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METAGENOMIC ANALYSIS OF INTESTINAL MICROBIOME AND BIOCHEMICAL COMPOSITION OF BROILER MEAT UPON USE OF Quercus cortex EXTRACT DIETARY ADDITIVE

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Abstract

Today, the use of antibiotics in veterinary medicine, as well as growth stimulants in animal husbandry, is considered the main reason for the development of bacterial resistance to antibiotics. Plant-based water extracts can provide simple new approaches to control pathogenic bacteria. Active search for natural alternative sources of antimicrobials, including wild plants, are almost unlimited source of phytochemicals. Plant-based water extracts can also provide simple new approaches to controlling pathogenic bacteria. Some authors suggest that an increase in broiler growth after adding active components of plants may be associated with an improvement in the microbial composition of the intestine and metabolic function. Other plant substances can improve the profile of unsaturated fatty acids and amino acids in meat. Thus, in order to get a more complete picture of the potential use of plant extracts for the prevention or control of bacterial infections, the most significant studies concern the evaluation of the activity of plant extracts in relation to the quality of products and the intestinal microbiome of farm animals and poultry. The aim of our experiment was to study the effect of Quercus cortex extract on biochemical composition of broiler chicken meat and intestinal microbiomes. The studies were carried out with Smena 8 broiler chickens (the Common Use Center for the scientific equipment of the BST RAS, in 2019). In the experiment, 120 broiler chickens aged 7-day were randomly assigned to 4 groups (n = 30 each, in 4 repetitions). The control broilers were fed the Basic Diet (BD); group I — BD + Quercus cortex extract 1 (1 ml/kg lw); group II — BD + Quercus cortex extract 2 (2 ml/kg lw); group III — BD + Quercus cortex extract 3 (3 ml/kg lw). Analysis of chemical composition of broiler chicken meat showed that the additional inclusion of oak bark extract at a dose of 1 ml/kg of live weight in the diet of the studied poultry helps to improve the quality of meat due to a 27.3 % ($p \leq 0.01$) increase in moisture, crude protein and ash, while reducing the level of crude fat. The dietary oak bark extract contributed to an increase in the amount of essential amino acids, for lysine by 1.63-3.43 % ($p \leq 0,01$, for leucine-isoleucine by 2.20-5.00 % ($p \leq 0.05$), for methionine by 0.55-1.93 % ($p \leq 0.05$), for valine by 1.06-1.95 % ($p \leq 0.05$), for phenylalanine (group I and II) by 0.45 % and 1.14 %, respectively ($p \leq 0.05$), for threonine (group I and II) by 1.07 % ($p \leq 0.05$) and 1.82 % ($p \leq 0.01$). Levels of non-essential amino acids in the pectoral muscles of broiler chickens compared to control also changed, with the maximum observed for a dosage of 2 ml/kg lw of oak bark extract. The content of unsaturated fatty acids in groups I and III increased compared to the control (for palmitoleic acid by 1.00 and 0.70 %, respectively, $p \leq 0.05$). Different dosages of dietary Quercus cortex extract have a significant effect on microbiota of the blind intestine. Changes affect phyla *Firmicutes* and *Bacteroidetes*, involved in metabolic energy resorption and degradation of proteins and polysaccharides. The abundance of phylum *Bacteroidetes* increased 3.96-fold and 2.10-fold in groups I and III compared to the control ($p \leq 0.05$), while in group II these bacteria were not found. The

number of members of *Firmicutes* phylum decreased 3.60-fold and 1.47-fold (in groups I and III vs. the control, $p \leq 0.05$) while increased 1.26 times in group II vs. the control, $p \leq 0.05$) Thus, broilers fed 1-3 ml/kg dietary Quercus cortex extract were superior to other birds in terms of amino acid and unsaturated fatty acid levels in carcass due intensification of digestion in intestine, which improves consumer quality of meat.

Keywords: microbiome, broiler chickens, oak bark extract, fatty acids, amino acids

Today, the use of antibiotics as drugs in veterinary medicine and stimulants in animal husbandry is considered the main reason for emerging bacterial resistance to antibiotics. The World Health Organization (WHO) has compiled a list of antibiotic-resistant priority pathogenic microorganisms requiring new infection control strategies [1]. This stimulates an active search for natural alternatives to antimicrobial drugs [2]. Wild plants are a virtually unlimited source of phytochemicals [3]. Assessment of the therapeutic potential of plant extracts against poultry bacteria revealed sensitivity of *Salmonella enteritidis* (63.64%) [4], *Pseudomonas aeruginosa* (81.81%) and *Escherichia coli* (27.27%) to leaf extracts from *Mangifera indica* L. cv. Julie, *Euadenia eminens* Hook f. and the bark of *Euadenia trifoliata* (Vahl) Oliv. [5, 6]. Aqueous extracts of plant origin can provide new simple approaches to combat pathogenic bacteria [7].

An increase in growth rates in broilers fed active plant components may be associated with an improved intestine microbiome composition and, thence, better metabolic function [8, 9]. Also, plant substances are known which improve the profiles of unsaturated fatty acids [10, 11] and amino acids [12, 13] in meat.

Addressing the problems of bacterial infections of farm animals and poultry necessitates more complete elucidation of the potential effects of plant extract on intestinal microbiomes and the quality of the food products. This information is expected to be helpful in the development of more efficient and simple application of natural therapeutic agents against bacteria, which additionally will increase livestock and poultry productivity in general.

Currently, studies of various plant extracts indicate the broad prospects [14-16]. However, papers concerning such effects in oak bark are relatively few [17-19], although oak bark is known to possess antibacterial properties and anti-quorum effects [20]. Previously, we showed that Quercus cortex extract combined with a probiotic [21] and an enzyme preparation [22] and depending on its composition [23] has a positive effect on the immunity and productivity of broilers.

Here, we present data characterizing the effect of Quercus cortex extract on the productive performance, amino acid and fatty acid profiles of meat, and on the structure and abundance of the cecum microbiocenosis in broiler chickens. We are not aware of such studies in the available literature.

The aim of the work was a metagenomic analysis of the intestinal microbiota of broiler chickens fed various doses of dietary Quercus cortex extract to link the microbiome profiles with the broiler productivity and meat quality parameters.

Materials and methods. The work was performed in an experimental biological clinic (vivarium) (Federal Research Center of Biological Systems and Agrotechnologies RAS, 2019). One hundred and twenty 7-day-old broiler chickens of Smena 8 cross were assigned to four treatments in a 35-day experiment: 30 broilers were fed basal diet (BD) throughout the experiment (control group), 30 broilers were fed BD + Quercus cortex, 1 ml/kg live weight (group I), 30 broilers were fed BD + oak bark extract (Quercus cortex), 2 ml/kg live weight (group II), and 30 broilers were fed BD + Quercus cortex), 3 ml/kg live weight (group III). Poultry housing and manipulations during the experiments complied with the requirements of the instructions and recommendations of the Russian regulations (Order of the Ministry of Health of the USSR № 755 of 08/12/1977), as well as “The Guide for Care and Use of Laboratory Animals (National Academy Press, Washington,

DC, 1996)". Carcass quality parameters were assessed at the end of the experiment in accordance with the state standard GOST 31962-2013.

For extract preparation, oak bark was crushed, and heated with distilled water (1:1) in a water bath for 30 min, then the bark fragments were separated and crude extract was filtered (ash-free filters "white ribbon", d = 70 mm, Reakon Plus LLC, Russia).

The microbial biodiversity in bird cecum was assessed on day 42. The collected samples of cecum contents were incubated at 37 °C for 30 min in 300 μl of sterile lysis buffer (20 mM EDTA, 1400 mM NaCl, 100 mM Tris-HCl, pH 7.5; 50 μl of a 100 mg/ml stock solution of lysozyme). Proteinase K (Thermo Fisher Scientific, Inc., USA, 10 μl of a 10 mg/ml solution) and SDS (the final concentration of 1.0%) were added, and the mixture was incubated for 30 min at 60 °C. DNA was purified with a mixture of phenol and chloroform (1:1), precipitated by sodium acetate (3 M, up to 10% by volume) with three volumes of absolute ethanol at –20 °C for 4 hours. After extractions with mixtures of phenol: chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1) DNA was precipitated from the aqueous phase with 1 M ammonium acetate (up to 10% by volume) and 3 volumes of anhydrous ethanol overnight at –20 °C. The DNA precipitate was separated by centrifugation (12000 rpm, 10 min), washed twice with 80% ethanol, dried and dissolved in TE buffer (1 ml of 1 M Tris-HCl, pH 8.0, 200 μl of 0.5 M EDTA, pH 8.0, H_2O to 100 ml; Evrogen, Russia). The purity during DNA extraction was assessed by the negative control (100 μl of autoclaved deionized water). The purity of the obtained DNA preparations was controlled electrophoretically in 1.5% agarose gel with photometry (NanoDrop 8000, Thermo Fisher Scientific, Inc., USA). The DNA concentration was measured fluorometrically (Qubit 2.0 instrument with high sensitivity for dsDNA measurement, Life Technologies, USA).

DNA libraries for sequencing were constructed as per the Illumina, Inc. (USA) protocol with primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 to the variable region V3-V4 of the 16S rRNA gene [24]. NGS sequencing was performed using a MiSeq platform (Illumina, Inc., USA) with MiSeq Reagent Kit V3 PE600 (Illumina, Inc., USA) (the Center for Shared Use of Scientific Equipment "Persistence of Microorganisms", Institute of Cellular and Intracellular Symbiosis, UB RAS). The operational taxonomic units (OTU) were classified using an interactive VAMPS tool and the RDP database (http://rdp.cme.msu.edu). Some OTUs were aligned using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the databases of nucleotide sequence nr/nt (National Center for Biotechnological Information, NCBI, https://www.ncbi.nlm.nih.gov/) and aligned sequences of ribosomal RNA genes SILVA (https://www.arb-silva.de).

Chemical analysis of meat samples included assay of fat fraction according to GOST 23042-86, of ash according to GOST 15113-77, of protein by the Kjeldahl method according to GOST 23327-78 with preliminary mineralization, of amine nitrogen content by formol titration, and moisture mass fraction according to GOST 15113.8-77.

To assess meat biological value, the content of nonessential and essential amino acids was determined in a bulk sample and in the longissimus muscle by capillary electrophoresis (Kapel-105 M system, Lumex, Russia; GOST R 55569-2013) in accordance with the manufacturer's protocol. Sample preparation included tissue homogenization, drying at 60-70 °C, grinding, acid or alkaline (only for tryptophan) hydrolysis at 110 °C for 14-16 hours. After acid hydrolysis, the samples were filtered (upon alkaline hydrolysis no filtration required). The hydrolysates were evaporated in warm air stream. The dry residue was diluted in

distilled water and clarified by centrifugation. The supernatant was used for analysis by capillary electrophoresis.

The fatty acid composition of muscle tissue lipids was assayed by gas chromatography method (Kristall Lux 4000 chromatograph, OOO NPF Meta-chromium, Russia; GOST R 55483-2013). The analytical standard was a mixture of fatty acid esters Supelco® 37 Component FAME Mix (Sigma-Aldrich, USA).

The data were statistically processed with the Statistica 10.0 program (StatSoft, Inc., USA). Results are presented as arithmetic mean (*M*) and standard error of the mean (±SEM). Differences were considered statistically significant at $p \leq 0.05$ [25]. For bioinformatic processing of sequencing results, the USEARCH v8.0.1623_win32 software package (https://www.drive5.com/usearch/download.html) was used. The procedure included the fusion of paired reads in operational taxonomic units, read filtering by quality and length (300 bp minimum size), removal of chimeras, doubletones and singletones, and clustering of reads in OTUs at a similarity level of 97% [26].

Results. Dietary biological additives directly affect quality of poultry meat, which ultimately can allow production of muscle tissue with high nutritional and/or biological value, and, if necessary, fatty tissue production [27, 28. In broiler growing and when assessing chicken meat for sale, the quality of both carcasses as a whole and their parts is accounted, for which the energy value is determined and the economic effect of the feed additives is calculated (Table 1).

1. Carcass quality parameters of Smena 8 broiler chicken fed plant extract Quercus cortex (vivarium, Federal Research Centre of Biological Systems and Agrotechnologies RAS, Orenburg, 2019)

Parameter	Control	Group I	Group II	Group III
Slaughter weight, g	2448.5±104.4	2597.3±204.9*	2257.6±100.7**	2176.5±78.5*
Semi-eviscerated weight, g	2053.0±101.9	2188.7±184.9	1899.2±99.5	1856.5±73.3*
Semi-eviscerated percentage	83.8±2.30	84.3±3.10	84.1±2.60	85.3±4.60*
Eviscerated weight, r	1595.5±98.8	1725.3±165.3*	1490.0±77.3	1444.0±71.1*
Dressing out percentage	65.2±1.61	67.4±1.18*	66.0±1.08	66.4±1.15
Meat quality index	2.39	2.36	2.27	2.31
Meat to bone index	2.10	2.08	2.23	2.49

N o t e. For description of groups, see *Materials and methods.*
*, ** Differences between the treatment and control are statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Upon good development of muscle tissue, the yield of edible parts of carcasses will be higher, as a result, the meat quality index (the ratio of fat and total protein content) and the meat to bone index (the ratio of muscle and bone weight) will change. In all test groups, the index of meat qualities was inferior to that in the control, but at the same time in group I it was higher than in group II and group III. The meat to bone index was maximum in group III (15.7% was higher than in the control), while in group I it was 0.95% lower than in the control. In general, the dietary oak bark extract contributed to an increase in the total meat yield compared to the control group, which is consistent with data of Jamroz et al. [29].

The maximum moisture level was noted in group I (1.01% higher than the control). The crude fat in group I significantly decreased compared to the control (by 27.3%, $p \leq 0.01$), while in group II it significantly increased (1.54 times, $p \leq 0.01$) compared to the control. The crude protein levels in test groups varied from 18.78 to 19.90% with the maximum values also in test group I as compared to the control and other test groups ($p \leq 0.05$). For ash content, distribution was similar with unreliable growth in group I (by 0.03% compared to the control).

One of the important chicken broiler meat quality criteria is its biological value determined by the amino acid and fatty acid composition. A decrease or a sharp increase in the accumulation of amino acids may indicate a negative effect of the introduced drug or biologically active substance, which, in turn, will affect the organoleptic properties of products which will no longer meet the requirements of GOST.

2. Amino acid content (%) in pectoral muscles of Smena 8 broiler chicken fed plant extract Quercus cortex (vivarium, Federal Research Centre of Biological Systems and Agrotechnologies RAS, Orenburg, 2019)

Amino acid	Control	Group I	Group II	Group III
Essential amino acids				
Lysine	9.67±0.31	11,30±0,28*	13,10±0,18**	11,1±0,09*
Phenylalanine	3.58±0.21	4,03±0,19*	4,72±0,25*	3,83±0,17
Leucine Isoleucine	10.7±0.21	14,10±0,15*	15,70±0,60*	12,9±0,11*
Methionine	2.51±0.19	3,06±0,14*	4,44±0,21**	3,48±0,15*
Valine	3.46±0.15	4,55±0,31*	5,41±0,32*	4,52±0,21*
Threonine	3.97±0.24	5,04±0,33*	5,79±0,32**	4,96±0,26
Total	33.9±4.60	42,10±3,70	49,20±4,70	40,80±6,20
Nonessential amino acids				
Arginine	4.98±0.19	6,83±0,24*	7,41±0,36*	7,16±0,41*
Tyrosine	3.87±0.40	5,77±0,32*	6,17±0,31*	5,26±0,24*
Giscidin	2.45±0.31	3,27±0,21	3,68±0,25*	2,89±0,12
Proline	2.63±0.16	3,30±0,23	3,75±0,32*	3,23±0,16
Serine	2.96±0.18	4,10±0,21**	5,24±0,24**	4,48±0,24*
Alanin	5.94±0.41	8,35±0,36*	9,68±0,34*	8,11±0,51*
Glycine	3.82±0.21	4,97±0,29	5,70±0,37*	4,82±0,32
Total	26.7±3.80	36,60±4,50	41,60±7,50	35,95±4,90

N o t e. For description of groups, see *Materials and methods.*

*, ** Differences between the treatment and control are statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

The accumulation of essential acids in the pectoral muscles of broilers in the test groups increased. So, for lysine, the indicators in the experimental groups exceeded the control ones, by 1.63% ($p \leq 0.05$) in group I, by 3.43% ($p \leq 0.01$) in group II, and by 1.43% ($p \leq 0.01$) in group III (Table 2). A similar pattern was observed for leucine-isoleucine, methionine and valine, on average, by 2.20-5.00% ($p \leq 0.05$), 0.55-1.93% ($p \leq 0.05$), and 1.06-1.95% ($p \leq 0.05$), respectively, compared to control. The phenylalanine level significantly exceeded the control, by 0.45% ($p \leq 0.05$) in group I, and by 1.14% ($p \leq 0.05$) in group II. The threonine accumulation in group I and group II significantly exceeded the control, by 1.07% ($p \leq 0.05$) and 1.82% ($p \leq 0.01$), respectively. In group II (see Table 2) there was a significant ($p \leq 0.05$) increase in the content of all nonessential amino acids compared to control, by 2.43% for arginine, by 2.30% for tyrosine, by 1.23% for histidine, by 1.12% for proline, by 2.28% for serine, by 3.74% for alanine, and by 1.88% for glycine. In group I and group III, the amount of the following amino acids significantly increased ($p \leq 0.05$) compared to control, by 1.85 and 2.18%, respectively, for arginine, by 1.90 and 1.39% for tyrosine, by 1.14 and 1.52% for serine, and by 2.41 and 2.17% for alanine ($p \leq 0.05$).

Thus, dietary oak bark extract as a rule contributed to an increase in the amount of both essential and nonessential amino acids in the pectoral muscles, with the maximum positive effect in the group with a dosage oak bark extract of 2 ml/kg live weight.

In the thigh muscles, the concentration of essential amino acids did not differ significantly from the control. Thus, in group II, only the content of lysine and leucine-isoleucine was significantly lower than the control (by 1.84 and 2.16%, respectively, $p \leq 0.05$). In group III, only lysine accumulation significantly decreased compared to the control (by 1.66%, $p \leq 0.05$), all other changes were unreliable. The patterns for nonessential amino acids were similar, a 1.65% decrease in lysine

($p \leq 0.05$) in group I, a 2.13% decrease in lysine ($p \leq 0.01$) and an 0.65% decrease in glycine ($p \leq 0.05$) in group II as compared to the control. As to pectoral muscles, it should be noted that the amino acid levels in the test groups did not exceed the maximum permissible concentration, remaining within the normal range.

Unsaturated fatty acids, which play an important role in metabolism in animals and humans, are of a special biological value [30]. In our experiment (Fig. 1), in poultry fed dietary oak bark extract at 1 ml/kg of live weight the accumulation of arachidic acid significantly increased (by 0.60% at $p \leq 0.01$) compared to the control, and the dose of 2 ml/kg increased the concentrations of stearic and arachidic acids by 1.39 and 1.00%, respectively ($p \leq 0.05$) compared to the control.

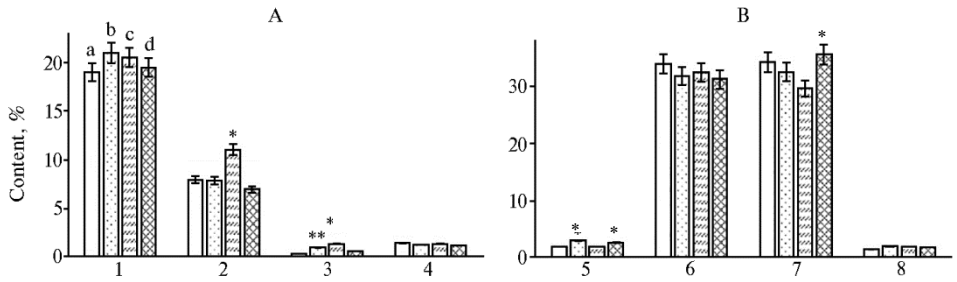

Fig. 1. Fatty acid profiles of pectoral muscles of Smena 8 broiler chicken fed plant extract Quercus cortex (vivarium, Federal Research Centre of Biological Systems and Agrotechnologies RAS, Orenburg, 2019): A — saturated fatty acids, B — unsaturated fatty acids; a — control, b — group I, c — group II, d — group III; 1 — palmitic, 2 — stearic, 3 — arachidic, 4 — gondoic, 5 — linoleic, 6 — oleic, 7 — palmitoleic, 8 — linolenic. Asterisks (*, **) mark statistically significant differences vs. the control at $p \leq 0.05$ and $p \leq 0.01$, respectively.

In the unsaturated fatty acid profiles of the pectoral muscles, the palmitoleic acid levels significantly went up, by 1.0 and 0.7% ($p \leq 0.05$), respectively, in groups I and III compared to the control. In group II, we noted only a significant decrease in linoleic acid, by 4.6% ($p \leq 0.05$).

The fatty acid composition also changed in the thigh muscles of broiler chickens (Fig. 2).

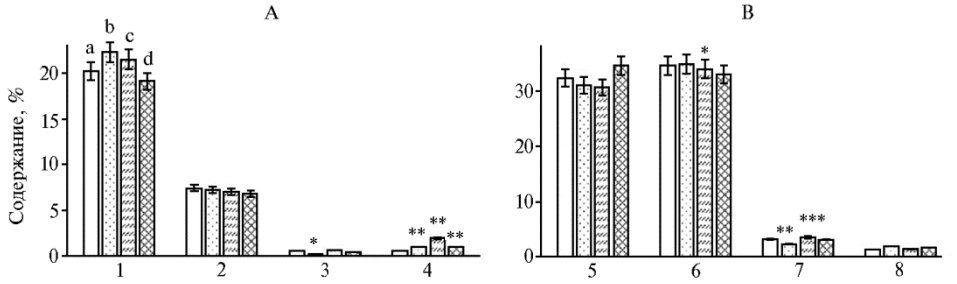

Рис. 2. Fatty acid profiles of thigh muscles of Smena 8 broiler chicken fed plant extract Quercus cortex (vivarium, Federal Research Centre of Biological Systems and Agrotechnologies RAS, Orenburg, 2019): A — saturated fatty acids, B — unsaturated fatty acids; a — control, b — group I, c — group II, d — group III; 1 — palmitic, 2 — stearic, 3 — arachidic, 4 — gondoic, 5 — linoleic, 6 — oleic, 7 — palmitoleic, 8 — linolenic. Asterisks (*, ** and ***) mark statistically significant differences vs. the control at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

Changes in concentration of saturated fatty acids, such as palmitin and stearic, were insignificant and unreliable. The level of arachidic acid in group I significantly declined, by 0.3% compared to the control ($p \leq 0.05$). A significant increase in the amount of gondoic acid upon all treatments should also be mentioned (by 0.5-1.5%, $p \leq 0.01$). S.T. Ahmed et al. [31[noted similar pattern when

used pomegranate processing by-products as feed additives.

Profiling chicken cecum microbiota by metagenomic sequencing (Table 3) allows us to classify a total of 425 OTUs in the control. Comparison with the SILVA database showed that these OTUs belonged to 19 phyla, 34 classes, 71 orders, 146 families, 247 genera and 297 species.

For group I, we identified 277 OTUs. Comparison with the SILVA database showed that these OTUs belonged to 21 phyla, 38 classes, 74 orders, 145 families, 230 genera and 284 species. In group II, bacteria accounted for 99.8%, and remaining 0.2% of microorganisms were unclassified**.** Here, 389 OTUs were found, of which 2 OTUs were removed as contaminants. Comparison with the SILVA database assigned the remaining 387 OTUs to 19 phyla, 30 classes, 64 orders, 130 families, 227 genera and 310 species. In group III, 406 OTUs were classified, which belong to 19 phyla, 34 classes, 70 orders, 142 families, 240 genera and 294 species.

These results showed that with an increase in the dose of oak bark extract, the phylum *Bacteroidetes* first increased in abundance (group I), then were not detected (group II) and increased again (group III) (see Table 3). Of the members of the phylum *Bacteroidetes*, the *Rikenellaceae* was dominant in group I and group III. Note that other studies [32, 33] note an opposite effect for *Ruminococcaceae* and *Lachnospiraceae*. In groups I and III, the *Clostridia* class was less abundant. Previous studies have also indicated the positive effect of tannin-containing substances, which inhibit the development of microorganisms of the Clostridia class [34, 35].

3. Taxonomic profile (%) of cecum microbiome of Smena 8 broiler chicken fed plant extract Quercus cortex (*M*±SEM, vivarium, Federal Research Centre of Biological Systems and Agrotechnologies RAS, Orenburg, 2019)

Phylum	Class	Family	Genus
Control (n = 5)			
Bacteroidetes (16.7±0.75)	*Bacteroidia* (16.7±0.75)	*Rikenellaceae* (14.6±0.61)	*Alistipes* (14.6±0.61)
		Bacteroidaceae (2.1±0.08)	*Bacteroides* (2.1±0.08)
Firmicutes (73.8±2.68)	*Bacilli* (2.68±0.13)	*Lactobacillaceae* (2.67±0.12)	*Lactobacillus* (2.67±0.12)
	Clostridia (71.0±2.5)	*Ruminococcaceae* (11.1±0.47)	–
		Clostridiaceae (20.8±0.84)	*Clostridium* (17.5±0.59)
			Faecalibacterium (3.1±0.23)
		Lachnospiraceae (3.88±0.14)	*Blautia* (2.19±0.05)
		Другие (35.2±0.11)	–
Proteobacteria (3.22±0.13)	*Deltaproteobacteria* (2.32±0.09)	*Desulfovibrionaceae* (2.32±0.09)	–
Group I (n = 5)			
Bacteroidetes (66.2±3.20*)	*Bacteroidia* (66.2±3.20*)	*Rikenellaceae* (56.2±2.44*)	*Alistipes* (56.2±2.44*)
		Bacteroidaceae (9.95±0.56*)	*Bacteroides* (9.95±0.56*)
Firmicutes (20.5±0.92*)	*Clostridia* (19.9±0.88*)	*Ruminococcaceae* (8.85±0.51)	–
		Clostridiaceae (2.85±0.12*)	*Faecalibacterium* (2.85±0.12)
		Lachnospiraceae (2.19±0.16)	–
Actinobacteria (2.1±0.14)	*Actinobacteria* (2.1±0.14)	*Micrococcaceae* (2.1±0.14)	*Rothia* (2.1±0.14)
Proteobacteria (2.59±0.36)	*Gammaproteobacteria* (2.59±0.36)	–	–
Group II (n = 5)			
Firmicutes (93.7±3.78*)	*Clostridia* (75.1±2.69)	*Ruminococcaceae* (24.5±0.87*)	*Subdoligranulum* (2.64±0.09)
			Ruminococcus (8.24±0.35)
		Clostridiaceae (22.7±0.94)	*Faecalibacterium* (13.4±0.53*)
			Clostridium (7.17±0.28)
			Butyricicoccus (2.17±0.10)
		Lachnospiraceae (24.4±0.76*)	*Blautia* (3.77±0.14)
			Coprococcus (2.56±0.21)
			Fusicatenibacter (7.65±0.36)
	Bacilli (18.6±0.77*)	*Lactobacillaceae* (17.7±0.72*)	*Lactobacillus* (17.7±0.72*)

Continued Table 3

Group III (n = 5)			
Proteobacteria (2.09±0.24)	*Gammaproteobacteria* (2.01±0.24)	–	–
Firmicutes (49.9±2.47)*	*Clostridia* (46.8±2.41)	*Ruminococcaceae* (18.6±0.83)	*Subdoligranulum* (4.59±0.17) *Ruminococcus* (2.28±0.31)
		Clostridiaceae (20.4±0.98)	*Clostridium* (2.39±0.21*) *Faecalibacterium* (17±0.72*)
		Lachnospiraceae (2.85±0.18)	–
	Bacilli (2.2±0.27)	*Lactobacillaceae* (2.2±0.27)	*Lactobacillus* (2.09±0.23)
Bacteroidetes (35.2±1.21*)	*Bacteroidia* (35.1±1.21*)	*Rikenellaceae* (30.7±1.12)	*Alistipes* (30.7±1.12)
		Bacteroidaceae (4.36±0.08)	*Bacteroides* (4.36±0.08)

N o t e. For description of groups, see *Materials and methods*. Dashes indicate that the marked taxa have not been classified. Microorganisms assigned to other unidentified taxa were not counted.
* Differences between the treatment and control are statistically significant at $p \leq 0.05$.

Phylum *Firmicutes*, with the classes *Clostridia* and *Bacilli*, dominated in group II while the phylum *Bacteroidetes* was absent. Similar effects were observed when supplementing broiler feed with grape extract [36]. Therefrom, it can be concluded that the dietary Quercus cortex directly affects microbial profiles of the broiler chicken cecum with regard to taxa *Firmicutes*, *Bacteroidetes*, *Bacilli*, and *Clostridia*, which can change metabolic processes in the body, in particular energy resorption or degradation of proteins and polysaccharides.

There were reports that the increase in broiler weight correlated with changes in the gut microbiota composition, especially with the abundance of *Bacteroidetes* and *Firmicutes* members [37]. With an increase in growth rates, a sharp increase in the abundance of *Bacteroidetes* representatives and a decrease in the proportion of *Firmicutes* were observed. This is due to the fact that the bacteria of the phylum *Bacteroidetes* are mainly able to stimulate digestion in the intestine, since they play a central role in the hydrolysis of complex molecules to simpler ones [38]. It is also important that bacteria of the genus *Alistipes* (family *Rikenellaceae*) are resistant to bile and necessary for the intestine, as they are capable of producing fibrinolysin, digesting gelatin, and fermenting carbohydrates to form acetic acid [39]. The genus *Odoribacter* (family *Porphyromonadaceae*) can ferment water carbohydrates to form short-chain fatty acids, which is important for the growth of both microorganisms and epithelial cells of the host organism [40]. Consequently, the improvement in the growth performance of broilers may be associated with an increase in the relative proportion of *Bacteroidetes* phylum bacteria in the cecum microbiota. Note also that the bacteria of the phylum *Firmicutes*, in turn, are important for feed digestion [41].

Our results of taxonomic analysis based on sequencing of the 16S rRNA gene are consistent with the data of a number of studies. Thus, high-throughput sequencing showed that phyla *Firmicutes* [42] and *Bacteroidetes* [43] predominate in the cecum of broiler chickens. However, it has also been reported that the predominance of the phylum *Proteobacteria* is possible [44]. Such variability in the dominance of representatives of different phyla can be associated with both the peculiarities of the sequencing methodology (different studies can use different primers, which, despite their universality, are more specific to certain sequences of the 16S rRNA gene in microorganisms), and with external factors (changes in climatic conditions, diet, age and breed of the studied poultry). It is worth noting that the *Proteobacteria* taxon includes representatives of the opportunistic group of intestinal bacteria. In our study, there was a decrease in the counts of *Proteobacteria* representatives, by 19.56% in group I and by 35.09% in

group III as compared to the control. Though these differences are not statistically significant, the trend we revealed may be of significant interest.

It is assumed that phenolic compounds contained in plant extracts, including oak bark extract, can damage the cell membrane of bacteria by interacting with membrane proteins, or be involved in interaction with cellular enzymes, which directly or indirectly causes metabolic dysfunction and death of bacteria [45]. Phenolic compounds are also able to inhibit the Quorum Sensing signal receptors and reduce toxin secretion [46]. Nevertheless, the control of pathogenic intestinal microflora with plant extracts, according to a number of reports, may differ from the direct action of antibacterial drugs and involve other mechanisms. Thus, it was shown that a dietary extract containing capsaicin, carvacrol and cinnamaldehyde significantly increases the number of *Lactobacillus* spp. [47]. In rats, this was associated with the stimulation of *Lactobacillus* growth and the production of lactic acid in the presence of carvacrol [48].

Discussing the increase in threonine content, it should be noted that, as reported [49], endogenous losses of some amino acids, if assessed by their amount in excrement, increases under the influence of tannic acid found in plant extracts. This was the case for methionine, histidine and lysine, while excretion was the smallest for threonine, cysteine and valine. Tannins are also suggested to influence the reabsorption of amino acids from the intestinal lumen. There are reports of adverse in vitro and in vivo effects of pure tannins and plant extracts on intestinal absorption and transport of amino acids such as proline, methionine, alanine, and phenyalanine [50].

The increase in the content of polyunsaturated fatty acids revealed by us is consistent with the results of Koreleski et al. [51]. They showed that the use of sage extract increases the content of araquinonic acid in the pectoral muscles of chickens. In addition, these same authors have described the different effects of sage and rudbeckia extracts. Thus, a dose of 560 mg added to poultry feed contributed to a change in the fatty acid profile. The addition of sage extract to the diet reduced the accumulation of polyunsaturated fatty acids in the pectoral muscles compared to control.

It is known that some medicinal plants have a positive effect on the productive and economic indicators in growing poultry, in particular on lipid metabolism in the liver and the antioxidant status of the body [52]. It was reported [53] that in the groups that received supplements containing polyphenols, the protein content in poultry pectoral muscles increased. Metabolites of phenolic compounds, the tannins and other substances, including those contained in Quercus cortex extract, possess antioxidant properties [54] which can affect the profile of fatty acids in muscle tissue. The low lipid content in poultry meat and the relatively high content of polyunsaturated fatty acids are recognized as one of the main beneficial properties of this valuable food product [55].

However, it should be noted that some bioactive substances contained in plant extracts and performing protective functions in plant tissues can have an ambiguous effect on the animal body [56], in particular on such essential processes as protein and fat metabolism. It is important to remember that in the composition of diets, trace elements interact with each other [57]. The property of tannins to bind to enzymes is known, and differences in the chemical structure of these polyphenols can affect such interactions [58, 59] and, as a consequence, metabolic processes which also change in different periods of poultry growth. Nevertheless, at a certain dose, tannins have a positive effect on productivity [60, 61].

So, our studies have shown that when using oak bark extract as a feed additive, the content of amino acids and unsaturated fatty acids in broiler meat

increases compared to the control poetry not fed the additive. At 1 ml/kg live weight, oak bark extract reduces the carcass fat content. The extract also has a positive effect on the cecum microbiota, increasing the abundance of microorganisms from the phyla *Bacteroidetes* and *Firmicutes*, which are necessary for the normal digestion of feed in the intestines of poultry. Thence, the use of oak bark extract in feeding broiler chickens at a dosage of 1-3 ml/kg live weight improve digestion processes in the intestine and promotes an increase in the content of amino acids and unsaturated fatty acids in the carcass meat, which, in turn, improves the consumer properties of the meat products.

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