AGRICULTURAL BIOLOGY,
2022, V. 57, Iss. 4, pp. 730-742
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Experimental mycotoxicoses

UDC 636.52/.58:619:616-099:591.1

doi: 10.15389/agrobiology.2022.4.730eng
doi: 10.15389/agrobiology.2022.4.730rus

EFFICACY OF A COMPLEX PREPARATION TO CORRECT DIGESTION IN BROILER CHICKENS (*Gallus gallus* L.) IN EXPERIMENTAL MYCOTOXICOSIS

V.G. VERTIPRAKHOV[1] ✉, A.A. GROZINA[2], E.A. YILDIRIM[3, 4], N.N. GOGINA[2], I.V. KISLOVA[2], N.V. OVCHINNIKOVA[2], M.V. KOSHCHEYEVA[2]

[1]*Timiryazev Russian State Agrarian University—Moscow Agrarian Academy,* 49, ul. Timiryazevskaya, Moscow, 127550 Russia, e-mail Vertiprakhov63@mail.ru (✉ corresponding author);
[2]*Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS,* 10, ul. Ptitsegradskaya, Sergiev Posad, Moscow Province, 141311 Russia, e-mail alena_fisinina@mail.ru, n.n.gogina@mail.ru, irina.kislova1606198@yandex.ru, natalya.o90@mail.ru, vlk.733@mail.ru;
[3]*JSC Biotrof+,* 19, korp. 1, Zagrebskii bulv., St. Petersburg, 192284 Russia, e-mail deniz@biotrof.ru;
[4]*Saint Petersburg State Agrarian University,* 2, lit A, Peterburgskoe sh., St. Petersburg—Pushkin, 196601 Russia
ORCID:
Vertiprakhov V.G. orcid.org/0000-0002-3240-7636
Grozina A.A. orcid.org/0000-0002-3088-0454
Yildirim E.A. orcid.org/0000-0002-5846-4844
Gogina N.N. orcid.org/0000-0003-1937-286X
Kislova I.V. orcid.org/0000-0001-6399-6886
Ovchinnikova N.V. orcid.org/0000-0001-9877-2161
Koshcheyeva M.V. orcid.org/0000-0002-0744-1883

The authors declare no conflict of interests
Acknowledgements:
Supported financially by the Russian Science Foundation, grant No 20-76-10003 "The study of the effects of T-2 and HT-2 toxins on digestion in birds, the development of diagnostic methods and the creation of a new comprehensive drug for the prevention of mycotoxicosis"
Received April 27, 2022

Abstract

Mycotoxins have a negative effect on the health and productivity of farm animals. The main criterion for the diagnosis of mycotoxicoses in the absence of a pronounced clinical picture of the disease is the presence of toxins in the feed. Different preparations are used to prevent mycotoxicoses, but their effect on the bird organism has not been fully studied. In the present work, the peculiarities of digestive function, metabolism and haematological values in broiler chickens of Smena 8 cross from 34- to 48-days old when using protease combined with sorbent in the case of experimental mycotoxicosis caused by T-2 toxin were shown first. The investigation was aimed at determining the effect of sorbent Zaslon 2+ and enzyme preparation Axtra Pro on duodenal enzymes activity, protein metabolism and morphobiochemical blood parameters in broiler chickens of Smena 8 cross with chronic intestinal fistula in experimental mycotoxicosis caused by T-2 toxin. The experiments were performed according to requirements of the European Convention on protection of vertebrate animals used for experiments or other scientific purposes (ETS № 123, Strasburg, 1986). The broiler chickens were kept in the vivarium from 1- to 48-day-old chickens (All-Russian Research Institute for Scientific and Technical Studying of Poultry Farming, 2021) respecting the regime of feeding and keeping according to the requirements for the definite age group and cross of poultry. Surgical operations of fistula implantation into the duodenum were carried out on 25 birds at the age of 20-25 days. A cannula was implanted opposite the place where the pancreatic and bile ducts ran into the intestine. Five groups of 5 birds were formed of clinically healthy birds. each group was formed according to the principle of analogues: Group I (control) was kept on the basic diet (OR) without the addition of mycotoxins, experimental group II received OR + T-2 toxin (0,1 mg/kg) + sorbent Zaslon 2+ (BIOTROF, Ltd., Russia) (2 g/kg food), group III — OR + T-2 toxin (0.4 mg/kg) + Zaslon 2+ (2 g/kg food), group IV — OR + T-2 toxin (0.1 mg/kg) + Zaslon 2+ (2 g/kg food) + Axtra Pro enzyme (DuPont de Nemours, Inc., USA) (0,1 g/kg feed), group V — OR + T-2 toxin (0.4 mg/kg) + Zaslon2+ (2 g/kg feed) + Axtra Pro (0.1 g/kg feed). The feed was contaminated with T-2 toxin to MAC levels (groups II and IV) and 4 MAC levels (groups III and V) by mechanical means in compliance with personnel safety requirements. Standard T-2 toxin (powder with mass fraction of main substance 99.7±0.3 %; Romer Labs, Austria, LOT No. S17052T) was used. The preparation period lasted from 26- to 33-day-old birds, the experiment period lasted 14 days (from 34- to 48-day-old birds). Chyme (1.0-2.0 ml) and litter (5.0 g)

samples were collected daily from each bird in the morning. Blood (2 ml) was taken 1 day before slaughter (at the age of birds 47 days) from the sub wing vein. When using Zaslon 2+ sorbent in combination with Axtra Pro protease to prevent mycotoxicosis, the enzymatic activity of duodenal chyme was increased compared with that of the basic preparation (sorbent): protease activity in duodenal contents by 15.5 % ($p < 0.05$), trypsin by 12.8 % ($p < 0.05$), alkaline phosphatase by 46.1 % ($p < 0.05$), total phosphorus content by 25.6 % ($p < 0.05$), at a toxin dose of 0.1mg/kg feed. amylase activity increased by 9.6 and 14.7 % at the dose of T-2 toxin 0.1 and 0.4 mg/kg, respectively, compared with using a single sorbent. There was a statistically significant increase in total proteolytic activity, trypsin (at the dose of 0.1 mg/kg toxin), and lipase activity (at the dose of T-2 toxin 0.4 mg/kg). The activity of enzymes in the litter of birds of experimental groups did not increase compared to the control group, indicating the positive role of the preparations in normalizing digestion when using the toxic feed in the bird's diet. The use of contaminated feed with T-2 toxin for 14 days adversely affected the state of protein metabolism, which was manifested in the reduction of nitrogen use by poultry in all experimental groups; there was also a negative trend in the availability of amino acids, especially when the toxin dose was 0.4 mg/kg. Biochemical blood parameters of broiler chickens in experimental mycotoxicosis showed impairment of protein, fat and carbohydrate metabolism, as well as signs of stress response due to the action of the toxin on digestive organs (pancreas, liver).

Keywords: T-2 toxin, HT-2 toxin, T-2 toxicosis, broilers, chyme, droppings, digestive enzymes, feed additive Zaslon 2+, enzyme Axtra Pro

Mycotoxins adversely affect the health and productivity of farm animals [1-3]. Studying the biochemistry and properties of mycotoxins, developing methods for their detection, identifying symptoms of diseases and complying with regulatory guidelines established by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) are the main areas of research and practice aimed at preventing or minimizing mycotoxin contamination of food and feed, reducing toxicity, and reducing economic losses [4-6].

It has been shown that activation in pancreatic tissues of genes of regulatory molecules for the development of an inflammatory response (*IL6* and *PTGS2*), genes associated with cell death (*Casp6*), as well as genes of antimicrobial factors (primarily *AvBD10*) can serve as an early prognostic marker of T-2 toxicosis in broilers [7]. Using principal component analysis (PCA), it was demonstrated that the expression of *PTGS2* genes in the pancreas, *IL6*, *PTGS2*, *IL8*, *IRF7*, *AvBD9*, *AvBD10* and *Casp6* in the caecum of the intestines of Smena 8 cross broilers, as well as the content in the blood total protein, glucose, triglycerides, activity of alkaline phosphatase and trypsin and the ratio of the activity of these enzymes were in close relationship [7].

A large number of different preparations have been proposed for the prevention of mycotoxicoses in animals [8-10]. The Russian Federation has patented a number of feed additives designed to reduce the negative impact of mycotoxins on animals. Methods for obtaining these drugs and their composition are very diverse. Mineral substances such as clay, zeolites, silicates are used [11)]. Enrichment of adsorbents with humic acids, yeast cell walls is possible (12). It is proposed to carry out the biological neutralization of mycotoxins using enzyme preparations of hydrolases, polypeptide esterases, multicomponent mixtures of enzymes and bacteria [13]. The effectiveness of the proposed drugs in relation to the sorption of mycotoxins in in vitro experiments and the effect on the productivity of animals and poultry in in vivo experiments has been proven, however, the mechanism of the observed positive effect on the digestion and body of the bird has not yet been sufficiently studied.

In the present work, it was shown for the first time that the mechanism of the positive effect of a complex preparation containing a sorbent and a protease on broiler chickens of the Smena 8 cross aged from 34 to 48 days with experimental T-2 toxicosis is associated with modulation of the activity of duodenal content proteases, trypsin in blood plasma. and the number of lymphocytes in the blood.

The aim of the work was to determine the effect of the Zaslon 2+ sorbent and the Axtra Pro enzyme preparation on the activity of duodenal enzymes, protein metabolism, and morphobiochemical blood parameters in broiler chickens of the Smena 8 cross with chronic intestinal fistula in experimental T-2 toxicosis.

Materials and methods. Physiological experiments were carried out in 2021 on broiler chickens (*Gallus gallus* L.) of the Smena 8 cross in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes (ETS No. 123, Strasbourg, 1986) [14]. Chickens from 1 to 48 days of age were kept in a vivarium (FSC All-Russian Research and Technological Institute of Poultry Farming RAS); feeding and maintenance regimes met the requirements for the age group and cross [15].

Surgical operations for implantation of fistulas into the duodenum were performed on 25 birds at 20-25 days of age, the cannula was implanted opposite the place where the pancreatic and bile ducts enter the intestine according to the author's method [16]. During the first day after the operation, the bird was limited in food, and then the feeding was normalized, following the passage of chyme in the intestine. The sutures were removed on day 5 after the operation. From clinically healthy birds, five groups were formed (5 broilers each) according to the principle of analogues. Group I (control) was kept on the basal diet (BD) without the addition of mycotoxins. Group II received BD + T-2 toxin (0.1 mg/kg) + sorbent Barrier 2+ (2 g/kg feed). Group III received BD + T-2 toxin (0.4 mg/kg) + Barrier 2+ (2 g/kg feed). Group IV received BD + T-2 toxin (0.1 mg/kg) + Zaslon 2+ (2 g/kg feed) + Axtra Pro enzyme (0.1 g/kg feed). Group V received BD + T-2 toxin (0.4 mg/kg feed) kg) + Barrier 2+ (2 g/kg feed) + Axtra Pro (0.1 g/kg feed). Feed additive Zaslon 2+ (OOO BIOTROF, Russia) consisted of a sorbent material of diatomite, bacteria *Bacillus* sp., a mixture of natural essential oils of eucalyptus, thyme, garlic and lemon. The proteolytic activity of the enzyme preparation Axtra Pro (DuPont de Nemours, Inc., USA) was 897.0±47.5 $mg \cdot g^{-1} \cdot min^{-1}$. Feed was contaminated with T-2 toxin up to 1 MPC (groups II and IV) and 4 MPC (groups III and V) mechanically in compliance with personnel safety requirements. We used standard T-2 toxin (powder with a mass fraction of the main substance 99.7±0.3%; Romer Labs, Austria, LOT No. S17052T). Fresh feed was given to the birds daily, access to water was not limited.

The preparatory period lasted from 26 to 33 days of age of the birds, the period of the experiment lasted 14 days (from 34 to 48 days of age). Samples of chyme (1.0-2.0 ml) and droppings (5.0 g) were collected daily during the experiment from each bird in the morning, placed in a refrigerator at –20 °C, samples (5 g each) were dried in freeze dryer of the TFD series (ilShinBioBase Co., Ltd., South Korea) for 34 h at –77,8 °C and a pressure of 5 mTorr (removal of 97% moisture from the substrate with the preservation of biologically active substances). In duodenal chyme and litter, the activities of digestive enzymes, alkaline phosphatase, mineral content were determined, the balance of nutrients, nitrogen absorption, and the availability of amino acids were assessed.

Blood (2 ml) was taken 1 day before the slaughter of a bird (at the age of 47 days) from the axillary vein (cutanea ulnaris) on the inner side of the wing above the elbow joint. The puncture site was clamped with a sterile swab for several minutes. Samples for biochemical studies were taken into sterile vacuum tubes with lithium heparin (4.0 ml; Shandong Weigao Group Medical Polymer Co., Ltd., China), for morphological studies with anticoagulant K3-EDTA (2.0 ml; SOYAGREENTEC Co., Ltd., South Korea). To separate plasma from formed elements, the samples were centrifuged in a Hettich EBA 200 centrifuge (Andreas Hettich GmbH & Co. KG, Germany) at 5000 rpm for 5 min.

Feed samples for analysis were taken according to GOST 13496.0-2016 [18] from bags. The arbitration sample was prepared from an average sample weighing 1 kg. Each food sample was analyzed 3 times. Sample preparation was carried out according to GOST 34140-2017 [17]. The quantitative content of T-2 and HT-2 toxins in the original compound feed was measured twice in each analyzed sample by tandem high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) (Agilent 1260 Infinity chromatographic system, Agilent Technologies, Germany; a mass spectrometer AB SCIEX Triple Quad™ 5500, Applied Biosystems, USA; Gemini® C18 chromatographic column with a reverse-phase sorbent based on silica gel with an organic polymer, particle size 5 μm, 150×4.6 mm, Phenomenex, USA) [18].

Amylase in duodenal contents and litter was determined according to Smith-Roy in the modification for high enzyme activity [19], protease activity was determined by hydrolysis of casein purified according to Hammersten (colorimetric control at $\lambda = 450$ nm), lipase, alkaline phosphatase, calcium and phosphorus content were measured (a semi-automatic biochemical analyzer SINNOWA BS-3000P, SINNOWA Medical Science & Technology Co., Ltd, China) with a set of veterinary diagnostic reagents (DIAKON-VET, Russia). Biochemical blood tests were performed on a Sinnowa BS-3000P semi-automatic biochemical analyzer (SINNOWA Medical Science & Technology Co., Ltd., China) with a kit for determining total protein, alkaline phosphatase, glucose, cholesterol, triglycerides, lipase (DIAKON-VET, Russia). Trypsin activity in blood plasma was measured on a semiautomatic biochemical analyzer BS-3000P by the kinetic method [20] using Na-benzoyl-DL-arginine-p-nitroanilide (BAPNA, Acros Organics, Switzerland) as a substrate. Morphological blood tests were performed on a DF-50 automatic hematological analyzer for veterinary medicine (Dymind Biotech, China) using branded reagents.

The JMP Trial 14.1.0 software (https://www.jmp.com/en_us/software/data-analysis-software.html) was used for statistical processing of the results. The results are presented as arithmetic means (*M*) and standard deviations (±SD). Significance of differences was determined by Student's t-test, differences were considered statistically significant at $p < 0.05$.

Results. Preparations for the neutralization of mycotoxins affected the enzymatic activity in the duodenal contents (Table 1). Amylolytic activity increased in group II by 34.5% ($p < 0.05$), in group III by 40.9%, in group IV by 44.1%, in group V by 55.6% compared to the control. Proteolytic activity increased statistically significantly ($p < 0.05$) in groups IV and V by 38.5 and 22.9%, respectively. Trypsin activity increased only in group IV (by 22.8%, $p < 0.05$). The lipolytic activity of the duodenal contents tended to increase in all experimental groups, but the indicator changed statistically significantly ($p < 0.05$) only in group V by 19.3% compared to group I. It should be noted that the increase in protease activity in the duodenal chyme in group IV was more pronounced than in group II, where the enzyme preparation was not used (indicators differed by 15.5%, $p < 0.05$).

Alkaline phosphatase produced during the destruction of cells of bone tissue, liver, intestines, performs the function of hydrolysis of monoester compounds of phosphoric acid with the formation of alcohol [21]. In groups treated with T-2 toxin, the activity of the enzyme significantly increased compared to the control, in group II by 13.9%, in group III by 22.3% ($p < 0.05$), in group IV by 66.5% ($p < 0.05$), in group V by 68.7% ($p < 0.05$). This indicates degenerative processes in the intestinal tissue, aimed at adaptation to the action of the toxin [22]. The total phosphorus in the duodenal contents increased in group IV by 62.1% ($p < 0.05$). Therefore, the studied preparations have the ability to neutralize the negative effect of mycotoxin on the intestines.

1. Activity of duodenal enzymes in broiler chickens (*Gallus gallus* L.) Smena 8 cross fed additive Zaslon 2+ and the Axtra Pro enzyme preparation under experimental T-2 toxicosis (absolutely dry matter, $n = 5$, $M \pm SD$; vivarium, FSC ARRTPI RAS, 2021)

Parameter	Group				
	I (control)	II	III	IV	V
Amylase, mg · ml · min^{-1}	2722±278.4	3662±244.9*	3837±234.7*	3922±131.0*	4237±240.3*
Proteases, mg · ml · min^{-1}	161±15.3	193±6.4	203±19.2	223±2.9*	198±2.7*
Trypsin, U/l	6770±339.2	7371±201.4	7609±408.5	8313±206.5*	7202±215.4
Lipase, U/l	17482±1225.2	18784±1731	18289±911.2	21941±2172.7	20863±312.4*
Alkaline phosphatase, U/l	130085±6466.6	148260±7699.4	159106±6269.9*	216656±14191.5*	219429±8676.0*
Calcium, mmol/l	243±5.5	258±14.4	248±9.9	245±3.5	224±4.5*
Phosphorus, mmol/l	161±29.5	172±22.6	180±20.3	261±6.5*	226±12.6

N o t e. For a description of the groups, see the Materials and methods section.
* Differences from control are statistically significant at $p < 0.05$.

Determination of the activity of digestive enzymes in poultry litter serves as a diagnostic test for assessing intestinal health [31]. The activity of total proteases and alkaline phosphatase in the litter of broiler chickens of the Smena 8 cross did not change significantly when the feed sorbent Zaslon 2+ and the enzyme preparation Axtra Pro were added to the diet against the background of experimental mycotoxicosis (Table 2). The exception was the activity of alkaline phosphatase, which decreased by 28.1% ($p < 0.05$) in group IV vs. control. In the litter, ther was a statistically significant ($p < 0.05$) decrease of amylolytic activity (by 46.7% in group II, by 66.7% in group III, by 73.3% in group IV, and by 40.0% in group V), the activity of lipase (by 48.9, 73.1, 49.4, 80.4%, respectively) and trypsin (respectively by 34.7, 18.8, 30.2, 26.8%) compared to group I.

2. Activity of digestive enzymes in the litter of broiler chickens (*Gallus gallus* L.) Smena 8 cross fed additive Zaslon 2+ and the Axtra Pro enzyme preparation under experimental T-2 toxicosis (absolutely dry matter, $n = 5$, $M \pm SD$; vivarium, FSC ARRTPI RAS, 2021)

Parameter	Group				
	I (control)	II	III	IV	V
Amylase, mg · ml · min^{-1}	900±69.4	480±0.1*	300±23.1*	240±0.1*	540±3.1*
Proteases, mg · ml · min^{-1}	45±5.8	36±6.3	35±1.9	49±5.3	35±5.8
Lipase, U/l	9586.5±678.03	4902.0±135.64*	2585.0±193.44*	4852.0±343.35*	1881.5±301.54*
Alkaline phosphatase, U/l	72977±3803.5	60833±6603.1	67559±3075.7	52479±3840.8*	62528±3410.4
Trypsin, U/l	3883.5±146.60	2537.0±94.02*	3154.5±90.75*	2712.0±112.90*	2842.0±29.28*

N o t e. For a description of the groups, see the Materials and methods section.
* Differences from control are statistically significant at $p < 0.05$.

The decrease in the activity of digestive enzymes in the feces could be due to several reasons: the return of enzymes from the small intestine to the blood [23], the degradation of enzymes by serine proteinases [24], the inactivation of enzymes by specific inhibitors, or their absorption by the intestinal microflora [25].

In general, the results of our experiment allow us to conclude that the drugs used to neutralize toxins correct the cycles of digestive enzymes [26] and alkaline phosphatase in the intestine and have a positive effect on enteral homeostasis.

How efficient the process of protein digestion is and what is the availability of nitrogen in experimental T-2 toxicosis can be judged by the balance of the protein components of the feed and amino acids [27]. With a high feed contamination with T-2 toxin (0.4 mg/kg), the digestibility of crude protein in group III was lower by 2.8% (with the addition of a sorbent to the feed), in group V by 2.7% (with the addition of sorbent and protease to the feed) vs. control (Table 3). Nitrogen uptake decreased in the groups receiving the sorbent by 14.1% for 0.1 mg/kg T-2 toxin and 20.1% for 0.4 mg/kg, when using the sorbent and the enzyme preparation by 14.7 and 22.3% ($p < 0.05$), respectively. The greatest decrease in

crude protein digestibility and nitrogen availability occurred in groups where for feed contamination 0.4 mg/kg T-2 toxin was used.

3. Crude protein digestibility and nitrogen assimilation in broiler chickens (*Gallus gallus* L.) Smena 8 cross fed additive Zaslon 2+ and the Axtra Pro enzyme preparation under experimental T-2 toxicosis (absolutely dry matter, $n = 5$, M±SD; vivarium, FSC ARRTPI RAS, 2021)

Parameter	Group				
	I (control)	II	III	IV	V
Crude protein, %	90.75±0.35	90.57±1.26	88.20±1.34	90.33±1.02	88.31±1.32
Nitrogen assimilation, %	62.96±1.43	54.08±3.75	50.34±5.68	53.69±4.88	48.90±3.98*

N o t e. For a description of the groups, see the Materials and methods section.
* Differences from control are statistically significant at $p < 0.05$.

4. Availability of amino acids in the intestines of broiler chickens (*Gallus gallus* L.) Smena 8 cross fed additive Zaslon 2+ and the Axtra Pro enzyme preparation under experimental T-2 toxicosis (absolutely dry matter, $n = 5$, M±SD; vivarium, FSC ARRTPI RAS, 2021)

Amino acid	Group				
	I (control)	II	III	IV	V
Aspartic	78.56±0.82	78.78±2.84	74.52±2.91	77.79±2.34	75.79±2.73
Threonine	80.35±0.75	80.90±2.55	75.36±2.81	79.81±2.13	77.70±2.51
Serene	80.02±0.77	80.59±2.60	74.95±2.86	79.89±2.12	77.36±2.55
Glutamine	89.74±0.39	89.39±1.42	88.26±1.34	89.66±1.09	88.43±1.30
Proline	87.39±0.48	87.03±1.73	84.19±1.80	86.54±1.42	84.81±1.71
Glycine	54.56±1.75	51.95±6.43	41.92±6.64	39.50±6.39	32.33±7.63*
Alanine	80.54±0.75	80.02±2.67	78.68±2.43	80.20±2.09	78.39±2.43
Valine	81.29±0.72	80.77±2.57	77.76±2.54	70.02±2.11	78.65±2.41
Isoleucine	83.82±0.62	83.65±2.18	80.95±2.17	82.72±1.82	81.71±2.06
Leucine	84.36±0.60	84.20±2.11	81.74±2.08	83.54±1.74	82.19±2.00
Tyrosine	81.53±0.71	81.89±2.42	77.78±2.54	80.27±2.08	79.33±2.33
Phenylalanine	84.43±0.60	84.27±2.10	81.99±2.05	84.16±1.67	82.71±1.95
Histidine	77.08±0.88	79.16±2.79	70.71±3.34	76.14±2.52	72.58±3.09
Lysine	84.81±0.58	84.65±2.05	80.58±2.21	83.47±1.74	82.04±2.02
Arginine	86.20±0.53	86.32±1.83	84.35±1.78	86.37±1.44	84.98±1.69
Cystine	77.15±0.88	77.72±2.98	72.82±3.10	76.44±2.48	74.86±2.83
Methionine	91.24±0.33	89.98±1.34	89.56±1.19	89.40±1.11	89.97±1.13

N o t e. For a description of the groups, see the Materials and methods section.
* Differences from control are statistically significant at $p < 0.05$.

The pattern was similar to the assimilation of amino acids in the intestines of broilers (Table 4). Despite the trend towards a decrease in the availability of amino acids in groups III and V, only the assimilation of the amino acid glycine in group V decreased statistically significantly (by 40.8%, $p < 0.05$). This may affect the processes of inhibition in the central nervous system, since glycine serves as a mediator in the transmission of nerve impulses [28]. Consequently, contamination of feed with T-2 toxin for 14 days had a negative effect on protein metabolism, reducing the digestibility of crude protein and the availability of glycine in group V.

We did not observe deviations in the general condition of broilers during the experiment, but we performed a morphobiochemical blood test in order to identify changes in the body during experimental mycotoxicosis (Tables 5, 6).

In groups IV and V, there was an increase in trypsin activity in blood plasma, respectively, by 75.7 and 58.4% ($p < 0.05$) compared with the control (see Table 5). These indicators exceeded the physiological norm and could indicate an inflammatory process in the intestine and pancreatic tissues [29], which is associated with the presence of PARs (proteinase-activated receptors) receptors that regulate cellular signaling and can cause an immune inflammatory response [30]. With mycotoxicoses in the digestive organs of broilers, apoptosis phenomena may occur when caspase is activated in the pancreatic tissue [7]. Trypsin activity increased in

groups IV and V which received a protease supplement along with the sorbent, by 110.3% ($p < 0.05$) and 103.2% ($p < 0.05$), respectively, compared to groups II and III. The activity of alkaline phosphatase decreased ($p < 0.05$) when using dietary sorbent, in group II by 39.5% and in group III by 60.8%. The phosphatase-protease index decreased 2.0-fold compared to control. Total protein in group III increased by 10.3% ($p < 0.05$) while in group IV it decreased by 19.3% ($p < 0.05$), that is, it depended on the blood trypsin activity. The change in protein metabolism occurred in group V with an increase in the amount of uric acid by 175.9% ($p < 0.05$), which indicates the inefficient use of nitrogen in birds when the feed was contaminated with T-2 toxin at maximum dosage (0.4 mg/kg). In other experimental groups, there was a negative trend in the use of amino acids. This indicates a violation of protein metabolism confirmed by a decrease in the assimilation of nitrogen by birds in the experimental groups (see Table 3).

5. Blood biochemical parameters in broiler chickens (*Gallus gallus* L.) Smena 8 cross fed additive Zaslon 2+ and the Axtra Pro enzyme preparation under experimental T-2 toxicosis (absolutely dry matter, $n = 5$, $M \pm SD$; vivarium, FSC ARRTPI RAS, 2021)

Parameter	Group				
	I (control)	II	III	IV	V
Trypsin, U/l	244.4±15.72	204.2±1.38	190.5±3.20	429.4±3.67*	387.2±29.90*
Alkaline phosphatase, U/l	3525±564.3	2133±167.3*	1382±34.3*	2384±28.9	4710±416.9
Phosphate-protease index	14.4	10.4	7.2	5.5	12.1
Total protein, g/l	44.5±1.18	46.1±1.68	49.1±0.67*	35.9±1.72*	46.2±1.71
Uric acid, rmol/l	177.1±18.52	223.4±15.25	217.0±8.12	202.7±10.70	488.4±25.68*
Glucose, mmol/l	8.2±0.58	10.8±0.05*	9.6±0.09*	11.6±0.17*	12.4±0.24*
Cholesterol, mmol/l	2.8±0.14	1.8±0.04*	2.5±0.16	2.6±0.20	3.7±0.05*
Triglycerides, mmol/l	0.28±0.02	0.57±0.06*	0.33±0.01*	0.43±0.01*	0.35±0.04
Calcium, mmol/l	2.6±0.07	3.6±0.06*	2.8±0.05	1.9±0.01*	2.4±0.13
Phosphorus, mmol/l	2.0±0.07	1.6±0.07*	2.2±0.03*	1.6±0.04*	1.8±0.09

N o t e. For a description of the groups, see the Materials and methods section.
* Differences from control are statistically significant at $p < 0.05$.

6. Hematological parameters in broiler chickens (*Gallus gallus* L.) Smena 8 cross fed additive Zaslon 2+ and the Axtra Pro enzyme preparation under experimental T-2 toxicosis ($n = 5$, $M \pm SD$; vivarium, FSC ARRTPI RAS, 2021)

Parameter	Group				
	I (control)	II	III	IV	V
Leukocytes, 10^9/l	41.2±4.36	37.2±1.95	62.4±7.79*	58.0±2.09*	38.5±4.87
Heterophiles, %	51.7±3.58	65.1±1.79*	54.3±0.61	58.2±4.29	64.3±4.92*
Eosinophils, %	8.4±0.97	8.8±2.29	8.5±1.04	7.3±0.37	7.2±1.68
Basophils, %	0.1±0.01	0.1±0.02	0.2±0.05	0.1±0.01	0.1±0.02
Lymphocytes, %	37.6±1.37	22.6±2.29*	35.6±1.35	34.3±1.79	27.8±2.70*
Monocytes, %	2.2±0.30	3.4±0.52	1.4±0.13	0.2±0.02	0.1±0.04
Erythrocytes, $\times 10^{12}$/l	3.5±0.15	3.3±0.11	4.4±0.34*	4.1±0.05*	3.3±0.24
Hemoglobin, g/l	165.3±6.15	154.3±4.71	200.5±12.34	200.8±4.56	164.5±14.44
Hematocrit, %	44.3±2.20	42.2±1.30	52.6±3.58	54.7±1.31	43.5±4.65

N o t e. For a description of the groups, see the Materials and methods section.
* Differences from control are statistically significant at $p < 0.05$.

A clear parameter to characterize the state of carbohydrate metabolism in broiler chickens was the blood glucose concentration which increased statistically significantly ($p < 0.05$) in all test broilers, in group II by 31.7%, in group III by 17.1%, in group IV by 41.5%, and in group V by 51.2%. This can be explained by the stress developed under an experimental mycotoxicosis [31].

The state of lipid metabolism is determined by the amount of cholesterol and triglycerides in the blood. The greatest increase in cholesterol levels in our experience was observed in group V by 32.1% compared to the control ($p < 0.05$). All experimental groups showed an increase in the amount of lipids in blood plasma but the most significant changes occurred in groups II (by 103.6%, $p < 0.05$)

and IV (by 53.6%, $p < 0.05$).

Hematological parameters of broilers reflected the metabolic and immune status of the bird (see Table 6). The number of leukocytes in test group III increased by 51.4% ($p < 0.05$) and in group IV by 40.8% ($p < 0.05$) compared to the control. Analysis of the leukoformula showed that in groups II and V the number of heterophils increased by 25.9 and 24.4%, respectively, which indicated an increase in resistance under the action of T-2 toxin [32]. The decrease in the number of lymphocytes in the experimental groups can be explained by changes in the lymphoid tissue of the intestines of chickens under the influence of T-2 toxin [33]. The most noticeable changes we observed in groups II (by 39.9%, $p < 0.05$) and V (by 26.1%, $p < 0.05$). In chickens treated with a protease sorbent as an additive, the number of monocytes was significantly lower (by 94.1 and 92.9%, $p < 0.05$) than in those receiving only the sorbent, which was apparently associated with a decrease in inflammatory response. The number of erythrocytes in the blood of a bird is closely related to oxidative processes in the body. It can be assumed that in groups III and IV, metabolism was activated due to an increase in the number of erythrocytes in the blood, hemoglobin and hematocrit. With an increase in these indicators, blood oxygen saturation increased markedly.

The mechanisms of action of mycotoxins on the digestive system of animals remain poorly understood, although the relevance of this problem is associated with the health of people who consume meat, including poultry meat [34]. Earlier, in a chronic experiment in vivo on broilers, we first studied the physiological and biochemical processes during the development of T-2 toxicosis and proposed methods for diagnosing the disease [24]. In this work, we considered the effect of the sorbent as a means of preventing mycotoxicoses in various ways of its application. An increase in the efficiency of the sorbent in combination with the protease indicates the promise of these studies and the expediency of their continuation [35].

It is known that cellular enzymes play an important role in detoxification in birds. S.Yu. Gulyushin and V.O. Kovalev [36] note that the generation of free radicals (free forms of oxygen) and antiradical (antioxidant) protection are in dynamic equilibrium. Under the equilibrium disturbance in poultry fed mixed feed contaminated with mycotoxins, an oxidative stress occurred together with the inhibition of the main enzymes of antiradical protection (catalase, peroxidase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase) and the accumulation of a larger amount of hydroperoxide products. As a result, there was a destabilization of biological membranes, a violation of homeostasis, oxygenation and tissue trophism, and a potentiation of various cytopathogenic effects. The authors suggest selenium preparations to increase the activity of serum and cellular antiradical protection factors, to normalize physiological and biochemical processes and, as a result, to provide a prolonged effect of productivity correction in the combined chronic mycotoxicoses. The results of studies [36] showed that Selexen and DAFS 25 had the most effective prophylactic properties in the experiments while the mineral salt Na_2SeO_3 was slightly inferior. The authors also came to the conclusion that monotherapy with the use of selenium preparations is not effective enough. It seems appropriate to combine it with other available methods of nonspecific prophylaxis that stimulate protein synthesis and/or excretion of xenobiotics.

The results of our study of a complex preparation for broiler chickens showed for the first time that the activity of digestive enzymes in the duodenal chyme and blood can serve as a criterion for evaluating the effectiveness of this preparation. Taking into account the observed decrease in the phosphatase-protease index in the test groups, it can be argued that the transition of the T-2 toxin

to the HT-2 metabolite [19] is slowed down due to the sorption capacity of the drug. The negative effect of T-2 toxin on protein metabolism follows from a decrease in nitrogen utilization in all test groups and a trend towards a decrease in the availability of amino acids, especially at 0.4 mg/kg T-2 toxin. The blood biochemical parameters indicate a violation of protein, fat and carbohydrate metabolism of broiler chickens, as well as signs of stress caused by the influence of the toxin on digestive organs, the pancreas and liver. The immune status of birds in experimental T-2 toxicosis changed mainly due to an increase in the number of heterophils capable of phagocytosis [37]. The number of lymphocytes, immunocompetent cells that counteract pathogenic biological agents, decreased, which makes the bird less protected from infectious diseases [38, 39].

Thus, in the experimental T-2 toxicosis of Smena 8 cross broiler chickens, the Zaslon 2+ sorbent in combination with the Axtra Pro enzyme preparation containing protease lead to more pronounced changes in the activity of duodenal enzymes than the sorbent alone. There was a statistically significant increase in total proteolytic activity (by 15.5%, $p < 0.05$), trypsin activity (by 12.8%, $p < 0.05$), alkaline phosphatase activity (by 46.1%, $p < 0.05$), total phosphorus (by 25.6%, $p < 0.05$). Amylase activity in the litter decreases 2.0-fold. The activity of blood trypsin in the groups fed the protease supplement along with the sorbent increased by 110.3% ($p < 0.05$) and 103.2% ($p < 0.05$) compared to the birds that received only Zaslon 2+. Also, the combined use of the sorbent and protease provides a higher number of blood lymphocytes (by 51.8%, $p < 0.05$). The obtained results suggest that in mycotoxicoses the correction of protein metabolism by the toxin sorbent in combination with proteases is associated with the stimulation of digestive enzyme activity and metabolism. We plan to continue these studies to gain a more complete understanding the described processes and to develop a new drug for the prevention and complex therapy of mycotoxicosis.

REFERENCES

1. Awuchi C.G., Ondari E.N., Ogbonna C.U., Upadhyay A.K., Baran K., Okpala C.O.R., Korzeniowska M., Guiné R.P.F. Mycotoxins affecting animals, foods, humans, and plants: types, occurrence, toxicities, action mechanisms, prevention, and detoxification strategies — a revisit. *Foods*, 2021, 10(6): 1279 (doi: 10.3390/foods10061279).
2. Ivanov A.V., Fisinin V.I., Tremasov M.Ya., Papunidi K.Kh. *Mikotoksikozy (biologicheskie i veterinarnye aspekty)* [Mycotoxicoses (biological and veterinary aspects)]. Moscow, 2010 (in Russ.).
3. den Hollander D., Croubels S., Lauwers M., Caekebeke N., Ringenier M., Meyer F.D., Reisinger N., Immerseel F.V., Dewulf J., Antonissen G. Biomonitoring of mycotoxins in blood serum and feed to assess exposure of broiler chickens. *Journal of Applied Poultry Research*, 2021, 30(1): 100111 (doi: 10.1016/j.japr.2020.10.010).
4. Adhikari M., Negi B., Kaushik N., Adhikari A., Al-Khedhairy A.A., Kaushik N.K., Choi E.H. T-2 mycotoxin: toxicological effects and decontamination strategies. *Oncotarget*, 2017, 8(20): 33933-33952 (doi: 10.18632/oncotarget.15422).
5. Kepinska-Pacelik J., Biel W. Mycotoxins—prevention, detection, impact on animal health (review). *Processes*, 2021, 9(11): 2035 (doi: 10.3390/pr9112035).
6. Yang X., Liu P., Zhang X., Zhang J., Cui Y., Song M., Li Y. T-2 toxin causes dysfunction of Sertoli cells by inducing oxidative stress. *Ecotoxicology and Environmental Safety*, 2021, 225: 112702 (doi: 10.1016/j.ecoenv.2021.112702).
7. Yyldyrym E.A., Grozina A.A., Vertiprakhov V.G., Il'ina L.A., Filippova V.A., Laptev G.Yu., Brazhnik E.A., Kalitkina K.A., Tarlavin N.V., Dubrovin A.V., Novikova N.I., Tyurina D.G. Effect of T-2 toxin on expression of genes associated with immunity in tissues of the blind processes of the intestinal and pancreas of broilers *(Gallus gallus* L.). *Sel'skokhozyaystvennaya biologiya*, 2021, 56(4): 664-681 (doi: 10.15389/agrobiology.2021.4.664rus).
8. Rychen G., Aquilina G., Azimonti G., Bampidis V., de Lourdes Bastos M., Bories G., Chesson A., Cocconcelli P.S., Flachowsky G., Gropp J,. Kolar B., Kouba M., Lopez-Alonso M., Mantovani A., Mayo B., Ramos F., Saarela M., Villa R.E., Wallace R.J., Wester P., Martelli G., Renshaw D., Lopez Puente S. Scientific opinion on the safety and efficacy of microorganism DSM 11798 as a technological additive for all avian species. *EFSA Journal*, 2017, 15(1): e04676 (doi: 10.2903/j.efsa.2017.4676).

9. Kotsyumbas I.Y., Fediakova O.I. Impact of sorbents on a concentration of T-2 and HT-2 toxins within a digestive system of the chickens. *The Animal Biology*, 2013, 15(3): 44-48
10. Polak-Śliwińska M., Poszczyk B. Trichothecenes in food and feed, relevance to human and animal health and methods of detection: a systematic review. *Molecules*, 2021, 26(2): 454 (doi: 10.3390/molecules26020454).
11. Balakrishnan U., Moortkhi R. *Primenenie beta-tseolita v kachestve veshchestva, svyazyvayushchego mul'titoksiny v korme dlya zhivotnykh. Patent na izobretenie 2670917 S9, 06.03.2020. Zayavka № 2016137785 ot 24.02.2015* [The use of beta zeolite as a multitoxin binder in animal feed. Patent 2670917 C9, 03/06/2020. Appl. No. 2016137785 02/24/2015] (in Russ.).
12. Yuy S., Li Ch., Yuy M., Yao Ts., Tan' B., Chzhu Ts. *Sorbent biotoksina i sposob ego polucheniya. Patent na izobretenie 2426445 S1, 26.06.2020. Zayavka № 2010108252/13 ot 06.08.2008* [Biotoxin sorbent and method for its preparation. Patent 2426445 C1, 06/26/2020. Appl. No. 2010108252/13 of 08/06/2008] (in Russ.).
13. Malkov M.A., Dan'kova T.V. *Sposob polucheniya biopreparata dlya ustraneniya mikotoksinov iz kormovogo syr'ya i biopreparat, poluchennyy etim sposobom. Patent na izobretenie 2420565 S1, 10.06.2011. Zayavka № 2010105445/10 ot 15.02.2010* [Method for obtaining a biological product for the elimination of mycotoxins from feed raw materials and a biological product obtained by this method. Patent 2420565 C1, 06/10/2011. Appl. No. 2010105445/10 dated February 15, 2010] (in Russ.).
14. *European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS № 123)* (Strasburg, 18.03.1986). Available: https://www.coe.int/en/web/conventions/full-list?module=treaty-detail&treatynum=123. Accessed: 20.08.2021.
15. *Rukovodstvo po optimizatsii retseptov kombikormov dlya sel'skokhozyaystvennoy ptitsy* /Pod red. V.I. Fisinina [Guidelines for optimizing compound feed recipes for poultry. V.I. Fisinin (ed.)]. Sergiev Posad, 2014: 3-4 (in Russ.).
16. Fisinin V.I., Vertiprakhov V.G., Grozina A.A., Kislova I.V., Koshcheeva M.V. The effects of chromium microadditive in different diets for laying hens (*Gallus gallus* L.) on the intestinal digestion and certain biochemical blood parameters. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2019, 54(4): 810-819 (doi: 10.15389/agrobiology.2019.4.810rus).
17. *GOST 13496.0-2016. Kombikorma, kombikormovoe syr'e. Metody otbora prob.* Available: https://docs.cntd.ru/document/1200140597 [GOST 13496.0-2016. Compound feed, compound feed raw materials. Sampling methods]. Accessed: 14.02.2022 (in Russ.).
18. *GOST 34140-2017. Produkty pishchevye, korma, prodovol'stvennoe syr'e.* Available: https://docs.cntd.ru/document/1200146270 [GOST 34140-2017. Food products, feed, food raw materials]. Accessed: 14.02.2022 (in Russ.).
19. Vertiprakh**ov** V.G., Grozina A.A., Gogina N.N., Kislova I.V., Ovchinnikova N.V., Koshcheeva M.V. The intestinal T-2 and HT-2 toxins, intestinal and fecal digestive enzymes, morphological and biochemical blood indices in broilers (*Gallus gallus* L.) with experimentally induced T-2 toxicosis *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2021, 56(4): 682-694 (doi: 10.15389/agrobiology.2021.4.682rus).
20. Batoev Ts.Zh. *Fiziologiya pishchevareniya ptits* [Physiology of bird digestion]. Ulan-Ude, 2001 (in Russ.).
21. Vertiprakhov V.G., Grozina A.A. *Veterinariya*, 2018, 12: 51-54 (doi: 10.30896/0042-4846.2018.21.12.51-54) (in Russ.).
22. Vimalraj S. Alkaline phosphatase: structure, expression and its function in bone mineralization. *Gene*, 2020, 754: 144855 (doi: 10.1016/j.gene.2020.144855).
23. Luo J.-J., Zhang Y., Sun H., Wei J.-T., Khalil M.M., Wang Y.-W., Dai J.-F., Zhang N.-Y., Qi D.-S., Sun L.-H. The response of glandular gastric transcriptome to T-2 toxin in chicks. *Food and Chemical Toxicology*, 2019, 132: 110658 (doi: 10.1016/j.fct.2019.110658).
24. Vertiprakhov V.G., Gogina N.N., Ovchinnikova N.V. *Veterinariya*, 2020, 7: 56-59 (doi: 10.30896/0042-4846.2020.23.7.56-59) (in Russ.).
25. Zamolodchikova T.S. Serine proteases in immune protection of the small intestine. *Biochemistry Moscow*, 2013, 78(3): 213-220 (doi: 10.1134/S0006297913030012).
26. Cao G.T., Zhan X.A., Zhang L.L., Zeng X.F., Chen A.G., Yang C.M. Modulation of broilers' caecal microflora and metabolites in response to a potential probiotic *Bacillus amyloliquefaciens*. *J. Anim. Physiol. Anim. Nutr.*, 2018, 102(2): e909-e917 (doi: 10.1111/jpn.12856).
27. Rothman S.S., Liebow C., Isenman L. Conservation of digestive enzymes. *Physiological Review*, 2002, 82(1): 1-18 (doi: 10.1152/physrev.00022.2001).
28. Wei J.T., Wu K.T., Sun H., Khalil M.M., Dai J.F., Liu Y., Liu Q., Zhang N.Y., Qi D.S., Sun L.H. A novel modified hydrated sodium calcium aluminosilicate (HSCAS) adsorbent can effectively reduce T-2 toxin-induced toxicity in growth performance, nutrient digestibility, serum biochemistry, and small intestinal morphology in chicks. *Toxins*, 2019, 11(4): 199 (doi: 10.3390/toxins11040199).
29. Harsing L.G., Matyus P. Mechanisms of glycine release, which build up synaptic and extrasynaptic glycine levels: the role of synaptic and non-synaptic glycine transporters. *Brain Research Bulletin*, 2013, 93: 110-119 (doi: 10.1016/j.brainresbull.2012.12.002).

30. Ramachandran R., Hollenberg M.D. Proteinases and signalling: pathophysiological and therapeutic implications via PARs and more. *British Journal of Pharmacology*, 2008, 153(S1): 263-282 (doi: 10.1038/sj.bjp.0707507).
31. Chen Y., Han S., Wang Y., Li D., Zhao X., Zhu Q., Yin H. Oxidative stress and apoptotic changes in broiler chicken splenocytes exposed to T-2 toxin. *BioMed Research International*, 2019, 2019: 5493870 (doi: 10.1155/2019/5493870).
32. Wu Q., Wang X., Nepovimova E., Miron A., Liu Q., Wang Y., Su D., Yang H., Li L., Kuca K. Trichothecenes: immunomodulatory effects, mechanisms, and anti-cancer potential. *Arch. Toxicol.*, 2017, 91(12): 3737-3785 (doi: 10.1007/s00204-017-2118-3).
33. Wojtacha P., Trybowski W., Podlasz P., Żmigrodzka M., Tyburski J., Polak-Śliwińska M., Jakimiuk E., Bakuła T., Baranowski M., Żuk-Gołaszewska K., Zielonka Ł., Obremski K. Effects of a low dose of T-2 toxin on the percentage of T and B lymphocytes and cytokine secretion in the porcine ileal wall. *Toxins*, 2021, 13(4): 277 (doi: 10.3390/toxins13040277).
34. Streit E., Schatzmayr G., Tassis P., Tzika E., Marin D., Taranu I., Tabuc C., Nicolau A., Aprodu I., Puel O., Oswald I. Current situation of mycotoxin contamination and co-occurrence in animal feed—focus on Europe. *Toxins*, 2012, 4(10): 788-809 (doi: 10.3390/toxins4100788).
35. Borisenko K.V., Vertiprakhov V.G. *Ptitsevodstvo*, 2018, 10: 20-23 (in Russ.).
36. Gulyushin S.Yu., Kovalev V.O. State of antiradical protection system in broilers during use of selenium containing preparations on the background of toxic feed (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2009, 4: 14-25 (in Russ.).
37. Liew P.X., Kubes P. The neutrophil's role during health and disease. *Physiol. Rev.*, 2019, 99(2): 1223-1248 (doi: 10.1152/physrev.00012.2018).
38. Yohannes T., Sharma A.K., Singh S.D., Sumi V. Experimental haematobiochemical alterations in broiler chickens fed with T-2 toxin and co-infected with IBV. *Open Journal of Veterinary Medicine*, 2013, 3(5): 252-258 (doi: 10.4236/ojvm.2013.35040).
39. Liu W., Wu D., Li S., Xu J., Li P., Jiang A., Zhang Y., Liu Z., Jiang L., Gao X., Yang Z., Wei Z. Glycolysis and reactive oxygen species production participate in T-2 toxin-stimulated chicken heterophil extracellular traps. *J. Agric. Food Chem.*, 2021, 69(43): 12862-12869 (doi: 10.1021/acs.jafc.1c05371).