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INFLUENCE OF ANTIBIOTICS, GLYPHOSATE AND A *Bacillus* sp. STRAIN ON PRODUCTIVITY PERFORMANCE AND GENE EXPRESSION IN CROSS ROSS 308 BROILER CHICKENS (*Gallus gallus* L.)

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Abstract

The combination of antibiotics and pesticide residues can compromise the therapeutic and production benefits of antibiotics in the poultry industry. These effects may be reflected in changes of gene expression. The present work, for the first time, shows that the stimulation of poultry meat productivity with veterinary antibiotics enrofloxacin and colistin is probably associated with the induced expression of *MYOG* gene which is known to promote the development and differentiation of muscles, genes of antimicrobial (*Gal9*, *Gal10*) and antiviral (*IRF7*) protection, and pro-inflammatory genes *IL6*, *IL8* and *PTGS2*. In addition, it was shown for the first time that glyphosate suppresses the expression of antimicrobial and antiviral genes in broilers of the Ross 308 cross. The aim of the study was to evaluate the change in the expression spectrum of key genes in broiler fed antibiotics, glyphosate and a biodestructor strain. The experiments were carried out on broilers of the Ross 308 cross from 1 to 35 days of age (the vivarium of BIOTROF$^+$ LLC, 2022). The broilers were divided into 4 groups of 40 birds each. Group I (control) was fed a diet without additives, group II received a diet with the addition of veterinary antibiotics enrofloxacin and colistin; group III experienced dietary antibiotics and glyphosate; group IV received dietary antibiotics, glyphosate and a strain of the microorganism-biodestructor *Bacillus* sp. GL-8. Glyphosate content was measured by ELISA using a STAT FAX 303+ analyzer (Awareness Technology, LLC, USA) and a Glyphosate ELISA Microtiter Plate test system (Abraxis, USA). Reverse transcription quantitative PCR was performed to evaluate gene expression of the caecum and pectoral muscle tissues. Total RNA was isolated from samples using the Aurum™ Total RNA mini kit (Bio-Rad, Hercules, USA). Specific primers were selected for immunity genes *IL6* (interleukin 6), *IL8* (interleukin 8), *IRF7* (interferon regulatory factor7), *PTGS2* (prostaglandin-endoperoxide synthase), *AvBD9* (*Gal9*) (β-defensin 9), *AvBD10* (*Gal10*) (β-биотро,bjnhdefensin 10). For productivity genes, *LGF-I* (insulin-like growth factor 1), *MYOG* (myogenin), *MYOZ2* (myosenin) and *GSTA3* associated with resistance to toxic and medicinal substances were tested. Amplification reactions were carried out using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, USA)

using a DTlight amplifier (DNA-Technology, Russia). The body weight of broilers was assessed at 7, 14, 21, 28 and 35 days of age. Mathematical and statistical data processing was performed using multivariate analysis of variance in Microsoft Excel XP/2003, R-Studio (Version 1.1.453) (https://rstudio.com). The results showed a 4.8-23.3 %-stimulated productivity ($p \leq 0.05$) of broilers from 14 days of life until the end of the experiment due to dietary antibiotics (group II vs. group I). At the end of the experiment, a negative effect of glyphosate on broiler productivity occurred (group III vs. group II, $p \leq 0.05$). In broilers of groups II and IV, the expression of *MYOG* gene was 2.0 and 2.1 times higher than in group I ($p \leq 0.05$). In the group fed glyphosate combined with antibiotics without a biodestructor strain added (group III), no activation of the *MYOG* gene expression occurred compared to group I ($p > 0.05$), which indicates a negative effect of glyphosate on the expression of productivity genes. Glyphosate (group III) also acted as a suppressor of the antimicrobial and antiviral genes *Gal9*, *Gal10* and *IRF7* as compared to group II ($p \leq 0.05$). The dietary biodestructor strain co-fed with glyphosate and antibiotics (group IV) provided an increase in *Gal9* expression compared to group III ($p \leq 0.05$). There was a tendency for a sharp increase in the expression of pro-inflammatory genes *IL6*, *IL8* and *PTGS2* (by 4.6, 11.2 and 6.6 times, respectively) in group II fed antibiotics vs. control group I ($p \leq 0.05$). Our findings once again confirms the effect of antibiotics on immune processes. For *GSTA3* gene associated with resistance to toxic and medicinal substances, it was shown that the introduction of antibiotics into feeds had some stimulating effect on the level of *GSTA3* gene expression in the caeca tissues of broilers (group II vs. group I, $p \leq 0.05$). Thus, the mechanism providing positive effects of antibiotics on productivity performance is probably partly due to the fact that they act as inducers of a set of important genes. Glyphosates fed in an amount corresponding to 1MPC reduced the stimulating effect of antibiotics. Glyphosates act, among other things, through the disruption of the activity of some key bird genes. The positive dynamics of the expression of various genes, including those involved in antimicrobial and antiviral defense, under the action of a biodestructor strain indicates the prospects for using probiotics as a means of smoothing out physiological imbalances caused by drugs and food contamination with toxic substances.

Keywords: mycotoxins, antibiotic, glyphosate, broilers, gene expression

Antibiotics play an important role in the fight against infectious diseases and are also used to stimulate the growth of poultry [1-3]. Metaphylactic administration of antibiotics, such as enrofloxacin, to chicks during the first few days of life, and sometimes during further rearing, is considered common practice among many poultry meat producers [4, 5]. Antibiotics can adversely affect the defense mechanisms of birds, which are determined by the functioning of the main organs of the immune system [6]. There is evidence that although enrofloxacin inhibits humoral immune mechanisms [7], it may promote cellular immune response in chickens [5].

Interestingly, the mechanism of growth stimulation of farm animals and poultry under the influence of antibiotics is still not clear. All hypotheses are reduced mainly to the modulation of the composition of the microbiota against their background [8]. H. Eyssen et al. [9] hypothesized that antibiotics stimulate chick growth through their antibacterial action against gram-positive micro-organisms that interfere with nutrient absorption. According to another hypothesis [10], a decrease in the population of lactobacilli in animals treated with antibiotics reduces the activity of bile salt hydrolase, which increases the relative abundance of conjugated bile salts, promotes lipid metabolism and energy synthesis. As a result, the weight gain of animals increases.

However, the use of antibiotics tends to compromise the immune system [11]. It has been shown in pigs [12] and poultry [13] that dietary antibiotics can interfere with gene expression.

In addition to antibiotics, many other factors affect broiler immunity, health and productivity [14]. For example, bird feeds, especially those based on genetically modified soybeans, contain a significant amount of glyphosate herbicide residues [15, 16], which can have a negative effect on the body [17, 18].

The digestive system serves as a protective barrier against exposure to pesticides and pathogens [19, 20]. Lymphoid tissues in the gastrointestinal tract of birds are well developed [21, 22] and are involved in the activation of immune responses [23-25]. It is important to note that the study of the effect of glyphosates

on the expression of genes in farm animals and birds has not been previously carried out.

The widespread use of antibiotics and the presence of glyphosates in feed can jeopardize the therapeutic and production effects of the use of antibacterial drugs. Glyphosate exposure has previously been shown to increase the tolerance of *Escherichia coli* and *Salmonella enterica* serovar *Typhimurium* to kanamycin and cephalosporin [26]. However, the combined effects of antibiotics and glyphosates have not been previously studied in animal models.

The search for agents that positively affect the bird gut microbiota by stimulating protective mechanisms and reducing the need for prophylactic and therapeutic use of antibiotics has been going on for many years. Beneficial microorganism strains undoubtedly rank first among such agents [27-29] and are widely used in poultry nutrition [30]. It is not uncommon for beneficial bacteria to be used concomitantly with antibiotics to prevent side effects of the latter [31]. The effect of microorganisms on immunity [32] and expression of host genes [33] has been proven. Strains of microorganisms-biodestructors were used for prophylaxis in cases of feed contamination with mycotoxins [34] and glyphosates [35]. In this regard, it is advisable to investigate whether the introduction of microorganism strains into diets can be a tool to smooth out the immunosuppression that has arisen against the background of antibiotics.

This paper is the first to report that the stimulation of the meat productivity of Ross 308 cross broiler chickens under the influence of the veterinary antibiotics enrofloxacin and colistin is probably associated with an induced expression of the *MYOG* gene mRNA which promotes the development and differentiation of muscles, antimicrobial genes (*Gal9*, *Gal10*), antiviral (*IRF7*) protection, and pro-inflammatory genes *IL6*, *IL8* and *PTGS2*. In addition, it has been shown for the first time that glyphosate suppresses the expression of antimicrobial and antiviral genes in broiler chickens.

Our goal was to evaluate the productivity and changes in the expression of genes associated with immunity, productivity, and resistance to toxic and medicinal substances in broiler chickens under the influence of antibiotics, including against the background of fodder contamination with glyphosate and the introduction of Bacillus sp. into the diet.

Materials and methods. The experiments were carried out in 2022 in the vivarium of OOO BIOTROF+ on the Ross 308 cross broilers (*Gallus gallus* L.) from 1 to 35 days of age; the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes (ETS No. 123, Strasbourg, 1986) [36] were complied with. Feeding and keeping conditions corresponded to recommendations for cross-country [37]. From day 1 to day 28 of growth, PK 5 compound feed was used, from day 29 to day 35 PK 6 compound feed was used.

The birds were divided into 4 groups of 40 birds each. In intact group I (control), broilers received a diet without the introduction of antibiotics, glyphosate, and a microorganism strain. In group II, a diet was fed with the addition of veterinary antibiotics enrofloxacin and colistin in the form of Enroflon K (OOO VIK — animal health, Russia) at a dosage of 1 ml/l of water from day 1 to day 5 of growth and florfenicol (OOO Agrovetzashchita S.-P. NVTs, Russia) from day 17 to day 20 at a dosage of 1 ml/l of water. In group III, the diet was added with Enroflon K according to the scheme described above, as well as glyphosate in the amount of 20 mg/kg of feed, which corresponded to 1 MPC for feed [38]. In group IV, the diet was added with enrofloxacin, colistin, florfenicol, glyphosate, and the strain *Bacillus* sp. GL-8. The bacterial preparation was used at a concentration of 106 cells/kg of feed.

To analyze the ability to biodegrade glyphosate in vitro, 11 strains of Bacillus sp. incubated with glyphosate in the form of the herbicide Tornado, BP (ZAO Firma Avgust, Russia) containing glyphosate N-(phos-phonomethyl)-glycine (isopropylamine salt) (360 g/l) for 2 days. The drug was added to the medium in an amount of 20 μl, which corresponded to 144 mg/l of pure glyphosate. The strains were cultured in a semi-synthetic nutrient medium (molasses 2%; NaCl 0.02%; K_2HPO_4 0.2%; $MgSO_4 \cdot 7H_2O$ 0.05%; $CaCO_3$ 0.01%) in glass flasks with cotton stoppers on a shaker at 230 rpm and a temperature of 32±1.2 °C without additional aeration. The concentration of bacteria at the beginning of growth in all variants was 1.0×10^4 cells/ml, the duration of cultivation was 2 days. The concentration of bacteria at the end of cultivation ranged from $1.9\times10^7\pm7.9\times10^5$ to $8.7\times10^8\pm6.3\times10^6$ cells/ml. The decrease in the content of mycotoxins in the nutrient medium with the inoculated culture of live bacterial cells compared to the control was conditionally considered as the biodegradation of glyphosate.

The strain *Bacillus* sp. GL-8 isolated from the intestines of broilers was obtained from the collection of OOO BIOTROF+. The strain was aerobic immobile spore-forming rods 1.2-1.5 μm wide and 2-5 μm long. It formed elliptical spores of a central location. To obtain preliminary conclusions that the *Bacillus* sp. GL-8 does not have virulence factors and etiological significance in the development of infectious processes; its hemolytic activity was determined. It was established after 24 hours when viewing colonies grown on 5% blood agar.

In a production experiment, glyphosate was used as part of the preparation Agrokiller (ZAO Firma Avgust, Russia) containing 500 g/l of glyphosate (isopropylamine salt). For this, a working solution was prepared from the Agrokiller preparation, which was applied by spraying feed, 5 ml of working solution per 1 kg feed, to a final content of pure glyphosate in the feed of 20 mg/kg. Mixing was carried out mechanically in compliance with personnel safety requirements. Feed intake by broilers averaged 150 g/day, i.e., broilers of the experimental groups received glyphosate daily in the amount of 3 mg/bird. After the introduction of glyphosate, its concentration in the feed was monitored by enzyme immunoassay (ELISA). 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, a STAT FAX 303+ strip enzyme immunoassay analyzer (Awareness Technology Co LLC, USA) and a Glyphosate ELISA test system, Microtiter Plate (Abraxis, USA) were used. The test is based on a direct competitive ELISA reaction between glyphosate, which is present in the sample, and a glyphosate labeled enzyme to bind rabbit anti-glyphosate antibodies and goat anti-rabbit immunoglobulins immobilized in microwells. After the enzyme immunoassay, the intensity of the color signal of the solution in the wells was inversely proportional to the concentration of glyphosate present in the samples.

To determine the expression of genes at the end of the experiment, tissue samples of caecum and pectoral muscles were taken. The samples were stabilized with the RNAlater reagent (Thermo Fisher Scientific, Inc., USA) and immediately sent to the OOO BIOTROF+ for RNA isolation.

Gene expression analysis was performed using quantitative PCR. To obtain RNA, tissues were mixed with liquid nitrogen and homogenized. Total RNA was isolated using the Aurum™ Total RNA mini kit (Bio-Rad, USA) following the manufacturer's instructions. The reverse transcription reaction was performed to obtain cDNA from an RNA template using iScriptTM Reverse Transcription Supermix (Bio-Rad, USA) [39]. The following specific primers were selected using the NCBI toolkit (https://www.ncbi.nlm.nih.gov) for expression analysis:

Gene, protein	Primers (5′→3′)
IL6, interleukin 6	F: AGGACGAGATGTGCAAGAAGTTC R: TTGGGCAGGTTGAGGTTGTT
IL8, interleukin 8	F: GGAAGAGAGGTGTGCTTGGA R: TAACATGAGGCACCGATGTG
IRF7, interferon regulatory factor 7	F: ATCCCTTGGAAGCACAACGCC R: CTGAGGCAACCGCGTAGACCTT
PTGS2, prostaglandin endoperoxide synthase 2	F: TCGAGATCACACTTGATTGACA R: TTTGTGCCTTGTGGGTCAG
AvBD9 (*Gal9*), β-defensin 9	F: AACACCGTCAGGCATCTTCACA R: CGTCTTCTTGGCTGTAAGCTGGA
AvBD10 (*Gal10*), β-defensin 10	F: GCTCTTCGCTGTTCTCCTCT R: CCAGAGATGGTGAAGGTG
LGF1, insulin-like growth factor 1	F: GCTGCCGGCCCAGAA R: ACGAACTGAAGAGCATCAACCA
MYOG , myogenin	F: GGAGAAGCGGAGGCTGAAG R: GCAGAGTGCTGCGTTTCAGA
MYOZ2, миозенин ()	F: CAACACTCAGCAACAGAGGC R: GTATGGGCTCTCCACGATTTCT
GSTA3 gene associated with resistance to toxic and drug substances	F: TACATCGCAGGGAAATACA R: GGAGAGAAAGGAAACACCA

Primers for amplification of the housekeeping gene encoding the ACTB beta actin protein were used as a reference control: F, 5'-CTGTGCCCATCT-ATGAAGGCTA-3', R, 5'-ATTTCTCTCTCGGCTGTGGTG-3' [40]. The reaction was carried out using a DTlight amplifier (DNK-Technology, Russia) and a SsoAdvancedTM Universal SYBR® Green Supermix kit (Bio-Rad, USA) according to the manufacturer's protocol [41]. Amplification mode and conditions were as follows: 5 min at 95 °C (preheating); 30 s at 95 °C, 30 s at 60 °C 30 s at 70 °C (40 cycles) [42]. Relative expression was assessed by the $2^{-\Delta\Delta CT}$ method [43]. The live weight of broilers was determined at the age of 7, 14, 21, 28 and 35 days [44].

Mathematical and statistical processing of the results was carried out by the method of multivariate analysis of variance (ANOVA) in Microsoft Excel XP/2003, R-Studio (Version 1.1.453) (https://rstudio.com). Results are presented as means (*M*) and standard errors of the means (±SEM). Significance of differences was established 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 (https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/TukeyHSD).

Results. In 6 out of 11 studied *Bacillus* strains we revealed the ability to biodegrade glyphosate in vitro, the most pronounced in the strain *Bacillus* sp. GL-8 compared to others (53.0±4.10%) (Table). This fact suggests the presence of *Bacillus* sp. GL-8 enzymes associated with the biodegradation of xenobiotics. In the study of GL-8 for hemolytic activity on blood agar, we did not observe zones of enlightenment around the colonies.

The data obtained may be of great practical importance for the use of *Bacillus* sp. GL-8 as a probiotic in poultry populations exposed to glyphosates. Many bacteria have been shown to be able to metabolize glyphosate to non-toxic compounds. Its biodegradation leads to the formation of metabolites, which are used as a source of carbon, nitrogen and phosphorus, elements necessary for the development of organisms [45].

Bacterial degradation of glyphosate occurs via two metabolic pathways. The first pathway is carried out with the participation of the enzyme glyphosatoxidoreductase, which breaks down the glyphosate molecule into two metabolites: glyoxylate, which enters the tricarboxylic acid cycle and forms carbon dioxide due to complete oxidation, and aminomethylphosphonic acid which is hydrolyzed by the enzyme carbon-phosphorus lyase (C-P-lyase) to phosphate and methylamine. The latter is converted into ammonia (a direct source of nitrogen) and formaldehyde, which enters the tetrahydrofolate cycle. The second degradation pathway

involves the enzyme C-P-lyase, which, due to its hydrolytic activity, forms phosphate and sarcosine. At the next stage, due to the activity of the enzyme sarcosine oxidase, sarcosine is converted into the amino acid glycine, which is used directly for metabolism and microbial biosynthesis, and formaldehyde, which is introduced into the tetrahydrofolate cycle [46]. It was shown that *Arthrobacter* sp. GLP-1, *Alcaligenes* sp. GL, *Pseudomonas pseudomallei* 22 and *Flavobacterium* sp. GD1 use glyphosate as a source of phosphorus [47]. Probiotic strains of microorganisms have long been used as biodegraders of toxic compounds in the gut [48, 49]. Nevertheless, it cannot be ruled out that in our experiment a certain proportion or the entire volume of glyphosate could be subjected to sorption rather than biodegradation. Therefore, more extensive and detailed studies are required for conclusions.

Glyphosate biodegradation under the influence of *Bacillus* sp. from the collection of OOO BIOTROF+ ($n = 3$, $M \pm$SEM; in vitro test, OOO BIOTROF+, St. Petersburg, 2022)

Strain	Biodegradation rate, %
Bacillus sp. GL-1	15.4±2.40
Bacillus sp. GL-2	0
Bacillus sp. GL-3	19.2±3.90
Bacillus sp. GL-4	6.3±0.52
Bacillus sp. GL-5	0
Bacillus sp. GL-6	0
Bacillus sp. GL-7	0
Bacillus sp. GL-8	53.0±4.10
Bacillus sp. GL-9	0
Bacillus sp. GL-10	13.9±2.30
Bacillus sp. GL-11	25.6±2.40

According to the analysis of the increase in the live weight of poultry, antibiotics stimulated ($p \leq 0.05$) productivity from day 14 of life until the end of the experiment by 4.8-23.3% (group II compared to group I) (Fig. 1). An increase in the live weight gain of broilers under the influence of antibiotics has long been known [8]. At the end of the experiment, against the background of antibiotics, a negative effect of glyphosate on the productivity of broilers (group III compared to group II) was manifested ($p \leq 0.05$). This also seems logical since, firstly, glyphosate can cause intracellular changes and cytotoxicity [18]. Glyphosates are known to affect mitochondrial activity and likely increase DNA damage [17]. Second, at the tissue and body levels, glyphosates can interfere with neurotransmitter function and likely act as endocrine disruptors [50]. Recent studies in mammalian models have shown changes in hormone levels [51], impaired puberty and reproduction [52]. Third, glyphosates can affect organisms through changes in microbial communities. The shikimate pathway is present in most bacteria, and in many bacteria, its key enzyme, enolpyruvylshikimate 3-phosphate synthase (EPSPS), is sensitive to glyphosate [53, 54]. Recently, glyphosates have been found to adversely affect intestinal bacterial communities in several model organisms as well as in vitro cultures [55-57].

Application of Bacillus sp. GL-8 in combination with antibiotics and glyphosate did not have a statistically significant effect on broiler productivity (see Fig. 1). This may be due to various reasons, in particular, the negative effect of antibiotics on survival and gene expression in the biodegrading microorganism strain.

In connection with the revealed differences in the productivity of broilers, we analyzed the expression of genes associated with the growth and formation of muscle fibers in response to the introduction of antibiotics, glyphosate, and a strain of a biodegrading microorganism into the diets. The most significant changes concerned the *MYOG* gene, which promotes muscle development and differentiation. Expression of mRNA of the *MYOG* gene was 2.0 and 2.1 times higher in groups

II and IV, respectively, compared to group I ($p \le 0.05$) (Fig. 2).

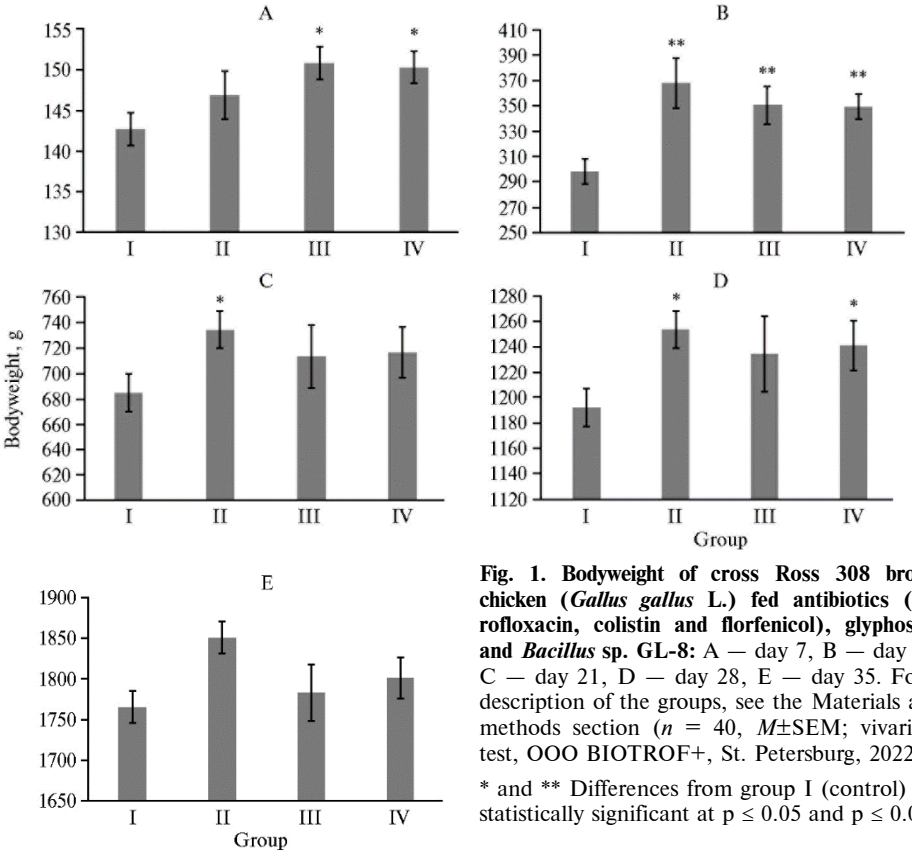

Fig. 1. Bodyweight of cross Ross 308 broiler chicken (*Gallus gallus* L.) fed antibiotics (enrofloxacin, colistin and florfenicol), glyphosate and *Bacillus* sp. GL-8: A — day 7, B — day 14, C — day 21, D — day 28, E — day 35. For a description of the groups, see the Materials and methods section (n = 40, M±SEM; vivarium test, OOO BIOTROF+, St. Petersburg, 2022).

* and ** Differences from group I (control) are statistically significant at $p \le 0.05$ and $p \le 0.01$.

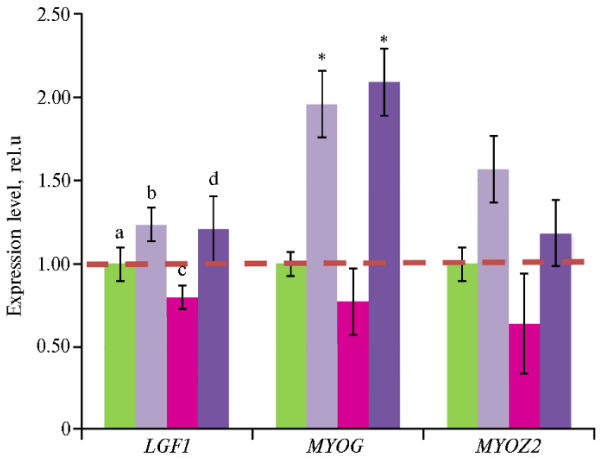

Fig. 2. mRNA expression levels of the genes *LGF1, MYOG* and *MYOZ* associated with growth and breast muscle formation in cross Ross 308 broiler chicken (*Gallus gallus* L.) fed antibiotics (enrofloxacin, colistin and florfenicol), glyphosate and *Bacillus* sp. GL-8: a — group I, b — group II, c — I group II, d — group IV; rel. u corresponds to the multiplicity of changes in expression compared with control group I, in which expression was taken as 1 (dashed red line corresponds to the the control expression). For a description of the groups, see the Materials and methods section (day 35, n = 3, M±SEM; vivarium test, OOO BIOTROF+, St. Petersburg, 2022).

* Differences from group I (control) are statistically significant at $p \le 0.05$.

The *MYOG* (myogenin) gene is a key regulatory transcription factor involved in muscle development during myogenesis [58]. There are also data on the role of *MYOG* after the completion of myogenesis. For example, a positive relationship has been reported between an increase in pectoral muscle mass and an increase in *MYOG* mRNA expression in 38-day-old broilers [59)]. Myogenin is known to play an important role in maintaining mitochondrial activity during exhausting exercise [60].

We believe that the increased expression of *MYOG* in our experiment

played a role in the increase in body weight of broilers in the variant with the antibiotic. Although *MYOG* functions are primarily associated with the induction of myogenesis, this gene also contributes to avian energy metabolism. Increased transcriptional activity of *MYOG* in experimental groups II and IV could be a factor contributing to the enhancement of mitochondrial function and increased energy accumulation. In group III, no activation of *MYOG* expression was noted ($p > 0.05$), which indicates a negative effect of glyphosate on the expression of bird productivity genes. In general, the data obtained indicated some smoothing of the negative effect of glyphosate during the introduction of a microorganism strain.

In the expression of the *LGF1* (insulin-like growth factor 1) and *MYOZ2* (myosenin) genes, we did not find any differences between the groups ($p > 0.05$).

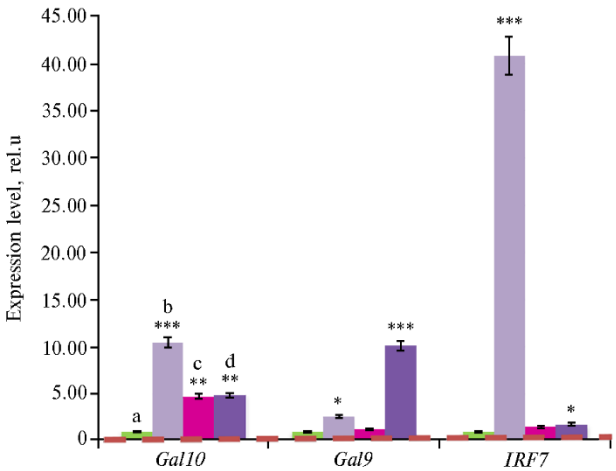

Fig. 3. mRNA expression levels of the genes of antimicrobial and antiviral defence *Gal9*, *Gal10* and *IRF7* in caecum of cross Ross 308 broiler chicken (*Gallus gallus* L.) fed antibiotics (enrofloxacin, colistin and florfenicol), glyphosate and *Bacillus* sp. GL-8: a — group I, b — group II, c — group III, d — group IV; rel. u corresponds to the multiplicity of changes in expression compared with control group I, in which expression was taken as 1 (dashed red line corresponds to the the control expression). For a description of the groups, see the Materials and methods section (day 35, $n = 3$, $M \pm$SEM; vivarium test, OOO BIOTROF+, St. Petersburg, 2022).

*, ** and *** Differences from group I (control) are statistically significant at $p \le 0.05$; $p \le 0.01$ и $p \le 0{,}001$.

With adding antibiotics in group II, the expression of antimicrobial and antiviral genes *Gal9*, *Gal10* and *IRF7* increased by 2.6, 10.5 and 40.8 times, respectively, compared to the control ($p \le 0.05$) (Fig. 3). *Gal9* (*AvBD9*) and *Gal10* (*AvBD10*) are genes associated with the synthesis of avian β-defensins [61]. Defensins promote adaptive immunity through the selective recruitment of monocytes, T-lymphocytes, immature dendritic and mast cells to infection sites [62, 63]. These compounds increase poultry resistance to many pathogens, including *Klebsiella pneumonia*, *Streptococcus bovis*, *Enterococcus faecalis*, *and Salmonella typhimurium* [64]. The *IRF7* gene, in turn, is associated with the synthesis of the regulatory factor interferon 7, a member of the family of regulatory interferon transcription factors [65]. Through its key role in immunity, *IRF7* has been implicated in increasing host resistance to many viruses through a variety of strategies [66]. We suggest that the expression of genes associated with antimicrobial and antiviral protection could be modulated both directly by antibiotics and by the luminal microbiota altered under their influence living in the caecum of broilers.

An increase in the expression of the described genes can also contribute to an increase in the live weight of broilers against the background of antibiotics due to a possible decrease in the pathogen load. Earlier T. Terada et al. [67] studied the effect of dietary antibiotics (penicillin and streptomycin) on gene expression in the caecum of broiler chickens. It was shown that on day 7 the expression of *AvBD1* and *AvBD2* decreased. However, on day 14, in the group treated with antibiotics, the expression of *TLR21* (toll-like receptor involved in antimicrobial protection) and antimicrobial peptide genes increased compared to the

control. In another study on 6-day-old chickens treated with enrofloxacin during the first 5 days of life, the antibiotic did not have a suppressive effect on the lymphocyte subpopulation [11].

In our experiment, glyphosate combined with antibiotics (group III) acted as a suppressor of the *Gal9*, *Gal10*, and *IRF7* expression compared to group II ($p \leq 0.05$). The decrease in the expression of the *Gal9* and *IRF7* genes in group III corresponded to the control without antibiotics ($p > 0.05$). The data obtained may indicate that glyphosate, present in feed even at the level of 1MPC (maximum permissible concentration), negatively affects the immune system, while reducing the therapeutic and zootechnical effects of antibiotics. This may partly explain the negative effect of glyphosate on broiler performance at the end of the experiment. Previously, similar data were obtained using the organochlorine pesticide dieldrin in rats [68]. Treatment of dopaminergic neuronal cells with dieldrin significantly reduced the expression of many genes, including antiviral response (*IFN*) genes [68].

The addition of *Bacillus* sp. GL-8 in feed against the background of glyphosate and antibiotics (group IV) led to increased expression of *Gal9* compared to group III (administration of glyphosate with antibiotics without a bacterial strain) ($p \leq 0.05$). Such results may indicate a certain prospect of reducing the negative impact of glyphosate on the mechanisms of antimicrobial and antiviral defense when using microorganisms with beneficial properties.

As for the antimicrobial and antiviral protection genes, there was a tendency to a sharp increase in the expression of pro-inflammatory genes *IL6*, *IL8* and *PTGS2* (4.6-fold, 11.2-fold, and 6.6-fold, respectively, in group II compared to control) ($p \leq 0.05$), which once again confirms the effect of antibiotics on immune processes (Fig. 4).

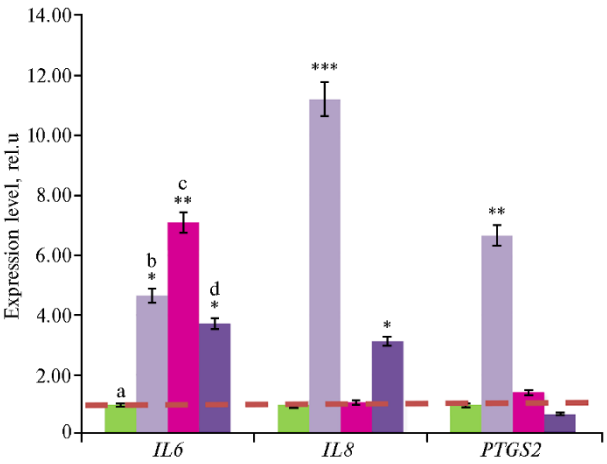

Fig. 4. mRNA expression levels of the proinflamatory genes *IL6, IL8* and *PTGS2* in caecum of cross Ross 308 broiler chicken (*Gallus gallus* L.) fed antibiotics (enrofloxacin, colistin and florfenicol), glyphosate and *Bacillus* sp. GL-8: a — group I, b — group II, c — I group II, d — group IV; rel. u corresponds to the multiplicity of changes in expression compared with control group I, in which expression was taken as 1 (dashed red line corresponds to the the control expression). For a description of the groups, see the Materials and methods section (day 35, $n = 3$, $M \pm SEM$; vivarium test, OOO BIOTROF+, St. Petersburg, 2022).
*, ** and *** Differences from group I (control) are statistically significant at $p \leq 0.05$; $p \leq 0.01$ и $p \leq 0{,}001$.

It is interesting that fluoroquinolones, which include enrofloxacin used in our experiment, affect the gene expression of many cytokines [69]. It has been noted that most fluoroquinolone derivatives superinduce the synthesis of interleukin 2 in vitro, but at the same time inhibit the synthesis of interleukin 1. Increased expression of pro-inflammatory genes when fed with an antibiotic can have various health consequences. On the one hand, interleukins (including *IL6*, *IL8*) are part of important innate protective immune responses, attracting additional leukocytes to the site of infection, which increase the resistance of epithelial cells [70, 71]. On the other hand, overproduction of pro-inflammatory cytokines is involved in the pathogenesis of a number of human diseases, including COVID-19 [72], and

is also associated with a decrease in the productivity of farm animals [73]. It has been proven [74, 75] that the administration of cytokine-based preparations to healthy animals provoked undesirable symptoms. Activation of pro-inflammatory cytokines is closely associated with *PTGS2* gene expression, since cytokines are able to induce it [76]. The *PTGS2* gene is associated with the synthesis of prostaglandin endoperoxide synthase (cyclooxygenase 2), which catalyzes the oxidative conversion of arachidonic acid to prostaglandin. Prostaglandin is subsequently metabolized to various biologically active metabolites such as prostacyclin and thromboxane A2, taking part in both local and systemic inflammatory responses [77].

In our experiment, the effect of glyphosate added to feed on pro-inflammatory genes manifested itself in different ways. Thus, the expression of *IL6* increased in group III compared to group II ($p \leq 0.05$), while the expression of *IL8* and *PTGS2* decreased ($p \leq 0.05$). The fact that pesticides in most cases serve as inducers of *IL6* expression has been reported in most previously published studies. For example, chronic exposure of rats to dichlorvosome (an organophosphorus insecticide) induces microglia activation with induction of NADPH oxidase and pro-inflammatory cytokines, including TNF-α, IL-1β, and IL6 [78]. Y. Zhang et al. [79] revealed an increase in malonic dialdehyde and IL6 in the muscles of rats exposed to omethoate, an insecticide widely used in developing countries. The authors concluded that omethoate may cause insulin resistance. In addition, a cross-sectional study has shown that farmers exposed to organophosphorus pesticides as a result of occupational activity are at risk of developing diabetes [79]. Cytokines produced by adipose tissue, such as TNF-α and IL6, control the secretion of C-reactive protein from the liver [80, 81]. Stimulation of this inflammatory mechanism appears to trigger insulin resistance in peripheral tissues [82].

The use of a microorganism-biodestructor had a positive effect on the expression of pro-inflammatory genes. Thus, *IL6* expression decreased in group IV compared to group III, while *IL8*, on the contrary, increased ($p \leq 0.05$). Indeed, some beneficial bacteria ferment dietary fiber to produce short-chain fatty acids such as acetate, propionate, and butyrate which are absorbed by intestinal cells and used as an energy source for their metabolism [83]. Short chain fatty acids, such as butyrate, have been shown to inhibit NO production and reduce the expression of cytokine genes such as *IL-1β*, *IL6*, *IFN-γ*, and *IL-10* [84].

The introduction of antibiotics into the diet of broilers had some stimulating effect on the expression of the *GSTA3* gene ($p \leq 0.05$) (Fig. 5). Interestingly, the addition of glyphosate to the diet against the background of antibiotics did not change the expression of this gene compared to that in group II ($p > 0.05$). The results obtained seem to be quite natural. The *GSTA3* gene is associated with the synthesis of glutathione-S-transferase, an enzyme responsible for the body's resistance to carcinogens, therapeutic drugs, environmental toxins, and oxidative stress products [85]. This enzyme catalyzes the nucleophilic scavenging of xenobiotics by glutathione, which neutralizes free radicals due to the high electron donating capacity of its sulfidryl (−SH) group and prevents damage to important cellular components, thereby participating in cellular defense against toxic substances. Glutathione S-transferase is abundant in the liver, gastrointestinal tract, lungs, and kidneys [85]. It is well known that coumarin, ethoxykin, aflatoxin B_1 and other compounds such as phenolic antioxidants and isothiocyanates act as inducers of xenobiotic metabolism enzymes [86-88]. Probably, in our experiment, antibiotics acted as inducers of the *GSTA3* gene, being substances foreign to the body.

The strain *Bacillus* sp. GL-8 had a positive effect on the expression of *GSTA3* (group IV compared to group III) ($p \leq 0.05$), it decreased to control values.

Previously, it was reported that the intestinal microbiota can influence the synthesis of enzymes that metabolize xenobiotics, in the large intestine and liver. The highest concentrations of enzymes that metabolize xenobiotics are observed in nonmicrobial animals [89]. Perhaps the effect of the introduction of the microorganism was associated with a decrease in the toxic load under the influence of antibiotics.

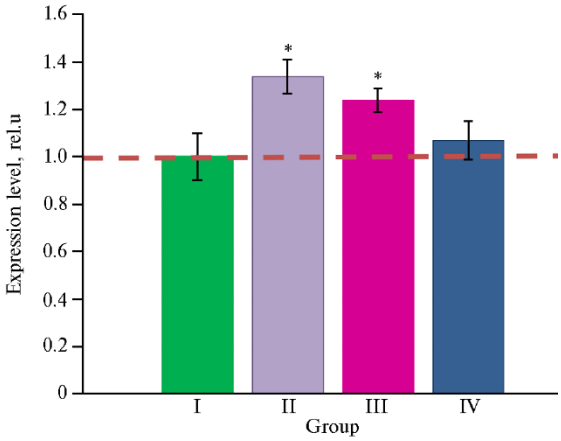

Fig. 5. mRNA expression levels of the *GSTA3* gene associated with esistance to toxic and drag subsyances in caecum of cross Ross 308 broiler chicken (*Gallus gallus* L.) fed antibiotics (enrofloxacin, colistin and florfenicol), glyphosate and *Bacillus* sp. GL-8. Rel. u corresponds to the multiplicity of changes in expression compared with control group I, in which expression was taken as 1 (dashed red line corresponds to the the control expression). For a description of the groups, see the Materials and methods section (day 35, $n = 3$, $M \pm$SEM; vivarium test, OOO BIOTROF+, St. Petersburg, 2022).
* Differences from group I (control) are statistically significant at $p \leq 0.05$.

Discussing the results obtained, it should be noted that information on the cellular and molecular processes by which antibiotics improve animal growth is limited, and the proposed hypotheses are reduced mainly to the possibility of microflora modulation. Understanding the biological mechanism of the action of antibiotics on the stimulation of the growth of farm animals and poultry is necessary to create effective alternatives to antibacterial drugs. Developed preparations should have similar stimulatory activity, but will avoid the problems of developing resistance to antimicrobial agents.

In the presented study, we revealed a positive effect of antibiotics on the performance of broilers, which corresponded to the level of expression of a number of genes, in particular, those associated with the development and differentiation of muscles. In all likelihood, the mechanism of the positive effect of antibiotics on productivity is partly due to the fact that they act as inducers of a number of important genes.

In practice, poultry is exposed not only to medicinal substances, but also to toxicants contained in feed, in particular pesticide residues. The health effects resulting from the synergistic action of antibiotics and pesticides are unpredictable. In our experiment, against the background of glyphosates applied in an amount corresponding to 1MPC, a decrease in the effect of productivity stimulation under the influence of antibiotics was observed in broilers. We have shown that glyphosate exposure occurs, among other things, through disruption of the activity of some key bird genes. The data obtained indicate the need to draw attention to the problem of the content of glyphosates in poultry feed and to clarify the limits of the MPC of glyphosates in feed.

The strain *Bacillus* sp. GL-8, which exhibits the properties of a biodestructor in vitro, did not contribute to a significant improvement in growth rates in broiler chickens with experimental fodder contamination with glyphosate. This indicates the need for selection of microorganisms, taking into account a complex of properties, including survival in the gastrointestinal tract, adhesion and other probiotic characteristics. Nevertheless, the observed positive changes in the transcription of a number of genes, including the genes of antimicrobial and antiviral protection, under the influence of a strain of a biodegrading microorganism indicate

the promise of using probiotics as a tool to mitigate the physiological imbalance against the background of the use of drugs and food contamination with toxic substances. In the future, it is of interest to accurately identify species and study other important probiotic properties and technological characteristics of the *Bacillus* sp. strain. GL-8, such as its resistance to antibiotics and drugs used to feed poultry.

This study presents the results of a complex multicomponent experiment in which we used three additives in the feed of broiler chickens with different effects and purposes (antibiotics, pesticide, *Bacillus* sp. strain). Of course, this complicates the interpretation of the results. For example, a bacterial strain could affect the productivity and expression of some genes in birds regardless of the administration of antibiotics, and antibiotics, in turn, could prevent colonization of the bird's intestines by this strain. In subsequent experiments, it is important to establish the exact effect of bacterial destructor strains on the productivity and expression of certain genes in birds, as well as the direct effect of antibiotics on the ability of destructor strains to colonize the intestines of birds. It is also of interest to study the colonization of the intestine by strains of probiotic microorganisms.

So, out of 11 studied strains of bacilli, *Bacillus* sp. GL-8 possesses the most pronounced ability to biodegrade glyphosate (53.0±4.10%). Antibiotics enrofloxacin, colistin and florfenicol stimulated the increase in body weight in Ross 308 cross broiler chickens from day 14 of life until the end of the experiment by 4.8-23.3%. By the end of the experiment, the negative effect of glyphosate on the productivity of broilers against the background of antibiotics was manifested. Expression of the *MYOG* gene mRNA which promotes muscle development and differentiation was 2.0 times and 2.1 times higher in broilers treated with antibiotics alone or in combination with *Bacillus* sp. GL-8, respectively, compared to control. When glyphosate was added to the feed against the background of an antibiotic without the introduction of a biodegrading microorganism strain, no changes in the expression of the *MYOG* gene were noted. With the introduction of antibiotics, the expression of antimicrobial (*Gal9*, *Gal10*) and antiviral (*IRF7*) protection genes increased by 2.6 times, 10.5 times and 40.8 times, respectively, compared to control. Glyphosate suppressed the expression of antimicrobial and antiviral genes. Dietary *Bacillus* sp. GL-8, when glyphosate and antibiotics were used, increased the expression of *Gal9*. Similar to antimicrobial and antiviral protection genes, the pro-inflammatory genes *IL6*, *IL8*, and *PTGS2* showed a tendency to a sharp increase in expression (by 4.6; 11.2 and 6.6 times, respectively) with the use of antibiotics. The introduction of antibiotics into the diet also had some stimulating effect on the expression of the *GSTA3* gene associated with resistance to toxic and medicinal substances.

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