AGRICULTURAL BIOLOGY,
2018, V. 53, № 4, pp. 799-810
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.5:636.086.78

doi: 10.15389/agrobiology.2018.4.799eng
doi: 10.15389/agrobiology.2018.4.799rus

ADDITION OF Quercus cortex EXTRACT TO BROILER DIET CHANGES SLAUGHTER INDICATORS AND BIOCHEMICAL COMPOSITION OF MUSCLE TISSUE

V.A. BAGIROV[1], G.K. DUSKAEV[2], N.M. KAZACHKOVA[2], Sh.G. RAKHMATULLIN[2], E.V. YAUSHEVA[2], D.B. KOSYAN[2], SH.A. MAKAEV[2], Kh.B. DUSAEVA[3]

[1]*Ernst Federal Science Center for Animal Husbandry,* Federal Agency of Scientific Organizations, 60, pos. Dubrovitsy, Podolsk District, Moscow Province, 142132 Russia, e-mail vugarbagirov@mail.ru;
[2]*Federal Research Centre of Biological Systems and Agrotechnologies RAS,* Federal Agency of Scientific Organizations, 29, ul. 9 Yanvarya, Orenburg, 460000 Russia, e-mail gduskaev@mail.ru (✉ corresponding author), vniims.or@mail.ru, vasilena56@mail.ru, shahm2005@rambler.ru, kosyan.diana@mail.ru;
[3]*Orenburg State University*, 13, prosp. Pobedy, Orenburg, 460018 Russia, e-mail albordusaeva@mail.ru
ORCID:
Bagirov V.A. orcid.org/0000-0001-5398-8815
Duskaev G.K. orcid.org/0000-0002-9015-8367
Kazachkova N.M. orcid.org/0000-0002-0871-736X
Rakhmatullin Sh.G. orcid.org/0000-0003-0143-9499
Yausheva E.V. orcid.org/0000-0002-1589-2211
Kosyan D.B. orcid.org/0000-0002-2621-108X
Makaev Sh.A. orcid.org/0000-0001-5836-0330
Dusaeva Kh.B. orcid.org/0000-0001-9176-0694

Acknowledgements:
Samples were analyzed in the Shared Use Center FRC BSAT RAS (Ros.RU № 000121 PF59 of 12.10.15) and in testing laboratory of the Center for Biotic Medicine NPO (Registration Certificate of ISO 9001: 2000, Number 4017-5.04.06).
Supported financially by Russian Science Foundation (project № 16-16-10048)
Received February 17, 2018

Abstract

Following world trends in feeding farm animals, it should be noted an increasing interest in polyphenolic substances derived from plant extracts. Recent studies indicate the ability of these substances to stimulate growth of animals and poultry, to reduce the risk of diseases and to improve consumer properties of products. This paper is the first our report to show that dietary Quercus cortex extract, when used separately or in combination with amylolytic and cellulolytic enzymes, promotes slaughter indices of Smena 8 cross broilers and can change meat composition on fatty acids and some microelements. For the experiment, 7-day chicken broilers were divided by analogs' method into 4 groups (n = 30, a total of 120 birds). Control group received main diet, Quercus cortex extract was added to the diets of groups I and II (2.5 ml/kg LW), group II and group III were also fed with an enzymatic preparation containing glucoamylase and concomitant cellulolytic enzymes (5 g/10 kg of feed). Dietary Quercus cortex extract led to an increase in pre-slaughter weight by 4.4-16.6 % over the entire experiment as compared to the control broilers, and caused changes in small intestine microbiota. The counts of *Bacteroidetes* and *Firmicutes* phyla were 5.1 % and 4.0 % higher, respectively, while *Proteobacteria* counts decreased by 3.2 %. Enzyme supplement increased the abundance of *Bacteroidetes*, *Firmicutes* and *Proteobacteria* phyla by 7.3 %, 6.5 % and 5.8 %, and combination of the enzyme preparation with the extract of oak bark increased the counts of *Actinobacteria* phyla by 9.0 %. In group II, the dry matter content in pectoral muscle was 1.29 % higher ($p \leq 0.05$) and crude fat was 1.35 % higher ($p \leq 0.05$), in group I and group III these values increased by 0.76 and 0.87 %, and by 0.08 and 0.33 %, respectively. The total concentration of unsaturated fatty acids was almost at the same level, and some peculiarities have been identified for only certain acids. The most obvious one was a decline in monounsaturated myristoleinic acid level in the experimental groups ($p \leq 0.05$) while the level of palmitoleic acid was rising ($p \leq 0.05$). In group I, the concentration of linolenic acid was higher ($p \leq 0.05$) compared to that in all test groups and the control group. The total concentration of saturated fatty acids in muscle samples of control and test groups also remained practically unchanged and leveled due to long-chain fatty acids which content in the test groups increased vs. a decrease in myristic acid level ($p \leq 0.05$). As a result of Quercus cortex extract supplement, a significant increase in magnesium level ($p \leq 0.05$) was found in group I. In all test groups, we observed a decrease in the level of calcium ($p \leq 0.05$) and iron, zinc, copper, cobalt and iodine, the trace elements ($p \leq 0.05$), in the broilers' muscle tissue as compared to the control group. Thus, the dietary Quercus cortex extract contributes to the increase in slaughter indices of broilers, can change fatty acid and elemental profile of muscle tissue and influences the microbiome of small intestine.

Keywords: Quercus cortex extract, broilers, muscle tissue, chemical composition, fatty acids, bioelements

The development of resistance to antimicrobial drugs and discussed possibility of transferring the resistance genes from animals to humans causes the growing concern that leads to the refusal of using feed antibiotics. However, the exclusion of them from diets decreases the efficiency of technologies in livestock and poultry farming [1].

One of the global trends in the practice of feeding farm animals and poultry is the growing interest to the perspectives of using plant extracts, including those containing tannins. Thus, the performed studies point out to the growth-promoting effect of these compounds to various animals and birds species [2, 3], while medicinal plants and tannins can reduce the risk of emergence of animal diseases [4-6] and change the consumer properties of a product [7-9]. The positive effect of the tannin derivatives of *Emblica officinalis* (Emblica, Indian Gooseberry) on the baby chickens' humoral immune responses against the coccidiosis infection [10] has been noted. The ability of garlic and pennywort to improve the growth of broiler chickens and to cause the positive changes the intestinal microbial communities and the fatty acid composition of the pectoral muscles has been studied; also these plants exhibit the immunomodulating effect and control the intestinal enteropathogens that influence on the results of feed conversion [11]. It is known that some medicinal and edible plants have a positive effect on the growth and economic effectiveness when growing poultry, for example, the positive effect of certain medicinal herbal mixtures on the lipid metabolism in the liver and on the organism's antioxidant status has been reported [12-15].

Poultry meat has a lot of beneficial nutritional properties, the important one of which is low content of lipids and relatively high content of polyunsaturated fatty acids [16-18].

The application of multienzyme complexes causes the active growth of intestinal microflora and the loss of a part of the substances reduced by enzymes. The suppression of microflora enables an increase in productive effect of enzymes significantly (by 30-35%). The combined use of an antibiotic and an enzyme preparation was discussed as a promising approach [19, 20]. It was reported that nitrogen digestibility increased by 37.0% and the pronounced synergism of these substances has been noted [19].

In the present paper, it was shown for the first time that the inclusion of the extract of Quercus cortex in the enzyme-containing diet of broilers contributes to the improvement of the slaughter qualities of poultry, changes in the fatty acid and elemental profile of muscle tissue and in the structure of the intestinal microbiome.

Our objective was to study the effect of Quercus cortex extract on the productive and quality parameters of poultry with enzyme containing diet.

Techniques. The researches were conducted in vivarium conditions (Orenburg region, 2017) on the chickens of the Smena 8 cross (n = 120). The 7-day-old broiler chickens selected for the experiment were divided into 4 groups using the analog method (n = 30). During the experiment, all the birds were in the same living conditions. The basal diets (BD) have been composed with taking into account the recommendations of the All-Russian Research and Technological Institute of Poultry (V.I. Fisinin, I.A. Egorov, T.N. Lenkova and others Guidelines for Optimizing the Recipes of Combined Feeds for Poultry. Moscow, 2009).

According to the scheme of experiment, the control group had been receiving the BD, the experimental group I — BD + extract of Quercus cortex (2.5 ml per 1 kg of body weight), experimental group II — BD + extract of

Quercus cortex (2.5 ml/kg of body weight) + enzyme preparation (5 g per 10 kg of feed), experimental group III — BD + enzyme preparation (5 g per 10 kg of feed). The enzyme preparation (GlucoLux-F, Sibbiopharm, Russia) containing glucoamylase and accompanying cellulolytic enzymes (xylanase, β-glucanase, cellulase) has been applied according to the manufacturer's standards. The birds were fed twice a day; the feed intake was accounted daily. The birds' living conditions and the procedures when conducting the experiments met the requirements of the instructions and recommendations of the Russian Specifications (Order of the Ministry of Health of the USSR No. 755 dated August 12, 1977) and of The Guide for Care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996). Every effort to minimize the birds' suffering and to reduce the number of used samples has been made. The birds' decapitation under the Nembutal ether has been made on the 42nd day.

To prepare extract of Quercus cortex, 50 g of crushed bark (dosage form) were poured with 500 ml of hot (70 °C) distilled water, heated in a boiling water bath for 30 min, percolated and filtered (White Ribbon ashless filters, d 70 mm, ApexLab, Russia). The filtered extract was analyzed by the chromatography-mass spectrometry using a GQCMS 2010 Plus gas chromatograph with the mass-selective detector (Shimadzu, Japan) on the HP-5MS column. For the results interpreting the GCMS Solutions and GCMS PostRun Analysis (Shimadzu, Japan) software was used; for the compound identification the set of CAS spectra libraries (https://www.cas.org), NIST08 (https://www.nist.gov), Mainlib (http://catalog.mainlib.org), Wiley9 (http://www.sisweb.com) has been used. The number of identified components was evaluated by the relative value (%) correlating the peak area of a component with the total peak area of the extract.

The chemical composition of the broiler tissues after the slaughter was determined by standard methods (State Standards GOST 13496.15-97, GOST 51479-99, GOST 23042-86, GOST 25011-81, GOST R 53642-2009), for analysis of fatty-acid composition of the muscle tissue a Crystal-4000 Lux gas chromatograph (NPF Meta-Chrom LLC, Russia) and the Lyumakhrom liquid chromatograph (Lyumeks, Russia) (GOST 51486-99) were used.

In analyzing the elemental composition of the tissues, the biosubstrates were ashed in the MD-2000 microwave decomposition system (PerkinElmer, Inc., USA). The element content in ash was determined (Elan 9000 mass-spectrometer, Optima 2000 V atomic emission spectrometer, PerkinElmer, Inc., USA).

The samples content of the broiler small intestine were placed into the sterile Eppendorf microtubes with the snap-on lid (Nuova Aptaca S.R.L., Italy); DNA was extracted and purified according to the modified method [21]. DNA purity (according to OD_{260}/OD_{280}) was assessed with a NanoDrop spectrophotometer (Thermo Scientific, USA), for measuring concentration (ng/μl) a Qubit 2.0 fluorimeter (Invitrogen/Life Technologies, USA) was used. DNA concentration was measured 3 times: after extraction, after the first polymerase chain reaction (PCR) with the specific 16S prokaryotic primers, and after the second PCR with the adapters and indices of Nextera XT protocols. The analysis of microflora was made by metagenomic sequencing (Illumina MiSeq, Illumina, USA) with the MiSeq® Reagent Kit v3 (600 cycles). The bioinformatic processing of the results was performed with PEAR software (Pair-End AssembeR, PEAR v0.9.8) [22].

Filtering, dereplicating, removing chimeric sequences, clustering, sorting (single-tons cutoff) and removing the contamination were performed in the USEARCH program (usearch v8.0.1623_i86linux32, http://drive5.com/usearch).

We used -fastq_filter algorithm for filtering, -derep_prefix algorithm for replication, and -cluster_otus algorithm for clustering and deleting chimeric sequences [23-26]. The VAMPS resource (Visualization and Analysis of Microbial

Population Structures, https://vamps.mbl.edu/) [27] was used for visualization. The sequencing results were processed with Microsoft Excel software package.

The experiment was completed with the balance experiments for determining the feed digestibility and nutrients utilization by the birds [28]. Ammonia in the average sample of the bird manure was fixed with the 0.1 N oxalic acid (4 ml per 100 g of manure). Upon the completion of a balance experiment, the samples were dried at 60-70 °C and kept in airtight container. The loss of substances and the amount of assimilated feed were calculated from daily accounting of the manure weight and composition.

The statistical processing was performed using SPSS Statistics 20 software (IBM, USA). The mean (M) and root-mean-square deviations ($\pm\sigma$) as well as the standard deviation errors ($\pm$SEM) were calculated. Nonparametric Wilcoxon test was used for comparison of the variants. The differences were considered statistically significant at $p < 0.05$.

Results. In the prepared oak bark extract, we have identified 35 compounds (Table 1) among which we detected the substances (10%) exhibiting the activity against the first-type Quorum Sensing system. The data on the investigation of the biological properties of these substances are partly presented by us earlier [29, 30].

1. Chemical compounds identified in the prepared extract of the Quercus cortex oak's bark

Name of the identified compound (under IUPAC))	Peak area, %
Propantriol-1,2,3*	3.56
Decane*	0.30
2-furancarboxylic acid*	0.30
1,3,5-triazin-2,4,6-triamine*	0.59
Pentadecane*	0.25
2,3-dihydroxypropanal*	0.35
Butanedioic acid*	0.30
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one*	1.19
2-amino-9-[3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3H-purin-6-one*	0.59
Cyclopentane-1,2-diol**	0.30
1,2:5,6-dinghydrogalaccitol**	0.89
5-hydroxymethylfurfurol*	1.98
Acetylcysteine, (R)-2-acetamido-3-mercaptopropanoic acid*	0.89
1-methylundecyl ester of 2-propenoic acid**	1.39
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one**	0.79
1-(2-hydroxyethyl)-4-methylpiperazine**	1.33
6-(4-hydroxy-6-methoxy-2-methyl-tetrahydro-pyran-3-yloxy)-2-methyl-dihydro-pyran-3-one**	0.79
1,2,3-trihydroxybenzene*	0.99
2-methyl-5-nitropyrimidine-4,6-diol**	0.99
4-hydroxy-3-methoxybenzaldehyde*	0.53
2-amino-9-[3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3H-purin-6-one*	25.6
1,6-anhydro-β-D-glucopyranose*	6.14
1-(β-D-arabinofuranosyl)-4-O-trifluoromethyluracil**	0.99
4-hydroxy-3-methoxybenzoic acid**	0.69
1,6-anhydro-β-D-glucofuranose*	0.89
4-propyl-1,3-benzenediol*	1.38
1,2,3,4,5-cyclohexanpentol*	36.38
4-(hydroxymethyl)-2,6-dimethoxyphenol*	0.37
4-(3-hydroxy-1-propenyl)-2-methoxyphenol*	4.45
9 -[(2R, 3R, 4S, 5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3H-purin-2,6-dione*	0.30
7-hydroxy-6-methoxy-2H-1-benzopyran-2-one*	0.48
methyl-α-D-glucopyranoside*	1.19
2H-1-benzopyranone-2*	0.30
2-ethoxy-6-(methoxymethyl)-phenol**	0.75
3,4,5-trimethoxyphenol**	1.79

* The components identified with the probability > 90%
** The components identified with the probability < 90%

The dietary Quercus cortex extract in group I led to the increase in preslaughter weight by 4.4% compared to the control, and in the weight of the semi-eviscerated carcass by 4.7% (by the end of the experiment). In the experi-

mental group II, with enzyme containing diet, the pre-slaughter weight increased by 16.6% ($p \leq 0.05$) and the weight of the semi-eviscerated carcass by 21.5%. In the groups, the share of muscle tissue in the weight of the semi-eviscerated carcass amounted to 55.5-57.3%. The positive effect of tannin containing preparations on the slaughter parameters of broiler chickens is known [31]; in particular, it has been noted upon the inclusion in the diet of the chestnut-based additives with 77%tannin content. These data have been confirmed in our research, and when comparing the obtained results, it is necessary to take into account that the concentration of tannins in the Quercus cortex extract is less than 20%.

The investigation of the muscle tissue chemical composition (Table 2) revealed the increase in the content of dry matter by 1.29% ($p \leq 0.05$) and of crude fat by 1.35% ($p \leq 0.05$) compared to the control in test group II, and by 0.76 and 0.87%, 0.08 and 0.33%, respectively, in test groups I and III. In tes groups there was a tendency to increase the protein content in pectoral muscles. This is consistent with the data of the previously performed assessment of the influence of polyphenolic substances on the chemical composition of meat and the results of feeding broilers with exogenous amylase in combination with xylanase [32, 33].

2. Chemical composition (%) of pectoral muscle tissue of 42-day-old broiler chickens of Smena 8 cross fed with dietary oak bark extract and biopreparation with amylolitic and cellulolytic activity ($M \pm$SEM, $n = 11$, the experiment in vivarium conditions)

Parameter	Group			
	control	I	II	III
Mass fraction of dry matter	25.19±0.17	25.61±0.27	26.48±0.42*	25.72±0.89
Mass fraction of crude fat	4.54±0.64	5.56±1.02	5.89±0.40*	5.81±0.85
Mass fraction of protein	20.10±0.51	20.57±0.50	20.77±0.11	20.63±0.71
Mass fraction of ash	0.96±0.01	0.96±0.01	0.97±0.01	0.97±0.02

N o t e. Control group was fed with basal diet (BD); test group I received BD + extract of Quercus corte; test group II received BD + extract of Quercus cortex + enzyme preparation; test group III received BD + enzyme preparation.

* Differences with the control group are statistically significant at $p \leq 0.05$

3. Fatty-acid profile (%) of the pectoral muscle tissue of 42-day-old broiler chickens of Smena 8 cross fed with dietary oak bark extract and biopreparation with amylolitic and cellulolytic activity ($M \pm$SEM, $n = 11$, the experiment in vivarium conditions)

Fatty acids	Group			
	control	I	II	III
Unsaturated fatty acids in total	69.78	70.09	69.49	70.18
of which the monounsaturated fatty acids	36.52	36.09	37.23	36.99
Saturated fatty acids in total	29.36	30.69	30.09	30.17
Myristic $C_{14:0}$	0.63±0.23	0.53±0.15*	0.50±0.10*	0.59±0.17
Myristolein $C_{14:1}$	0.13±0.13	0.10±0.13	0.10±0.12*	0.12±0.15
Palmitic $C_{16:0}$	19.03±1.69	20.13±0.35	20.76±0.73	19.81±0.98
Palmitoleic $C_{16:1}$	2.46±0.61	3.53±0.51*	4.10±0.54*	3.77±0.71
Stearic $C_{18:0}$	9.70±1.02	10.03±0.58	8.83±0.97	9.77±0.83
Oleic $C_{18:1}$	33.93±0.89	32.46±0.27	33.03±0.69	33.10±0.77
Linoleic $C_{18:2}$	1.50±0.15	1.50±0.21	1.56±0.19	1.49±0.18
Linolenic $C_{18:3}$	31.76±1.43	32.50±0.32**	30.70±0.79	31.70±0.97

N o t e. See the groups' description in Table 1.

* Differences with the control group are statistically significant at $p \leq 0.05$

** Differences with the experimental group II are statistically significant at $p \leq 0.05$

The energy content in the diet and feed intake are the most important factors influencing the accumulation of lipids in the muscle tissue. The additional energy in the diets of the experimental groups contributed to the increase of the feed utilization efficiency and could be deposited as fat in the muscles. The analysis of the fatty-acid profile of the broiler muscle tissue (Table 3) showed that in the control and test groups the sum of unsaturated fatty acids was almost

the same. At the same time, the peculiarities of accumulation of some fatty acids have been revealed. In particular, the decrease in the content of monounsaturated myristoleic acid by 23.1% both in the test group I ($p \leq 0.05$) and in II ($p \leq 0.05$) upon the increase of the share of palmitoleic acid ($p \leq 0.05$) by 43.5 and 66.7% respectively was the most obvious. The content of polyunsaturated long-chain fatty acids has not being changing significantly. Previously, the reduction of the share of monounsaturated fatty acids in the bird muscle tissue upon the inclusion in the diet of phenolic compounds (tannin, gallic acid, thymol) has been noted by other authors [34]. There is the data on the increasing of the content of polyunsaturated fatty acids in the broiler chicken muscle after the addition of thymol, carvacol and other polyphenols to the feed [11, 35]. Our data are consistent with this, i.e. the content of linolenic acid in test group I was 2.3-5.8% higher ($p \leq 0.05$) compared to control and other test groups. The content of saturated fatty acids in muscle tissues of the control and test groups also remained almost unchanged. It should be noted that such leveling of the content occurred mostly owing to the long-chain fatty acids vs. a 15.8-20.6% reduction of myristic acid in test groups ($p \leq 0.05$). The metabolites of phenolic compounds (tannins and other substances) including those contained in the extract of Quercus cortex have antioxidant properties [36-38] that could influence the fatty-acid profile of the muscle tissue.

It is quite probably that some of the biologically active substances contained in the plant extracts and performing protective functions in the plant tissues may ambiguously influence birds [39, 40], in particular, assimilation of microelements, therefore we investigated the elemental status of the broiler muscle tissue (Table 4).

4. Content (μg/g) **of essential elements in pectoral muscle tissue of 42-day-old broiler chickens of Smena 8 cross fed with dietary oak bark extract and biopreparation with amylolitic and cellulolytic activity** (M±SEM, n = 11, the experiment in vivarium conditions)

Element	Group			
	control	I	II	III
Ca	202±20	151±15*	156±16*	172±15
P	2608±261	2741±274	2515±251	2687±270
K	4231±423	4556±456	4600±460*	4495±431
Mg	315±31	375±37*	344±34	361±29
Na	775±78	638±64	539±54*	597±65
Zn	17.1±1.71	9.54±0.95	7.26±0.73*	8.93±0.98
Mn	0.207±0.025	0.135±0.016*	0.138±0.017*	0.143±0.020
Cu	0.694±0.083	0.309±0.037*	0.317±0.038*	0.433±0.044
Fe	13.76±1.38	6.25±0.62*	6.85±0.68*	7.55±0.95
Co	0.0019±0.00037	0.0008±0.00023*	0.0007±0.00022*	0.0010±0.00033*
Se	0.181±0.022	0.148±0.018	0.134±0.016*	0.157±0.020
I	0.116±0.014	0.039±0.006*	0.011±0.002*	0.088±0.007

N o t e. See the groups' description in Table 1.

* Differences with the control group are statistically significant at $p \leq 0.05$.

After the inclusion of the Quercus cortex extract in the diet, the accumulation of magnesium in test group I increased significantly ($p \leq 0.05$). This is probably related to its high concentration in the extract itself (1628 rg/g), as well as to the ability to form the weak complexes with chemical elements in the gastrointestinal tract and to more efficient reabsorption [41].

In test groups the calcium content in muscle tissue decreased ($p \leq 0.05$) compared to control. This can be explained by the formation of tannin-calcium complexes in the gastrointestinal tract of chickens that is implicitly confirmed by the literature data [42]. The calcium oxalate formation was found to be inhibited in laboratory animals fed with the extract of *Sargassum wightii* Greville ex J. Agardh, which also contains phenolic metabolites and tannins.

Magnesium and calcium are antagonists, and the increase in consumption of one of them causes the increased excretion of another one; moreover, a lot of researches have shown that as the calcium consumption increases, its absorption decreases [41]. In our experiment, the calcium concentration in the extract of Quercus cortex was the highest, 0.012 mg/g.

In test groups, the accumulation of three microelements, iron, zinc and copper, decreases ($p \leq 0.05$) that coincides with the data about decreasing of the zinc and copper content in the liver of monogastric animals fed with vegetable products (extract of grape marc) containing polyphenolic substances [43]. In other research [44], the authors suggested that the absorption of Ca, P, Mg, Na, K, Fe, and Co decreases when a high tannin content in the diet (1.36% of the dry matter of the diet). The results of our experiment agree with this. In addition, it is known that tannin fixes iron in the intestinal lumen that, in turn, affects the growth of microorganisms [45]. The fact of interaction with each other of the microelements contained in the broiler diets is no less important [46, 47].

Exogenous enzymes in the broiler diet had no apparent effect on the muscle tissue's chemical composition.

In the starting period, the digestibility of crude protein in test group I was 4.0% lower than in the control, but in the second growing period, this parameter, on the contrary, increased by 4.2%. The highest digestibility in both periods was noted in group II, i.e. in the first period, the difference with control was in raw protein 3.6%, in crude fiber 0.7%, in crude fat 5.7% ($p \leq 0.05$), in the second period these were 1.7; 7.7 and 3.8%, respectively. The tannin ability to bind with enzymes is known, and differences in the chemical structure of these polyphenols can influence on such interactions [48] and, as a result, on metabolic processes which are being changing during different periods of poultry growing. At a certain dose, tannins have a positive effect on productivity [49]. A similar effect (fluctuations in the digestibility of substances during growth) is observed in our experiment.

The use of the oak bark extract in the diet led to the changes in the quantitative composition of microorganisms in the small intestine of broiler chickens. The increase in the number of the gram-negative, non-sporeforming anaerobic rod-shaped bacteria of the *Bacteroidetes* phylum (by 5.1%) and of the bacteria of *Firmicutes* phylum, which have mostly gram-positive cell wall type (by 4.0%) and decrease in the number of the *Proteobacteria* group (by 3.2%) were noted. The changes were mainly related with the increase in the number of microorganisms of *Bacteroidia* (by 5.2%) and *Bacilli* (by 6.5%) classes and with the decrease in the representation of the *Gammaproteobacteria* (by 3.3%) and *Clostridia* (by 4.3%) classes. The investigation of the species composition showed the increase in the number of bacteria of the *Bacteroides* (by 4.9%), *Clostridium* (by 8.4%) and *Lactobacillus* (by 7%) genera as compared to control.

The enzyme additive caused the increase in the number of microorganisms of the *Bacteroidetes* (by 7.3%), *Firmicutes* (by 6.5%) and *Proteobacteria* (by 5.8%) phyla. The changes were mainly related with the increase in the number of representatives of the *Bacteroidia* (by 6.1%), *Bacilli* (by 14.4%) and *Clostridia* (by 17.1%) classes. The counts of bacteria of *Bacteroides* and *Clostridium* genera increased by 9.5% and 7.8%, respectively.

The combination of the enzyme additive with the oak bark extract increases the number of the *Actinobacteria* phylum bacteria (by 9.0%) and reduces *Bacteroidetes* phylum microorganisms (by 6.7%) that is natural when changing of the population of *Actinobacteria* and *Bacteroidia* classes. In the *Firmicutes* taxon, the increase in the number of the *Bacilli* bacteria (by 35.5%) and the decrease in the number of *Clostridia* microorganisms (by 36.8%) were observed. The number

of bacteria of *Lactobacillus* genus increased by 34.7%, of *Corynebacterium* increased by 9.0% and of *Clostridium* genus increased by 5.7%. The other investigations have also demonstrated the inhibition of the clostridia growth by the tannin containing substances [31] and the large changeability of the microbiome of the broiler' small intestine influenced by tannins [7[.

5. Representation of bacterial taxa in the small intestine of 42-day-old broiler chickens of Smena 8 cross fed with dietary oak bark extract and biopreparation with amylolitic and cellulolytic activity (M±SEM, n = 5, the experiment in vivarium conditions)

Phylum, class	Family	Genus
	Control	
Phylum *Bacteroidetes* (8.8±0.12 %):		
Bacteroidia (8.8±0.06 %)	*Bacteroidaceae* (7.95±0.11 %)	*Bacteroides* (7.95±0.09 %)
Phylum *Actinobacteria* (7.89±0.22 %):		
Actinobacteria (7.89±0.12 %)	*Microbacteriaceae* (2.86±0.08 %)	No data
	Nitriliruptoraceae (4.6±0.13 %)	*Nitriliruptor* (4.6±0.10 %)
Phylum *Firmicutes* (76.1±0.13 %):		
Clostridia (56.0±1.02 %)	*Lachnospiraceae* (16.3±0.23 %)	No data
	Ruminococcaceae (21.1±0.17 %)	*Ruminococcus* (15.8±0.14 %)
	Clostridiaceae (17.5±0.17 %)	*Clostridium* (3.2±0.12 %)
		Faecalibacterium (4.2±0.44 %)
Bacilli (19.6±0.3 %)	*Lactobacillaceae* (18.7±0.08 %)	*Lactobacillus* (18.7±0.13 %)
Phylum *Proteobacteria* (6.13±0.56 %):		
Gammaproteobacteria (5.7±0.87 %)	*Moraxellaceae* (5.64±0.01 %)	*Acinetobacter* (5.6±0.12 %)
	Group I	
Phylum *Firmicutes* (80.1±0.23 %):		
Bacilli (26.1±0.14 %)	*Lactobacillaceae* (25.7±0.18 %)	*Lactobacillus* (25.7±0.13 %)
Clostridia (51.7±0.12 %)	*Clostridiaceae* (25.7±0.81 %)*	*Faecalibacterium* (4.54±0.13 %)
		Clostridium (8.7±0.33 %)
	Ruminococcaceae (14.6±0.11 %)	*Pseudoflavonifractor* (5.03±0.10 %)
		Ruminococcus (3.02±0.08 %)
	Lachnospiraceae (11.1±0.14 %)	*Blautia* (2.06±0.06 %)
Phylum *Proteobacteria* (2.95±0.09 %)*:		
Gammaproteobacteria (2.35±0.14 %)*	No data	No data
Phylum *Bacteroidetes* (14±0.12 %):		
Bacteroidia (14.0±0.02 %)	*Bacteroidaceae* (12.9±0.11 %)	*Bacteroides* (12.9±0.19 %)
Phylum *Actinobacteria* (2.73±0.17 %)*:		
Actinobacteria (2.23±0.05 %)*	No data	No data
	Group II	
Phylum *Firmicutes* (74.3±0.25 %):		
Bacilli (55.1±0.42 %)*	*Lactobacillaceae* (53.4±1.21 %)	*Lactobacillus* (53.4±0.98 %)
	Aerococcaceae (2.34±0.12 %)	No data
Clostridia (19.2±0.09 %)*	*Clostridiaceae* (13.1±0.18 %)	*Clostridium* (8.9±0.35 %)*
	Lachnospiraceae (2.28±0.13 %)	No data
Phylum *Actinobacteria* (16.9±0.12 %)*:		
Actinobacteria (16.9±0.44 %)	*Corynebacteriacea* (17.9±0.11 %)	*Corynebacterium* (16.8±0.22 %)
Phylum *Proteobacteria* (5.2±0.23 %):		
Gammaproteobacteria (5.2±0.75 %)	*Peptostreptococcaceae* (5.4±0.12 %)	*Romboutsia* (2.63±0.51 %)
Phylum *Bacteroidetes* (2.18±0.78 %)*:		
Bacteroidia (2.18±0.41 %)*	*Bacteroidaceae* (2.18±0.19 %)	*Bacteroides* (2.18±0.74 %)
	Group III	
Phylum *Bacteroidetes* (16.1±0.05 %)*:		
Bacteroidia (14.9±0.12 %)	*Bacteroidaceae* (9.9±0.31 %)	*Bacteroides* (17.4±0.09 %)
Phylum *Actinobacteria* (8.9±0.16 %):		
Actinobacteria (7.8±0.18 %)	*Microbacteriaceae* (3.8±0.12 %)	No data
	Nitriliruptoraceae (4.8±0.77 %)	*Nitriliruptor* (2.3±0.04 %)
Phylum *Firmicutes* (82.6±0.12 %):		
Clostridia (73.1±0.17 %)	*Lachnospiraceae* (14.1±0.09 %)	No data
	Ruminococcaceae (21.1±0.08 %)	*Ruminococcus* (11.6±0.12 %)
	Clostridiaceae (29.5±0.07 %)*	*Clostridium* (11±0.06 %)*
		Faecalibacterium (5.0±0.10 %)
Bacilli (34.0±0.11 %)*	*Lactobacillaceae* (28.3±0.08 %)	*Lactobacillus* (19.9±0.22 %)
Phylum *Proteobacteria* (11.9±0.08 %)*:		
Gammaproteobacteria (5.7±0.07 %)	No data	No data

N o t e. See the groups' description in Table 1.

* Differences with the control group are statistically significant at $p \leq 0.05$.

Thus, the slaughter parameters of broilers improve both upon the addition of the Quercus cortex extract to the diet and when using it with enzyme containing diet. The content of some saturated and unsaturated fatty acids (pal-

mitoleic and linolenic) in the muscle tissue increases when feeding the birds with the said extract, but the fatty acid profile remains almost the same. Also, in the test groups, the content of dry matter and fats in the pectoral muscle increases, and the feed digestibility changes depending on the growing period. The detected decrease in the amount of certain macro- and microelements (Ca, Fe, Zn, Cu, Co and I) in the muscle tissue is conditioned by the peculiarities of the extract composition and dosage as well as by the synergistic interactions of chemical elements. After the inclusion of the Quercus cortex extract in the diet, the number of *Bacteroidetes* and *Firmicutes* phyla microorganisms in the thin intestine increases, and the count *Proteobacteria* decreases. Combination of the extract and enzyme containing diet increases the abundance of *Actinobacteria* phylum, and the enzyme containing diet without adding the extract increases abundance of *Bacteroidetes, Firmicutes* and *Proteobacteria* phyla.

REFERENCES

1. Redondo L.M., Chacana P.A., Dominguez J.E., Fernandez Miyakawa M.E. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. *Front. Microbiol.*, 2014, 5: 118 (doi: 10.3389/fmicb.2014.00118).
2. Artem'eva O.A., Pereselkova D.A., Fomichev Yu.P. Dihydroquercetin, the bioactive substance, to be used against pathogenic microorganisms as an alternative to antibiotics. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2015, 50(4): 513-519 (doi: 10.15389/agrobiology.2015.4.513eng).
3. Shuhao W., Lin Z., Jiaolong L., Jiahui C., Feng G., Guanghong Z. Effects of dietary marigold extract supplementation on growth performance, pigmentation, antioxidant capacity and meat quality in broiler chickens. *Asian-Australas. J. Anim. Sci.*, 2017, 30(1): 71-77 (doi: 10.5713/ajas.16.0075).
4. Van Parys A., Boyen F., Dewulf J., Haesebrouck F., Pasmans F. The use of tannins to control *Salmonella typhimurium* infections in pigs. *Zoonoses Public Health*, 2010, 57: 423-428 (doi: 10.1111/j.1863-2378.2009.01242.x).
5. Anderson R.C., Vodovnik M., Min B.R., Pinchak W.E., Krueger N.A., Harvey R.B., Nisbet D.J. Bactericidal effect of hydrolysable and condensed tannin extracts on *Campylobacter jejuni* in vitro. *Folia Microbiol.*, 2012, 57: 253-258 (doi: 10.1007/s12223-012-0119-4).
6. Huyghebaert G., Ducatelle R., Van Immerseel F. An update on alternatives to antimicrobial growth promoters for broilers. *Vet. J.*, 2011, 187(2): 182-188 (doi: 1016/j.tvjl.2010.03.003).
7. Tosi G., Massi P., Antongiovanni M., Buccioni A., Minieri S., Marenchino L., Mele M. Efficacy test of a hydrolysable tannin extract against necrotic enteritis in challenged broiler chickens. *Italian Journal of Animal Science,* 2013, 12(3): e62 (doi: 10.4081/ijas.2013.e62).
8. Castaneda M., Hirschler E., Sams A. Skin pigmentation evaluation in broilers fed natural and synthetic pigments. *Poultry Sci.*, 2005, 84: 143-147 (doi: 10.1093/ps/84.1.143).
9. Duskaev G.K., Kazachkova N.M., Ushakov A.S., Nurzhanov B.S., Rysaev A.F. The effect of purified Quercus cortex extract on biochemical parameters of organism and productivity of healthy broiler chickens. *Veterinary World*, 2018, 11(2): 235-239 (doi: 10.14202/vetworld.2018.235-239).
10. Qari M.K., Masood A., Mian M.A., Muhammad S., Muddassar Z., Zafar I., Faqir M., Anwar M.I. Studies on *Emblica officinalis* derived tannins for their immunostimulatory and protective activities against coccidiosis in industrial broiler chickens. *The Scientific World Journal*, 2014, 2014: Article ID 378473 (doi: 10.1155/2014/378473).
11. Suriya K.R., Idrus Z., Nordiana A.A.R., Mahdi E., Meng G.Y. Effects of two herbal extracts and Virginiamycin supplementation on growth performance, intestinal microflora population and fatty acid composition in broiler chickens. *Asian-Australas. J. Anim. Sci.*, 2014, 27(3): 375-382 (doi: 10.5713/ajas.2013.13030).
12. Patel A.P., Bhagwat S.R., Pawar M.M., Prajapati K.B., Chauhan H.D., Makwana R.B. Evaluation of *Emblica officinalis* fruit powder as a growth promoter in commercial broiler chickens. *Veterinary World*, 2016, 9(2): 207-210 (doi: 10.14202/vetworld.2016.207-210).
13. Kassa S., Mengistu U., Animut G. Effect of different levels of *Lepidium sativum* L. on growth performance, carcass characteristics, hematology and serum biochemical parameters of broilers. *SpringerPlus*, 2016, 5(1): 1441 (doi: 10.1186/s40064-016-3118-0).
14. Shokraneh M., Ghalamkari G., Toghyani M., Landy N. Influence of drinking water containing Aloe vera (*Aloe barbadensis* Miller) gel on growth performance, intestinal microflora, and humoral immune responses of broilers. *Veterinary World*, 2016, 9(11): 1197-1203 (doi: 10.14202/vetworld.2016.1197-1203).
15. Shirzadegan K., Falahpour P. The physicochemical properties and antioxidative potential of raw

thigh meat from broilers fed a dietary medicinal herb extract mixture. *Open Vet. J.*, 2014, 4(2): 69-77.
16. Bourre J. Where to find omega-3-fatty acids and how feeding animals with diet enriched in omega-3-fatty acids to increase nutritional value derived products for human: What is actually useful? *J. Nutr. Health Aging*, 2005, 9(4): 232-242.
17. Wood J.D., Enser M., Nute G., Richardson R., Sheard P. Manipulating meat quality and composition. *Proc. Nutr. Soc.*, 1999, 58(2): 363-370 (doi: 10.1017/S0029665199000488).
18. Yalcin S., Onbaslar I., Sehu A., Yalcin S. The effect of dietary garlic powder on the performance, egg traits and blood serum cholesterol of laying quails. *Asian-Australas. J. Anim. Sci.*, 2007, 20: 944-950 (doi: 10.5713/ajas.2007.944).
19. Nechipurenko L.I., Dyukarev V.V. *Byulleten' VNII fiziologii, biokhimii i pitaniya sel'skokhozyaistvennykh zhivotnykh*, 1973, 2(28): 26-29 (in Russ.).
20. Miles R.D., Brown R.D. Jr., Comer C.W., Oelfke E. Influence of an enzyme and an antibiotic on broiler performance. *Journal of Applied Animal Research*, 1996, 9(2): 105-117 (doi: 10.1080/09712119.1996.9706112).
21. Andronov E.E., Pinaev A.G., Pershina E.V., Chizhevskaya E.P. *Nauchno-metodicheskie rekomendatsii po vydeleniyu vysokoochishchennykh preparatov DNK iz ob"ektov okruzhayushchei sredy* [Methodological recommendations on the isolation of highly purified DNA preparations from environmental objects]. St. Petersburg, 2011 (in Russ.).
22. Zhang J., Kobert K., Flouri T., Stamatakis A. PEAR: A fast and accurate Illumina Paired-End reAd merger. *Bioinformatics*, 2014, 30(5): 614-620 (doi: 10.1093/bioinformatics/btt593).
23. Edgar R.C., Flyvbjerg H. Error filtering, pair assembly and error correction for nextgeneration sequencing reads. *Bioinformatics*, 2015, 31(21): 3476-3482. (doi: 10.1093/bioinformatics/btv401).
24. Edgar R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 2010, 26(9): 2460-2461 (doi: 10.1093/bioinformatics/btq461).
25. Edgar R.C., Haas B.J., Clemente J.C., Quince C., Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 2011, 27(16): 2194-2200 (doi: 10.1093/bioinformatics/btr381).
26. Edgar R.C. UPARSE: highly accurate OTU sequences from microbial amplicon reads *Nature Methods*, 2013, 10(10): 996- 998 (doi: 10.1038/nmeth.2604).
27. Huse S.M., Mark Welch D.B., Voorhis A., Shipunova A., Morrison H.G., Eren A.M., Sogin M.L. VAMPS: a website for visualization and analysis of microbial population structures. *BMC Bioinformatics*, 2014, 15: 41 (doi: 10.1186/1471-2105-15-41).
28. Fisinin V.I., Egorov I.A., Okolelova T.M., Imangulov Sh.A. *Kormlenie sel'skokhozyaistvennoi ptitsy* [Feeding of poultry]. Sergiev Posad, 2010 (in Russ.).
29. Duskaev G.K., Deryabin D.G., Karimov I., Kosyan D.B., Notova S.V. Assessment of (in vitro) toxicity of Quorum Sensing inhibitor molecules of Quercus cortex. *Journal of Pharmaceutical Sciences and Research*, 2018, 10(1): 91-95.
30. Karimov I., Duskaev G., Inchagova K., Kartabaeva M. Inhibition of bacterial Quorum sensing by the ruminal fluid of cattle. *International Journal of GEOMATE*, 2017, 13(40): 88-92 (doi: 10.21660/2017.40.65948).
31. Schiavone A., Guo K., Tassone S., Gasco L., Hernandez E., Denti R., Zoccarato I. Effects of a natural extract of chestnut wood on digestibility, performance traits, and nitrogen balance of broiler chicks. *Poultry Sci.*, 2008, 87(3): 521-527 (doi: 10.3382/ps.2007-00113).
32. Starčević K., Krstulović L., Brozić D., Maurić M., Stojević Z., Mikulec Ž., Bajić M., Mašek T. Production performance, meat composition and oxidative susceptibility in broiler chicken fed with different phenolic compounds. *J. Sci. Food Agric.*, 2015, 95(6): 1172-1178 (doi: 10.1002/jsfa.6805).
33. Amerah A.M., Romero L.F., Awati A., Ravindran V. Effect of exogenous xylanase, amylase, and protease as single or combined activities on nutrient digestibility and growth performance of broilers fed corn/soy diets. *Poultry Sci.*, 2017, 96(4): 807-816 (doi: 10.3382/ps/pew297).
34. Hashemipour H., Kermanshahi H., Golian A., Veldkamp T. Effect of thymol and karvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens.. *Poultry Sci.*, 2013, 92: 2059-2069 (doi: 10.3382/ps.2012-02685).
35. Okuda T. Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochemistry*, 2005, 66(17): 2012-2031 (doi: 10.1016/j.phytochem.2005.04.023).
36. Brenes A., Viveros A., Goci I., Centeno C., Sáyago-Ayerdy S.G., Arija I., Saura-Calixto F. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poultry Sci.*, 2008, 87(2): 307-316.
37. Chamorro S., Viveros A., Rebolé A., Rica B.D., Arija I., Brenes A. Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace. *Food Research International*, 2015, 73: 197-203 (doi: 10.1016/j.foodres.2014.11.054).
38. Hashemi S.R., Davoodi H. Herbal plants and their derivatives as growth and health promoters in animal nutrition. *Vet. Res. Commun.*, 2011, 35(3): 169-180 (doi: 10.1007/s11259-010-9458-2).
39. Smulikowska S., Pastuszewska B., Święch E., Ochtabińska A., Mieczkowska A., Nguyen V.C., Buraczewska L. Tannin content affects negatively nutritive value of pea for monogastrics. *J.*

Anim. Feed Sci., 2001, 10(3): 511-523 (doi: 10.22358/jafs/68004/2001).
40. Oberlis D., Kharland B., Skal'nyi A. *Biologicheskaya rol' makro- i mikroelementov u cheloveka i zhivotnykh* [The biological role of macro- and microelements in humans and animals]. St. Petersburg, 2008 (in Russ.).
41. Sujatha D., Kiranpa Singh, Mursalin Vohra, Kumar K.V., Sunitha S. Antilithiatic activity of phlorotannin rich extract of *Sarghassum wightii* on calcium oxalate urolithiais — in vitro and in vivo evaluation. *Int. Braz. J. Urol.*, 2015, 41(3): 511-520 (doi: 10.1590/S1677-5538.IBJU.2014.0357).
42. Fiese A., Ehrmann M, Geßner D.K., Most E., Eder K. Effects of polyphenol-rich plant products from grape or hop as feed supplements on iron, zinc and copper status in piglets. *Archives of Animal Nutrition*, 2015, 69(4): 276-84 (doi: 10.1080/1745039X.2015.1057065).
43. Hassan I.A., Elzubeir E.A., El Tinay A.H. Growth and apparent absorption of minerals in broiler chicks fed diets with low or high tannin contents. *Tropical Animal Health and Production*, 2003, 35(2): 189-196 (doi: 10.1023/A:1022833820757).
44. Chung K.-T., Lu Z., Chou M.W. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. *Food and Chemical Toxicology*, 1998, 36(12): 1053-1060 (doi: 10.1016/S0278-6915(98)00086-6).
45. Andjelkovia M., Van Camp J., De Meulenaer B., Depaemelaere G., Socaciu C., Verloo M., Verhe R. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chem.*, 2006, 98(1): 23-31 (doi: 10.1016/j.foodchem.2005.05.044).
46. Bao Y.M., Choct M., Iji P.A., Bruerton K. Trace mineral interactions in broiler chicken diets. *Brit. Poultry Sci.*, 2010, 51(1): 109-117 (doi: 10.1080/00071660903571904).
47. Mondal S., Haldar S., Saha P., Ghosh T.K. Metabolism and tissue distribution of trace elements in broiler chickens' fed diets containing deficient and plethoric levels of copper, manganese, and zinc. *Biol. Trace Elem. Res.*, 2010, 137(2): 190-205 (doi: 10.1007/s12011-009-8570-z).
48. Hofmann T., Glabasnia A., Schwarz B., Wisman K.N., Gangwer K.A., Hagerman A.E. Protein binding and astringent taste of a polymeric procyanidin, 1,2,3,4,6-penta-O-galloyl-beta-D-glucopyranose, castalagin, and grandinin. *J. Agric. Food Chem.*, 2006, 54(25): 9503-9509 (doi: 10.1021/jf062272c).
49. Schiavone A., Guo K., Tassone S., Gasco L., Hernandez E., Denti R., Zoccarato I. Effects of a natural extract of chestnut wood on digestibility, performance traits, and nitrogen balance of broiler chicks. *Poultry Sci.*, 2008, 87(3): 521-527 (doi: 10.3382/ps.2007-00113).