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MIXTURES OF BIOLOGICALLY ACTIVE SUBSTANCES OF OAK BARK EXTRACTS CHANGE IMMUNOLOGICAL AND PRODUCTIVE INDICATORS OF BROILERS

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Abstract

To date, numerous studies are focused on searching for alternatives to antibiotics with similar antimicrobial and growth-stimulating effects that do not cause bacterial resistance and potential side effects for animals. Promising phytogenic compounds have been also recognized as potential alternatives to antibiotics in feeds. One of the problems of phytogenic compound use is the unstable chemical composition of plant extracts, depending on the conditions of growth, distribution area and other factors, so the question arises of extracting some substances with the known properties or designing their compositions. In this paper, it has been shown for the first time that a dietary composition of biologically active substances of *Quercus* cortex helps to maintain productivity and improves the immunomodulating state of Smena 8 poultry cross broilers. In our experiment, a composition of substances (CS) extracted from *Quercus* cortex and chemically synthesized («Acros Organics B.V.B.A.», Belgium) was used, including 2-n-propylresorcinol (98 %, AVH27024); 4-hydroxy-3-methoxybenzaldehyde (99 %, AC14082-1000); 7-hydroxycoumarin (99 %, AC12111-0250); 3,4,5-trimethoxyphenol (98.5 %, AC18914-0050); scopoletin (95 %, AC30290-0010); coniferyl alcohol (98 %, AL22373-5) with a confirmed anti-QS effect. A total of 120 broiler chickens aged 7 days were divided into 4 groups (n = 30) by analogue method. Control group was fed with the basic diet (BD). BD + CS 1 (1 ml/kg of live weight), BD + CS 2 (2 ml/kg of lw), and BD + CS 3 (3 ml/kg lw) were used for group 1, group 2, and group 3, respectively. In the experimental groups, as compared to the control, the number of blood leukocytes increased by 19.2-28.5 % ($P \le 0.05$), blood lymphocytes were higher by 24.4, 36.2 % ($P \le 0.05$) and 44.0 % ($P \le 0.05$), blood monocytes were higher by 23.5, 23.5 and 29.4 % ($P \le 0.05$), and blood granulocyte counts were higher by 12.3 % ($P \le 0.05$), 5.7 and 9.5 %. The blood ALT activity in the group 2 and group 3 exceeded the control value by 13.2 % ($P \le 0.05$). The level of GGT tended to decrease in the group 2 and group 3, along with a significant decrease in LDH by 17.6-22.5 % ($P \le 0.05$). The intake of the CS as a feed additive was accompanied by an increase in blood SOD levels in the test groups, the highest concentration being observed in the group 1 (95.3 %). The catalase indices had similar patterns. Dietary composition of biologically active substances promoted a 16.4 % increase ($P \le 0.05$) in blood β-lysine levels in the group 1. Within 4 weeks, the dietary CSs led to an increase in the live weight of the poultry of the group 2 and groups 3 by 12.6-15.0 % ($P \le 0.05$) when compared to the group 1. In the group 1, the birds grew more rapidly with a 100 % survival rate of the herd, in contrast to the remaining groups with the survival index of 71 to 85 %. These contributed to an insignificant increase in the bird live weight at the end of the experiment (day 42) by 1.9 % ($P \ge 0.05$) compared to the control group. Thus, feeding broiler chickens with the composition of bioactive substances in the initial concentration helps to maintain productivity and improve the immunomodulating state of body.

Keywords: biologically active substances, oak bark, broiler chickens, biochemical and mor-

phological parameters of blood, blood enzymes, growth rate

Use of antibiotics (AB) as growth stimulants in animal feed is limited by growth of AB resistance of bacterial pathogens that poses a threat for the national health [1, 2]. To date, numerous studies are focused on searching for alternatives to antimicrobial substances with preventative and growth stimulating effects that do not cause bacterial resistance and potential side effects in animals.

Phytogenic compounds [1, 3-5] are widely recognized as potential antibiotic alternative in feeds. These are plant-based biologically active compounds with positive effect on animal growth and health, which are often used in form of essential oils and plant extracts [6]. Results of studies aimed at understanding of the phytogenic compound action mechanisms, possible side effects and economic effectiveness, are not convincing yet. There is information confirming the ability of phytogenic compounds, including extracts from alfalfa seeds [7], grasses [8], and essential oils [9, 10] to affect quorum sensing (QS) of bacteria. All of them are produced in studies in vitro and relate to food products. QS inhibition for prevention and combat with bacterial infections in farm animals remains the less studied problem, except for the aquaculture [11].

One of obstacles for use of phytogenic compounds is instable chemical composition of plant extracts depending on growing conditions, ecosystem, and other factors. Earlier, several compounds from oak bark extract (*Quercus* cortex) having both antibacterial and anti-QS activity [12, 13] was found. These substances were used in tests on agricultural poultry.

In present study we for the first time had shown that inclusion of the composition of biologically active compounds from *Quercus* cortex extract into the ration of broiler chickens cross Smena 8 promotes maintenance of productivity and strengthening of immune-modulating body state.

Purpose of our research is to study effect of various dosages of biologically active compounds from *Quercus* cortex extract on productive indicators and immunity in broiler chickens, and to assess perspectives of use of such combinations and possible mechanism of their action.

Techniques. Chemical substances were identified by gas chromatography with a mass-selective detector GQCMS 2010 Plus (Shimadzu, Japan) in tube HP-5MS. GCMS Solutions, GCMS PostRun Analysis software was used at interpretation of research results, set of mass spectra libraries CAS, NIST08, Mainlib, Wiley9 and DD2012 Lib was used for identification of compounds. Each identified component was quantified as the percentage of its peak area from the total area of the extract peaks.

In tests we used mixture of substances earlier extracted from the oak bark [12, 13] and artificially synthesized (Acros Organics B.V.B.A., Belgium). These are 2-n-propylresorcinol (98 %), 4-hydroxi-3-metoxibenzaldehyde (99 %), 7-hydroxi coumarine (99 %), 3,4,5-trimetoxiphenol (98.5 %), scopoletin (95 %), and coniferyl alcohol (98 %). Anti-QS-effect of this composition (SC) was tested on *Chromobacterium violaceum* strain CV026 by agar diffusion method (qualitatively) and by serial dilution method in liquid medium (quantitatively).

Broiler chickens aged 7 days (cross Smena 8, $n = 120$) divided by analogue method into 4 groups, $n = 30$ each, were selected for tests in vivarium conditions. During the tests, all poultry was in similar feeding and keeping conditions. Rations were made accounting for recommendations of the Russian Research and Technological Institute of Poultry (VNITIP) [14]. Control group ate the main diet (MD); group I (trial) was fed MD + substance composition (SC) 1 (1 ml/kg of live weight); group II (trial) was fed MD + SC 2 (2 ml/kg of live weight); group III (trial) — MD + SC 3 (3 ml/kg of live mass). Chemical substances in the composition for the group I were 2-n-propylresorcinol (1.5

mg/ml), 4-hydroxi-3-metoxibenzaldehyde (0.5 mg/ml), 7-hydroxi coumarine (0.5 mg/ml), 3,4,5-trimetoxi-phenol (2 mg/ml), scopoletin (0.3 mg/ml), coniferyl alcohol (4.5 mg/ml); for the group II — 3; 1; 1; 4; 0.6; 9 mg/ml, respectively, for the group III — 4.5; 1.5; 1.5; 6; 0.9; 13.5 mg/ml. Poultry was fed 2 times daily, feed consumption was accounted daily, SC solution was provided individually. Poultry was provided unlimitedly with water. Growth and development of chickens (inspection and individual weightings were carried out daily, in the same time in morning hours) was assessed. Decapitation was performed under nembutal ester on day 42. Housing and procedures during tests were in line with instructions and recommendations of the Russian regulation (Decree of the Ministry of Health of USSR№ 755 dated 12.08.1977) and The Guide for Care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996). All endeavors were taken to minimize the agony in animals and to reduce the number of used samples.

Blood samples for hematologic studies were collected into vacuum vials with anticoagulant (EDTA-K3), for biochemical studies — in vacuum vials with coagulating activator (thrombin). Hematological indicators (number and types of leucocytes) were estimated (an automated analyzer URIT-2900 Vet Plus, URIT Medical Electronic Group Co., Ltd", China).

Data was statistically processed in software IBM SPSS Statistics Version 20 (https://www-01.ibm.com). Mean (*M*) and standard errors of the mean (±SEM) were calculated. Differences are statistically significant at $p < 0.05$.

Results. Analysis of morphological and biochemical blood indicators allows identification of changes in trial groups (Table 1). Number of leucocytes was 19.2-28.5 % higher ($P \leq 0.05$), counts of lymphocytes in groups I, II and III increased by 24.4; 36.2 ($P \leq 0.05$) and 44.0 % ($P \leq 0.05$), respectively, of monocytes — by 23.5; 23.5 and 29.4 % ($P \leq 0.05$), and of granulocytes — by 12.3 ($P \leq 0.05$); 5.7 and 9.5 %.

1. Count ($\times 10^9$/л) **of white cells in blood of cross Smena 8 broiler chickens fed bioactive composition (extract from oak bark + artificially synthesized substances)** (*M*±SEM, vivarium conditions)

Indicator	Group			
	control (n = 15)	I (n = 15)	II (n = 15)	III (n = 15)
Leucocytes	24.9±1.21	29.7±1.33*	30.5±1.08*	32.0±1.51*
Lymphocytes	12.7±0.91	15.8±1.18	17.3±1.58*	18.3±1.11*
Monocytes	1.7±0.77	2.1±1.81	2.1±0.54	2.2±0.53*
Granulocytes	10.5±0.91	11.8±0.21*	11.1±0.94	11.5±1.34

N o t e. See description of groups in section "Methodology".
* Differences with control are statistically significant at $P \leq 0.05$.

Hematologic parameters serve convenient indicator for assessment of physiological state and health in animals and may be useful to control effects of feed additives [15]. Our data correlates with earlier research results [16, 17]. Other tests on animals in vitro also showed that plant-based bioactive compounds increase immune activity by increase of phagocytosis [18]. This can also explain growth of blood indicators in trial groups.

Activity of blood alanine aminotransferase (ALAT) in groups II and III trial was 13.2 % higher ($P \leq 0.05$) compared to control. Activity of aspartate aminotransferase (ASAT) was the least in group I ($P \geq 0.05$) (Table 2).

In earlier paper [19] dietary polyphenols did not significantly affect ASAT and ALAT, however, the researchers did not performed exact chemical identification of the bioactive components. In the composition used in our experiment, the coniferyl alcohol which possesses cytotoxicity [20] is high in level and can affect hepatic cells, as follows from ASAT and ALAT indicators in groups II and III. Content of γ-glutamyl transpeptidase (γ-GT) decreases in

groups II and III at a decrease of lactate dehydrogenase (LDG) by 17.6-22.5 % ($P \leq 0.05$) as compared to control. Obtained data indirectly testify positive effect of the substance composition in the initial concentration on protein and carbohydrate metabolism. Similar findings are reported by H.Y. Qiao et al. [20]. In their study dietary 4-hydrxi-3,5-dimetoxi-cinnamic acid, a polyphenolic compound, had no effect ($P > 0.05$) on blood activity of creatine kinase and lactate dehydrogenase in broilers. Besides, as is known [21], oak bark extract promotes better glucose tolerance and significantly decreases its absorption through the gastric epithelium.

2. Activity of blood enzymes and non-specific immunity indicators in cross Smena 8 broiler chickens fed bioactive composition (extract from oak bark + artificially synthesized substances) (M±SEM, vivarium conditions)

Indicator	Group			
	control (n = 15)	I (n = 15)	II (n = 15)	III (n = 15)
ALAT, IU/l	3.8±0.76	4.0±0.36	4.3±0.43*	4.3±0.37*
ASAT, IU/l	228.8±21.84	219.6±12.53	229.9±13.23	231.8±15.51
γ-GT, IU/l	16.5±1.04	18.3±2.85	15.0±1.08	16.0±2.00
LDG, IU/l	14.2±3.68	14.0±5.57	11.7±2.99*	11.0±2.97*
SOD, %	218.6±54.02	427.1±52.52*	391.1±38.80*	378.1±51.33*
Catalase, μmol $H_2O_2 \cdot l^{-1} \cdot min^{-1}$	989.4±46.30	1438.0±57.73*	1701.6±53.33*	1468.5±52.91*
BSLA, %	47.1±0.39	45.3±0.91	44.9±1.2	45.1±0.55
β-Lysine, %	72.9±0.40	89.3±0.80*	69.6±0.52	84.5±0.51

N o t e. ALAT — alanine aminotransferase, ASAT — aspartate aminotransferase, γ-GT — γ-glutamyl transpeptidase, LDG — lactate dehydrogenase, SOD — superoxide dismutase, BSLA — blood serum lysozyme activity. See description of groups in section "Methodology".

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

SC intake with feed leads to increased antioxidant activity, in particular, due to higher blood content of superoxide dismutase in chickens of trial groups, with the highest concentration in group I (95.3 %). Catalase content in poultry in trial groups was higher than in control by 45.3-71.9 % ($P \leq 0.05$). Antioxidant properties of oak bark extracts [22, 23] are also shown in our tests. Increased total antioxidant activity and SOD level in blood of broilers [24, 25] were due to feed supplementation with gallic acid, an oak bark component. Increased blood catalase concentration also occurred in broiler chickens after use of grape seed powder as a source of polyphenolic substances in diet [19].

SC in the diet promotes 16.4 % higher blood β-lysine content in group I as compared to control ($P \leq 0.05$). Weight gain in poultry of group I was also higher than in groups II and III, i.e. by 15.0 % ($P \leq 0.05$) in 1 week, by 14.3 % in 2 weeks and by 12.6-13.9 % in weeks 3 and 4. In group I, more rapid growth combined with 100 % survival while in other groups this indicator was 71-85 % (see Table 3).

3. Weigh (g) **dynamics in cross Smena 8 broiler chickens fed bioactive composition (extract from oak bark + artificially synthesized substances)** (M±SEM, vivarium conditions)

Group	Week 1	Week 2	Week 3	Week 4
Control	299.5±15.4	652.5±28.9	1164.0±21.5	1787.0±22.2
I	317.2±12.5	618.8±23.5	1134.4±17.2	1660.0±23.5
II	275.6±20.1	541.0±23.2*	1007.0±22.7	1571.5±26.8
III	291.6±10.7	558.4±11.3	1015.2±12.4	1456.8±19.5*

N o t e. See description of groups in section "Methodology".

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

Use of bioactive substances at initial concentration (group I) resulted in 1.9 % ($P \geq 0.05$) weight gain at the end of test (on day 42) as compared to control group. This is in line with data on growth stimulation by dietary gallic acid and grape seeds rich in polyphenols [19, 20]. The composition we used also contains phenolic compounds and, thus, these results are objectively comparable.

However, there are studies in which β-resorcylic acid as antimicrobial feeding additive has no effect on weight gain in broiler chickens [26]. In our study, weight of poultry in groups II and III decreased as concentration of bioactive substances increased, and, note, propyl resorcinol close in origin to resorcylic acid was present in the composition. As to growth stimulation effect, several mechanisms are possible, e.g. a decrease in total bacterial load, suppression of pathogenic microorganisms, thinning mucosa layer, and direct immune modulation [27]. It is known that tannings may participate in modulation of the composition and activity of intestinal microflora and its interaction with the entering compounds. It is reported that gram-positive bacteria are more sensitive to plant-based extracts rich in tannin-like substances [28, 29]. It is also confirmed by properties of compounds earlier found in *Quercus* cortex extract [12, 13].

Therefore, dietary additive composition of oak bark extract + artificially synthesized substances at a dose of 1 ml/kg live weight favorably modulates immune state and antioxidant activity in vivo in broiler chickens that is followed by increased blood levels of β-lysine, superoxide dismutase and catalase, higher survival (up to 100 %) and improved productivity (up to 15 %).

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