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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

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Abstract

At present over 400 mycotoxins have been identified with mutagenic, teratogenic, embryotoxic, allergenic, and immunosuppressing properties, suppressing cellular and humoral immunity. Trichothecene mycotoxins are prevalent on the territory of Russian Federation, the most abundant and hazardous being T-2 and HT-2 toxins inducing gastroenterites, necroses of skin and mucosa of the oral cavity, disturbances in the function of central nervous system. The exposure limit for T-2 toxin in feeds for poultry is established in SanPiN 2.3.2.1078-1 at 100 μg/kg. Mycotoxin induced injuries of the organs are preceded by functional disturbances affecting the hematological status and enzymatic reactions in the digestive tract; the most exact data on these disturbances can be obtained by *in vivo* experiments on fistulated birds. We used this approach in our study in combination with the analyses of mycotoxin concentrations in lyophilized samples of duodenal chyme and feces by high-performance liquid chromatography and tandem mass spectrometry (HPLC MS/MS). The aim of the study was the investigation of the T-2 toxin in the gastrointestinal tract and the effects of the toxin on the intestinal activities of the digestive enzymes and morphological and biochemical blood indices in cross Smena 8 broilers with experimentally induced T-2 toxicosis. Birds with chronic duodenal fistulae were fed the feeds contaminated with T-2 toxin in doses from 100 μg/kg (corresponding to the exposure limit statutorily set for chicken) to 400 μg/kg for 2 weeks. The measurements of the T2 toxin in the duodenal chyme and feces evidenced the transformation of T-2 to HT-2 toxin in the intestine and intense absorption of the latter into the bloodstream since its concentration in feces was significantly lower in compare to the duodenal chyme. All dietary doses of T-2 increased the duodenal activities of the total proteases (by 46.3-96.6 %, $p < 0.05$), lipase (by 16.8-25.5 %, $p < 0.05$), and amylase (by 99.7 %) at T-2 dose 400 μg/kg while activity of alkaline phosphatase decreased by 23.8-27.9 % ($p < 0.05$). In the feces the increase in proteolytic activity by 76.0-169.1 % ($p < 0.05$) and decreases in the activities of lipase (by 23.2 % at T-2 dose 400 μg/kg) and amylase (by 55.1-57.2 %) were found. The activity of trypsin in blood serum decreased by 12.2-22.2 % ($p < 0.05$) while the increases in the activity of alkaline phosphatase varied from 52.4 % to 5-fold. As a result the phosphatase-protease index increased from 31 to 92 in average. At T-2 dose 400 μg/kg the decreases in the concentrations in blood serum of total protein (by 13.2 %, $p < 0.05$), glucose (by 13.5 %, $p < 0.05$), and triglycerides (4-fold) were found. The total leukocyte number in blood decreased by 15.8-16.6 %. At T-2 doses 100 and 200 μg/kg the trend to higher percentage of the lymphocytes was found though the percentage of neutrophils remained unaffected, evidencing the activation of the specific antitoxic protective mechanisms. The inversed correlations between the degree of the transformation of T-2 toxin into HT-2 toxin and tryptic activity in duodenal chyme and phosphatase-protease index in blood serum were found; these correlations can be used in the diagnostic test for T-2 toxicosis.

Keywords: T-2 toxin, HT-2 toxin, T-2 toxicosis, broilers, duodenal chime, digestive enzymes, proteases, lipase, amylase, biochemical blood indices, trypsin, alkaline phosphatase, triglycerides, cell blood composition, leukocytic formula

Foods and feeds often contain mycotoxins, which are secondary metabolites produced by imperfect fungi (formal class *Fungi imperfecti*) of the genera *Fusarium, Aspergillus*, *Myrothecium*, *Trichoderma*, *Trichothecium*, *Penicillium*, etc. Mycotoxins vary in chemical structure, toxicity, and mechanism of action [1, 2]. Today, there are over 400 known mycotoxins; the most common ones that are toxic to humans and animals are aflatoxins (aflatoxin B_1), fumonisins, zearalenone, type B trichothecenes (deoxynivalenol), type A trichothecenes (T-2 toxin), and ochratoxin A [3].

Trichothecenes are synthesized by the genus *Fusarium* fungi; however, *Cephalosporium*, *Myrothecium*, *Stachybotrys*, and *Trichoderma* are also capable of producing these compounds [4]. There are over 150 known structurally related compounds that are classified into four types, A to D, by their chemical structure [5]. The most common toxins are Group A (HT-2 and T-2 toxins) and Group B (deoxynivalenol (DON), 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, nivalenol). T-2 and HT-2 toxins, although a small group, are the most toxic trichothecenes [6]. These toxins can cause slower live weight gain, hemorrhagic lesions in mucous membranes, abortions, lower milk productivity and egg production [7].

Monitoring feeds for mycotoxin presence is a major area of focus around the world. Corn [8], compound feed [9], and barley are recognized to have the highest contamination with type A trichothecenes. Type A trichothecene mycotoxins (T-2 toxin, etc.), Type B trichothecenes (DON, etc.), zearalenone, ochratoxin, and fumonisins are the most common trichothecenes in Russia's climate [11, 12].

T-2 contamination is a grave threat to industrial poultry farming, as this toxin has multiple negative effects on birds' physiology due to its ability to inhibit protein synthesis, alter the expression of inflammatory genes [13], which later causes necrosis and desquamation of epithelial cells in the glandular stomach mucosa [14]. Since T-2 toxin has low bioavailability, most of its transforms into metabolites, in particular HT-2 toxin [15].

Large T-2 and HT-2 intake results in lower productivity, alters blood biochemistry [16], restructures hepatocytes [17], splenocytes [18], and the bursa of Fabricius [19]. T-2 toxins also exhibit additive toxicity if the feed contains ochratoxin A [20], fumonisin B and deoxynivalenol [21], cyclopiazonic acid [22], or aflatoxin [23], making it a source of substantial harm to animal husbandry.

Complete compound feeds are the staple diet for farm animals. Feed monitoring studies [24] report T-2 to be prevalent in all Russian regions. It is most commonly found in compound feeds for poultry and pigs (79.1% of the tested samples) [25]. T-2 toxin was also found in 32% of wheat samples, 70.9% of barley samples, and 94% of corn samples [26], which must be borne in mind when making complete compound feeds. Enzyme immunoassay for mycotoxin contamination showed poultry compound feeds [27] to contain all major groups of mycotoxins, with T-2 toxin detected in 88.2% of the tested samples.

For a long time, mycotoxicosis risk evaluations were based on testing the feeds for safety. Biomaterials such as blood, urine, or feces are the best candidate for testing in terms of informativeness with respect to a mycotoxin-affected animal's condition [28]. High-performance liquid chromatography-mass spectrometry (HPLC-MS) accurately detects mycotoxins and their metabolites in blood, heart, liver, spleen, lung, kidney, glandular stomach, muscular stomach, small intestine, muscle, bone, and brain samples [29].

Pathogenesis in poultry fed with T-2 and HT-2-contaminated feeds remains an under-investigated issue; to date, there are no effective methods for diagnosis and prevention of these mycotoxicoses. Before mycotoxins take their toll on organs, the organs develop functional disorders that affect the enzymatic status of the digestive canal and the morphobiochemistry of blood. Once in the body, mycotoxins undergo biochemical transformations. In vivo experiments on fistulated animals with HPLC-MS testing for mycotoxin contamination of samples can provide the most complete insight into the patterns of these processes.

Here, using this approach, we showed the T-2 toxin-contaminated feed to result in both T-2 and HT-2 appearance in broilers' intestines. The latter toxin is intensively absorbed into the blood stream and seems to pass into tissues as well, as it is far less present in feces than in the duodenum. The activity of the enzymes in the duodenal chyme, blood enzyme activity, and WBC differential change, a sign of increased hepatic exposure to toxins and of an emerging pathology. The authors propose the phosphatase-protease index (PPI) as a marker for early diagnosis of mycotoxicosis, with blood PPI > 20 being indicative of digestive disorders [30].

The goal hereof was to quantify the presence of T-2 and HT-2 toxins in the chyme and feces, the activity of digestive enzymes, and the morphological and biochemical blood parameters in broilers affected experimentally with T-2 toxicosis.

Material and methods. The experiment involved cross Smena 8 broiler chicks (*Gallus gallus* L.) in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123, Strasbourg, 1986) [31]. From Day 1 to Day 47 of age, all the birds involved in the experiment were kept in a vivarium (Russian Poultry Research Center of the Russian Academy of Sciences — VNITIP, 2020); their feed and housing were compliant with the requirements applicable to this age and cross [32]. Twenty specimens were fistulated on Days 20 to 25; the researchers used their own method to cannulate the site opposite to the location of pancreatic and bile inflows into the intestines [33]. After a single postoperative no-feed day, the poultry was switched to rationed feeding; chyme passage through the intestines was carefully monitored. Stitches were removed on post-op Day 5. The birds, clinically healthy, were split into four five-specimen groups: Group 1 for control, no T-2 toxin in the diet; Group 2 had its diet T-2 toxin-contaminated at MPC levels (100 μg/kg), Group 3 received 2MPC (200 μg/kg), and Group 4 had 4MPC (400 μg/kg). T-2 toxin was added to feeds mechanically until reaching the required concentration; all personnel safety requirements were complied with. Standard T-2 toxin (powder, mass fraction of the toxin 99.7±0.3%, Romer Labs, Austria. cat. N 10000310, LOT #S17052T). Fresh feed was accessible every day; water access was not restricted.

The preparatory phase lasted from Day 26 to Day 33 of age; the experiment itself was 14 days long (Days 34 to 47 of age). Duodenal chyme and fecal samples for testing for T-2 and HT-2 presence, as well as for enzymatic activity, were sampled every day throughout the experiment (including the preparatory phase) and placed in a freezer at –20 °C. Combined samples were further made for each broiler (combined preparatory-phase sample + combined sample for each week of the experiment, i.e., samples taken up until Day 40 and up until Day 47 of age); they were subject to subsequent preparations and testing. For T-2 and HT-2 quantifications, the research team further merged samples in each group. Chyme samples (1.0 to 2.0 ml) and fecal samples (5.0 g) were taken every morning and placed in a

refrigerator at –20 °C; 5-g samples were dried in a TFD lyophilic drier (ilShinBioBase Cl., Ltd., South Korea) over 34 hours at –77.8 °C and 5 mTorr to remove 97% of moisture from the substrate while preserving bioactive compounds. Two milliliters of blood was sampled before the experiment, then at the end of every week (on Days 33, 40, and 47 of age) from the vena cutanea ulnaris on the inner side of the wing above the ulnar joint. The puncture site was covered with a sterile swam for a few minutes. Samples for biochemical testing were collected into sterile vacuum tubes with lithium heparin (4.0 ml, Shandong Weigao Group Medical Polymer Co., Ltd., China); samples for morphological testing were collected into tubes with the K3-EDTA anticoagulant (2.0 ml, SOYAGREENTEC Co., Ltd., South Korea). The samples were centrifuged at 5000 rpm for 5 minutes to separate the plasma from the corpuscles.

Feed was sampled for testing per GOST 13496.0-2016 Compound feeds, feed raw materials. Methods of sampling (Moscow, 2020) from bags. The arbitration sample was made from an average 1 kg sample. Each feed sample was tested thrice. Samples were prepared per GOST 34140-2017 Food products, feed, food raw materials. Method for determination of mycotoxins by high-performance liquid chromatography – mass spectrometry (Moscow, 2020).

T-2 and HT-2 in the feedstock, the duodenal chyme, and feces was measured twice for each tested sample by HPLC-MS (an Agilent 1260 Infinity chromatographic system, Agilent Technologies, Germany; an AB SCIEX Triple Quad™ 5500 mass spectrometer, Applied Biosystems, USA; a Gemini® C18 5 μm 150×4.6 mm column, a reverse-phase sorbent based on silica gel with an organic polymer, Phenomenex, USA). Standard Biopure series T-2 and HT-2 solutions (Romer Labs, Austria) were used as internal standards, as well as for plotting the calibration curves. Tests were designed per GOST 34140-2017. For testing, the research team used methanol, acetonitrile (HPLC-MS purity, min. 99.9% mass fraction of the substance, Merk, Germany); glacial acetic acid, ammonium acetate (min. 99.0% mass fraction of the substance, Sigma, Germany). Eluent A: 890 cm^3 of deionized water, 100 cm^3 of methanol, 10 cm^3 of acetic acid, and 0.384 g of ammonium acetate (5 mmol/l). Eluent B: 970 cm^3 of methanol, 20 cm^3 of deionized water, 10 cm^3 of acetic acid, and 0.384 g of ammonium acetate (5 mmol/l). Extraction solution: acetonitrile:water:acetic acid at 79:20:1. Chromatographic separation was performed by binary gradient elution: 1.5 min at 100% Eluent A, linear increase in Eluent B up to 50% over 1.5 min, followed by a linear increase in Eluent B up to 100% over 9 minutes. Chromatographic separation at 100% Eluent B for 5 min, followed by column balancing at 100% Eluent A for 3 min. Column temperature: 25 °C; mobile phase velocity: 1 cm^3/min; sample injection temperature: 10 °C, the volume of injected samples: 5 mm^3 each. The retention time was 10.30 min for T-2 toxin, 9.79 min for HT-2 toxin; the detection limit was 3.25 and 2.70 μg/kg, respectively; the quantification limit was 5.23 to 129.20 and 3.5 to 129.20 μg/kg, respectively. The estimated m/z values (positive ionization by spraying in an electric field) were 305.1 for T-2, 345.1 for HT-2.

Amylase was determined by the high-activity modification of the Smith-Roy method [34]; protease activity was determined by hydrolysis of Hammarsten-purified casein (calorimetric control at $\lambda = 450$ nm); lipase was quantified on a SINNOWA BS-3000P semi-automatic biochemical analyzer (SINNOWA Medical Science & Technology Co., Ltd, China) using veterinary diagnostic reagents from DIACON-VET, Russia.

Biochemical blood tests were run on a BS-3000P analyzer (China) using a kit for total protein, alkaline phosphatase, glucose, cholesterol, triglycerides, and lipase (DIACON-VET, Russia). Trypsin activity in plasma was measured on a BS-

3000P analyzer kinetically [35] using Na-benzoyl-DL-arginine-p-nitroanilide as substrate (BAPNA, ACROS ORGANICS, Switzerland).

Blood morphology was tested on a DF-50 automatic hematological analyzer for veterinary applications (Dymind Biotech, China) using the manufacturer's reagents.

Statistical tests were run (JMP Trial 14.1.0 (https://www.jmp.com/en_us/software/data-ana-lysis-software.html). The results are reported as $M \pm SD$, where M is the arithmetic mean, $\pm SD$ is the standard deviation. Significance was tested by Student's t-test with a threshold $p < 0.05$.

Results. Mycotoxins are metabolized in the digestive canal, and the metabolites end up in the blood. T-2 metabolism is known to involve deacetylation, hydroxylation, and conjugation [2, 16]. The authors of this paper earlier showed T-2 toxin to convert into HT-2 toxin in birds and to significantly affect the concentration of nitric oxide and the activity of pancreatic enzymes [36]. In continuation of that effort, this paper complements chemical, toxicological, physiological, and biochemical findings with the results of general and biochemical blood tests as markers of the clinical status in poultry sustaining experimental T-2 toxicosis. Besides, lyophilic drying was used to better prepare the samples.

1. Mycotoxin concentration (μg/kg) **in the duodenal chyme and feces of Smena 8 broiler chicks (*Gallus gallus* L.) upon T-2 toxicosis experiment** ($M \pm SD$, vivarium of the Federal Scientific Center VNITIP RAS, 2020)

Микотоксин	Group (n = 5)			
	I (control without T-2 toxin)	II (T-2 toxin 100 μg/kg feed)	III (T-2 toxin 200 μg/kg feed)	IV (T-2 toxin 400 μg/kg feed)
Duodenal chyme				
T-2 toxin	0.5±0.13	20.2±2.75*	49.6±6.00*	41.9±6.36*
HT-2 toxin	30.0±4.90	47.9±1.60*	57.9±3.20*	53.9±1.45*
Feces				
T-2 toxin	2.5±0.26	2.3±0.40	4.8±0.68*	19.2±6.20*
HT-2 toxin	40.6±8.40	44.6±3.26	53.9±3.60	38.5±5.67

Note. Measured concentrations of toxins in the original non-contaminated feed were 4.3±0.13 μg/kg for T-2, 5.6±0.47 μg/kg for HT-2. Chyme and feces were sampled from each broiler daily throughout the 14-day experiment and combined into weekly samples (one for 40 days of age, one for 47 days of age; the experiment began on Day 34 of age). Weekly samples were further merged into combined lyophilized samples for each group. Analyte concentrations were determined by tandem high-performance liquid chromatography and mass spectrometry (HPLC-MS), each test being run thrice on an Agilent 1260 Infinity system (Agilent Technologies, Germany) and an AB SCIEX Triple Quad™ 5500 mass spectrometer (Applied Biosystems, USA). Means (M) for weekly samples are reported on a group-by-group basis.

* Difference from the control group is statistically significant at $p < 0.05$.

T-2 and HT-2 testing of feed, duodenal chyme, and feces of the fistulated controls (Table 1) showed that prior to contamination, T-2 and HT-2 toxins were present in the feeds in comparable amounts (4.3±0.19 and 5.6±0.47 μg/kg, respectively). Chicks on a non-contaminated diet had these toxins in the chyme and in feces in other ratios. On average, the control group's feces contained 5 times more T-2 toxin than was found in the duodenum, $p < 0.05$, which shows that most of the toxin would be removed unaltered. However, the sixfold presence of HT-2 in the duodenum compared to T-2 concentrations indicated that the digestive canal actively metabolized T-2 toxin and converted it into a blood-absorbable metabolite. HT-2 toxin amounts in feces were the same as in the duodenal chyme except in Group 4. Raising the T-2 toxin presence in the feed to 100 μg/kg (1MPC) drastically increased 40-fold in the chyme compared to the controls; however, its concentrations in feces remained on part with the controls. This could be due to the T-2 to HT-2 conversion and further absorption in blood and tissues, as the HT-2 presence in feces did not change significantly. The dosage increase to 200 μg/kg resulted in a 2.4-fold increase in T-2 toxin in the chyme ($p < 0.05$), 2.1-fold increase in feces compared to the 1MPC group. HT-2 presence increased by 20.9% ($p < 0.05$) and

20.8% ($p > 0.05$), respectively. Therefore, the T-2 to HT-2 ratio in the intestines and feces indicates intensive absorption of HT-2 toxin into blood. When dosed at 400 μg/kg, a 7.7 times greater fecal presence of T-2 toxin was found in comparison with the controls, a sign of intestinal adaptation towards more intensive removal of the toxin, probably by faster chyme movement in the caudal direction. Clinical manifestations included digestive disorders and enteritis with signs of diarrhea.

The authors' earlier experiment showed high mycotoxin doses to negatively affect the activity of digestive enzymes [37]. To complement these findings, the authors further studied the activity of digestive enzymes in lyophilized duodenal contents and in feces for this research, see Table 2. T-2 contamination of the feed affected the proteolytic activity of the duodenal chyme: it rose by 78.9% in Group 2 ($p < 0.05$), by 46.3% in Group 3 ($p < 0.05$), and by 96.6% in Group 4 ($p < 0.05$) compared to the preparatory-phase readings. Higher lipase activity in Groups 2 and 3 constituted a significant difference of 25.5% and 16.8%, respectively ($p < 0.05$). Amylase activity rose by 99.7% ($p < 0.05$) in Group 4. Unlike digestive enzymes, the activity of alkaline phosphatase showed a downward trend, as it dropped by 23.8% in Group 2, by 27.9% in Group 3, did not change in Group 4.

2. Activity of digestive enzymes in duodenal chyme and feces of Smena 8 broiler chicks (*Gallus gallus* L.) upon T-2 toxicosis experiment (*M*±SD, vivarium of the Federal Scientific Center VNITIP RAS, 2020)

Metric	Group (n = 5)					
	II (T-2 toxin 100 μg/kg feed)		III (T-2 toxin 200 μg/kg feed)		IV (T-2 toxin 400 μg/kg feed)	
	preparatory phase	experiment	preparatory phase	experiment	preparatory phase	experiment
Duodenal chyme						
Amylase, mg/(g·min)	6433±446.0	8100±861.2	8550±1670.0	5450±437.5	5033±314.1	10050±875.2*
Lipase, μmol/(g·min)	29.4±2.59	36.9±1.66*	25.6±0.49	29.9±1.29*	22.0±3.43	26.1±0.35
Proteases, mg/(g·min)	190±18.7	340±21.2*	229±22.8	335±27.6*	233±13.5	458±56.3*
Trypsin, μmol/(g·min)	4.92±0.364	6.86±0.354	5.27±0.581	5.84±0.196	6.47±0.589	9.33±2.045
Alkaline phosphatase, μmol/(g·min)	147.5±13.64	112.4±5.19*	134.6±1.88	97.1±7.18*	140.0±2.11	188.8±37.62
Phosphatase-protease index	29.9	16.4	25.5	16.6	21.6	20.2
Feces						
Amylase, mg/(g·min)	1413±111.0	635±67.1*	1890±275.1	810±82.0*	1145±153.1	1140±94.2
Lipase, μmol/(g·min)	1802±54.0	1946±316.0	913±6.6	880±43.0	1225±43.0	941±33.0*
Proteases, mg/(g·min)	77±10.3	182±22.1*	71±10.0	125±6.5*	68±6.3	183±4.6*
Trypsin, μmol/(g·min)	4.38±0.349	2.72±0.175	3.79±0.233	3.32±0.242	3.39±0.313	3.00±0.382
Alkaline phosphatase, μmol/(g·min)	211.6±10.08	140.8±30.98*	104.9±8.55	77.5±4.92*	82.8±4.19	143.1±14.08*
Phosphatase-protease index	48.3	51.8	31.6	23.3	24.2	47.7

Note. Measured concentrations of toxins in the original non-contaminated feed were 4.3±0.13 μg/kg for T-2, 5.6±0.47 μg/kg for HT-2. For the physiological experiment (with breakdown by groups and period), chyme and fecal samples were collected from each broiler on a daily basis before the experiment began (the preparatory phase from Day 26 to Day 33 of age), then throughout the 14-day experiment (from Day 34 to Day 47 of age); the experimental samples were combined into weekly samples (Day 40 and Day 47).
* Difference from the preparatory-phase readings is significant at $p < 0.05$.

Individual changes in the activity of proteases, amylase, trypsin, and lipase in the duodenal chyme sampled from broiler chicks were indicative of their response to different T-2 doses in the feed; data can be found on http://www.agrobiology.ru, Fig. 1. In most specimens, increased proteolytic enzyme activity was the characteristic response to the toxin in the feed.

Feces were tested biochemically (see Table 2) after lyophilic drying that preserved the digestive enzymes in their active state. Amylase activity was found to have decreased in Groups 2 and 3 by 55.1% and 57.2%, respectively, compared to the preparatory-phase readings. Lipase activity dropped by 23.2% ($p < 0.05$) at maximum dosage. Proteolytic activity in feces rose significantly with the T2 toxin presence in the feed: by 136.4%, 76.0%, and 169.1% against the preparatory-phase

readings, respectively.

Thus, protease activity in the duodenal contents was far higher than in feces: 2.5-fold difference in the preparatory phase and 1.9-fold difference during the experiment in Group 2; 3.2-fold and 2.7-fold in Group 3; 3.4-fold and 2.5-fold in Group 4. Enzymes return to the blood as the enzyme recirculation hypothesis stipulated [38, 39], or intestinal microflora destroys them; either suggestion explains why protease activity decreases significantly further down the digestive canal. Notably, mycotoxins stimulate the activity of proteases in the duodenal contents. Besides, fecal matter also shows a high proteolytic activity, a sign of digestive disorders, as elevated intestinal motility removes excess enzymes by defecation. Thus, should the body receive a small dose of the toxin in the feed, its initial adaptation consists in increased proteolytic activity of the intestinal enzymes.

Given that intestinal protease activity correlates with the enzymatic activity of the blood, clinical diagnosis of T-2 toxicosis could measure the change in the trypsin activity in broiler plasma in addition to testing fecal proteolytic activity, see Table 3.

3. Biochemical blood metrics of Smena 8 broiler chicks (*Gallus gallus* L.) upon T-2 toxicosis experiment (*M*±SD, vivarium of the Federal Scientific Center VNITIP RAS, 2020)

Metric	Group (n = 5)			
	I (control without T-2 toxin)	II (T-2 toxin 100 μg/kg feed)	III (T-2 toxin 200 μg/kg feed)	IV (T-2 toxin 400 μg/kg feed)
Trypsin, U/l	93.3±4.36 77.5±6.43	81.9±1.58* 68.6±3.02	82.5±1.12 60.4±3.89*	72.6±0.31* 66.3±7.65
Alkaline phosphatase, U/l	4179±456.1 1298±192.5	4310±609.7 754±37.7	4370±476.2 1304±118.8	6369±757.8* 6487±570.5*
Phosphatase-protease index	45 17	53 11	53 21	87 98
Total protein, g/l	40.1±0.71 36.3±1.20	40.3±1.23 36.4±0.13	40.4±0.19 35.5±0.21	41.4±0.16 40.1±0.85*
Glucose, mmol/l	12.4±0.24 10.4±0.32**	11.3±0.33 9.8±0.16**	12.9±0.07 10.0±0.16**	13.0±0.21 9.0±0.31*. **
Triglycerides, mmol/l	0.3±0.01 0.8±0.04**	0.3±0.02 0.4±0.02	0.4±0.02 0.4±0.15	0.4±0.28 0.2±0.01
Cholesterol, mmol/l	3.4±0.04 2.8±0.14	3.1±0.05* 3.1±0.60	3.6±0.25 3.1±0.26	3.6±0.21 2.8±0.04**

N o t e. Boilers aged 40 days above the line, 47 days below the line.
* Difference from the Group I (control) is statistically significant at $p < 0.05$.
** Difference between 47-day old and 40-day old chickens is statistically significant at $p < 0.05$.

Biochemical blood tests showed experimental T-2 toxicosis to suppress trypsin activity at higher T-2 doses; the effect was most noticeable at 4MPC, see Table 3. Alkaline phosphatase had the opposite dynamics: at 4MPC, its activity pentupled over two weeks, and PPI rose from an average of 31 to 92, a sign of decreased secretory function of the pancreas, lower availability of nutrients, and higher toxic load on the liver [40]. At 4MPC, total blood protein rose by 13.2% but glucose dropped by 13.5% and triglycerides decreased fourfold, a sign of stress response to T-2 toxin intake.

Further morphological test results were indicative of the hematological abnormalities attributable to experimental mycotoxicosis, see Table 4. The leukocyte count decreased by 15.8% on Day 40, 16.6% on Day 47 at 2MPC. However, this was still within the normal range, so it would be too early to diagnose immunity deficiency. WBC differential showed higher lymphocyte counts compared to pseudo-eosinophil counts at 2MPC and 4MPC, which is attributable to specific defenses against infectious and non-infectious pathologies. The eosinophil percentage was lower at 2MPC and higher in other groups compared to the controls. The monocyte percentage was reduced to a third at

1MPC, then stabilized at the same level. At 2MPC, monocyte counts dropped more significantly (6-fold and 2-fold, respectively) compared to the controls. The maximum toxin dosage (4MPC) multiplied monocyte counts by 1.5 on Day 40 compared to the controls. The basophil percentage showed downward trends in Day 47 samples in Groups 2 and 3 (the proportions were halved compared to the controls).

4. Blood leukocytes and WBC differential of Smena 8 broiler chicks (*Gallus gallus* L.) upon T-2 toxicosis experiment (M±SD, vivarium of the Federal Scientific Center VNITIP RAS, 2020)

Metric	Group (n = 5)			
	I (control without T-2 toxin)	II (T-2 toxin 100 μg/kg feed)	III (T-2 toxin 200 μg/kg feed)	IV (T-2 toxin 400 μg/kg feed)
Leukocytes, 10^9/l	32,2±0,90	27,1±0,61*	29,6±0,70	32,7±1,65
	30,8±1,25	25,7±0,23*	28,1±0,90	28,6±2,88
Pseudo-eosinophils, %	44,8±1,49	33,9±1,95*	36,0±1,23*	47,8±3,05
	41,1±2,46	34,2±0,98	45,2±6,24	36,6±1,70
Lymphocytes, %	48,8±1,70	62,3±2,24*	58,9±1,73*	41,0±4,02
	52,4±2,22	59,9±0,41*	47,7±7,56	55,2±0,29
Monocytes, %	0,6±0,08	0,2±0,03*	0,1±0,05*	0,9±0,05*
	0,4±0,07	0,3±0,10	0,2±0,06	0,5±0,09
Eosinophils, %	5,5±0,42	3,1±0,28*	4,7±0,48	9,7±1,05*
	5,7±0,75	5,3±0,50	6,6±1,24	7,2±1,36
Basophils, %	0,4±0,04	0,4±0,05	0,2±0,01*	0,4±0,03
	0,4±0,06	0,2±0,03*	0,2±0,03*	0,3±0,03

N o t e. Boilers aged 40 days above the line, 47 days below the line.
* Difference from the Group I (control) is statistically significant at $p < 0.05$.

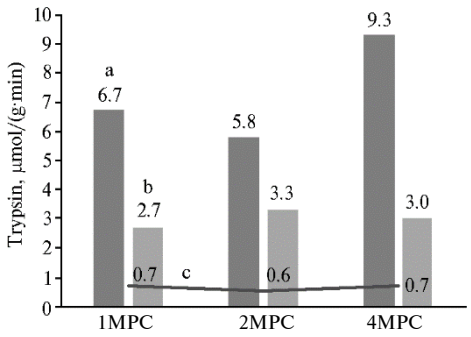

Fig. 2. Trypsin activity in various biological materials of Smena 8 broiler chicks (*Gallus gallus* L.) upon T-2 toxicosis experiment (average values throughout the experiment per doses): a — duodenal chyme, b — feces, c — blood; 1MPC — 100 μg/kg feed, 2MPC — 200 μg/kg feed, 4MPC — 400 μg/kg feed (vivarium of the Federal Scientific Center VNITIP RAS, 2020).

T2 toxin actively converted into a different form as indicated by the above-noted increase in HT-2 amounts in chyme and feces sampled from broiler chicks fed with 2MPC of T-2 toxin, see Table 1. Duodenal chyme contained 2.5 times T-2 and 1.2 times HT-2 amounts found in Group 1 (1MPC). At the same time, the activity of trypsin, which is involved in both protein digestion and metabolism regulation [35], decreased in the duodenum and increased in feces whilst not changing significantly in blood, see Fig. 2. This indicates a protective response of the digestive glands to the toxin, which manifests as increased secretion of pancreatic and intestinal juices to preserve enteral homeostasis when the body is sustaining a large fluid loss. As intestinal chyme moves faster due to adaptation to the toxin, it disrupts enzyme recirculation, and some of the enzymes are excreted with feces [36]. At 4MPC, the T-2 toxin presence was reduced 2.6-fold in the duodenal chyme and increased by a factor of 8.4 in feces. Intestinal adaptation to 4MPC of T-2 differed significantly from adaptation to lower doses: trypsin activity in the duodenal chyme was rising whereas the fecal presence of the enzyme was virtually constant, see Fig. 2.

Co-analysis of T-2 to HT-2 metabolization (HT-2 and T-2 ratio) and chyme trypsin activity revealed an inverse relationship between the two processes, which was more apparent at 4MPC, see Fig. 3. Thus, our research

revealed an inverse relationship between blood PPI and the quantitative ratio of HT-2 to T-2 in the duodenal chyme at 4MPC, which could be useful for diagnosing T-2 mycotoxicosis, see Fig. 3.

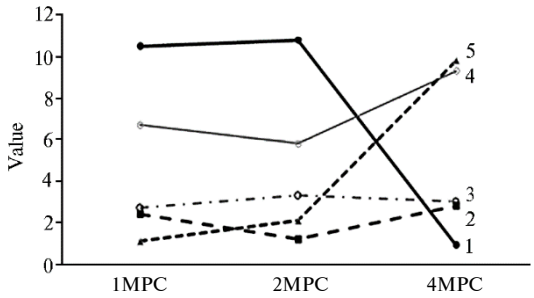

Fig. 3. The HT-2 toxin/T-2 toxin concentration ratio in duodenal chyme (1) **and feces** (2)**, activity of trypsin** (U/ml) **in feces** (3) **and duodenal chyme** (4)**, and phosphatase-protease index** (5) **of Smena 8 broiler chicks (*Gallus gallus* L.) upon T-2 toxicosis experiment:** 1MPC — 100 μg/kg feed, 2MPC — 200 μg/kg feed, 4MPC — 400 μg/kg feed (vivarium of the Federal Scientific Center VNITIP RAS, 2020).

Thus, experimental T-2 toxicosis showed T-2 toxin to convert into HT-2 toxin in the digestive canal of broilers; HT-2 absorbs intensively into the blood and seemingly ends up in tissues, as it is far less present in feces than in the duodenum (by 28.7%). T-2 poisoning of feed (1MPC = 100 μg/kg, 2MPC = 200 μg/kg, 4MPC = 400 μg/kg) increased the proteolytic activity of the duodenal chyme by 78.9% ($p < 0.05$), 46.3% ($p < 0.05$), and 96.6% ($p < 0.05$), respectively; that is, the greatest effect was observed at 4MPC. That concentration also yielded maximum amylase activity (twofold). An increase in lipase activity (less substantial but still significant at 25.5% and 16.8%, $p < 0.05$) was observed at 1MPC and 2MPC dosage. On the contrary, alkaline phosphatase activity either tended to decrease (1MPC and 2MPC) or did not change. Trypsin activity in the blood was 12.2–22.2% lower (Groups 2, 4) on Day 40 of age, 22.1% lower (Group 3) on Day 47, whereas alkaline phosphatase activity was pentupled over the two weeks of the 4MPC diet. The PPI increase indicated a higher toxic load on the liver. Leukocyte counts were 16% lower on average at 1MPC. WBC differential showed a higher percentage of lymphocytes at 1MPC and 2MPC in contrast to pseudoeosinophils, which is attributable to specific antitoxin defenses of the body. The PPI of blood was found to be inversely related to the quantitative ratio of HT-2 and T-2 in the chyme at 4MPC T-2 presence in the feed. PPI was found to increase at high doses of T-2 in the feed. Thus, PPI testing could be used in the diagnosis of T-2 toxicosis in poultry.

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