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BIOCHEMICAL AND MOLECULAR GENETIC INDICATORS OF ANTIOXIDANT PROTECTION AND IMMUNITY IN MALE CHICKS (*Gallus gallus domesticus*) OF DIFFERENT GENOTYPES

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Abstract

A comparative study of the relationship between the antioxidant protection (AOP) and immunity in poultry of various genotypes is relevant for clinical and physiological assessment of health status and the search for combinations of genotypes to obtain new crosses. In this work, for the first time, differences in biochemical and molecular genetic indicators of antioxidant protection and immunity were established for the Russian White, Ross 308 cross, and Russian White × Cornish cockerels. Correlations were revealed between the expression of some genes for AOP and immunity enzymes in caecum and liver tissues, and the average daily weight gain. The aim of the work was to assess the factors of immunity and antioxidant status, nonspecific immunity indicators, and gene expression levels for enzymes involved in antioxidant protection and immune response in male chickens (*Gallus gallus domesticus*) of different genotypes. The studies were carried out in 2022 at physiological yard of the Ernst Federal Research Center for Animal Husbandry. Blood samples were taken from Russian White cockerels (RW, $n = 28$), Ross 308 cross broilers ($n = 9$) and Russian White × Cornish cockerels (CORN × RW, $n = 128$) at slaughter at the age of 9 weeks. The TBA test with thiobarbituric acid to measure the TBA-active products (TBA-AP) was performed with Agat-Med kits (Russia). The activity of ceruloplasmin (CP) was determined by the Revin method, the amount of total water-soluble antioxidants (TWSA) amperometrially (a TsvetYauza-01-AA with an amperometric detector, Khimavtomatika, Russia), the ratio of TBA-AP to the CP was calculated. TAWSA was evaluated as equivalents to gallic acid using calibration solutions with a mass concentration of 0.2, 0.5, 1.0 and 4.0 mg/dm^3 prepared from 100 mg/dm^3 gallic acid. A solution of orthophosphoric acid (0.0022 mol/dm^3) was used as an eluent. Other indicators of antioxidant status were determined with commercial kits (Elabscience Elabscience Biotechnology, Inc., China). Reduced glutathione (E-BC-K096-M), superoxide dismutase (SOD) (E-BC-K020-M), catalase (E-BC-K031-M) and total antioxidant status (TAS) (E-BC-K219-M) were measured by ELISA test (an Immunochem-2100 microplate photometer, High Technology Inc., USA). Nonspecific immunity (i.e., bactericidal activity BA and lysozyme activity LA) of RW ($n = 12$), CORN × RW ($n = 68$) and Ross 308 ($n = 9$) male chicks were determined (a microbiological analyzer Multiskan FC, ThermoFisher Scientific Inc., Finland). Analysis of relative gene expression was performed using real-time PCR. Tissue samples of the caecum and liver were taken from RW ($n = 10$), Ross 308 ($n = 9$), and CORN × RW ($n = 11$) cockerels, 30 samples of each tissue. The relative expression of the genes responsible for antioxidant protection (catalase *CAT*, glutathione peroxidase *GSH-Gpx*, heme oxygenase 1 *HO-1*, superoxide dismutase *SOD*, related transcription factor 2, NF-E2 *Nrf2*) and involved in the immune response (avian beta defensin 9 *AvBD9*, interleukin 6 *IL6*, interleukin 8 *IL8*) was assessed. Total antioxidant status (TAS) of broilers was lower

than that of analogues, which was confirmed by the maximum content of TBA-AP, 4.27 vs. 3.04 μmol/l for RW ($p < 0.05$) and 2.79 μmol/l ($p < 0.01$) for CORN × RW, with a minimum content of ceruloplasmin (37.78 mg/l), and, accordingly, a higher TBA-AP/CP ratio. In the blood of Ross 308 cross males, the maximum TWSA was detected (49.78 mg/l at $p < 0.001$ compared to RW), which was due to the maximum amount of reduced glutathione among analogues (38.26 μmol/l at $p < 0.001$ compared to RW and $p < 0.001$ compared to CORN × RW). The blood activity of catalase in broilers was also high (100.50 U/l at $p < 0.05$ compared to RW and $p < 0.01$ compared to CORN × RW). However, their antioxidant system must work at the maximum to neutralization of reactive oxygen species (ROS). Our data on the expression of AOP and immunity genes confirmed these conclusions. In the caeca of broilers, the genes *CAT* and *GSH-Gpx* expression was 5 times higher compared to egg breed cockerels ($p = 0.0007$ and $p = 0.0008$, respectively), *HO-1* 2 times higher ($p = 0.01$), *SOD* higher by 40 %. In the liver of broilers, there was a decrease in the genes *SOD* and *GSH-Gpx* expression by 5-6 times compared to RW ($p = 0.005$ for both genes), *CAT* expression increased by 27 %, and *HO-1* by 42 times ($p = 0.001$). In broilers, the blood lysozyme concertation and activity were the highest (0.47 μg/ml and 3.14 AU/TP, $p < 0.001$) with a decrease in the percentage of lysis (36.1 vs. 45.6-48.7% in other cockerels, $p < 0.05$) with the minimum BA among analogues. This is confirmed by the fact that the expression of pro-inflammatory cytokines (primarily IL-8) which inhibit humoral immunity was generally lower in the studied broiler tissues while it increased in males of other genotypes. This could lead to a decrease in the humoral response. The average daily weigh gain of poultry highly correlated with the *CAT* ($r = 0.998$ at $p = 0.03$) and *AvBD-9* ($r = 0.999$ at $p = 0.016$) expression in the caecum. In the caecum, high correlations were found between the expression of *CAT* and *AvBD-9* ($r = 0.999$ at $p = 0.014$), *IL6* and *HO-1* ($r = 0.999$ at $p = 0.1$), which confirms the relationship between AOP and bird health. Ross 308 cross broilers showed a higher accumulation of lipid peroxidation products. This highlights the feasibility of using nutritional factors to reduce oxidative stress and increase the antioxidant potential of the body to improve the quality of poultry products.

Keywords: antioxidant status, immunity, chickens, broilers, genotypes, gene expression, *CAT*, *GSH-Gpx*, *HO-1*, *SOD*, *Nrf2*, *AvBD9*, *IL6*, *IL8*

Poultry of modern genotypes have a high productivity potential, but it is often not fully realized because of stresses of various etiologies. Oxidative stress, which results from an imbalance in the formation and detoxification of free radicals as a result of disturbances in feed, climatic, technological and biological growing conditions, negatively affects health, growth performance and product quality. The effects of various stresses on the bird's body were described in our previous work [1]. It has been established that climatic and other maintenance factors determine behavioral, physiological and immune reactions in the body, affect antioxidant and biochemical status, and productivity. The works of various authors are devoted to studying the influence of stress of various etiologies, including oxidative stress, on the poultry body and product quality [2-5]. It has been shown that oxidative stress can impair health status, growth performance, and meat quality [6-9].

Reactive oxygen species (ROS) are produced in mitochondria [10]. During normal physiological processes in the body, the production and clearance of ROS are in dynamic equilibrium [11]. However, when this equilibrium is shifted, ROS can lead to oxidative damage, pathological processes, and even cell death [12]. Oxidative stress induces sensitivity to ROS, signaling through certain pathways, and activation of target genes, such as those encoding antioxidant defenses and certain inflammatory mediators.

There is a relationship between antioxidant defense systems and the body's natural resistance. Thus, an increase in free radical reactions of lipid peroxidation (LPO) leads to disruption of the function of processing antigenic information and the synthesis of antibodies. By damaging cellular structures, oxidative stress can trigger or enhance the inflammatory response caused by immune cells and mediators [13]. A number of immunomodulators block the lipid peroxidation of plasma and subcellular membranes, protecting them from the action of peroxides and free radicals, which are mostly formed in metabolically active cells (macrophages, neutrophils), and thereby maintaining the normal structure and function of membranes [14].

The body contains a variety of antioxidant molecules that counteract and neutralize ROS and other oxidants. Antioxidant defense systems can be divided into two categories, the enzymatic and non-enzymatic. They can also be classified as endogenously produced or exogenously produced dietary antioxidants [15].

It is known that the antioxidant protection and immunity status are influenced by exo- and endogenous factors. The features of physiological and biochemical processes in the body, which determine the intensity of metabolism and growth rate of a bird, largely depend on its origin and direction of productivity [16]. Many researchers associate the antioxidant properties of poultry products with the poultry breed [17, 18]. However, little is known about the effect of genotype on antioxidant defense in poultry. Of interest is a comparative study of the relationship between the processes of antioxidant protection and immunity in poultry of different genotypes, which is important in the clinical and physiological assessment of health status and the search for combinations of genotypes to obtain new crosses. An integrated approach, including biochemical, microbiological and molecular genetic methods, provides a deeper understanding of the mechanisms of immunity formation and antioxidant protection, which is necessary to obtain high-quality poultry products.

In this work, for the first time, differences in biochemical and molecular genetic indicators of antioxidant protection and immunity were established in males of the White Russian breeds, the Ross 308 cross and crosses of the Russian White and Cornish breeds. Correlations were revealed between the expression of some genes of AOD enzymes and immunity in the cecum of the intestine, liver tissue and the average daily increase in live weight, as well as between the relative expression of genes.

The purpose of the work was to reveal factors invlolved in the formation of immunity and antioxidant status, indicators of nonspecific immunity, gene expression for enzymes involved in antioxidant protection and the development of the immune response in cockerels (*Gallus gallus domesticus*) of different genotypes.

Materials and methods. The study was carried out on cockerels (*Gallus gallus domesticus*) of the Russian White breed (RW, $n = 28$), broilers of the Ross 308 cross ($n = 9$) and Russian White × Cornish crossbreds (CORN × RW, $n = 128$). The birds were kept in the same conditions (the physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022). For the biochemical analysis of pro- and antioxidant status, blood samples were collected at slaughter at the age of 9 weeks.

In blood serum, the concentration of products that react with thiobarbituric acid (TBA-AP) was determined using Agat-Med kits (Russia), ceruloplasmin activity (CP) according to the Revin method [19], the total content of water-soluble antioxidants (TWSA) amperometrically (a TsvetYauza-01-AA device with an amperometric detector, Khimavtomatika, Russia), the ratio of TBK-AP to CP by calculation.

To determine TWSA, the strength of the electric current that occurs during the oxidation of molecules on the surface of the electrode at a certain potential was measured. TWSA was estimated in equivalents of gallic acid. For this purpose, working solutions for calibration with a mass concentration of 0.2, 0.5, 1.0 and 4.0 mg/dm^3 were prepared from 100 mg/dm^3 solution of gallic acid. Orthophosphoric acid solution (0.0022 mol/dm^3) was an eluent. Other indicators of antioxidant status, namely the content of reduced glutathione (E-BC-K096-M), the activity of superoxide dismutase (SOD) (E-BC-K020-M), catalase (E-BC-K031-M) and total antioxidant status (TAS) (E-BC-K219-M) was determined by ELISA test using an Immunochem-2100 microplate photometer (High Technology, Inc., USA) and commercial kits (Elabscience Biotechnology, Inc., China) according to

the protocols suggested by the manufacturers.

Indicators of nonspecific immunity of males RW (n = 12), CORN × RW (n = 68) and Ross 308 (n = 9), the blood serum bactericidal (BSBA) and lysozyme activity were determined using a Multiskan FC microbiological analyzer (ThermoFisher Scientific, Inc., Finland) [20]. Serum lysozyme activity (LA) was characterized by the percentage of lysis, the amount of lysozyme in 1 ml of blood serum (lysozyme, μg/ml) and specific activity units (AU) per 1 mg of protein (AU/TP) [21].

Relative gene expression analysis was performed by real-time PCR. Tissue samples of the caeca and liver were collected from males RW (n = 10), Ross 308 (n = 9) and CORN × RW (n = 11) (a total of 30 samples of each tissue). The relative expression was determined for genes responsible for antioxidant protection (catalase — *CAT*, glutathione peroxidase — *GSH-Gpx*, heme oxygenase 1 — *HO-1*, superoxide dismutase — *SOD*, related transcription factor 2 NF-E2 — *Nrf2*) and involved in the immune response (avian beta-defensin 9 — *AvBD9*, interleukin 6 — *IL6*, interleukin 8 — *IL8*). Samples were prepared as per the Instructions for sanitary and microbiological control of carcasses, poultry meat, poultry products, eggs and egg products at poultry farming and processing enterprises (https://meganorm.ru/Data2/1/4293751/4293751517.pdf). Samples were placed in IntactRNA solution (Evrogen, Russia) and stored at –20 °C. The studies were carried out in 3 repetitions.

Total RNA from the samples was isolated using the Aurum Total RNA kit (Bio-Rad, USA) according to the manufacturer's instructions. Homogenization of samples were homogenized (a Precellys Evolution homogenizer, Bertin Technologies SAS, France). Using the iScript™ RT Supermix kit (Bio-Rad, USA), a reverse transcription reaction was performed to obtain cDNA on an RNA template.

Amplification was carried out using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, USA) in accordance with the manufacturer's protocol [22] (a DTlight detecting amplifier, NPO DNA-Technology, Russia). Amplification mode and conditions are 5 min at 95 °C (pre-denaturation); 30 s at 95 °C, 30 s at 60 °C, 30 s at 70 °C (40 cycles) [23]. Relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method [24]. The bird beta-actin protein gene *ACTB* was a reference gene. The primer sequences (5′ → 3′) were for *SOD* F: CGGGCCAGTAAAGGTTACTGGAA, R: TGTTGTCTCCAAATTCATGCACATG; for *GSH-Px* F: GCATCCGCTTCCACGACTTCCT, R: CCGCTCATCCGGGTCCAACAT; for *HO-1* F: GGTCCCGAATGAATGCCCTTG, R: ACCGTTCTCCTGGCTCTTGG, for *CAT* F: ACCAAGTACTGCAAGGCGAA, R: TGAGGGTTCCTCTTCTGGCT; for *Nrf2* F: AAAACGCTGAACCACCAATC, R: GCTGGAGAAGCCTCATTGTC; for *AvBD-9* F: AACACCGTCAGGCATCTTCACA, R: CGTCTTCTTGGCTGTAAGCTGGA; for *IL6* F: AGGACGAGATGTGCAAGAAGTTC, R: TTGGGCAGGTTGAGGTTGTT; for IL8 F: GGAAGAGAGGTGTGCTTGGA, R: TAACATGAGGCACCGATGTG.

The average daily weight gain was determined by weighing performed strictly before feeding. We used analytical scales PR224 (Ohaus Corp., USA) for 1-day-old chickens, electronic kitchen scales VT-8008 (Vitek, Russia) for chickens aged from 1 day to 3-4 weeks, and an electronic steelyard MT-1645 (MARTA, China) for adults.

Mathematical processing of the results was carried out with the software packages Microsoft Office Excel 2003, STATISTICA 10 (Statistica 13RU, StatSoft, Inc., USA) using descriptive statistics and correlation analysis methods. Mean values (M), standard errors of means (±SEM), standard deviations (±SD), coefficients of variation (Cv), and Pearson correlation coefficients (r) were calculated. Correlations at r up to 0.2 were considered very weak, 0.2-0.5 weak, 0.5-

0.7 medium, 0.7-0.9 high, more than 0.9 very high. The scatter of data values was considered insignificant at $Cv < 10\%$, medium at $Cv = 10\text{-}20\%$, and significant at $20\% < Cv \leq 33\%$. Differences were statistically significant at $p < 0.05$, highly significant at $p < 0.01$, $p < 0.001$.

Results. The intensity of lipid peroxidation is determined both by radical and peroxide formation and by the state of endogenous antioxidant defense, therefore, determining the antioxidant activity of these systems is of practical importance [25]. Often, to assess the state of lipid peroxidation, the reaction with thiobarbituric acid (TBA) is widely used. The TBA test is based on the ability of TBA to react with malondialdehyde (MDA), a low molecular weight compound that serves as an intermediate in the enzymatic oxidation of arachidonic acid and as an end product in the oxidative degradation of lipids [26, 27].

LPO products, in particular MDA, exhibit cytotoxic, mutagenic and carcinogenic properties. The consequences of their exposure include, for example, loss of cell proliferation potential, changes in gene expression, mutations, molecular heterogeneity, disruption of intercellular communication, and organ dysfunction [28]. In addition, MDA is one of the end products of the peroxidation of polyunsaturated fatty acids in the human body and a marker of oxidative stress.

1. Parameters of antioxidant protection and lipid peroxidation in cockerels (G*allus gallus domesticus*) of different genotypes (physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022)

Parameter	*M*	min	max	±SEM	±SD	*Cv*, %
Russian white breed ($n = 28$)						
TBA-AP, μmol/l	3,04*	2,26	4,10	0,08	0,43	14,22
CP, mg/l	62,53	38,00	117,00	3,43	18,14	29,00
TWSA, mg/l	45,20	29,43	75,54	2,02	10,67	12,61
Reduced glutathione, μmol/l	23,84***	11,47	37,74	2,04	7,62	31,96
SOD, U/ml	19,45***	17,58	20,19	0,22	0,83	4,25
Catalase, U/l	52,25*	6,67	196,06	14,16	52,97	101,37
TAS, mmol/l	0,69	0,28	0,95	0,04	0,15	22,09
TBA-AP/CP	0,05					
Cross Ross 308 broilers ($n = 9$)						
TBA-AP, μmol/l	4,27	1,95	6,87	0,57	1,70	39,78
CP, mg/l	37,78†††	25,00	66,00	3,95	11,84	31,34
TWSA, mg/l	49,78	42,28	59,46	1,61	4,82	9,69
Reduced glutathione, μmol/l	38,26	29,83	48,84	2,23	6,69	17,48
SOD, U/ml	15,22	10,89	18,03	0,76	2,27	14,90
Catalase, U/l	100,50	46,06	217,27	17,69	53,07	52,80
TAS, mmol/l	0,64	0,36	0,99	0,07	0,21	33,15
TBA-AP/CP	0,11					
Crosses of Russian White and Cornish breeds						
TBA-AP, μmol/l	2,79**	1,33	5,23	0,06	0,72	25,72
CP, mg/l	41,94††	23,00	78,00	0,99	11,16	26,62
TWSA, mg/l	41,03***	22,80	73,55	0,97	11,00	26,81
Reduced glutathione, μmol/l	22,02***	7,86	44,91	2,01	10,26	46,59
SOD, U/ml	19,30***	16,57	21,21	0,20	1,04	5,41
Catalase, U/l	32,94**	11,81	61,81	6,68	17,69	53,72
TAS, mmol/l	0,78†	0,59	1,37	0,03	0,16	20,27
TBA-AP/CP	0,07					

N o t e. TBA-AP — thiobarbituric acid reacting products, TWSA — total amount of water-soluble antioxidants, CP — ceruloplasmin, SOD — superoxide dismutase, TAS — total antioxidant status

*, **, *** Differences from broilers are statistically significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; †, ††, ††† — compared to the Russian White breed at $p < 0.05$, $p < 0.01$, and $p < 0{,}001$, respectively.

In our study, the maximum blood content of TBA-AP was in broilers, 4.27 μmol/l vs. 3.04 μmol/l in RW males ($p < 0.05$) and 2.79 μmol/l in CORN × RW ($p < 0.01$) (Table 1).

Attention to ceruloplasmin is due to the fact that it is one of the main scavengers of extracellular free radicals. Ceruloplasmin specifically inhibits damage to various biomolecules [29]. The blood content of ceruloplasmin in RW birds was 62.53 mg/l, in CORN × RW crossbreds 41.94 mg/l, and in broilers 37.78 mg/l. The difference was statistically significant between RW and broiler

groups ($p < 0.001$), as well as between RW and CORN × RW ($p < 0.01$).

TWSA in birds ranged within 45.20-49.78 mg/l with the maximum value in broilers (a significant difference with CORN × RW, $p < 0.001$). In these birds, the parameter showed low variability. In RW males, TWSA was 45.20 mg/l with moderate variability. In CORN × RW, TWSA was the minimum and highly variable (41.03 mg/l). The maximum TWSA in broilers vs. other genotypes was most likely associated with an increase in the amount of reduced glutathione and other water-soluble antioxidants the content of which we neglected.

The amount of reduced glutathione and catalase activity in broilers were the highest among the analyzed genotypes, 38.26 μmol/l (at $p < 0.001$ compared to RW and $p < 0.001$ compared to crossbreeds) and 100.50 U/l (at $p < 0.05$ and $p < 0.01$). SOD activity, on the contrary, turned out to be minimum, 15.22 U/ml (at $p < 0.001$ compared to RW and $p < 0.001$ compared to crossbreeds). TAS in broilers was lower than in analogues, which can be explained by the high consumption of various antioxidants for interaction with LPO products TBA-AP. When assessing the relationship between lipid peroxidation and antioxidant defense (AOD) based on the ratio of a number of components of these systems, we noted that the TBA-AP/CP ratio in broilers, due to low CP activity, was higher than in birds of other genotypes. That is, the antioxidant system in broilers of the Ross 308 cross is the most vulnerable, despite the high TWSA level and catalase activity. This fact is confirmed by the minimum TAS value which characterizes the state of all antioxidants in the body (30).

Thus, the high growth rate of broilers makes them more susceptible to oxidative stress, which can negatively affect meat quality. According to I.F. Gorlov et al. [31], the oxidative changes in chilled meat depends on the reactivity of the poultry antioxidant system and the formation of lipid peroxidation products. The weakening of antioxidant activity and activation of free radical oxidation of lipids in the blood of broiler chickens enhance the processes of meat oxidation [31]. In addition, stress increases the production of free radicals which can inactivate essential antioxidant enzymes, causing autocatalytic irreversible oxidation [32]. In this regard, the synthesis of new antioxidant enzymes is the most important response to stress conditions, which can explain the higher activity of catalase, the content of reduced glutathione and TWSA in broilers.

M. Madkour et al. [33] noted a decrease in the activity of catalase and superoxide dismutase in the liver tissues of broilers under heat stress at an early age compared to poultry raised under normal conditions.

K.S. Ostrenko et al. [34] propose to replenish the need for antioxidants with a decrease in the ability of laying hens to adapt to changing environment due to long-term selection for maximum egg productivity. In the work of these authors, when using the dietary water-soluble antioxidant dihydroethoxyquin, the content of lipid peroxidation products and holesterol in lipoprotein fractions of various densities decreased [34].

Nonspecific resistance is the ability to maintain optimal functioning of organs, systems, or the entire body, both under stereotypical conditions and all kinds of influences [35]. The decisive link in the chain of environment—body response—productivity should be considered the genotype [36]. When studying the indicators of nonspecific immunity in cockerels, we identified differences in indicators depending on the genotype of the bird (Table 2).

The resistance of a bird is largely determined by the humoral immunity level. Lysozyme, performing important biological functions in the body (bactericidal properties, stimulating effects on phagocytosis, neutralization of some microbial toxins, anti-inflammatory effect) serves as one of the key humoral factors

of innate immunity. The lysozyme gene is gradually activated in mature macrophages [37].

In broilers, we found the highest blood content and highest activity of lysozyme (0.47 μg/ml and 3.14 AU/TP, $p < 0.001$) and a decrease in the percentage of lysis (36.1% vs. 45.6-48.7 % in other cockerels, $p < 0.05$). That is, the mechanisms of humoral immunity activation in birds of different genotypes differ. V.G. Ovsyannikova et al. [38] reported that humoral factors of innate immunity are involved in the acute algogenic process, providing preventive protection against pervasion of microbial agents. This response does not last long, but it should be noted that in broilers it was stronger.

2. Parameters of nonspecific immunity in cockerels (G*allus gallus domesticus*) of different genotypes (physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022)

Parameter	*M*	min	max	±SEM	±SD	*Cv*, %
Russian white breed ($n = 12$)						
Lysis, %	48.70*	32.87	64.59	2.56	8.85	18.17
Lysozyme, μg/ml serum	0.36*	0.22	0.45	0.02	0.06	16.53
Lysozyme activity, AU/TP	2.42†	1.27	3.32	0.16	0.56	23.09
BSBA, %	55.32*†	37.10	66.00	2.44	8.47	15.31
Cross Ross 308 broilers ($n = 9$)						
Lysis, %	36.10	16.07	56.92	4.94	14.82	41.05
Lysozyme, μg/ml serum	0.47	0.34	0.80	0.05	0.15	31.71
Lysozyme activity, AU/TP	3.14	2.02	6.17	0.43	1.28	40.92
BSBA, %	35.14	5.20	54.00	7.09	21.27	60.51
Crosses of Russian White and Cornish breeds ($n = 68$)						
Lysis, %	45.60*	9.38	91.50	2.29	18.87	41.38
Lysozyme, μg/ml serum	0.25***	0.05	0.50	0.01	0.10	41.94
Lysozyme activity, AU/TP	1.34***	0.07	3.16	0.11	0.89	66.14
BSBA, %	37.99	16.40	69.90	1.44	11.90	31.33

Note. BSBA is blood serum bactericidal activity.
*, ** Differences from broilers are statistically significant at $p < 0.05$ and $p < 0{,}001$; † — compared to crossbred poultry at $p < 0{,}001$.

Cytokines are polypeptides or glycoproteins that are synthesized and secreted primarily by immune cells. Cytokines are involved in nonspecific resistance reactions, cellular and humoral immunity [39]. Interleukins, cytokines, and tumor necrosis factor are the main inflammatory mediators secreted by immune cells to control the inflammatory response. Proinflammatory cytokines (including IL-6 and IL-8) enhance cellular immunity and inhibit humoral immunity [40], while playing a major role in the formation of antiviral defense.

As will be shown below, in broilers the expression of genes for pro-inflammatory cytokines (primarily IL-8) was generally lower, and in Russian White males and crossbreds it increased in the tissues of the caecum and liver; this could reduce the humoral response, expressed as a decrease in BSBA (see Table 2). IL-6, by suppressing the secretion of pro-inflammatory cytokines, acts as an anti-inflammatory factor.

V.I. Fisinin et al. [41] showed that lysozyme titer, which correlates with the amount of cytokines and glucocorticoid hormones, could serve as a marker of stress. Cytokines mediate interactions between cells and perform various biological functions, in particular they regulate cell growth, differentiation and maturation, the immune response, and are involved in inflammation and wound healing. Decreased cytokine levels may slow down the differentiation of stem cells into mature immune cells and lead to decreased disease resistance [42]. Sanitary and bacteriological indicators of the microclimate at a poultry farm affect the general clinical condition and safety of the livestock. Resistance parameters should be considered depending on whether the poultry is used for meat or eggs To ensure optimal performance, it is important to monitor and, if necessary, modulate the immune response to maintain homeostasis.

3. Relative expression of genes in the cecum of the intestine and in the liver of cockerels (G*allus gallus domesticus*) of different genotypes (physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022)

Genotype	Gene							
	CAT	*GSH-Gpx*	*HO-1*	*SOD*	*AvBD9*	*IL6*	*IL8*	*NrF2*
Cecum								
RW (C) (n = 10)	1	1	1	1	1	1	1	1
CORN × RW (n = 11)	2.42±0.169	2.84±0.257	2.18±0.163	2.04±0.219	1.82±0.095	1.87±0.093	5.18±0.692	1.16±0.157
Ross 308 (n = 9)	5.06±0.274	4.73±0.270	2.05±0.216	1.41±0.155	3.22±0.189	1.76±0.215	0.67±0.052	1.58±0.210
Liver								
RW (C) (n = 10)	1	1	1	1	1	1	1	1
CORN × RW (n = 11)	1.14±0.110	1.57±0.155	119.63±9.005	1.33±0.132	25.52±4.294	3.43±0.371	1.58±0.178	0.52±0.100
Ross 308 (n = 9)	1.27±0.099	0.15±0.066	42.19±3.486	0.18±0.012	19.15±1.391	0.98±0.100	0.46±0.090	0.96±0.129

N o t e. RW is Russian White breed (control — C), Ross 308 are broilers of cross Ross 308, CORN × RW is cross between Russian White and Cornish breeds; *CAT* — catalase, *GSH-Gpx* — glutathione peroxidase, *HO-1* — heme oxygenase 1, *SOD* — superoxide dismutase, *NrF2* — related transcription factor 2, *AvBD9* — avian beta defensin 9, *IL6* — interleukin 6, *IL8* — interleukin 8. The results in graphical form are presented at http://www.agrobiology.ru.

Adaptation to stress occurs at the level of genes, which are called vitagens. These include genes responsible for the synthesis of protective molecules, including heat shock proteins, immunoglobulins, and antioxidant enzymes [43]. Studying the expression of these genes is a new approach to the diagnosis and prevention of stress at the molecular level [44]. Our studies have shown that the transcriptional activity of the analyzed genes depends not only on the genotype of the bird, but also on the organ and tissue in which these genes are expressed (Table 3).

The biological role of superoxide dismutase (SOD) is to catalyze the dismutation of superoxide radical into hydrogen peroxide. Gluthione peroxidase catalyzes the reaction and reduces hydrogen peroxide to water using reduced glutathione as a co-substrate. Tissue-specific expression and activity of *SOD* and *GSH-Gpx* are influenced by various factors, including genetic, nutritional, and stress-related [45]. Heme oxygenase 1, known as heat shock protein-32 (HSP32), is responsible for the degradation of heme to produce carbon monoxide, biliverdin and free iron. Like HSP70, HO-1 is a stress-inducible, one of the three HO isoforms described to date, providing a critical protective mechanism in systems that are responsible for adaptation to oxidative, inflammatory, and cytotoxic stress [46].

In most tissues, HO-1 is expressed at relatively low levels and can be induced by various insults associated with oxidative stress (heme, ultraviolet radiation, heavy metals, cytokines, hydrogen peroxide, nitric oxide NO, glutathione depletion) [47].

In the intestinal cecum of birds of three genotypes we studied, the expression of the *CAT*, *GSH-Gpx*, *HO-1*, and *SOD* genes differed. In CORN × RW compared to RW, the gene relative expression was higher, e.g., for *GSH-Gpx* 2.84-fold ($p = 0.006$), for *CAT* 2.42-fold ($p = 0.004$), for *HO-1* 2.18-fold ($p = 0.01$), and for *SOD* 2.0-fold ($p = 0.02$). In broilers, the relative expression of *CAT* and *GSH-Gpx* increased 5-fold vs. egg poultry ($p = 0.0007$ and $p = 0.0008$, respectively), for *HO-1* the relative expression was 2 times higher ($p = 0.01$), for *SOD* 40% higher. The data obtained are consistent with the blood catalase activity and reduced glutathione in the broilers.

Differences in the expression of genes associated with antioxidant defense were also found in the liver of coccerels. In CORN × RW, the expression was higher than in RW, for *HO-1* by 119 times ($p = 0.001$), for *SOD* by 33%, for *GSH-Gpx* by 57%, and for *CAT* by 14%. In the broilers, a 5-6-fold decrease in the relative expression of the antioxidant defense genes *SOD* and *GSH-Gpx* occurredv compared to the RW group ($p = 0.005$). The relative expression of *CAT* in the broilers turned out to be 27% higher, *HO-1* 42 times higher ($p = 0.001$).

The Nrf2 transcription factor is best known as one of the main participants in the development of cellular responses to xenobiotics and oxidative stress. Recent studies have identified new Nrf2 target genes and identified several additional functions of Nrf2 that extend beyond its redox properties, including regulation of inflammation, autophagy, metabolism, proteostasis, and protein denaturation responses. Nrf2 has become a major target of research related to inflammation, metabolism, cancer prevention, and treatment because its functions are more extensive than originally thought ([48].

In the cecum, the expression of the transcription factor Nrf2 in the broilers was 1.5 times higher than in the control. In the liver tissues of CORN × RW birds, *Nrf2* expression was 2 times lower than the control ($p = 0.02$), and in the broilers it was comparable to the control.

The nonspecific immune system includes β-defensins and interleukins. IL-6 is a multifunctional cytokine that is involved in inflammatory responses. IL-8 (a member of the CXC sub-family of chemokines) serves as a chemoattractant for leukocytes, the activation of which leads to proinflammatory responses such as

oxidative burst and increased cell death. IL-8 was first isolated from fibroblasts in chickens. It is known that under stress there is an increase in the amount of interleukins, which is caused by the development of inflammation. Stress can alter regulation of the immune system by increasing the activity of interleukins, namely IL-6, a major mediator of inflammatory and immune responses [49, 50].

In the cecum of CORN × RW cockerels compared to egg-bred birds, the expression of *AvBD9* and *IL6* genes was 1.8 times higher (p = 0.005 and p = 0.004, respectively), of *IL8* 5 times higher (p = 0.009). In the liver of CORN × RW, a similar pattern occurred, the relative expression of *IL6* was greater than that in RW by 3.43 times (p = 0.006), *AvBD9* by 25.50 times (p = 0.009), *IL8* by 1.56 times (p = 0.05).

In the ceca, the relative expression of *IL6* in broilers was higher than in RW by 1.76 times (p = 0.03), *AvBD9* by 3.22 times (p = 0.002). Moreover, the expression of *IL8* which is activated during infections, was approximately 30% lower in broilers compared to egg-laying birds. In the liver of broilers, the expression of *AvBD9* was 19.15 times higher than that in RW (p = 0.001), but the expression levels of the *IL6* and *IL8* genes were loer than in Russian White poultry.

4. Correlations (*r*) between the relative expression of antioxidant defense and immunity genes depending on their location and in connection with growth intensity of cockerels (G*allus gallus domesticus*) of different genotypes (*n* = 30, physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022)

Parameter	Gene							
	CAT	*GSH-Px*	*HO-1*	*SOD*	*AvBD-9*	*IL6*	*IL8*	*Nrf2*
				Cecum				
ADWG	0.998 (p = 0.03)	0.993	0.733	0.275	0.999 (p = 0.016)	0.722	–0.189	0.992
CAT		0.987	0.700	0.228	0.999 (p = 0.014)	0.688	–0.235	0.997
GSH-Px	0.987		0.807	0.384	0.990	0.798	–0.073	0.970
HO-1	0.700	0.807		0.855	0.715	0.999 (p = 0.10)	0.530	0.639
SOD	0.226	0.384	0.855		0.250	0.863	0.893	0.148
AvBD-9	0.999 (p = 0.014)	0.990	0.715	0.250		0.704	–0.214	0.995
IL6	0.688	0.798	0.999 (p = 0.1)	0.863	0.704		0.543	0.927
IL8	–0.235	–0.073	0.530	0.893	–0.214	0.543		–0.314
Nrf2	0.997	0.970	0.639	0.148	0.995	0.630	–0.314	
				Liver				
ADWG	0.989	–0.690	0.223	–0.776	0.622	–0.131	–0.587	0.049
CAT		–0.577	0.362	–0.677	0.728	0.014	–0.463	–0.096
GSH-Px	–0.577		0.552	0.992	0.139	0.808	0.991	–0.757
HO-1	0.362	0.552		0.441	0.903	0.937	0.658	–0.963
SOD	–0.677	0.991	0.441		0.012	0.726	0.966	–0.667
AvBD-9	0.728	0.139	0.903	0.012		0.696	0.270	–0.752
IL6	0.014	0.808	0.937	0.726	0.696		0.880	–0.997
IL8	–0.463	0.991	0.658	0.966	0.270	0.880		–0.837
Nrf2	–0.096	–0.757	–0.963	–0.667	–0.752	–0.997	–0.837	

Note. ADWG — average daily live weight gain, *CAT* — catalase, *GSH-Gpx* — glutathione peroxidase, *HO-1* — heme oxygenase 1, *SOD* — superoxide dismutase, *Nrf2* — related transcription factor 2, *AvBD9* — avian β-defensin 9, *IL6* — interleukin 6, *IL8* — interleukin 8.

We also calculated Pearson correlations between the relative expression of immunity genes and AOD in different tissues (in the cecum and in the liver) and in connection to the growth rate of birds of all three genotypes (Table 4).

Average daily live weight gain (ADWG) in poultry showed tight correlation with the expression of the *CAT* (*r* = 0.998 at p = 0.03) and *AvBD9* (*r* = 0.999 at p = 0.016) genes in the cecum. In the cecum, high correlations were also noted between the expression of the *CAT* and *AvBD9* (*r* = 0.999 at p = 0.014), *IL6* and *HO-1* (*r* = 0.999 at p = 0.1) genes. Thus, the genes for antioxidant protection and

immunity are closely related to each other, which confirms the connection between the body's AOD and poultry health. V.G. Narushin et al. [51] determined correlations between the expression of certain immune genes and biochemical and immunological blood parameters in laying hens. The most informative biochemical and immunological blood parameters were the content of urea, urea nitrogen, glucose and the activity of IgG2 immunoglobulin.

Our work did not establish significant correlations between ADWG and the expression of genes associated with AOD and immunity in the liver of birds (see Table 4). Here, we also did not find statistically significant correlations between the expression of these genes. This requires research, which we propose to conduct using additional dietary components to increase the antioxidant and immune status of poultry and improve product quality.

So, on cockerels of cross Ross 308, the Russian White breed and crosses of the Russian White and Cornish breeds, it is shown that the bird's genotype determines the state of the body's antioxidant system, which influences the accumulation of lipid peroxidation (LPO) products, the function of the enzymatic component of antioxidant defense (AOD), general antioxidant status and immune response. In broilers of the Ross 308 cross, a higher accumulation of lipid peroxidation products occurs which imposes special tension in the antioxidant system and can be compensated by a high total content of water-soluble antioxidants and catalase activity. Data on the expression of genes for antioxidant enzymes and immunity in the cecum and in the liver of birds confirm the results of biochemical blood tests. We are the first to establish correlations of the expression of some genes for AOD enzymes and immunity in the cecum and liver tissue with the average daily weight gain of cockerels, as well as between the relative expression of genes, which confirms the connection between AOD and poultry health. Our findings indicate that studying and applying ways to reduce oxidative stress and additionally protect the antioxidant system of intensively growing poultry are relevant. Next, we plan to explore ways to reduce the impact of stress (including oxidative stress) on poultry health and meat quality through nutritional factors.

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