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Efficacy of a complex plant-based preparation for poultry mycotoxicosis

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SUMMARY
The article presents results of efficacy studies for a complex plant-based preparation for poultry mycotoxicosis. Feed additive fibralin contains polysaccharides (dried sugar beet pulp) and phospholipids (rapeseed lecithin) in the proportion 4:1. Eighteen-day-old "Ross-308" broiler chickens with average weight of (665.10 ± 4.28) g were tested, since such mycotoxins as T-2 toxin, zearalenone and aflatoxin B_1 were detected in their feeds. Maximum admissible level of each toxin was not exceeded, however, their cumulative effect on poultry resulted in mycotoxicosis. Use of fibralin in the feed (3 kg per one ton) for 10 days reduced clinical signs of intoxication, increased flock survival by 13.5% and stimulated body weight gain by 15.8%. Pharmacological effect of fibralin was demonstrated by improvement of blood morphobiochemical parameters in poultry, i.e. reduction of leukocytes by 19.3% and cholesterol by 13.6%; and an increase in the number of erythrocytes by 19.4%, hemoglobin by 8.1% and calcium by 9.5%. Antitoxin therapy had a positive effect on liver structure and functions and that fact was confirmed by a decrease in aminotransferase level in serum and normal levels of total protein. The data obtained may justify the use of this natural bio-preparation as a product with antitoxic and hepatoprotective properties and the use of fibralin for mycotoxicosis treatment of poultry.

Key words: mycotoxicoses, poultry, fibralin, beet pulp, lecithin.

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Эффективность комплексного препарата на основе вторичных растительных ресурсов при микотоксикозе сельскохозяйственной птицы

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РЕЗЮМЕ
Представлены результаты изучения эффективности комплексного препарата на основе вторичных растительных ресурсов при микотоксикозе сельскохозяйственной птицы. Препарат фибралин содержит комплекс веществ полисахаридной (сухой свекловичный жом) и фосфолипидной (рапсовый лецитин) природы в соотношении 4:1. Исследования проведены на 18-суточных цыплятах-бройлерах кросса «Росс-308» со средней массой тела (665,10 ± 4,28) г, в кормовых рационах которых были обнаружены микотоксины: Т-2 токсин, зеараленон и афлатоксин B_1. Концентрация токсинов по отдельности не превышала максимально допустимого уровня, но их сочетанное воздействие на организм птицы обуславливало развитие микотоксикоза. Применение фибралина в дозе 3 кг на тонну корма в течение 10 дней привело к снижению клинических признаков интоксикации, повышению сохранности поголовья на 13,5% и интенсивности приростов массы тела на 15,8%. Фармакологический эффект фибралина проявился улучшением морфобиохимических параметров крови птицы за счет снижения концентрации лейкоцитов на 19,3% и холестерина на 13,6% при увеличении содержания эритроцитов на 19,4%, гемоглобина – на 8,1% и кальция – на 9,5%. Антитоксическая терапия оказала положительное действие на структурное и функциональное состояние печени, что подтвердилось снижением уровня аминотрансфераз в сыворотке крови и нормализацией концентрации общего белка. Полученные данные могут служить основанием для применения данного биологического комплекса природного происхождения в качестве препарата с антитоксическими и гепатопротекторными свойствами и использования фибралина при терапии микотоксикозов у птицы.

Ключевые слова: микотоксикозы, птица, фибралин, свекловичный жом, лецитин.

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INTRODUCTION
An increase in the number of mycotoxicoses cases in animals and poultry is reported now and this situation poses high economic and environmental risks. There are hundreds of various mycotoxins; they differ in their chemical structure and in their impact on animals. Most widespread mycotoxins are aflatoxin, zearalenone, T-toxin, fumonisin and ochratoxin. Many mycotoxins cause severe irreversible changes in animals that frequently result in death. This problem is aggravated by the fact that mycotoxins (transmitted to animals via feeds) can accumulate in food products of animal origin, thus posing a significant risk for human health [1, 2].

Taking into account the above, we believe that veterinary science faces now a crucial task to develop effective complex antitoxic preparations. A wide range of preparations that have antitoxic, hematoprotective, antioxidant properties includes fibralin based on such plant by-products as beet pulp and rapeseed lecithin.

Beet pulp fibers included into preparation normalize motor functions of biliary tract, i.e. stimulate bile excretion and prevent stases in hepatobiliary system. Beetroot contains many microelements and vitamins, and beet pectin found in its fibres has physicochemical properties (i.e. low etherification degree with a great number of free carboxyl groups) of the best natural adsorbent and complexing agent for different xenobiotics [3–5].

Lecithin, the second component of fibralin, is a complex of essential phospholipids with a range of different functions. It is a component of cellular membranes (in the form of phosphatidylcholine); an emulsifier and regulator of cholesterol crystallization; it contains omega-6 polyunsaturated fatty acids that normalize lipid transport in blood and improve intestinal lipid absorption; it plays an important role in immune protection and has an antioxidant effect etc. [6–8].

The purpose of the research is to analyze fibralin efficacy for mycotoxicosis in broiler chickens.

MATERIALS AND METHODS
Fibralin contains polysaccharides (dried beet pulp) and phospholipids (rapeseed lecithin) in the proportion 4:1.

Fibralin efficacy was tested in backyard "IP I. V. Remesnik" (the Dinsky Raion, the Krasnodar Krai). 440 eighteen-day-old "Ross-308" cross broilers with average weight of (665.10 ± 4.28) g were used. Deaths were reported in the backyard during 5 days before the experiment, when starter diet was being replaced by grower diet.

Mixed feed "Rost" was tested in ELISA for mycotoxins. For this purposes we used Stat Fax® 2600 (USA) analyzer and a test kit for indirect competitive ELISA (ZAO "Farmatekh", Russia). The test revealed mycotoxins in the feeds: T-2 toxin – 0.016 mg/kg; zearalenone – 0.018 mg/kg; aflatoxin B_1 – 0.002 mg/kg. Maximum admissible level of each toxin was not exceeded, however, their cumulative effect on poultry resulted in mycotoxicosis.

In order to assess fibralin efficacy we divided the chickens into 3 groups: experimental group No. 1 (200 chickens) received 3 kg of fibralin per ton of feed; experimental group No. 2 (200 chickens) received 1.5 kg of another preparation for comparison "AtoxBio Plus" (OOO "TekhnoFeed", Russia) per ton of feed; control group No. 3 (40 chickens) received no treatment. Thus, the preparations were given for 10 days.

Clinical observations were performed during the experiment; chickens were weighed at the beginning and at the end and dead birds were subject to post-mortem examination. On day 1 and day 10 of the experiment, we took 10 chickens from each group for general and biochemical blood tests. Biochemical blood tests were performed in automatic chemistry analyzer Vitalab Selectra Junior (the Netherlands) with chemical reagents from ELITech Clinical System (France) and Analyticon Biotechnologies AG (Germany), general blood test was performed in automatic analyzer Mythic 18 Vet (Switzerland).

Table 1
Body weight gain of broiler chickens treated for mycotoxicosis

Таблица 1
Динамика массы тела цыплят-бройлеров при лечении микотоксикоза

Groups	Body weight, g	
	Day 1 of the experiment	Day 10 of the experiment
Experimental No. 1	664.90 ± 4.26	1204.90 ± 11.3
Experimental No. 2	665.40 ± 4.41	1192.50 ± 12.9
Control No. 3	664.80 ± 4.18	1040.70 ± 10.7

The following benchmarks were used to evaluate treatment effectiveness: survival rate, clinical status, appetite, locomotor activity, results of general and biochemical blood tests, body weight gain.

All the animal tests were performed in compliance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 "On protection of animals used for scientific purposes".

Statistical analysis was performed using Statistica 6.0 software. Validation criterion was determined by Student's table.

RESULTS AND DISCUSSION

During the whole experiment 3 chickens were found dead (1.5%) in group No. 1 (received fibralin) on the first two days of the experiment; 5 chickens died in group No. 2 (2.5%) and 6 birds died in control group (15%).

Gravimetric measurements showed weight gain in all the three groups, however, weight gain rate was obviously lower in chickens from control group (received no treatment) than that in birds from experimental groups. Thus, on day 10 of the experiment the difference from experimental group No. 1 was 15.8% and from group No. 2 – 14.6% (Table 1).

Assessment of hematological status revealed that white blood cell count in chickens from all groups was close to upper normal level, on average $(37.10 \pm 1.27) \times 10^9$/L. At the end of the experiment white blood cell (WBC) count in chickens from group No. 3 increased by 9.1% and accounted for $(39.80 \pm 0.96) \times 10^9$/L, whereas WBC concentration in groups No. 1 and No. 2 decreased by 19.3 and 16.2%, respectively. Besides, the group that received no treatment demonstrated a progressive decrease in red blood cell (RBC) count by 12.1% – $(2.90 \pm 0.22) \times 10^{12}$/L and in hemoglobin level by 6.7% – (84.10 ± 2.08) g/L as compared to their original levels. Broiler chickens from experimental groups No. 1 and No. 2 demonstrated positive dynamics, i.e. an increase in RBC count by 19.4 and 12.5%; and hemoglobin by 8.1 and 11.6%, respectively, as compared to the original data.

Laboratory blood tests revealed that treatment for mycotoxicosis with antitoxic preparations was accompanied by positive changes in biochemical profile of chickens, with a number of better values reported in experimental group No. 1 (Table 2).

At the beginning of the experiment, the chickens demonstrated higher levels of liver enzymes, i.e. alanine aminotransferase (ALT) was higher than the reference value and aspartate aminotransferase (AST) activity was reported at the upper normal level. These data suggest there was a mild cytolytic process in liver caused by the given combination and concentration of mycotoxins. Antitoxin therapy in experimental groups had a positive effect on liver structure and functions as it was confirmed by a drop in aminotransferases level in chicken serum: in group No. 1 – ALT decreased by 30.5% ($p \le 0.01$) and AST by 15.1%; in group No. 2 – ALT decreased by 15.1% ($p \le 0.05$) and AST by 6.1%. On day 10, the enzyme activity in the control group increased as compared to the original values: ALT increased by 19.7% ($p \le 0.05$) and AST by 12.6% ($p \le 0.05$). These changes indicate an acceleration in hepatocyte cytolysis accompanied by mycotoxicosis that pushes the enzymes out into the intracellular space and increases their level in blood.

A baseline study revealed hypercholesterolemia that demonstrated early signs of liver damage in chickens. Hypercholesterolemia is a sign of acute liver dysfunction in the early stages of the disease. It is well-known that cholesterol concentration drops lower than the normal value, when an acute condition becomes a chronic one. These changes in cholesterol profile were as well observed during the experiment: those broiler chickens that had mycotoxins in their diet and received no treatment had cholesterol below lower limit of normal at the end of the experiment, i.e. (2.46 ± 0.09) mmol/L with the significant difference from the original data of 33.7% ($p \le 0.01$). Due to the treatment provided, cholesterol concentration was optimal in group No. 1 by 13.6% and in group No. 2 by 7.3% as compared to the background data.

Hepatic protein synthesis in chickens from experimental groups improved as it was indicated by normalization of total protein concentration that increased by 16.7% ($p \le 0.05$) in group No. 1 and by 15.8% in group No. 2 on day 10 of the experiment as compared to the original values. At the end of the experiment, hypoproteinemia became more evident in control chickens, with a decrease in total protein by 5.9%.

There was also a 10.1% drop in creatinine concentration in group No. 3 as compared to the original values; however, this parameter did not significantly change in experimental groups.

Pharmacological effect that fibralin has on protein metabolism is explained not only by improved hepatic protein synthesis influenced by a hepatoprotective component of the preparation, but also by the intake of beet pulp proteins represented by such amino acids as lysin, arginine, leucine, phenylalanine, threonine, valine, methionine and cystine.

In addition, it is the mineral content of beet pulp (a lot of calcium, potassium, sodium, magnesium, cuprum, cobalt) that leads to changes in calcium and phosphorus metabolism in chickens treated for mycotoxicosis. Total calcium in chicken blood reported in baseline studies was at the lower limit of normal – (2.10 ± 0.09) mmol/L on average. At the end of the experiment, the chickens from experimental group No. 1 (received fibralin) demonstrated an increase in calcium level up to (2.30 ± 0.06) mmol/L, which complies with the normal values. No significant changes were reported in mineral metabolism in the other groups.

Table 2
Blood chemistry values for broiler chickens treated for mycotoxicosis ($M \pm m$; $n = 10$)

Таблица 2
Биохимические показатели крови цыплят-бройлеров при лечении микотоксикоза ($M \pm m$; $n = 10$)

Values	Groups		
	Experimental No. 1	Experimental No. 2	Control No. 3
	Day 1 of the experiment		
ALT, u/L	22.60 ± 1.77	21.30 ± 1.67	23.90 ± 1.32
AST, u/L	296.50 ± 5.60	284.50 ± 3.50	291.70 ± 4.40
Cholesterol, mmol/L	3.74 ± 0.18	3.69 ± 0.11	3.71 ± 0.12
Glucose, mmol/L	8.70 ± 0.55	8.90 ± 0.46	9.10 ± 0.41
Creatinine, μmol/L	29.10 ± 1.32	28.50 ± 1.15	29.80 ± 0.79
Total protein, g/L	31.20 ± 0.85	29.80 ± 1.22	30.40 ± 0.43
Calcium, mmol/L	2.10 ± 0.12	2.10 ± 0.09	2.00 ± 0.05
Phosphorus, mmol/L	1.28 ± 0.16	1.34 ± 0.12	1.36 ± 0.18
	Day 10 of the experiment		
ALT, u/L	15.70 ± 0.39**	18.10 ± 0.48*	28.60 ± 0.54*
AST, u/L	251.50 ± 5.60	267.30 ± 3.40	328.90 ± 4.70*
Cholesterol, mmol/L	3.23 ± 0.07*	3.42 ± 0.13	2.46 ± 0.09**
Glucose, mmol/L	10.90 ± 0.63	9.40 ± 0.36	8.20 ± 0.27
Creatinine, μmol/L	32.30 ± 0.71	29.10 ± 0.62	26.80 ± 0.36
Total protein, g/L	36.40 ± 0.43*	34.50 ± 1.30	28.60 ± 0.74
Calcium, mmol/L	2.30 ± 0.06	2.20 ± 0.11	1.90 ± 0.13
Phosphorus, mmol/L	1.35 ± 0.15	1.39 ± 0.24	1.42 ± 0.17

* $p \le 0.05$;
** $p \le 0.01$ – differences are verified in relation to background data (различия достоверны по отношению к фоновым данным).

CONCLUSION

Consequently, the carried out research demonstrated that a complex use of substances with adsorptive, hepatoprotective, antioxidant and metabolism-stabilizing properties improves survival rate and productivity of poultry fed with mycotoxin-contaminated feeds. Use of fibralin against combined mycotoxicosis in broiler chickens (at a dose of 3 kg per ton of feed) reduces clinical signs of intoxication, normalizes morphological and biochemical parameters of blood, increases survival and weight gain rates in the flock.

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Prospects of betulin application in broiler farming

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SUMMARY

It is an urgent task today to seek and implement nature-like technologies in broiler production and obtain biologically complete and safe poultry products, thus refusing from antibiotic use in feed. Feed additives based on natural components can be an effective tool for implementation of preventive and therapeutic veterinary measures. The paper presents research study results of application of betulin-based phytobiotics in broiler farming. The tests were carried out on Ross-308 cross-breed broiler chickens within the production cycle at one of the poultry farms of the Sverdlovsk Oblast. Birds of the experimental group received compound feed supplemented with dry betulin at 2.5 mg/kg of live weight from day 21 to day 35 of growing. The introduction of betulin-based feed additive into the diet contributed to increase in live weight gain and 7.6% pectoralis muscle output as compared with the control group. It was established that the phytobiotic consumption resulted in reduced deposition of subcutaneous and abdominal fat, higher biological value of meat by increasing the ash content, improved technological properties of meat due to increasing water-holding capacity of muscle fiber and intensity of formation and maturation of muscle fiber. Histological studies of pancreatic tissue samples from broiler chickens showed increase in the mass of islets of Langerhans and insulin-producing cell complexes. The pancreas was activated due to effects of the betulin-based feed additive. The results obtained indicate that the use of betulin in broiler production is a promising trend.

Key words: broiler chicken, feed additive, betulin, phytobiotics, live weight, muscle fibre, fat, pancreas, islets of Langerhans.

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Перспективы применения бетулина в бройлерном птицеводстве

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РЕЗЮМЕ

Поиск и внедрение природоподобных технологий при выращивании цыплят-бройлеров и получении биологически полноценной и безопасной продукции птицеводства в условиях отказа от кормовых антибиотиков на сегодняшний день является актуальной задачей. При проведении профилактических и терапевтических ветеринарных мероприятий эффективным средством могут быть кормовые добавки на основе природных компонентов. В работе представлены результаты научного исследования по применению фитобиотика на основе бетулина в бройлерном птицеводстве. Испытания проведены в условиях технологического цикла на одной из птицефабрик Свердловской области на цыплятах-бройлерах кросса «Росс-308».