Expression of Prostaglandin-Related Genes in Broilers Under the Influence of Glyphosate and Probiotic

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Abstract. The presence of residues of the herbicide glyphosate in poultry feed is a fairly common problem. Our experiments were carried out in the vivarium of LLC "BIOTROF +" on broilers of the cross "Ross 308". For the experiment, the birds were divided into 3 groups: 1 control group, which received a diet without additives, diet with the addition of glyphosate and the strain of the microorganism Bacillus sp.GL-8.The analysis of gene expression of the caecum of the intestines of broilers was carried out using quantitative PCR with reverse transcription.For the analysis of mRNA expression, specific primers were selected for the following prostaglandin genes studied: PTGER3, PTGER4, PTGR1, PTGDS and PTGES Amplification reactions were performed using SsoAdvanced ™ Universal SYBR® Green Supermix (Bio-Rad) For the first time in our study, the impact of glyphosates on poultry occurs, including through changes in the activity of key genes associated with prostaglandin metabolism Glyphosate introduced into bird feed in an amount corresponding to I eat one MPC, acted as an inducer of prostaglandin receptor gene expression (PTGER3 and PTGER4), prostaglandin catabolism (PTGR1) and prostaglandin synthesis (PTGDS and PTGES) in the caeca of the intestine of broilers. Thus, the levels of mRNA expression of the PTGER3 gene and the PTGER4 gene when exposed to glyphosate (group 2) were activated 1.87 and 1.91 times compared with the control group 1, PTGDS and PTGES genes - 1.35 times, PTGR1 gene - 1 .87 times ($P \leq 0.05$). In the future, the PTGER3, PTGER4, PTGDS, and PTGR1 genes can be used as possible therapeutic targets for toxicosis caused, in particular, by feed glyphosates. The probiotic strain of Bacillus sp. GL-8 probably restored the microbiota, thereby enhancing its protective effect against toxicants, in particular, its metabolic functions of biodegradation. This was expressed in the “smoothing” of the level of PTGR1 gene activity to the level of the control group, as well as a decrease in the activity of PTGDS, PTGES, and PTGER3. Positive shifts in the change in transcription of prostaglandin genes under the influence of a strain of a probiotic microorganism indicate the prospect of using probiotics as a tool for leveling physiological imbalance against the background of feed contamination with toxic substances.

INTRODUCTION

One of the main goals of the poultry industry, like any other production system, will always be to prevent the risk and consequences of diseases. Infectious agents, toxins, nutritional imbalances, overcrowding or other external stressors can induce the activity of immune responses that, on the one hand, control the negative impact and provide protective reactions, and on the other hand, lead to damage to host tissues and cause cell proliferation, inflammation and apoptosis. As a link between the external world and the internal environment of the body, the digestive system is a protective barrier against the effects of substances that can disrupt the course of biological processes, such as pesticide residues. On the other hand, the intestine is particularly susceptible to damage, in particular, mediated by its own immune responses, and, as a rule, the overproduction of some immunity genes becomes the main cause of pathogenesis. The release of pro-inflammatory cytokines has been well studied to cause pyrexia, anorexia, weight loss, and apathy in birds. There are also separate reports regarding the relationship between intestinal diseases and

the activity of prostaglandin genes [1], however, this issue has been poorly studied and more questions than answers remain in this area. Interestingly, a number of researchers see the key role of prostaglandin gene expression in the occurrence of skeletal anomalies in birds, primarily dyschondroplasia of the tibia [2], leading to lameness, which reduces the gross profit of the sub-sector by about 10-40%. However, the exact etiology of the disease is still unknown. It follows from this that the study of the functioning of prostaglandins in birds is of fundamental and practical interest.

Prostaglandins such as E_2, D_2, I_2, $F_{2\alpha}$, thromboxane A_2 and others are lipids derived from 20-carbon fatty acids. They are found in all tissues and organs and mediate various physiological and pathological reactions. Prostaglandins are synthesized in animal, avian, and human cells from various essential fatty acid precursors, including arachidonic acid, acting in an autocrine/paracrine manner via G protein-coupled receptors (DP_1, DP_2, EP_1, EP_2, EP_3, EP_4 subtypes). Prostaglandins are produced in response to many factors, mainly as a result of the induction of cyclooxygenase-2.

So far, attempts to elucidate the exact function and mechanism of action of prostaglandins have not yet been successful, since they are induced by many factors that have a different effect on the body, at the same time, they are characterized by a temporary effect, and regulation occurs at several levels. The most remarkable fact is that they can have completely opposite effects on the body. For example, they are multifunctional regulators of bone metabolism, stimulating, on the one hand, resorption associated with inflammation and loss of bone tissue, and on the other hand, participating in the processes of bone formation associated with fracture healing and heterotopic ossification. By regulating many of the physiological functions of the intestine, including mucosal protection, gastrointestinal secretion and motility, under certain conditions, prostaglandins cause inflammatory diseases of the digestive system and the growth of cancerous tumors.

In our opinion, the observation of prostaglandin gene expression may help shed light on their physiological functions.

There are indications that exposure to certain pesticides alters the expression of key genes in the gut, primarily pro-inflammatory genes leading to digestive dysfunction. Separate works have been published [2] indicating that some pesticides, such as, for example, tetramethylthiuram disulfide (thiram), provoke the occurrence of tibial dyschondroplasia in birds through the regulation of prostaglandin expression. There is evidence that when exposed to a number of xenobiotics, some prostaglandins may be associated with increased xenobiotic toxicity [3]. Paradoxically, a number of prostaglandins are also associated with the processes of restoring the physiological balance against the background of exposure to toxicants on the body, including the stimulation of the cellular antioxidant program, which contributes to the detoxification of xenobiotics. However, similar experiments have not been carried out on birds before.

It is important that the presence of xenobiotics, including residual amounts of glyphosate herbicide, in feed for industrial poultry is a fairly common problem [4]. Evidence is emerging that glyphosates can have a negative impact on the health of animals, birds and humans [5]. However, there are no data in the literature on the effect of glyphosate on the expression of prostaglandin genes, which can contribute to an increase in their toxicity, and, conversely, participate in detoxification processes.

At the same time, it is important to develop methods to improve the health status of birds against the background of the presence of toxicants in feed. Probiotic strains of microorganisms can take part in the detoxification of xenobiotics and affect gene expression, and hence the activity of enzymes. This allows us to consider beneficial bacteria as a mechanism for ensuring the body's resistance to the action of toxicants.

The aim of our study was to evaluate the change in the expression of the spectrum of prostaglandin genes in broilers against the background of feed contamination with glyphosate and the introduction of a probiotic strain of *Bacillus* sp. into the diet. GL-8. For this, quantitative reverse transcription PCR was carried out using specific primers for genes.

MATERIALS AND METHODS

The experiments were carried out in the vivarium of LLC "BIOTROF+" on broilers of the cross "Ross 308" from 1 to 35 days of age in 2022 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). The conditions of feeding and keeping corresponded to the requirements for cross-country broilers. For feeding from the 1st to the 28th day of cultivation, PK 5 feed was used for broilers, from 29 to 35 days - PK 6 for broilers. In the diet

of all groups, according to the traditional production schemes for growing broilers, veterinary antibiotics enrofloxacin and colistin were introduced from 1 to 5 days and florfenicol from 17 to 20 days.

For the experiment, the birds were divided into 3 groups of 40 heads each:

- Control, receiving a diet without the introduction of glyphosate and a probiotic strain of a microorganism;
- Experimental - receiving a diet with the addition of glyphosate in the amount of 20 mg / kg of feed, which corresponded to 1 MPC for food (SanPiN 1.2.3685-21 "Hygienic standards and requirements for ensuring the safety and (or) harmlessness of environmental factors for humans");
- Experimental - receiving a diet with the addition of glyphosate in the amount of 20 mg/kg of feed, as well as a probiotic strain of the microorganism *Bacillus* sp. GL-8.

To conduct a production experiment, glyphosate was used as part of the preparation "Agrokiller" (CJSC firm "Avgust", Russia), containing 500 g/l of glyphosate acid (isopropylamine salt). To do this, a working solution was prepared from the drug "Agrokiller", the working solution was applied to the feed by spraying in a ratio of 1 kg of feed 5 ml of the working solution, to a final concentration of pure glyphosate in the feed of 20 mg/kg Mixing was carried out mechanically in compliance with personnel safety requirements .

After the introduction of glyphosate, its concentration in the feed was monitored by enzyme immunoassay (ELISA). In addition to the above, the diet of broilers practically did not contain background amounts of glyphosate, which indicates the purity of the experiment. To analyze the content of glyphosates by ELISA in feed and nutrient media, we used the STAT FAX 303+ strip enzyme immunoassay analyzer (Awareness Technology, LLC, USA) and the Glyphosate ELISA Microtiter Plate test system (Abraxis, USA).

To analyze gene expression in broilers, at the end of the experiment, tissues of the blind processes of the intestines of broilers were taken. The collected samples were immediately stabilized with the RNAlater reagent. All samples were immediately sent to the molecular genetic laboratory of the research and production company LLC "BIOTROF+" for RNA isolation.

The analysis of gene expression was carried out using quantitative PCR with reverse transcription, the preliminary step of which was the isolation of RNA. The tissues were crushed by mixing with liquid nitrogen and homogenized. Total RNA was isolated from tissue samples using the Aurum™ Total RNA mini kit (Bio-Rad, Hercules, CA, USA) following the manufacturer's instructions. A reverse transcription reaction was performed to generate cDNA from an RNA template using iScript™ Reverse Transcription Supermix (Bio-Rad). To analyze mRNA expression, specific primers were selected for the following protsaglandin genes: for the EP_3 gene, the prostaglandin E_2 receptor *PTGER3*, F:TCTCGGCAGAAACCCAAAGC, R:CGGAGCAGCAGATAAACCCAC; for the EP_4 subtype gene - prostaglandin receptor E_2*PTGER4* - F:TCAGGAAAGCCATCGAGAAG, R:CTGCGACCATCCACACAATT; for the prostaglandin reductase 1 gene *PTGR1*- F: AATAGAGGCTGGAGAACTCA, R: TCCCAAGTGCTAGAGATTTG; for the gene prostaglandin-H2 D-synthase PTGDS - F: GCACCTGCTGAAGATGTGTA, R: CTCTTTCTCGCACTGTTCAC; for the prostaglandin E synthase gene PTGES - F: TTCGCCTTCTACAGCACGAT, R: TTCTTCCTGAGCCTCACTTGT. As a reference control, primers for the "housekeeping" gene, the beta-actin protein (ACTB), were used.

Amplification reactions were carried out using SsoAdvanced ™ Universal SYBR® Green Supermix (Bio-Rad) in accordance with the manufacturer's protocol using a DTlight detecting amplifier (DNA-Technology, Russia). Amplification mode and conditions corresponded to each primer. Relative expression levels were assessed using method 2 "-ΔΔCT".

Mathematical and statistical processing of the results was carried out by the method of multifactor analysis of variance (multifactor ANalysis Of VAriance, ANOVA) in Microsoft Excel XP/2003, R-Studio (Version 1.1.453) (https://rstudio.com). Differences were considered significant at $P \leq 0.05$. Results are presented as means (M) and standard errors of means (±SEM). Significance of differences was determined by Student's t-test, differences were considered statistically significant at $p \leq 0.05$. Means were compared using the Tukey Significantly Significant Difference (HSD) test and the TukeyHSD function in the R Stats Package.

RESULTS

Results of analysis of prostaglandin receptor gene expression

Analysis of the relative levels of transcripts of the *PTGER3* and *PTGER4* prostaglandin receptor genes in the caeca tissues of broilers in response to the administration of glyphosate and the probiotic strain *Bacillus* sp. GL-8 are shown in Figure 1.

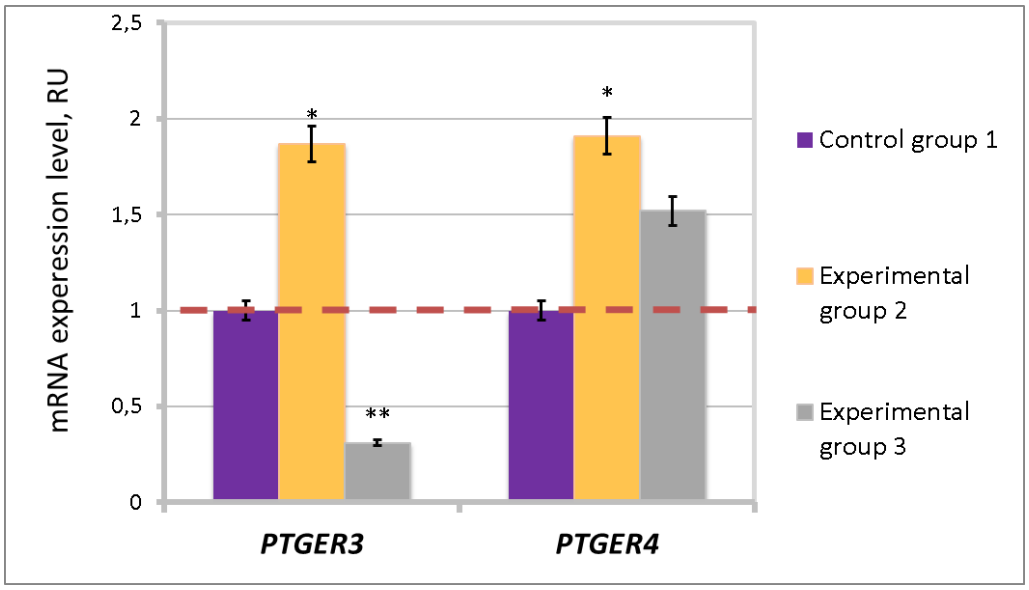

FIGURE 1. The level of expression of prostaglandin receptor genes in the cecum of the broiler intestines in response to feeding glyphosate and a probiotic strain of bacteria (data obtained at the end of the experiment on day 35, vivarium LLC "BIOTROF +", 2022), RU - the multiplicity of changes in expression levels in comparison with control group 1 taken as 1, * differences from control group 1 at P≤0.05, ** differences from experimental group 2 at P≤0.05, dashed red line shows the level of expression in the control. Results are presented as the mean (± SEM) of mRNA expression.

The expression levels of the *PTGER3* gene (EP_3 prostaglandin E_2 receptor) and the *PTGER4* gene (EP_4 prostaglandin E_2 subtype receptor) were activated by 1.87 and 1.91 times when exposed to glyphosate (group 2) compared with control group 1 (P≤0.05) . The induction of expression of the prostaglandin E_2 receptor *PTGER3* and *PTGER4* genes against the background of the presence of glyphosate in feed is natural. Previously, using an animal model of a guinea pig as an example, it was shown that in response to the toxic effect of ethanol in the cells of the gastric mucosa, EP_2 and EP_4 receptors were activated, which was associated with the cytoprotective effect of these receptors against ethanol-induced apoptosis [7]. It has also been demonstrated in mice that EP2 receptors play a role in preventing radiation-induced apoptosis of crypt epithelial cells in the small intestine [7].

Nevertheless, in our opinion, the activation of the expression of the *PTGER3* and *PTGER4* genes in the variant with the introduction of glyphosate into the diet most likely had negative consequences for the bird organism. This is because prostaglandin receptors, including EP_2 and EP_4 and others, are associated with stimulation of cAMP/protein kinase A (PKA) signaling through sequential activation of adenylate cyclase. PKA has been shown to phosphorylate and activate Akt-kinase, which in turn inhibits glycogen synthase kinase-3 (GSK-3). Inhibition of GSK-3 reduces inhibitory phosphorylation of cytosolic β-catenin, which promotes translocation of β-catenin to the nucleus and leads to cell proliferation [8]. In addition, the expression of prostaglandin receptors is significantly increased against the background of inflammatory bowel diseases. *In vitro* studies looking at early responses of prostaglandin receptors in various colonic epithelial cell lines demonstrate that they increase *IL-8* mRNA expression and protein secretion, indicating their pro-inflammatory role [9]. Of particular interest is the fact that EP_1 and EP_3 receptors counteract the increase in mRNA of the *BCRP* gene, a multidrug resistance protein that induces resistance of the body to many drugs and xenobiotics [10], and therefore can reduce the body's resistance to glyphosate. . Previously, it was also shown that glyphosate can contribute to the disruption of xenobiotic detoxification processes [11]. It was

found that glyphosate reduced the activity of cytochrome P450-dependent monooxygenase and aryl hydrocarbon hydroxylase enzymes in the intestine and liver. These enzymes are involved in the neutralization of many toxicants.

In our experiment, there was a decrease in the level of activity of the *PTGER3* gene in the variant with the use of a probiotic strain of a microorganism against the background of glyphosate (group 3 compared with group 2) (P≤0.05). This could make a positive contribution to the health of the birds.

The results of the analysis of the expression of genes associated with the synthesis of prostaglandins

Analysis of the relative levels of transcripts of genes associated with the synthesis of prostaglandins in the tissues of the blind processes of broilers in response to the introduction of glyphosate and a probiotic strain is shown in Figure 2.

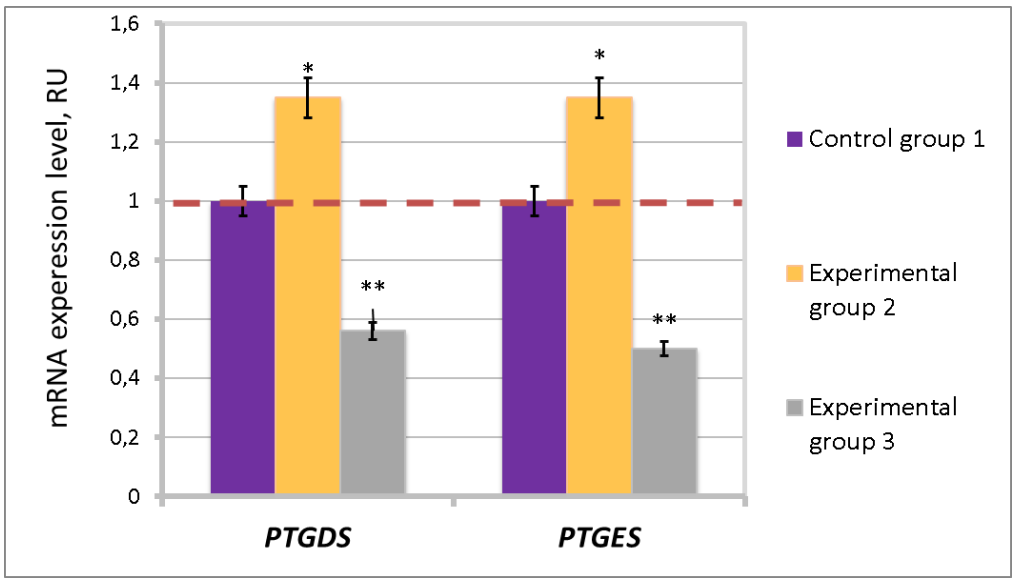

FIGURE 2. The level of expression of genes associated with the synthesis of prostaglandins in the caecum of the intestines of broilers, in response to feeding glyphosate and a probiotic strain of bacteria (data obtained at the end of the experiment on day 35, vivarium LLC "BIOTROF+", 2022), RU - multiplicity of changes expression levels compared to control group 1, taken as 1, * difference from control group 1 at P≤0.05, ** difference from experimental group 2 at P≤0.05, dashed red line shows the level of expression in the control. Results are presented as the mean (± SEM) of mRNA expression.

The levels of expression of the *PTGDS* gene (prostaglandin-H2 D-synthase) and the *PTGES* gene (prostaglandin-E-synthase) when feed was contaminated with glyphosate (group 2) increased by the same number of times (1.35 times) compared with control group 1 (P ≤0.05). This could have undesirable consequences for the health of birds and their level of resistance to toxicants. The protein encoded by the *PTGES* gene is a glutathione-dependent prostaglandin-E synthase. The expression of this gene has been shown to be induced by the pro-inflammatory cytokine interleukin 1β (*IL1β*). Its expression may also be induced by the TP53 tumor suppressor protein and may be involved in TP53-induced apoptosis. Prostaglandin-H2 D-synthase catalyses the conversion of PGH2 to PGD2-prostaglandin involved in smooth muscle contraction/relaxation, being a potent inhibitor of platelet aggregation. Interestingly, there is evidence that prostaglandin H-synthase has hydroperoxidase activity and, in the presence of arachidonic acid, can participate in the bioactivation of many chemical xenobiotics (polycyclic aromatic hydrocarbons and aromatic amines), which ultimately results in the enhancement of their mutagenic activity. , carcinogenic properties, pulmonary toxicity, teratogenicity, nephrotoxicity and myelotoxicity. At the same time, some carcinogens are metabolized into forms that covalently react with cellular macromolecules [3].

Inhibition of the activity of the *PTGDS* and *PTGES* genes was observed in the variant with the use of the probiotic strain *Bacillus* sp. GL-8 against the background of glyphosate (group 3 compared with group 2) (P≤0.05), which indicates a positive effect of the microorganism strain on gene expression. The fact of the effect of probiotics

on avian gene expression was shown by us in previous works [12]. This may contribute to strengthening the body's defense responses to toxicants and maintain homeostasis.

Results of the analysis of the expression of the PTGR1 gene associated with prostaglandin catabolism.

The data of the analysis of the relative levels of *PTGR1* gene transcripts in the tissues of the caeca of broilers in response to the introduction of glyphosate and the probiotic strain are shown in Figure 3.

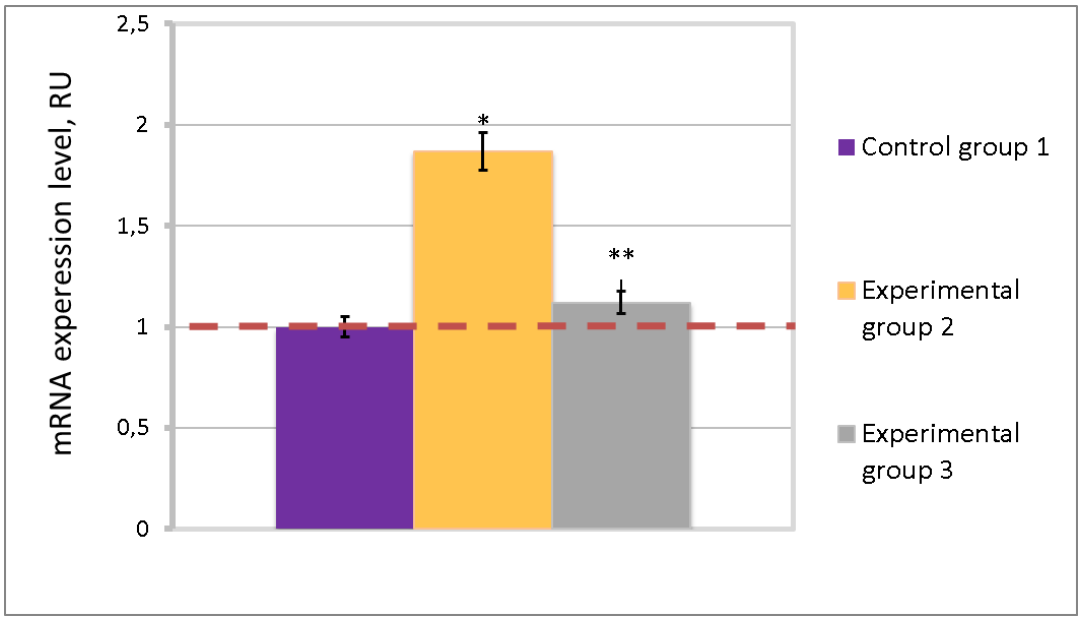

FIGURE 3. The level of expression of the *PTGR1* gene in the caecum of the intestines of broilers in response to feeding glyphosate and a probiotic strain of bacteria (data obtained at the end of the experiment on day 35, vivarium LLC "BIOTROF +", 2022), RU - the multiplicity of changes in expression levels compared to control group 1, taken as 1, * differences from control group 1 at P≤0.05, ** differences from experimental group 2 at P≤0.05, dashed red line shows the level of expression in the control. Results are presented as the mean (± SEM) of mRNA expression.

The level of transcription of the *PTGR1* gene (associated with the synthesis of prostaglandin reductase 1) under the influence of glyphosate (group 2) increased by 1.87 times compared with the control group 1 (P≤0.05). This is logical since prostaglandin reductase 1 is the key enzyme responsible for the biological catabolism of prostaglandins and some eicosanoids. However, the PTGR1 protein has been characterized as a cytoprotective enzyme that is induced in rat liver after treatment with anti-cancer chemotherapeutic agents such as dithiolethiones [13]. Dithiolethiones are a class of cancer chemoprevention agents that induce carcinogen detoxification enzymes such as NAD(P)H dehydrogenase, quinone 1, glutathione S-transferase, epoxide hydrolase, glutamate-cysteine ligase, and UDP-glucuronosyltransferase by activating the Keap1-Nrf2 pathway. *PTGR1* expression is regulated by the transcription factor Nrf2, which is constitutively activated by oncogenes, mutations, or other mechanisms, stimulating a cellular antioxidant program that promotes the detoxification of oxidative by-products. These observations support the notion that PTGR1 acts as a cytoprotective enzyme that has both antioxidant and anti-inflammatory functions, which may allow it to be considered as a protective agent whose synthesis is induced by feed glyphosates. That is, increased expression of *PTGR1* against the background of glyphosate contamination can be considered as a mechanism for enhancing resistance to xenobiotics to maintain the body's own homeostasis.

The effect of the probiotic strain on the expression of the *PTGR1* gene turned out to be "smoothing", i.e. the level of expression in the experimental group 3 was at the level with the control group 1 (P≤0.05). This may be due to an increase in the number of taxa in the intestinal microbiome that have the properties of biodegradation of toxins against the background of a probiotic. Earlier W. Meinl et al. [14] it was also shown that the intestinal microbiota can influence the expression of xenobiotic-metabolizing enzyme genes, including the glutathione-S-transferase gene,

in the colon and liver. Interestingly, the enzyme glutathione-S-transferase, which plays a key role in detoxification and protection against xenobiotics, also has a significant effect on the genes of prostaglandins and their receptors [15].

CONCLUSION

For the first time in the world, our study has shown that the impact of glyphosates on poultry occurs, among other things, through changes in the activity of key genes associated with prostaglandin metabolism. Glyphosate, introduced into poultry feed in an amount corresponding to one MPC, acted as an inducer of prostaglandin receptor gene expression (*PTGER3* and *PTGER4*), prostaglandin catabolism (*PTGR1*), and prostaglandin synthesis (*PTGDS* and *PTGES*) in caeca of broilers. Due to the fact that the *PTGER3* and *PTGER4* genes are associated with a decrease in the expression of the multidrug resistance protein, and the *PTGDS* gene is associated with an increase in the toxicity of xenobiotics, an increase in their expression in response to the administration of glyphosate can have negative consequences for the body, reduce resistance to toxicants, while simultaneously increasing their toxicity. On the other hand, the *PTGR1* gene is associated with xenobiotic detoxification processes. Therefore, we believe that the increased expression of the *PTGR1* gene in the intestine against the background of the intake of glyphosate may be associated with the body's resistance to the intake of the toxicant, be a mechanism for increasing the level of metabolic resistance and maintaining its own homeostasis. In the future, the *PTGER3*, *PTGER4*, *PTGDS* and *PTGR1* genes can be used as possible therapeutic targets for toxicosis caused, in particular, by feed glyphosates. The data obtained indicate the need to draw attention to the problem of glyphosate content in bird feed and to clarify the boundaries of the maximum permissible concentrations of glyphosate in feed. The probiotic strain of *Bacillus* sp. GL-8 probably restored the microbiota, thereby enhancing its protective effect against toxicants, in particular, its metabolic functions of biodegradation. This was expressed in the "smoothing" of the level of *PTGR1* gene activity to the level of the control group, as well as a decrease in the activity of *PTGDS*, *PTGES*, and *PTGER3*. Positive shifts in the change in transcription of prostaglandin genes under the influence of a strain of a probiotic microorganism indicate the prospect of using probiotics as a tool for leveling physiological imbalance against the background of feed contamination with toxic substances.

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REFERENCES

1. H. W. Murcia and G. J. Diaz, Protective effect of glutathione S-transferase enzyme activity against aflatoxin B1 in poultry species: relationship between glutathione S-transferase enzyme kinetic parameters, and resistance to aflatoxin B1. Poult Sci., **100**, **8**, 101235 (2021).
2. N. C. Rath, W. E. Huff, G. R. Huff and L. Kannan, Induction of tibial dyschondroplasia by carbamate and thiocarbamate pesticides, Avian Dis., **51**, **2**, 3–590 (2007).
3. B. J. Smith, J. F. Curtis and T. E. Eling, Bioactivation of xenobiotics by prostaglandin H synthase. Chem Biol Interact.;**79**, **3**, 245-64 (1991).
4. D. G. Tyurin, V. Kh. Melikidi and T. M. Okolelova, Glyphosate in compound feed for poultry, Poultry farming, **3**, 27–30 (2021).
5. B. Szekacs, Darvas Re-registration challenges of glyphosate in the European union Front Environ. Sc., **6**, 35 (2018).
6. T. Hoshino, S. Tsutsumi, W. Tomisato, H. J. Hwang, T. Tsuchiya and T. Mizushima, Prostaglandin E2 protects gastric mucosal cells from apoptosis via EP2 and EP4 receptor activation, J Biol Chem, **278**, 12752-12758 (2003).
7. C. W. Houchen, M. A. Sturmoski, S. Anant, R. M. Breyer and W. F. Stenson, Prosurvival and antiapoptotic effects of PGE2 in radiation injury are mediated by EP2 receptor in the intestine, Am J Physiol Gastrointest Liver Physiol, **284**, 490–498 (2003).
8. M. Li, X. Wang, M. K. Meintzer, T. Laessig, M. J. Birnbaum and K. A. Heidenreich, Cyclic AMP promotes neuronal survival by phosphorylation of glycogen synthase kinase 3beta, Mol Cell Biol, **20**, 9356–9363 (2000).

9. Y. Yu and K. Chadee, Prostaglandin E2 stimulates IL-8 gene expression in human colonic epithelial cells by a posttranscriptional mechanism, J Immunol, **161**, 3746–3752 (1999).
10. C. W. Mason, G. T. Lee, Y. Dong, H. Zhou, L. He and C. P. Weiner, Effect of prostaglandin E2 on multidrug resistance transporters in human placental cells, Drug metabolism and disposition: the biological fate of chemicals, **42**, **12**, 2077–2086 (2014).
11. A. Samsel and S. Seneff, Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance, Interdiscip Toxicol. Dec, **6**, **4**, 159-84 (2013).
12. G. Y. Laptev, E. Yildirim and L. A. Ilina, Effects of Essential Oils-Based Supplement and Salmonella Infection on Gene Expression, Blood Parameters, Cecal Microbiome, and Egg Production in Laying Hens, Animals, **11**, 360 (2021).
13. T. Primiano, Y. Li, T. W. Kensler, M. A. Trush and T. R. Sutter, Identification of dithiolethione-inducible gene-1 as a leukotriene B4 12-hydroxydehydrogenase: implications for chemoprevention Carcinogenesis, **19**, 999-1005 (1998).
14. W. Meinl, S. Sczesny, R. Brigelius-Flohé, M. Blaut and H. Glatt, Impact of gut microbiota on intestinal and hepatic levels of phase 2 xenobiotic-metabolizing enzymes in the rat, Drug Metab Dispos. Jun, **37**, **6**, 1179-86 (2009).
15. S. Niu, C. X. Wang, F. J. Jia, A. R. Jahejo, X. Li, G. B. Ning, D. Zhang, H. L. Ma, W. F. Hao, W. W. Gao, Y. J. Zhao, S. M. Gao, J. H. Li, G. L. Li, F. Yan, R. K. Gao, N. R. Huo, W. X. Tian and H. C. Chen, The expression of prostaglandins-related genes in erythrocytes of broiler chicken responds to thiram-induced tibial dyschondroplasia and recombinant glutathione-S-transferase A3 protein, Res Vet Sci., **124**, 112-117 (2019).