST 31653-12 "Feed. Enzyme-linked immunosorbent assay for mycotoxins", using test systems developed by the All-Russian Scientific Research Institute of Animal Health "Mycological studies of feed according to the Guidelines for the isolation and quantification of microscopic fungi in feed, feed additives and raw materials for feed production" (Moscow, 2003, No. 13-5 02/0827).

Statistical processing of the results was carried out using statistical programs; quantitative characteristics were evaluated by comparing the average values of two sample sets with the determination of Student's criterion and significance level (p).

3 Results

In the course of mycological, toxicological and biological as well as enzyme-linked immunosorbent assay of samples of mono feed taken at the farm, the concentration of spores of *Aspergillus flavus* fungi was revealed in amount of 2.5×10^4, *Fusarium sp.* 3.4×10^4, *Mucorsp.* – $2{,}0 \times 10^4$. The concentration of mycotoxins in the samples exceeded the maximum permissible level (T-2 toxin at the concentration of 0.4 mg/kg and aflatoxin B1 at the concentration of 0.2 mg/kg), which was confirmed by bioassay in laboratory animals (mice).

A clinical examination of the young bulls participating in the experiment was characterized by symptoms manifested by general inhibition, growth retardation, progressive emaciation against the background of decreased appetite, and increased thirst. In the gastrointestinal tract, disorders of the pancreas (hypotension and atony), stagnation of feed masses, slowing of intestinal motility, violation of bowel movements (alternating diarrhea with constipation) were noted. The mucous membranes were characterized by anemia, and the skin covering was characterized by exfoliation and patches of alopecia.

The results of the experiment found out that the use of tetra-p in combination with preparations with sorption activity had a pronounced therapeutic effect on the body of young bulls. An improvement in the clinical condition of the animals of the experimental groups was observed from 14–16 days of the experimental period and was manifested by an increase in appetite, normalization of the gastrointestinal tract, decrease in salivation and restoration of the colour of the mucous membranes of the oral and nasal cavities.

Starting from the second month of the experimental period, the syndrome of mycotoxicosis in animals was absent.

Against the background of positive dynamics in the experimental groups, the anamnestic data of control bulls were less pronounced. The use of bentonite allowed reducing the level of contamination of feed with mycotoxins, which was manifested by an improvement in general condition, increased appetite and weakening the diarrheal syndrome. However, until the end of the experiment, 13 animals from the group revealed some clinical signs of metabolic disturbance (dullness of the skin covering, exfoliation of the epidermis, dystonia of the pancreas with rhythm disturbance and rumination strength).

The positive effect of the preparations on the plastic and metabolic reactions in the body of the experimental young bulls facilitated the mobilization of anabolic processes, causing an increase in the animal's body weight (Table 2).

Table 2. Dynamics of the body weight of young bulls (n = 20)

Groups	Age, months			
	9	10	11	12
control	223.0±0.71	238.0±0.65	261.4±0.68	279.3±1.62
1 experimental	221.5±0.78	246.1±1.15*	275.4±1.32	305.4±2.43**
2 experimental	222.0±1.25	245.9±1.28	274.6±2.19*	302.3±2.34

Reliability in relation to the control group: ** – $p \geq 0.01$, * – $p \leq 0.05$

Gravimetric indicators throughout the entire period of research in young bulls of experimental groups determined a dynamic increase in body weight relative to the control analogues. Intergroup differences with the control for the first month of the experimental period amounted to 3.4 % (1st experimental group) and 3.3 % (2nd experimental group). In the following months, the difference in growth between the control and experimental groups was 5.4 and 5.0 % (after 60 days) and 9.3 and 8.2 % (after 90 days), respectively. The total increase in body weight of experimental bulls by the end of the studies exceeded the values of the control group by 11.7 and 10.3 %.

The use of tetra-p as the feed additive to the feed of young bulls in experimental groups against the background of the use of adsorbents made it possible to ensure homeostasis of the animal organism during the growth period, weaken the metabolic syndrome and normalize the processes of biological synthesis in liver.

Background studies of blood serum in young bulls showed deviations in a number of biochemical parameters, manifested by hypoproteinemia and hypoalbunemia against the background of a decrease in the protein synthesizing function of liver (the concentration of total protein and albumin fraction was 84 and 76 % of the species norm). Urea level was reduced in 68 % of cases in the examined samples, low glucose, triglycerides and carotene levels were noted. Transaminase activity (dominated by aspartate aminotransferase) was increased in 70 % cases in animals.

A blood biochemical analysis performed at the end of the experimental period (Table 3) showed an increase in the level of total protein in animals of all groups with priority in the 1st experimental group. There, the concentration of total protein in relation to the control analogues increased by 15.8 % (p ≤ 0.01), in the 2nd experimental group it increased by 12.4 %. Combination therapy contributed to the stabilization of proteinograms of experimental calves. Average albumin values were recorded in the range of normal values, exceeding the control indices by 21.3 and 18.0 %. On this background, the ratio of globulin fractions was optimized, an integral indicator of which was a decrease in γ-globulins in the experimental groups by 20.7 and 16.6 %, while they were simultaneously increased in control animals.

Table 3. Biochemical indicators of blood serum of young bulls (M±m; n=20)

Indicators	Background	End of the experiment		
		1 control	2 experimental	3 experimental
Total protein, g/l	62.4±1.8	72.3±1.7	83.7±4.4**	81.3±3.7*
Albumin, %	32.5±0.91	36.1±2.4	43.8±0.96	42.6±1.14
α- globulins, %	14.8±0.55	13.5±1.06	13.4±0.75	13.7±0.85
β- globulins, %	12.2±0.4	9.4±0.17	10.3±0.54	9.5±0.16
γ- globulins, %	40.5±2.16	41.0±1.3	32.5±0.88	34.2±1.0
Urea, mmol/l	3.11±0.43	3.2±0.09	4.9±0.11*	4.6±0.10**
Glucose, mmol/l	2.05±0.37	1.98±0.12	3.45±0.28	3.54±0.19**
Triglycerides, mmol/l	0.21±0.15	0.30±0.09	0.47±0.07	0.42±0.06
Cholesterol, mmol/l	2.8±0.56	3.7±0.12	5.9±0.10	5.7±0.14
ALT, Unit/l	42.4±1.58	36.0±4.2	28.3±3.8*	29.4±5.1
AST, Unit/l	146.3±3.28	132.0±5.4	96.2±4.0**	98.1±3.7*
Carotene, mg %	0.32±0.05	0.36±0.09	0.52±0.10**	0.50±0.08

Reliability in relation to the control group: ** – p ≥ 0.01, * – p ≤ 0.05

A significant increase in urea concentration was noted, by 1.53 (p ≤ 0.05) and 1.44 (p ≥ 0.01) times, while in the control this indicator changed within narrow boundaries and did not reach the norm (3.2 ± 0, 21 mmol/l). So, the complex use of the tetra-p feed additive with the detoxifying preparations in the treatment of mycotoxicosis contributed to the activation of the protein synthesizing function of liver on the background of a weakening of the “inflammatory syndrome” in hepatocytes.

The introduction of tetra-p into the feed additive scheme favorably affected the lipid metabolism of young bulls, which was confirmed by the normalization of triglycerides and cholesterol, the concentration of which in the experimental groups increased by 1.56 and 1.4 times (triglycerides) and by 1.59 and 1.54 times, respectively, in comparison with the control bulls.

The improvement in liver function under the influence of the preparations was accompanied by the optimization of serum transaminases belonging to the group of indicator enzymes recorded within the limits of reference values. In percentage terms, the significant difference between the indices of the animals of the experimental groups and the control group was 21.4 and 18.3 % for ALT and 27.1 and 25.7 % for AST, respectively.

The influence of the feed additive on the experimental animals was manifested by an increase by 1.44 times in the level of carotene in the first experimental group with a high significance of differences (p ≥ 0.01) with the control bulls, in which hypocarotinemia was recorded during the research. In the second experimental group, the carotene level exceeded the values of the control analogues by 38.9 %.

4 Conclusion

Thus, the results of the studies showed that the comprehensive scheme of pharmacotherapy aimed at the effective correction of various pathogenesis links of combined mycotoxicoses with the use of adsorbing preparations can reduce the toxic load on the body on the first line of defense. This is due to its high sorption activity and the additional use of the feed additive with a number of specific pharmacological properties.

The developed scheme improves the efficiency of mycotoxicosis treatment in animals due to the optimizing influence on metabolism, restoration of impaired homeostasis, activation of reparative processes of the liver tissue and enhancement of the deposition, activation and utilization of pollutants produced by microscopic fungi.

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