AGRICULTURAL BIOLOGY,
2021, V. 56, Iss. 4, pp. 664-681
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

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

Mycotoxicoses

UDC 636.52/.58:619:616-099:575

doi: 10.15389/agrobiology.2021.4.664eng
doi: 10.15389/agrobiology.2021.4.664rus

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.)

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The authors declare no conflict of interests
Acknowledgements:
Supported financially from the Russian Science Foundation, grant No. 20-76-10003
Received April 22, 2021

Abstract

A significant proportion of poultry feed is contaminated with T-2 toxin. The bird's immune system is one of the targets of this xenobiotic. However, the results of studying the effect of T-2 toxin on the expression of immunity genes in birds are extremely limited. In the present study, we have shown that contamination of broiler feed with T-2 toxin affects the level of expression of genes associated with the functioning of the immune system in the cecum and pancreas. The aim of the work was to assess the effect of T-2 toxin on the level of expression of genes involved in the immune system responses in the tissues of the intestine and pancreas cecum in broilers. The feeding trials with T-2 toxin added to the feed were carried out on broilers of the Smena 8 cross from 30 to 50 days of age (the vivarium of the Federal Research Center VNITIP RAS, 2020). Broilers were assigned to four treatments. The control group I received a diet with no T-2 toxin added, group II received a diet added with 100 μg/kg T-2 toxin, group III with 200 μg/kg, and group IV with 400 μg/kg. Gene expression was analyzed by quantitative PCR with reverse transcription (RT-qPCR). A reverse transcription reaction was performed to generate cDNA on an RNA template using the iScript™ Reverse Transcription Supermix (Bio-Rad, USA). The following primer pairs were used: for Interleukin 6 (*IL6*) F — 5′-AGGACGAGATGTGCAAGAAGTTC-3′, R — 5′-TTG-GGCAGGTTGAGGTTGTT-3′; for Interleukin 8 (*IL8*) F — 5′-GGAAGAGAGGTGTGCTTGGA-3′, R — 5′-TAAC-ATGAGGCACCGA-TGTG-3′; for Interferon 7 (*IRF7*) F — 5′-ATCCCTTGGAAGCACAACGCC-3′, R — 5′-CTGA-GGCAACCGCGTAGACCTT-3′; for Prostaglandin-endoperoxide synthase 2 (*PTGS2)* F — 5′-TC-GAGATCACACTTGATTGACA-3′, R — 5′-TTTGTGCCTTGTGGGTCAG-3′; for avian beta-defensin 9 (*AvBD9*(*Gal9*)) F — 5′-AACACCGTCAGGCATCTTCACA-3′, R — 5′-CGTCTTCTT-GGCTGTAAGCTGGA-3′, for avian beta-defensin 10 (*AvBD10*(*Gal10*)) F — 5′-GCTCTTCGCT-GTTCTCC-TCT-3′, R — 5′-CCAGAGATGGTGAAGGTG-3′; for Caspase 6 (*Casp6*) F — 5′-CAG-AGGAGACAAGTGCCAGA-3′, R — 5′-CCAGGAGCCGTTTACAGTTT-3′. The beta-actin protein gene was a reference control. Amplification reactions were performed using a SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, USA). The amplification mode and conditions corresponded to those proposed by the primer developers. The relative expression level was estimated by the $2^{-\Delta\Delta CT}$ method. Biochemical blood profiles of broilers were analyzed (a Sinnowa BS3000P semi-automatic biochemical analyzer, SINNOWA Medical Science & Technology Co., Ltd, China) with a set of

veterinary diagnostic reagents (DIAKON-VET, Russia). Principal component analysis (PCA) was used to compare gene expression levels and blood biochemical parameters. The expression of genes associated with the inflammatory response, apoptosis, antimicrobial and antiviral protection was evaluated. Activation ($p \leq 0.05$) of the expression of the pro-inflammatory genes *IL6* and *PTGS2* occurred in broilers fed T-2 toxin. This can adversely affect the health and productivity of the birds, since the overproduction of proinflammatory cytokines is involved in the pathogenesis of several diseases. An increase (up to 41.7-fold, $p = 0.0005$) in the *PTGS2* gene expression in the pancreas was characteristic of all groups fed T-2 toxin compared to the control. In the tissues of the intestinal cecum, there was a decrease (up to 12.5-fold, $p = 0.02$) in the expression level of the *Casp6* gene of the apoptosis factor regardless of the T-2 toxin dosage. In the pancreas, there was a reverse tendency of a sharp increase in the *Casp6* gene expression as the T-2 toxin concentration increased ($p \leq 0.0008$). In group II, the expression increased 22.4 times ($p = 0.0008$), in group III 715.8 times ($p = 0.0003$), in group IV 31288.3 times ($p = 0.0003$) compared to the control. The expression of *AvBD9* and *AvBD10* genes of avian β-defensins which are associated with a higher bacteriostatic activity against many pathogens decreased 2.1 to 5.3 times ($p \leq 0.05$) in the caecum of broilers fed 200 and 400 200 μg/kg T-2 toxin. In the pancreas, regardless of the T-2 toxin dosage, on the contrary, the expression of these genes significantly increased ($p \leq 0.04$). In the caecum, 100 μg/kg T-2 toxin exposure inhibited the *IRF7* gene expression 3-fold ($p = 0.03$) compared to the control. This can negative affect birds' health, since the *IRF7* gene of the interferon regulatory factor 7 participates in counteraction against many viruses. In general, the pancreas was found to be more sensitive to the effects of the T-2 toxin because the expression of almost all studied genes was significantly increased as compared to that in the cecum tissue. This difference in the immune response may be due to the functional divergence between the intestine and the pancreas. PCA method revealed a close relationship between the expression of the *PTGS2* gene in the pancreas, the *IL6*, *PTGS2*, *IL8*, *IRF7*, *AvBD9*, *AvBD10*, and *Casp6* genes in the cecum and the total blood protein, trypsin, glucose, alkaline phosphatase, triglycerides, and phosphatase/trypsin coefficient. Our findings indicate the effect of feed contamination with T-2 toxin on the immunological functions of the caecum and pancreas of broilers through modulation of the immunity genes expression. Quantitative PCR analysis of the expression of immunity genes can serve as an effective tool for the search for predictive markers of T-2 toxicosis of poultry to monitor the health status of livestock in poultry farms.

Keywords: T-2 toxin, mycotoxicosis, broilers, gene expression, bird immunity, cytokine, interferon, apoptosis, β-defensins

A significant part of poultry feed, primarily feed grains, is contaminated with mycotoxins – by-products of microscopic fungi. Among them, T-2 toxin has been acknowledged as one of the most toxic [1]. This compound belongs to the sesquiterpenoid trichothecene mycotoxins produced by molds of the genera *Fusarium*, *Myrothecium*, *Cephalosporium*, *Verticimonosporium*, and *Stachybotrys*. Micromycetes of *Fusarium* spp. are the main producers of T-2 toxin and the most common pathogens of agricultural crops in the temperate climate of Europe, Asia, and North America [2]. In Russia, a wide prevalence of T-2 toxin has been demonstrated in feed raw materials (grain feed, cake, meal) and in complete feed for poultry [3, 4]; data have been obtained on the high frequency of T-2 toxin contamination of sunflower meal and sunflower meal [5].

The toxicity of *Fusarium* mycotoxins is based on the ability to bind to eukaryotic ribosomes and inhibit protein synthesis, as well as the ability to generate free radicals [6]. As a result of the effect of T-2 toxin on animals, a change in metabolism in the tissues of the spleen, thymus, stomach, and liver was noted [7].

In poultry, when the feed is contaminated with T-2 toxin, feed refusal, deterioration of its conversion rates, bloody diarrhea, a decrease in live weight gain, egg production, and thinning of the eggshell are noted [8]. The action of mycotoxins is most susceptible to the epithelial surface of the digestive tract [9, 10], which manifests itself in ulcerative necrotic inflammation. In other animals, when exposed to T-2 toxin, acinar degeneration and necrosis in the pancreas were also noted [11].

A number of studies [12, 13] have confirmed the hypothesis that the immune system is one of the targets of mycotoxins. Trichothecene mycotoxins act in different directions and with unequal intensity on different links of the immune system, showing both immunostimulating and immunosuppressive

properties depending on the dose and frequency of exposure. The digestive tract is characterized by the function of the regional immune system [14, 15]. The tissues of the mucous membrane lining the gastrointestinal tract contain lymphoid structures, which represent the first line of defense against pathogens and xenobiotics entering the body [15]. In birds, lymphoid tissues in the gastrointestinal tract are well developed and consist of lymphoid cells proper, as well as specialized lymphoid structures – Peyer's patches [16]. These structures play an important role in the induction of immune responses [17]. Epithelial cells of the mucous membrane of the avian digestive system are equipped with TLR receptors associated with the induction of the synthesis of chemokines, cytokines, lysozymes, β-defensins, cathelicidins, and avidin [18–20]. In chickens, the proinflammatory cytokines interleukin-1β (IL1B), interleukin-6 (IL6), interleukin-17 (IL17A), interleukin-22 (IL22) have been described [21]. Among the main groups of antimicrobial defensin peptides in chickens, β-defensins (previously known as gallinacins) AvBD1 (Gal1, Gal1), AvBD2 (Gal2), AvBD4 (Gal4), AvBD10 (Gal10) were identified [22–24]. Due to the conformation, avian β-defensins exhibit more pronounced efficacy against gram-positive bacteria [25]. The enzymes of the caspase group play a key role in apoptosis. In addition to apoptosis, these enzymes are involved in modulating inflammatory responses [26].

Thus, poisoning with mycotoxins, in particular with T-2 toxin, can be accompanied by complications associated with a violation of the biological mechanisms of resistance to infections and other immune functions. The volume of studies of such changes is growing [1, 27]; however, information on the effect of the T-2 toxin on the expression of genes associated with immunity in birds remains extremely limited. The accumulation of these data will allow understanding the patterns of disorders of immune homeostasis, which is necessary for the search for effective immunostimulants or immunosuppressants for the prevention and treatment of post-intoxication infectious complications and diseases and the identification of biomarkers of T-2 toxicosis.

This work has shown for the first time that the expression of some genes associated with immunity serves as an early prognostic marker of T-2 toxicosis in broilers. This concerns the activation in the tissues of the pancreas of the genes of regulatory molecules that provide the initial stages of the development of the inflammatory response, in particular *IL6* and *PTGS2*, as well as genes associated with cell death, for example, *Casp6*. In addition, the activation of genes for antimicrobial factors, primarily *AvBD10*, can be a marker of the pathological process.

The aim of the study was to assess the effect of 20-day exposure to T-2 toxin when introduced with feed on the expression of genes associated with inflammatory response, apoptosis, antimicrobial and antiviral protection.

Materials and methods. Experiments were performed on broiler chickens (*Gallus gallus* L.) of the Smena 8 cross from 30 to 50 days of age (the vivarium of the Federal Research Center VNITIP RAS, 2020) in accordance with the European Convention for the protection of vertebrate animals used for experiments or other scientific purposes (ETS No. 123, Strasburg, 1986). The conditions of feeding and keeping corresponded to the requirements for the cross ("Methodology for conducting scientific and industrial research on feeding poultry. Molecular genetic methods for determining the intestinal microflora". Sergiev Posad, 2013).

The basis of the poultry ration was the PK-6 compound feed (Russia). The feed was mechanically contaminated with standard T-2 toxin (powder with a mass fraction of the main substance of 99.7 ± 0.3%, Romer Labs, Austria, cat. No. 10000310, LOT # S17052T) to the set MPC in compliance with personnel safety requirements. The amount of mycotoxins in the feed before contamination (background content) and after contamination was monitored by tandem high-

performance liquid chromatography-mass spectrometry (HPLC-MS/MS); a chromatographic system Agilent 1260 Infinity LC, (Agilent Technologies, USA), a mass spectrometer ABSCIEX TripleQuad™ 5500 (Sciex, USA), a column for reverse-phase separation Gemini® C18 110A 5 μm 150×4.6 mm (Phenomenex, USA). Chromatographic separation was performed in the binary gradient elution mode. When preparing mobile phases A and B and carrying out HPLC-MS/MS, the researchers were guided by GOST 34140-2017 "Food products, feed, food raw materials. Method for the determination of mycotoxins using high-performance liquid chromatography with mass spectrometric detection" (Moscow, 2020). Separation mode: 1.5 min – 100% eluent A, 1.5 min – a linear increase in the proportion of eluent B to 50%, 9 min – a linear increase in the proportion of eluent B to 100%, 5 min – chromatographic separation at 100% eluent B; column temperature 25 °C, mobile phase flow rate 1 cm^3/min, sample injection temperature 10 °C, sample volume 5 mm^3. The retention time for T-2 toxin is 10.30 min, for NT-2 toxin – 9.79 min. The detection limits are 3.25 rg/kg for the T-2 toxin and 2.70 rg/kg for the HT-2 toxin, the quantitative limits for the T-2 toxin are 5.23–129.20 rg/kg, for the HT-2 toxins – 3.50–129.20 rg/kg. The calculated ion (in the mode of positive ionization by spraying in an electric field) for T-2 toxin is 305.1 m/z, for NT-2 toxin – 345.1 m/z. Standard solutions of T-2 and HT-2-toxin of the Biopure series (Romer Labs, Austria) were used to construct calibration graphs and as internal standards.

The chickens were divided into four groups (5 animals in each): group I received a diet without the introduction of T-2 toxin (control), II — a diet with the addition of T-2 toxin in an amount of 100 rg/kg (1MPC), III — 200 rg/kg (2MPC), IV — 400 rg/kg (4MPC).

To analyze gene expression in broilers, at the end of the experiment, tissues of the caecum and pancreas were taken. IntactRNA fixative was used to stabilize RNA in biological samples (Evrogen, Russia). The tissues were homogenized in liquid nitrogen. To isolate total RNA, the Aurum™ Total RNA mini-kit (Bio-Rad Laboratories, Inc., USA) was used following the manufacturer's instructions. The reverse transcription reaction was performed using an iScript™ Reverse Transcription Supermix (Bio-Rad Laboratories, Inc., USA) [28].

Seven genes were selected for expression analysis. Specific primer pairs: for *IL6* F — 5′-AGGACGAGATGTGCAAGAAGTTC-3′, R — 5′-TTGGGCAGGTTGAGGTTGTT-3′; for *IL8* F – 5′-GGAAGAGAG-GTGTGCTTGGA-3′, R – 5′-TAACATGAGGCACCGATGTG-3′; for *IRF7* F – 5′-ATCCCTTGGAAGCACAACGCC-3′, R – 5′-CTGAGGCAACCGCG-TAGACCTT-3′; for *PTGS2* F – 5′-TCGAGATCACACTTGATTGACA-3′; R – 5′-TTTGTGCCTTGTGGGTCAG-3′; for *AvBD9 (Gal9)* F – 5′-AACACCGTCAGGCATCTTCACA-3′, R – 5′-CGTCTTCTTGGCTGTAAGCTGGA-3′; for *AvBD10 (Gal10)* F – 5′-GCTCTTCGCTGTTCTCCTCT-3′, R – 5′-CCAGAGATGGTGAAGGTG-3′, for *Casp6* F – 5′-CAGAGGAGACAAGTGCCAGA-3′, R – 5′-CCAGGAGCCGTTTACAGTTT-3′. The housekeeping gene *ACTB* (beta-actin protein) was used as a reference control with primers F – 5′-ATTGTCCACCGCAAATGCTTC-3′, R – 5′-AAATAAA-GCCATGCCAATCTCGTC-3′.

PCR was performed using a DT Lite-4 detecting amplifier (NPO DNA-Tekhnologiya, Russia) and a SsoAdvanced™ Universal SYBR® Green Supermix kit (Bio-Rad Laboratories, Inc., USA) in accordance with the manufacturer's protocol [29]. The amplification mode and conditions corresponded to each primer [30]. The relative expression level was assessed by the $2^{-\Delta\Delta CT}$ method [31].

Blood for biochemical analysis (2-3 ml) was taken at the end of the experiment after 14-hour fasting of birds from the axillary vein on the inner side

of the wing above the ulnar articulation ("General and Special Methods for Studying the Blood of Birds of Industrial Crosses". Yekaterinburg—St. Petersburg, 2009). Sodium citrate was an anticoagulant. The studies were performed on a Sinnowa BS3000P semi-automatic biochemical analyzer (SINNOWA Medical Science & Technology Co., Ltd, China) with a set of veterinary diagnostic reagents (DIAKON-VET, Russia).

To compare the levels of gene expression and blood biochemical parameters, the principal component analysis (PCA) was used with the construction of a correlation matrix. The number of significant principal components was determined on the basis of an eigenvalue criterion equal to 1, with the preservation of any component with an eigenvalue > 1 [32]. The quality of the presentation of the variables on the factorial map and the overall contribution to the principal components of individual traits were assessed based on the calculation of cos^2. Cos^2 visualization for variables in all dimensions was performed using the corrplot package (https://cran.r-project.org/web/packages/corrplot/corr-plot.pdf; https://github.com/taiyun/corrplot).

Mathematical and statistical processing of the results was carried out by multifactor ANOVA in Microsoft Excel XP/2003, R-Studio (Version 1.1.453) (https://rstudio.com). Mean values (*M*) and standard errors of means (±SEM) are shown. Mean values were compared using Tukey's significant difference test using the TukeyHSD function in the R Stats Package (https://cran.r-project.org/web/packages/TukeyC/Tu-keyC.pdf, https://github.com/jcfaria/TukeyC); differences were considered statistically significant at $p \leqslant 0.05$.

Results. Feed consumption by broilers averaged 150 g/day, that is, poultry of groups II-IV received daily toxin with T-2 feed in an amount of 15, 30, and 60 rg/bird, respectively.

Aflatoxins (B_1, G_1), fumonisins (B_1, B_2, B_3), and deoxynivalenol (DON) were not found in the feed used for contamination. The T-2 toxin was present in a background amount of 4.33 μg/kg, which could not significantly affect the results of the experiment, given that the MPC for T-2 toxin is 100 μg/kg. Ochratoxin A (2.27 μg/kg at MPC 50 μg/kg) and zearalenone (2.25 rg/kg at MPC 1000 μg/kg) were also detected in quantities that were much lower than the MPC.

Fig. 1 shows the effect of T-2 toxin on the expression of genes for interleukins *IL6*, *IL8*, as well as prostaglandin endoperoxide synthase *PTGS2*, which are associated with proinflammatory factors. T-2 toxin at a concentration of 100 μg/kg (1MPC) did not affect the expression of the *IL6, IL8,* and *PTGS2* genes in the caecum in group II of broilers. However, in group III (2MPC of T-2 toxin), the expression of the *PTGS2* gene increased by 3.0 times ($p = 0.03$), and in group IV (4MPC of T-2 toxin), the effect was even more pronounced: the level of expression of *PTGS2* increased by 5.9 times ($p = 0.001$); in addition, the level of expression of the *IL6* gene increased in comparison with the control ($p = 0.005$). In the tissues of the pancreas, the effect of exposure to T-2 toxin was more pronounced than in the tissues of the cecum of the intestine. For example, an increase in the expression of the *PTGS2* gene (up to 41.7-fold, $p = 0.0005$) was observed in all experimental groups compared with the control. T-2 toxin had a specific effect on the expression of proinflammatory genes in broilers, since no effect on the *IL8* gene was revealed (in contrast to the *IL6* and *PTGS2* genes) ($p > 0.05$). In general, an obvious dose-dependent effect of T-2 toxin occurred on the expression of proinflammatory genes in the tissues of the cecum of broilers. Previously, a similar effect with respect to *IL6* when exposed to toxic substances in animals was demonstrated by Brown et al. [33].

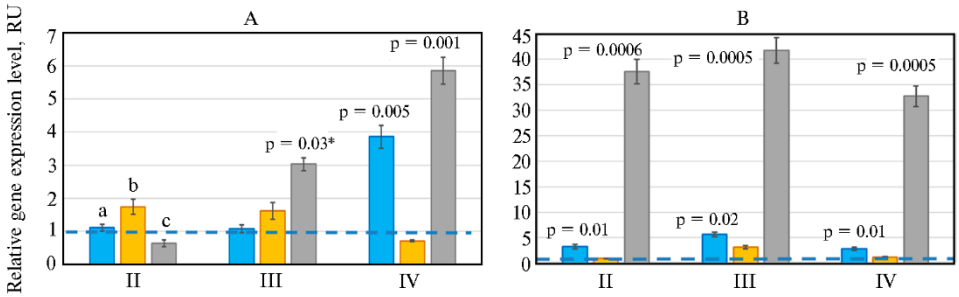

Fig. 1. The expression of genes associated with proinflammatory factors in the tissues of the epithelium of the cecal processes of the intestine (A) **and pancreas** (B) **in Smena 8 cross broiler chickens (*Gallus gallus* L.) under experimental T-2 toxicosis:** II, III, IV — the groups receiving 1MPC, 2MPC and 4MPC of T-2 toxin, respectively (1MPC — 100 μg/kg feed); a, b, c — genes *IL6*, *IL8* and *PTGS2*, respectively. Relative units are the frequency of changes in expression compared to group I (control), where the expression level was taken as 1. The dashed blue line shows the expression level in the control. The results are shown as *M*±SEM (the vivarium of the Federal Research Center VNITIP RAS, 2020).

* Differences with control are statistically significant at $p \leq 0.05$.

The activation of the expression of proinflammatory genes when fed with T-2 toxin may have adverse effects on the health and productivity of broilers. On the one hand, interleukins are important for innate protective immune responses, attracting additional leukocytes to the site of infection, which increases the resistance of epithelial cells [34, 35]. On the other hand, the overproduction of proinflammatory cytokines is involved in the pathogenesis of a number of diseases in animals and humans [36], and is also associated with a decrease in the productivity of farm animals [37]. It has been shown [38-40] that the administration of drugs based on cytokines to healthy animals provoked negative reactions of the organism. The activation of inflammatory cytokines is closely related to the expression of the *PTGS2* gene, since cytokines are able to induce this gene [41]. *PTGS2* is a gene for prostaglandin endoperoxide synthase (cyclooxygenase 2), which is involved in the oxidative conversion of arachidonic acid to prostaglandin, which in these reactions is also metabolized to biologically active prostacyclin and thromboxane A2. Prostaglandin, prostacyclin, and thromboxane A2 are involved in both local and systemic inflammatory reactions [42].

The obtained data indicating the activation of the expression of proinflammatory genes when feeding the T-2 toxin to broilers is consistent with the results of previous studies. It has been shown [43-45] that under the influence of toxic substances in animals, the production of pro-inflammatory factors, in particular, the cytokines *IL4*, *IL10,* and *IL13*, increases. It was noted [46] that exposure to trichothecene mycotoxins was capable of transcriptionally and post-transcriptionally enhancing the expression of genes associated with the inflammatory response.

Fig. 2 shows the level of expression of the *Casp6* gene associated with the apoptosis factor in the epithelial tissues of the digestive system of broilers in response to feeding with T-2 toxin. A decrease (up to 12.5-fold, $p = 0.02$) in the expression of the *Casp6* gene was noted in the tissues of the intestinal cecum in all groups compared with the control (Fig. 2). A dose-dependent effect was revealed: an increase in the concentration of the toxin in the food led to a more pronounced inhibition of expression. The opposite trend was observed in the tissues of the pancreas. With an increase in the concentration of T-2 toxin in feed, the expression of the *Casp6* gene sharply increased ($p \leq 0.0008$): in group II — by 22.4 times ($p = 0.0008$), in group III — by 715.8 times ($p = 0.0003$), in group IV — 31,288.3 times ($p = 0.0003$) compared to the control.

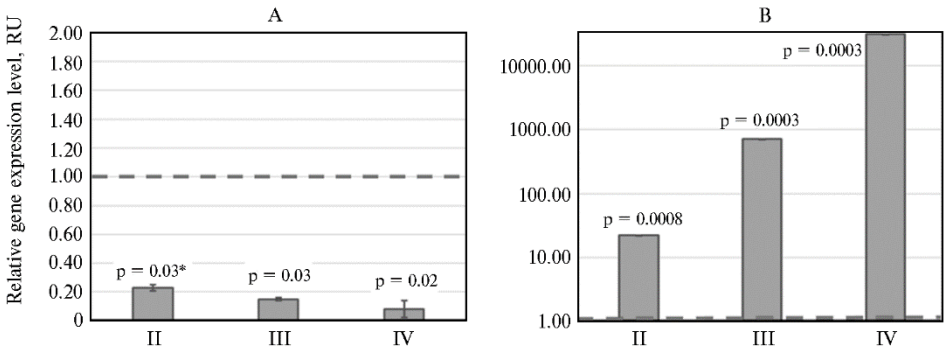

Fig. 2. The expression level of the *Casp6* gene associated with the apoptosis factor in the tissues of the epithelium of the cecal processes of the intestine (A) **and pancreas** (B) **in Smena 8 cross broiler chickens (*Gallus gallus* L.) under experimental T-2 toxicosis:** II, III, IV — the groups receiving 1MPC, 2MPC and 4MPC of T-2 toxin, respectively (1MPC — 100 μg/kg feed). RU is the multiplicity of changes in expression compared to group I (control), where the expression level is taken as 1. The dashed line shows the expression level in the control. The results are shown as *M*±SEM (vivarium of the Federal Scientific Center VNITIP RAS, 2020).

* Differences with control are statistically significant at $p \leq 0.05$.

Casp6 encodes the caspase 6 protein, which belongs to the family of cysteine-aspartate-specific proteases, the activation of which plays a central role in cell apoptosis [47]. On the one hand, apoptosis (genetically programmed cell death) is the most important mechanism of immunoregulation from the moment of maturation and differentiation of immunocompetent cells to the stage of implementation of the mechanisms of innate and adaptive immunity [48, 49]. On the other hand, in the tissues of the digestive system, toxin-induced apoptosis has negative consequences, since it is associated with damage to mitochondria and the release of cytochrome C from them (mitochondria-mediated apoptosis pathway) [50-52]. In addition, toxic substances can have a cytotoxic effect on macrophages and monocytes [52-54]. The activation of members of the caspase family is capable of inducing DNA damage in macrophages through the cleavage of the enzyme poly [ADP-ribose] polymerase 1 (PARP-1) [52, 55]. Studies have shown that certain toxic substances initiate apoptosis of B cells, which can aggravate the state of the immune system [56].

The obtained results, demonstrating the activation of *Casp6* gene expression in the broiler pancreas under the influence of T-2 toxin, coincide with the data published earlier. The activation of several caspases under the influence of trichothecenes has been reported [57-59]. It was shown [47] that high doses of trichothecene mycotoxins provoked apoptosis of leukocytes with concomitant suppression of immunity. In vivo administration of trichothecenes to rodents, including the T-2 toxin, led to apoptosis in the thymus, spleen, and bone marrow [60, 61].

Fig. 3 shows the level of expression of the *AvBD9* and *AvBD10* genes in the caecum and pancreas of broilers when exposed to T-2 toxin. *AvBD9* (*Gal9*) and *AvBD10* (*Gal10*) are avian β-defensin genes [62]. Defensins, selectively recruiting monocytes, T-lymphocytes, immature dendritic and mast cells to the foci of infection, participate in adaptive immunity reactions [63-65]. Defensins are associated with increased bacteriostatic activity against many pathogens, including *Klebsiella typhimurium*, *Streptococcus bovis*, *Enterococcus faecalis*, *Salmonella typhimurium* [24]. In the tissues of the cecum of the intestine in broilers of groups III and IV, the level of expression of the *AvBD9* and *AvBD10* genes decreased by 2.1–5.3 times ($p \leq 0.05$) compared to the control (see Fig. 3). On the contrary, the expression of these genes (especially *AvBD10*) in the pancreas tissues of broilers

from all groups increased significantly compared to the control ($p \leq 0.04$). Thus, in group IV, a 40.8-fold activation of the expression of the *AvBD10* gene ($p = 0.0002$) was noted.

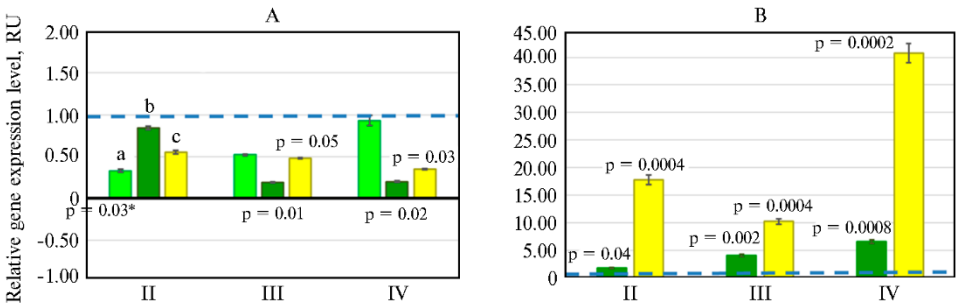

Fig. 3. The expression level of the genes associated with antimicrobial and antiviral factors in the tissues of the epithelium of the cecal processes of the intestine (A) **and pancreas** (B) **in Smena 8 cross broiler chickens (*Gallus gallus* L.) under experimental T-2 toxicosis:** II, III, IV — the groups receiving 1 MPC, 2MPC and 4MPC of T-2 toxin, respectively (1MPC — 100 μg/kg feed); a, b, c — genes *IRF7*, *AvBD9* and *AvBD10*, respectively. RU is the multiplicity of changes in expression compared to group I (control), where the expression level is taken as 1. The dashed line shows the expression level in the control. The results are shown as *M*±SEM (vivarium of the Federal Scientific Center VNITIP RAS, 2020).

* Differences with control are statistically significant at $p \leq 0.05$.

Note that the ability to both reduce and increase the resistance of animals to pathogens has been described by many researchers for various mycotoxins. Broiler chickens fed feed contaminated with ochratoxins were found to be more susceptible to Salmonella contamination [66]. In another study [67], the introduction of ochratoxin A into broiler feed did not affect the abundance of *S. typhimurium* in the caecum; however, the synergistic effect of salmonella and toxin led to an increase in poultry mortality by 13.2% and a decrease in body weight. In pigs with fumonisin B_1 contamination of feed, an increase in intestinal colonization of pathogenic *Escherichia coli* was associated [68], while feeding with T-2 toxin in the intestinal contents and other organs decreased the abundance of *Salmonella typhimurium* as compared to the control (feed without experimental contamination with T-2 toxin) [69].

In addition, in the caecum of the intestine of broilers from group II, the expression of the *IRF7* gene was inhibited by 3.0 times ($p = 0.03$) compared with the control (see Fig. 3). The *IRF7* gene encodes the regulatory factor interferon 7 (a member of the family of regulatory factors for interferon transcription) [70]. Due to its key role in host immunity, *IRF7* is involved in counteracting many viruses using various strategies [71, 72]. Pestka et al. [46] reported similar results regarding the expression of the *IRF7* gene in the tissues of the caecum of the intestine of broiler chickens with experimental T-2 toxicosis. Feeding broiler chickens with a mixture of mycotoxins, including DON, reduced IFN-γ mRNA synthesis. At the same time, the example of pigs [73] showed an increase in the production of interferon-γ upon contamination of feed with DON.

The comparison of the influence of T-2 toxin on the expression of genes associated with immunity in the cecum with the pancreas suggests that the tissues of the pancreas are more sensitive to the T-2 toxin, since the expression of almost all studied genes here was significantly enhanced compared to the tissues of the intestinal cecum. First of all, this concerns the activation in the tissues of the pancreas of the genes of regulatory molecules that provide the initial stages of the development of the inflammatory response, including *IL6* and *PTGS2*, genes associated with cell death, for example *Casp6*, and the synthesis of antimicrobial

factors, especially *AvBD10*. The authors believe that such a difference in the severity of the immune response may be due to functional differences between the intestine and the pancreas. It was shown [74] that the endocrine, as well as the exocrine, parts of the pancreas had enormous functional plasticity, which could also contribute to the enhancement of the expression of the studied genes when exposed to the T-2 toxin. This research confirms the data of Scaglia et al. [75], who described active apoptosis in the islets of Langerhans in the pancreas of 13-17-day-old rats. Autoimmune cytokines, in particular IL1β and TNFa, associated with additional insulin release, induce islet cell apoptosis [76]. Possibly, T-2 toxin may affect the synthesis of exocrine digestive enzymes by acinar cells or the synthesis of peptides by insulocytes of the islets of Langerhans of the pancreas. It was found that somatostatin, produced by delta cells of pancreatic islets, significantly affected the expression of genes involved in inflammatory reactions and leukocyte chemotaxis [77]. T-2 toxin could also cause damage to acinar cells in broilers [11] and, as a consequence, increased expression compared to the caecum. There is evidence that damage to acinar cells is accompanied by an increase in the synthesis of proinflammatory cytokines IL6, IL1β, TNFα, and IL8 [78].

In order to search for factors that influenced the expression of genes associated with immunity in experimental T-2 toxicosis of broilers, the researchers compared the data on *IL6, IL8*, *PTGS2*, *IRF7*, *AvBD9*, *AvBD10,* and *Casp6* in the cecum and pancreas with the biochemical parameters of blood (total protein, trypsin, glucose, alkaline phosphatase, cholesterol, triglycerides, AlPh/T — alkaline phosphatase/trypsin ratio) (Table), using PCA (Fig. 4).

Biochemical blood parameters in Smena 8 cross broiler chickens (*Gallus gallus* L.) under experimental T-2 toxicosis (*M*±SEM, vivarium of the Federal Scientific Center VNITIP RAS, 2020)

Parameter	Group (n = 5)			
	I (control)	II (1MPC)	III (2MPC)	IV (4MPC)
Total protein, g/l	39.5±0.83	36.8±1.94	39.9±0.17	41.5±1.31
	40.1±0.71	40.3±1.23	40.4±0.19	41.4±0.16
	36.3±1.20	36.4±0.13	35.5±0.21	40.1±0.85[b]
Trypsin, U/l	74.2±3.17	60.3±1.52	62.6±0.68	67.5±4.51
	93.3±4.36[a]	82.0±1.58[ab]	82.5±1.12[ab]	72.6±0.31[b]
	77.5±6.43	68.6±3.02	60.4±3.89	66.3±7.65
Glucose, mmol/l	12.1±0.19	11.1±0.32	11.1±0.13	10.9±0.09[b]
	12.4±0.24	11.2±0.33	12.8±0.07	13.0±0.21[a]
	10.4±0.32[a]	9.8±0.16[a]	10.0±0.16[a]	9.0±0.31[a]
Alkaline phosphatase (ALP)	6232±590.7	5456±313.1	7974±621.2	8157±296.5
(U/L)	4179±456.1[a]	4310±609.7	4370±476.2[a]	2987±757.8[ab]
	1298±192.5[a]	754±37.7[ab]	1303±118.8[a]	3695±114.7[ab]
Cholesterol, mmol/l	2.9±0.09	3.0±0.12	3.3±0.23	3.5±0.12
	3.4±0.04	3.1±0.05	3.6±0.25	3.6±0.21
	2.8±0.14	3.1±0.60	3.1±0.26	2.8±0.04
Triglycerides, mmol/l	0.4±0.01	0.3±0.01	0.3±0.02	0.4±0.02
	0.3±0.01	0.3±0.02	0.4±0.02	0.4±0.28
	0.8±0.04[a]	0.4±0.02	0.4±0.15	0.2±0.01[a]
AlPh/T (ALP/trypsin)	83.9	90.5	127.4	120.8
	44.8	52.6	52.9	41.1
	16.7	10.9	21.6	55.7

N o t e. For each parameter, the first row shows values for 33 days of age (before the start of the experiment), the second row for 40 days of age (received T-2 toxin for 7 days), and the third row for 47 days of age (received T-2 toxin for 14 days); the control group T-2 did not receive toxin; 1MPC corresponds to 100 μg/kg T-2 toxin concentration.

[a] Differences between indicators in chickens of different ages are statistically significant at $p < 0.05$.

[b] Differences between the parameters in control chickens and chickens receiving T-2 toxin at different doses are statistically significant at $p < 0.05$.

Fig. 5 characterizes the quality of the presented variables (cos^2) on the factor map (see Fig. 4). Variables including values of *PTGS2* gene expression in

the pancreas, *IL6, PTGS2, IL8*, *IRF7*, *AvBD9*, *AvBD10*, and *Casp6* in the caecum, as well as data on total protein, trypsin, glucose, alkaline phosphatase, triglycerides, FPI in the blood, made a high contribution to PC1 with a $\cos^2$ value of 0.44-0.88 (see Figs. 4, 5).

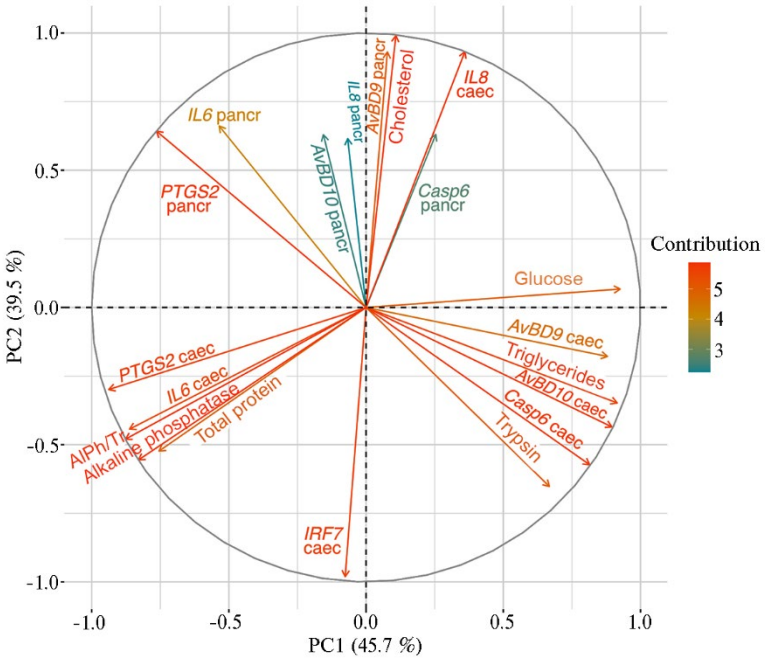

Fig. 4. Factor map (principal component analysis, PCA) **based on the expression levels of genes associated with immunity in tissues** (pancr — pancreas, caec — caecums of the intestine), **and biochemical blood parameters in Smena 8 cross broiler chickens (*Gallus gallus* L.) with experimental T-2 toxicosis.** The color gradient and closeness to the circle of correlations reflect the contribution of variables to the main components: red and close to the circle of correlations means high contribution, blue color and distance from the circle of correlations means low contribution. A variable on one side of another variable has a high value for that variable, and one on the diametrically opposite side of another variable has a low value for that variable. AlPh/T means the alkaline phosphatase/trypsin ratio (vivarium of the Federal Scientific Center VNITIP RAS, 2020).

The results obtained indicate that the studied indicators were in close connection with each other, which seems logical. Thus, the paper [79] showed a change in the activity of digestive enzymes in broilers under heat stress and a related change in the synthesis of mRNA of some genes, in particular *SGLT1*, *GLUT2, FABP1*. In experiments on experimental hyperstimulation of acute pancreatitis in animals [80, 81], it was found that inflammatory diseases of the pancreas were associated with the activation of trypsin synthesis in acinar cells. Therefore, the change in trypsin synthesis upon contamination of broiler feed with T-2 toxin could contribute to the regulation of the expression of proinflammatory genes, in particular *PTGS2*. In addition, it was reported [82] that trypsin was involved in the formation of so-called "fragile" membranes in organelles, through which cathepsin B and other enzymes enter the cytosol. The released cathepsin B induces apoptosis (through activation of caspases) and death of acinar cells of the pancreas [82]. In another study [83], it was shown that proteasomes that provide degradation of proteins in cells contain two trypsin-like and two caspase-like proteolytic regions, for which complex interactions have been proven: substrates of caspase-like sites allosterically inhibit chymotrypsin-like activity. These facts clarify the reason for the revealed relationship between the expression of the *Casp6* gene and the blood trypsin content.

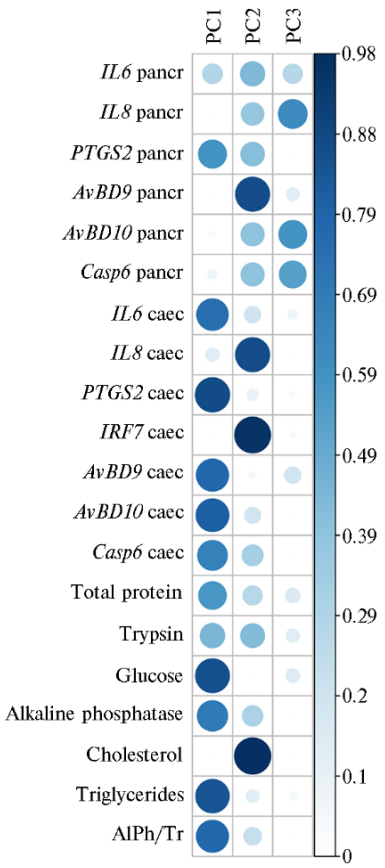

Fig. 5. Visualization of the quality of variables (cos^2) **on the factor map** (see Fig. 4, principal component analysis, PCA) **for the expression levels of genes associated with immunity in tissues** (pancr — pancreas, caec — caecums of the intestine) **and biochemical blood parameters in Smena 8 cross broiler chickens (*Gallus gallus* L.) with experimental T-2 toxicosis** (the corrplot package https://cran.r-project.org/web/packages/corrplot/corrplot.pdf; https://github.com/taiyun/corrplot). The diameter of the circle and the color intensity reflect the contribution of the variables (cos^2) to the principal components: large diameter and dark blue (high cos^2) indicate a high contribution of the variable, no circle and white (low cos^2) — the lowest contribution (vivarium of the Federal Scientific Center VNITIP RAS, 2020).

Expression of the genes *IL6*, *AvBD9* in the pancreas, *IL8*, *IRF7* in the caecum and blood cholesterol in broilers made a high contribution to PC2 with a cos^2 value of 0.44-0.98 (see Figs. 4, 5). The obtained results indicate the mutual influence of these variables. Indeed, it was reported [84] that the *IL8* gene, activating the expression of the *miR-183* gene in macrophages, which in turn inhibits the expression of the *ABCA1* gene, interferes with the *ABCA1*-dependent cholesterol efflux. In another work [85], it was found that a high level of intracellular cholesterol played a decisive role in the transmission of signals initiated by the proinflammatory gene product *IL-17A* in keratinocytes.

The results of the study show that increased levels of expression of genes that control the body's defense reactions and determine the nature of immunological reactions when exposed to T-2 toxin can be considered as early prognostic markers of T-2 toxicosis in broilers. First of all, this is the activation in the tissues of the pancreas of the genes of regulatory molecules that are involved in the initial stages of the development of inflammatory reactions (*IL6* and *PTGS2*), genes associated with cell death (*Casp6*), genes of antimicrobial factors (primarily *AvBD10*).

Therefore, the obtained data indicate the effect of contamination of broiler feed with T-2 toxin on the immune functions of the caecum and pancreas through modulation of the expression of genes associated with immunity. The observed activation ($p \leq 0.05$) of the expression of the proinflammatory genes *IL6* and *PTGS2* can create health risks for poultry and reduce its productivity, since the overproduction of proinflammatory cytokines is involved in the pathogenesis of various diseases. In the pancreas, the expression of the *PTGS2* gene increased even at 1MPC of the T-2 toxin in the food, reaching 41.7-fold values as compared with the control ($p = 0.0005$). In the cecum tissue, the expression of the gene *Casp6* for the apoptosis factor decreased, starting from 1MPC (up to a 12.5-fold decrease, $p = 0.02$), and in the pancreas, on the contrary, the activity of the *Casp6* gene with an increase in the concentration of T-2 toxin in feed increased sharply (by 22.4-31,288.3 times, $p \leq 0.0008$). The expression of the β-defensin genes *AvBD9* and *AvBD10* in the cecum tissues decreased compared to the control (2.1-5.3 times, $p \leq 0.05$) at 2MPC and 4MPC of T-2 toxin, and in the pancreas, regardless of the T-2 toxin dose, on the contrary, increased ($p \leq 0.04$) (to a greater extent, this

concerned *AvBD10*). The expression of the *IRF7* gene associated with the manifestation of antiviral activity was inhibited by 3.0 times in the blind processes of the intestine of birds at 1MPC T-2 toxin ($p = 0.03$). In general, in the tissues of the pancreas, the expression of almost all studied genes associated with immunity was more noticeably activated, and the frequency of apoptosis, which is closely related to the activation of caspase, significantly increased. That is, the pancreas turned out to be more sensitive to the T-2 toxin than the tissues of the cecum of the intestine, which may be due to the functional differences between the intestine and the pancreas. PCA showed that the expression of *PTGS2* genes in the pancreas, *IL6*, *PTGS2*, *IL8*, *IRF7*, *AvBD9*, *AvBD10*, and *Casp6* in the caecum of the intestine, as well as the level of total protein, trypsin, glucose, alkaline phosphatase, triglycerides, and the alkaline phosphatase/trypsin ratios in broiler blood, were closely related. Increased levels of expression of the genes *IL6*, *PTGS2*, *Casp6*, *AvBD10* can be considered as markers of the development of T-2 toxicosis in broilers and used to quantitatively characterize the potential effect of T-2 toxin and assess the sensitivity of poultry to it when monitoring the state of the livestock and carrying out preventive and therapeutic measures in poultry farms.

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