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Influence Of Food Factors On The Microbiome Composition And Amino Acids Digestion In Chickens.

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ABSTRACT

The purpose of the research was to study the intestinal microbiome composition of Cobb Avian 48 chicks and the digestibility level of amino acids with balanced and unbalanced by amino acids content rations, and also with introduction of *Bacillus subtilis* 1-85 probiotic strain into the intestine of poultry. The amino acids digestibility was determined based on a digestion trial. The amino acids amount in samples was determined by high-performance liquid chromatography method. The composition of microflora of chicken small intestine was observed using the T-RFLP method. The observe results have shown that digestibility of several amino acids in chicken small intestine was more dependent on the composition of the ration, rather than on the use of probiotic bacterial strain. More effective digestion of amino acids was noted in chickens that received rations balanced by amino acids digestibility. At the same time, the use of the T-RFLP molecular genetic method has shown that the composition of microbial communities of the small intestine of chickens was more dependent on the introduction of a factor adjusting the microflora – the probiotic bacterial strain, rather than on the composition of the ration. Introduction of the bacterial strain contributed to the elimination of chicken small intestine microflora dysbiosis. It has been established that the representatives of intestinal microbiome affect the amino acids digestibility in different ways. The representatives of some bacterial taxa in the small intestine can be competitors for the macroorganism in terms of consumption of amino acids, using them in the cellular anabolism. At the same time, our experiment has shown that some microorganisms were directly related to the increase in certain amino acids digestibility.

Keywords: limiting aminoacid lysine, lysine digestibility, poultry combi-feed, probiotics, T-RFLP for poultry intestine.

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INTRODUCTION

The prevailing thing in poultry feeding is food protein, because the level of liveweight gain and egg production depends on protein in the first place. It was proven a long ago that the use of feed with insufficient protein and imbalance of amino acids, especially the essential ones, could have a negative impact on the vital activity and productivity of poultry [1, 2]. At the same time, low protein digestibility can cause various diseases: nephritis, inflammation of leg joints, etc. Concentrated formula feeds for monogastric animals and poultry, which include only phytogenic components, are usually deficient in the content of the most important limiting amino acids, first, lysine [3]. Therefore, additives of synthetic amino acids are widely used to increase the adequacy of vegetable formula feeds [4-8]. However, it makes formula feeds significantly more expensive.

The poultry intestinal microbiome is a rather rich and complex community of symbiotic microorganisms [9-11], consisting of bacteria, archaea [12], micromycetes [13], protozoa [14] and viruses [15]. The main inhabitants of chicken intestinal microbiome are bacteria [16]. The appearance of 16S rRNA gene sequencing method allowed to find representatives of 13 bacterial phylum in chicken intestinal microbioecosystem, the dominant (>90 %) of which were *Firmicutes*, *Bacteroidetes* and *Proteobacteria*. In total, over 900 equivalents of operational taxonomic units (OTU) were found in the chicken intestine, 117 of them belonging to known bacteria. It has been proven that many representatives of intestinal normal microflora can have a positive effect on the macroorganism, including by active synthesis of enzymes (proteases, cellulases, etc.), contributing to activation of food digestion process, production of vitamins, bacteriocins and other biologically active substances. Interaction of bacteria with each other (antagonism, symbiosis, etc.) and the host macroorganism can affect the digestibility of nutrients in feed [17, 18]. Therefore, one of the ways to reduce feed consumption per unit and increase the efficiency of its digestion is the applying of technologies that use bacterial cultures with enzymatic and probiotic activity, correcting the composition of normal microflora representatives and having antimicrobial activity against pathogens [19-21].

Thus, the purpose of this research was to observe the chicken intestinal microbiome composition and the level of amino acids digestibility with rations that are balanced and unbalanced by amino acids content, and with introduction of a probiotic bacterial strain into the poultry intestine.

MATERIALS AND METHODS

The experiment was performed with Cobb Avian 48 chickens from 1 day to 37 days of age in the vivarium of the Federal Scientific Center All-Russian Research and Technological Institute of Poultry Farming (VNITIP). Eggs were incubated in the incubator house of VNITIP. Birds were kept in battery cages Big Dutchman (without separation by sex) in compliance with all technological parameters, corresponding to the regulations of VNITIP and the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes ETS N 123 (Strasbourg, March 18, 1986). Chicks were fed manually with plenty of dry formula feed in accordance with the cross standards established by VNITIP.

Experimental groups (Table 1) with 40 chicks each were formed at one day of chick age according to the analogue principal – with the same origin, age and general development. The poultry were individually weighed and distributed by groups randomly, according to recommendations for scientific research on the feeding of agricultural poultry [22]. Chickens in group I (Control I) received crumbled compound feed, balanced without considering the amino acids digestibility (CF 1). Chickens in group III were fed with similar compound feed with introduction of *Bacillus subtilis* 1-85 probiotic strain, obtained from a collection of non-pathogenic microorganism strains from BIOTROF+ Ltd. (St. Petersburg). In group II (Control II) chickens received crumbled compound feed, balanced with considering the amino acids digestibility (CF 2). Chickens in group IV were fed with similar compound feed with introduction of a *Bacillus subtilis* 1-85 probiotic strain. Introduction the bacterial strain into the feed started from the first day of life in the amount of 10^3 CFU per gram of compound feed.

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Table 1: The experimental design for chickens

Group	No of specimens	Diet characteristics
Control I	40	Combi-feed balanced regardless of amino acid digestibility (CF 1)
Control II	40	Combi-feed balanced with regard to amino acid digestibility (CF 2)
Experimental I	40	CF 1 + probiotic
Experimental II	40	CF 2 + probiotic

The compound feed composition received by chickens is presented in table 2.

Table 2: Combi-feed formulas for chickens

Components	Proportion of a component in the group diet, %			
	Control I M±m, n=3	Control II M±m, n=3	Experimental I M±m, n=3	Experimental II M±m, n=3
Wheat	58.19±2.8	57.86±2.1	58.19±1.8	57.96±0.9
Sunflower cake	7±0.31	7±0.28	7±0.35	7±0.25
Soybean meal	10±0.5	10±0.47	10±0.44	10±0.32
Fish meal	3±0.12	3±0.11	3±0.08	3±0.14
Sunflower oil	5.8±0.28	5.8±0.18	5.8±0.25	5.8±0.11
Gluten	3±0.11	3±0.15	3±0.12	3±0.12
Peas+soya	10±0.4	10±0.4	10±0.32	10±0.33
Chalk	0.6±0.02	0.6±0.01	0.6±0.03	0.6±0.02
Defluorinated phosphate	1.4±0.05	1.4±0.06	1.4±0.06	1.4±0.07
Salt	0.1±0.009	0.1±0.008	0.1±0.01	0.1±0.005
Lysine	0.28±0.05	0.35±0.006	0.28±0.01	0.35±0.009
Methionine	0.13±0.01	0.23±0.007	0.13±0.008	0.23±0.01
Threonine	-	0.1±0.005	-	0.03±0.0006
Premix	0.5±0.02	0.5±0.03	0.5±0.01	0.5±0.02
Probiotic	-	-	0.1±0.01	0.1±0.008
Metabolic energy, kcal	314.59±14.3	314.92±15.2	314.59±13.0	314.92±12.6
Crude protein	20.1±1.0	20.24±0.95	20.1±0.78	20.24±0.9
Crude fat	9.05±0.4	9.05±0.39	9.05±0.23	9.05±0.44
Crude fiber	4.51±0.2	4.5±0.21	4.51±0.19	4.5±0.15
Calcium	0.89±0.04	0.89±0.03	0.89±0.03	0.89±0.02
Total phosphorus	0.72±0.03	0.72±0.03	0.72±0.04	0.72±0.04
Available phosphorus	0.42±0.02	0.42±0.01	0.42±0.016	0.42±0.015
Sodium	0.16±0.009	0.16±0.005	0.16±0.007	0.16±0.005
Chlorine	0.18±0.007	0.2±0.003	0.18±0.002	0.2±0.01
Potassium	0.72±0.04	0.71±0.03	0.72±0.04	0.71±0.02

The strain of *Bacillus subtilis* 1-85 was obtained from a collection of non-pathogenic microorganism strains from BIOTROF+ Ltd. (Russia). Storage and passage of culture in living condition were performed according to the recommendations [23].

The amino acids digestibility was estimated based on a digestion trial data, performed according to the methodological recommendations of VNITIP, 2000 (Agricultural poultry feeding rationing by available amino acids, 2000). 5 analogue chickens were taken from each group for this purpose. Birds were kept in specially equipped cages for careful account of feed and excrements. The poultry feeding was equal the scientific trial feeding. Before the beginning of the accounting period, the feeders were cleared from remaining feed and the droppings pans were cleaned from excrements. The obtained mass was placed in glass jars with ground stoppers.

The amount of amino acids in excrements and feed samples was determined by high-performance liquid chromatography using the Knauer device (BioKhimMak ST, Russia), equipped with a modular system AZURA. The method included the separation of free amino acids on a cation exchanger in a step pH gradient with subsequent postcolumn derivatization with ninhydrin according to the methodology described in Speckmann et al. works of [24].

The samples of small intestine content for microflora analysis were collected with strict sterility and were immediately frozen (Instructions for sanitary and microbiological control of poultry carcasses, meat, products, eggs and egg products at poultry farms and processing enterprises, 2018) from birds at 37 days old – three replications from each group.

The microbiome composition observe of small intestine was performed by T-RFLP method in molecular-genetic research laboratory of BIOTROF+ Ltd. research and production company.

The total DNA of the samples was isolated using the DNA Purification Kit (Fermentas, Inc., Lithuania). 200 µl of sample genomic was mixed with 400 µl of lysis solution and incubated at 65°C for 5 min. Then the sample was incubated at 65°C for 10 min with occasional tube inverting. 600 µl of chloroform was added and the sample was centrifuged at 10,000 rpm for 2 min. The upper aqueous phase containing DNA was transferred to a new tube and add 800 µl of freshly prepared precipitation solution, and was centrifuged at 10,000 rpm for 2 min. The supernatant was completely removed and DNA pellet was dissolve in 100 µl of NaCl solution by gentle vortexing. 300 µl of cold ethanol was added, then the DNA was precipitated (10 min at –20°C) and spinned down (10,000 rpm, 3–4 min). The ethanol was removed. The pellet was washed once with 70 % cold ethanol and DNA was dissolved in 100 µl of sterile deionized water by gentle vortexing.

DNA amplification was performed using the DNA amplifier Verity (Life Technologies, Inc, USA) with eubacterial primers: 63F (CAGGCCTAACACATGCAAGTC) – with a label at the 5'-end (fluorophore D4 – WellRed) and 1492R (TACGGHTACCTTGTTACGACTT).

Fluorescently labeled amplicons of 16S rRNA gene were purified according to the standard methodology [25]. The concentration of purified fragments of 16S rRNA gene was determined using the fluorimeter Qubit 2.0 (Invitrogen, Karlsruhe, Germany) according to the manufacturer's recommendations. The restriction of 30–50 ng amplicons of 16S rRNA was performed using restrictases HaeIII, HhaI and MspI, following the manufacturer's recommendations (Fermentas, Lithuania). The restriction products were analyzed using the sequenator CEQ 8000 (Beckman Coulter, USA) according to the manufacturer's recommendations.

The bacterial phylogenetic group identification was provided using the program Fragment Sorter and the database (http://www.oardc.ohiostate.edu/trflpfragsort/index.php).

Mathematical and statistical processing of the results was performed using the standard methods of analysis of variance [26] using the software EXCEL XP/2010. The experimental data were processed using parametric (Student-Fisher test) and non-parametric (Wilcoxon-Mann-Whitney test) statistical methods. The biological diversity was assessed using Shannon-Weaver and Simpson indices in Past program (http://folk.uio.no/ohammer/past/). Pearson correlation coefficients were calculated in order to explain the cause-effect relationship between the microorganism content of small intestine and digested amino acids amount. They allowed to establish direct relationship between variables by their absolute values [26]. Correlation indices were analyzed if the amount of the observed microorganism in the total microbial community exceeded 1 %.

RESULTS AND DISCUSSION

Currently, the question of the real amino acids availability and digestibility in birds remains controversial, which is due to the difficulties in determining of endogenous amino acids, including due to the limitations of almost all existing methods of their determination [27]. However, we think that the method we used to observe the amino acids digestion processes in digestion trials allows to contemplate the digestion physiology of birds not by fragments, but at the level of the entire organism.

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Table 3 contains the results of amino acids digestibility analysis by the bird's organism, which were determined based on digestion trials.

Table 3: The amino acid digestibility in the avian body (%)

Parameter (%)	Group			
	Control I	Control II	Experimental I	Experimental II
Lysine	84.01	86.4	83.6	87.4
Histidine	78.0	78.1	75.3	77.6
Arginine	87.1	88.2	86.2	88.3
Aspartic acid	80.3	82.1	81.4	81.9
Threonine	75.2	78.9	78.5	82.0
Serine	79.4	79.7	80.0	81.7
Glutamic acid	90.4	91.9	90.4	90.8
Proline	82.3	86.5	82.3	84.8
Glycine	63.5	63.6	61.9	67.3
Alanine	75.8	71.2	65.9	68.8
Cystine	77.2	78.7	66.8	71.8
Valine	72.2	76.6	73.5	76.8
Methionine	87.3	88.1	84.1	86.0
Isoleucine	78.3	81.1	80.2	83.1
Leucine	82.3	85.2	83.8	84.8
Tyrosine	73.0	75.3	76.6	73.2
Phenylalanine	83.1	83.4	82.8	83.4

Table 3 shows that the digestibility of several amino acids was more dependent on the ration composition rather than on the probiotic bacterial strain of *B. subtilis* 1-85 appliance. Thus, the highest digestibility of lysine, aspartic acid, threonine, proline, valine, isoleucine, leucine and tyrosine was noticed in chicks that received compound feed balanced with considering the amino acids digestibility (Control II), compared to the group that received a ration balanced without considering the amino acids digestibility (Control I). Previously, similar results were obtained by Fisinin et al. [28]. Thus, the analysis results of amino acids balance in feed and in ileum contents of chickens indicated that the amino acids digestion process in poultry digestive tract was more effective with a more nutritious compound feed with smaller amount of poorly hydrolyzable components, more raw protein and exchange energy: the free amino acids amount in the ileum contents was higher by 2 %.

It should be noted that the introduction of *B. subtilis* 1-85 strain in the chickens' intestine in our experiment led both to the increase and to the decrease of some amino acids digestibility in both experimental groups. For example, the appliance of a probiotic bacterial strain in the group balanced considering the amino acids digestibility (Experimental IV) led to the increase in digestibility of threonine, proline, glycine and isoleucine and decrease in digestibility of alanine, cystine, methionine and tyrosine compared to a similar group without the probiotic usage (Control II).

Table 4 shows the results of poultry small intestine microbiocenosis composition, obtained by the T-RFLP analysis method.

Table 4: Taxonomic composition of microbiocenoses in small intestine of chickens

Taxon		Proportion in the bacterial community, %			
		Control I M±m, n=3	Control II M±m, n=3	Experimental I M±m, n=3	Experimental II M±m, n=3
Phylum *Firmicutes*	Family *Ruminococcaceae*	62.7±2.9	35.3±1.5***	77.7±3.2**	39.0±1.3***
	Family *Lactobacillaceae*	2.9±0.12	2.0±0.1**	2.7±0.11	1.8±0.08**
	Family *Carnobacteriaceae*	0.88±0.032	0.65±0.02**	0.81±0.03	0.67±0.02**
	Family *Enterococcaceae*	13.4±0.53	7.5±0.32***	16.6±0.74**	8.2±0.33***
	Family *Clostridiaceae*	0.24±0.011	0.55±0.01**	0.34±0.015*	0.54±0.02***

			*		
	Family *Eubacteriaceae*	4.43+0.07	10.99+0.3***	31.68+0.45***	29.93+0.9***
	Family *Veillonellaceae*	15.45+0.6	15.53+0.4	3.52+0.51***	4.66+0.72***
	Family *Bacillaceae*	0.5+0.02	14.96+0.6***	8.34+0.4***	16.16+0.51***
Phylum *Bacteroidetes*	Family *Flavobacteriaceae*	0.15+0.005	1.32+0.04***	1.38+0.02***	0.22+0.008*
	Family *Flexibacteraceae*	5.18+0.07	3.08+0.08***	0.24+0.001***	0.59+0.001***
	Family *Bacteroidaceae*	-****	0.3+0.002	1.15+0.06	1.53+0.06
	Family *Sphingobacteriaceae*	2.95+0.12	1.98+0.08***	1.36+0.04**	4.62+0.19**
Phylum *Actinobacteria*	Order *Actinomycetales*	0.11+0.008	1.45+0.03***	-	0.02+0.001*
	Family *Bifidobacteriaceae*	0.16+0.01	0.21+0.01**	-	0.73+0.03***
Phylum *Proteobacteria*	Family *Enterobacteriaceae*	0.46+0.02	0.30+0.01*	0.18+0.007**	0.40+0.02
	Burkholderia sp.	1.14+0.04	1.37+0.05	0.08+0.003***	3.27+0.14***
	Helicobacter sp.	0.20+0.01	-	0.21+0.009	-
	Unidentified *Proteobacteria*	4.06+0.08	19.32+0.6***	9.77+0.09***	16.13+0.5***
Phylum *Fusobacteria*	*Fusobacterium* sp.	0.43+0.01	1.77+0.05***	-	0.22+0.001
Phylum *Tenetricutes*	Genus *Mycoplasma* sp.	0.05+0.003	0.88+0.001***	0.93+0.03***	0.07+0.001
Uncultured bacterium		56.03±1.5	4.53+0.18	0.39+0.002***	-

* p≤0.001. **p≤0.01. ***p≤0.05 (when comparing experimental groups and control II with control I). -**** - below the T-RFLP detection limit

T-RFLP analysis showed that chicken small intestine microflora contained from 35.3±1.5 to 77.7±3.2 phylotypes of bacteria, depending on the experimental group. The calculation of biodiversity indices (Shannon, Simpson, Margalef) revealed more expressed taxonomic diversity and complexity of intestinal microbial communities organization in poultry that received a ration balanced without considering the amino acids digestibility (Control I and Experimental I). This indicates the uncertainty and heterogeneity of microbiocenosis, accumulation of entropy and a certain degree of disorganization in these chicks, compared to other groups that received rations balanced considering the amino acids digestibility (Control II and Experimental II).

It has been shown that most of microorganisms found in the small intestine could not be attributed to any existing taxon. The number of unidentified bacteria was from 7.69±1.03 to 56.03±1.5%, depending on the variant of experimental group. Similar results were obtained by the researchers earlier [29, 30].

According to table 4 data, the qualitative composition of identified microorganisms of chickens' intestines was generally similar in all variants. According to the results of taxonomic diversity analysis, intestinal microbiota in most of studied samples was attributed to 5 phylum: *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria* and *Fusobacteria*. The intestinal microbiome of group "Experimental II" chickens also included bacteria from the phylum *Tenetricutes*. Bacteria that prevailed in Control II, Experimental I and Experimental II belonged to the phylum *Firmicutes* and *Actinobacteria*, in Control I group – to the phylum *Firmicutes* and *Proteobacteria*. These data are partially consistent with the results obtained earlier [31-33]. Thus, Li et al. [34], using the method of 16S rRNA gene sequencing, showed that the microbial communities of chickens' intestines were dominated by representatives of phylum *Firmicutes* and *Proteobacteria*, accounting for over 90 % of the analyzed sequences.

The results of cluster analysis by Ward's method have shown that chickens small intestine microbial communities' composition was more dependent on the introduction of a factor adjusting the microflora – the

probiotic bacterium strain, rather than on the composition of the ration. In general, the introduction of *B. subtilis* 1-85 into the intestinal ecosystem in both groups contributed to the increase in the representatives of *Ruminococcaceae and Eubacteriaceae* families *and* decreased in the representatives of *Lactobacillaceae, Clostridiaceae* families *and* non-culturable microorganisms, compared to the groups without introduction of the bacterial strain. *Ruminococcaceae* family prevailed among the representatives of the phylum *Proteobacteria* in both groups with *B. subtilis* 1-85; while *Lactobacillaceae* family prevailed in the groups without additives. The revealed dependence is of interest because lactic acid bacteria in *Lactobacillaceae* family synthesize a lactate, which reduces the pH level of chyme, as the main product of metabolism. It is known that the representatives of *Ruminococcaceae* family [35, 36] are extremely sensitive reduction of pH, and, accordingly, and increase in the share of lactic acid bacteria can promote its competitive displacement from the intestine. Bacteria of *Ruminococcaceae* family can play an important role in the digestion of poultry, because a specific feature of this taxon is the ability to form a series of digestive enzymes, including cellulases, which allows the macroorganism to efficiently use the energy of feed rich in fiber.

The intestines of chickens in the groups that received a ration balanced considering the amino acids digestibility (Control II), had an increased number of families *Ruminococcaceae, Carnobacteriaceae, Eubacteriaceae, Bacillaceae, Enterobacteriaceae* and order *Actinomycetales* and reduction of families *Clostridiaceae* and *Veillonellaceae*, compared to the group that received a ration balanced without considering the amino acids digestibility (Control I). The decrease in the share of families *Clostridiaceae* and *Veillonellaceae* in the chickens' intestine of the group that received a ration balanced without considering the amino acids digestibility can indicate a microbiome imbalance, because these taxa often include intestinal species that play a positive role in the digestion of mammals and birds. Thus, for example, *Clostridium lochheadii,* a representative of *Clostridiaceae* family*,* are able to degrade cellulose to acetate, formate and butyrate. The representatives of *Veillonellaceae* family include such species as *Selenomonas ruminantium, Selenomonas lactilytica, Megasphaera elsdenii,* which form several volatile fatty acids in the intestine: propionic, butyric, valerian, etc. [36].

A Pearson's method was provided to carry out a correlation analysis between the dominant (at least 1 %) microorganism species presence and amino acids digestibility. Previously, a similar method was successfully applied by researchers [37] to study relations between the birds' metabolism and the content of microorganisms in their gastrointestinal tract. For example, figure 2 shows the values of correlation coefficients between the lysine digestibility and certain bacteria content in the small intestine. Lysine is one of the essential amino acids, which cannot be synthesized by poultry organism. At the same time, this amino acid actively participate in the synthesis of required for skeletal tissues formation proteins, enzymes and hormones, improves the digestion of calcium and its transport to the bone tissue, which has a positive effect on the growth and formation of bones, strengthens the immunity against viral infections, contributes to tissue regeneration, serves as a source of energy, regulates feed consumption. Lysine affects the oxidation-reduction reactions in the body, catalyzes the processes of interamination and deamination, affects the process of acylation, etc. Since the lysine content in phytogenic substrates is few (less than 6 % of protein amount), plant feed for poultry in the feed base existing in the Russian Federation is usually the most deficient in the content of this amino acid.

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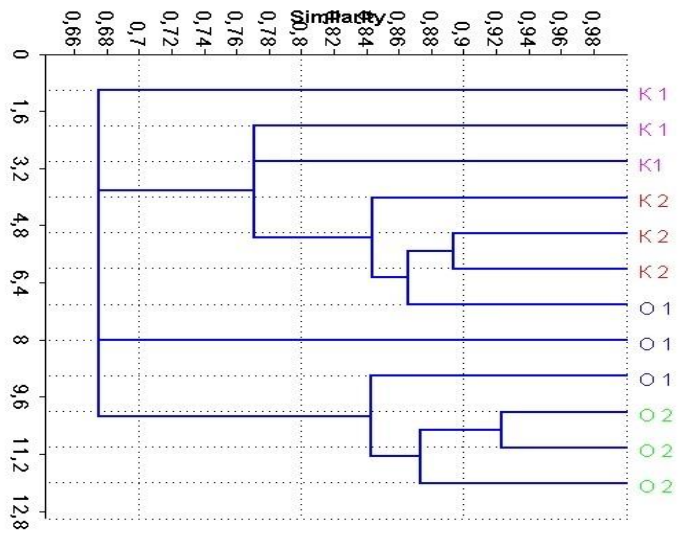

Figure 1: Dendrogram of similarity (based on the Ward method) of the microbiocenoses of the small intestine of chickens based on the results of T-RFLP analysis: K1 - Control I. K2 - Control II. O1 - Experimental I. O2 - Experimental II.

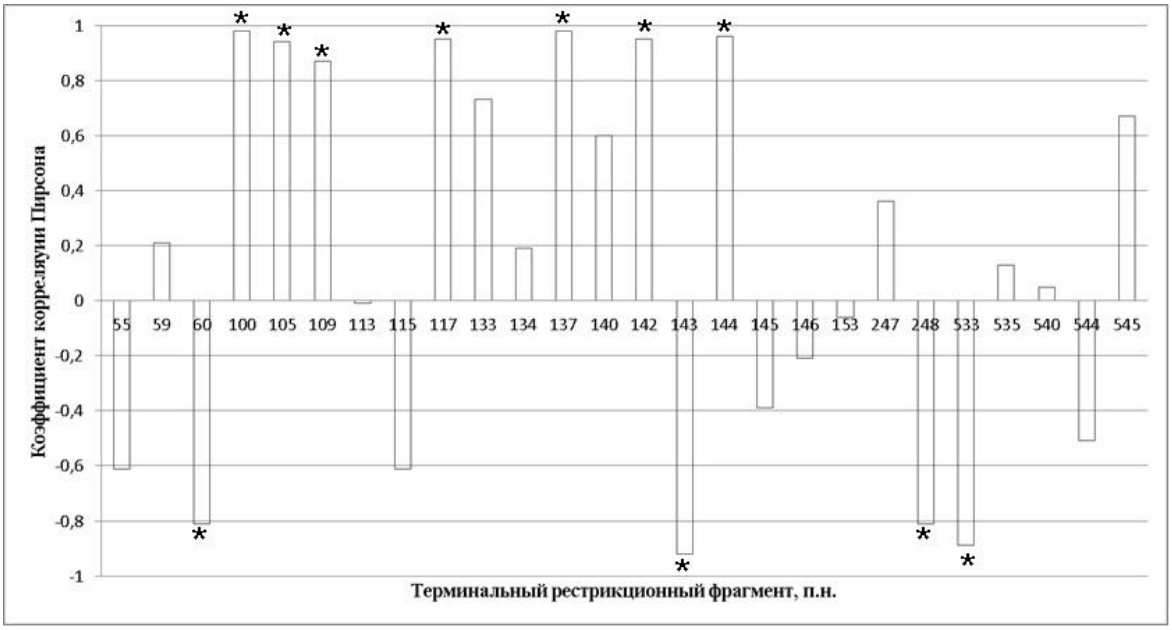

Figure 2: Pearson correlation coefficient between the presence of the dominant bacterial species and lysine digestibility. The numbers on the diagram are the size of the terminal restriction fragment (b.p. - a pair of nucleotides). corresponding to the bacterial phylotype: 55 b.p. - *Bacteroides plebeius*; 59 b.p. - *Bacteroides coprophilus*; 60 b.p. - *Bacteroides vulgatus*; 100. 140 b.p. - *Bifidobacterium* sp.; 105 b.p. - *Paenibacillus validus*; 109. 247. 248 b.p. - *Microbacterium* sp.; 113. 115 - *Bacillus* sp.; 117 b.p. - *Burkholderia* sp.; 133 b.p. - *Corynebacterium jeikeium*; 134 b.p. - *Paenibacillus* sp.; 137 b.p. - *Streptomyces* sp.; 142. 144 b.p. - *Lactobacillus delbrueckii*; 143. 145. 146. 533. 535. 540. 544. 545 b.p. - *Lactobacillus* sp.; 153 b.p. - *Sphingobacterium faecium*. *p≤0.05 (based on Student's t-test)

Figure 2 shows that several bacterial phylotypes had a significant impact on the lysine digestion by the bird's organism. It has been established that an increase in the number of *Bacteroides vulgatus* (60 bps), *Lactobacillus* sp. (143, 533 bps), *Microbacterium* sp. (248 bps) was due to a decrease in the lysine digestibility. The representatives of *Lactobacillus* sp. are extremely demanding to the amino acids in digestible form content, which are used by them in the processes of cellular anabolism [38]. The data obtained are consistent

with the results of Fack and Bäckhed [39], who observed different influence of *Lactobacillus reuteri* strains on the weight dynamics in mice. Thus, the introduction of *L. reuteri* L6798 strain into the mice's ration was associated with an increase in the mice's weight, while the introduction of *L. reuteri* ATCC PTA 4659 strain was associated with weight loss. It is also known that the representatives of *Bacteroides* are also able to digest amino acids from the external environment [40]. Consequently, certain representatives of the resident intestinal microbiota can compete with the host for amino acids in the rations. These data are consistent with the results of several researchers [41], who demonstrated that the use of antibiotics was associated with a decrease in the content of bacteria in the small intestine and with a liveweight gain in chickens.

It has been shown that an increase in the number of bacteria *Lactobacillus delbrueckii*, *Lactobacillus* sp. (142, 144 bps), bifidus bacteria *Bifidobacterium* sp. (100 bps), *Paenibacillus validus* (105 bps), *Microbacterium* sp. (109 bps), *Burkholderia* sp. (117 bps), *Streptomyces* sp. (137 bps) had a positive association with lysine digestion. It is known that such microorganisms as the representatives of *Lactobacillus*, *Bifidobacterium*, *Paenibacillus, Streptomyces* are able to synthesize organic acids and bacteriocins [36], having antimicrobial activity against pathogenic forms [42, 43]. It is known that active reproduction of bacterial pathogens in the intestinal lumen causes inflammatory reaction in intestinal epithelium, alteration, necrosis, damage of villi, etc., which has a negative impact on the amino acids digestion by the macroorganism.

It is interesting that the number of non-culturable phylotypes in the microbiome of the small intestine (data are not shows in the figure) had a positive (71, 72, 75, 76, 77, 80, 136, 151, 153 bps) or negative (57, 68, 69, 204 bps) association with the digestibility of lysine, aspartic acid, threonine, serine, proline, valine, leucine and other amino acids in birds ($p \leq 0.05$). This allows to expand the idea about the role of these microorganisms in poultry digestion.

CONCLUSIONS

Overall, digestibility of several amino acids in chicken's small intestine was more dependent on the ration composition, rather than on the of the *B. subtilis* 1-85 probiotic strain usage. More effective amino acids digestion was observed in chickens that received rations balanced by amino acids digestibility. At the same time, the use of the T-RFLP molecular-genetic method has shown that the microbial communities' composition of broiler small intestine was more dependent on the introduction of a factor adjusting the microflora – the probiotic bacterium strain, rather than on the ration composition. Introduction of the bacterial strain contributed to the elimination of small intestine dysbiosis. It has been established that the representatives of intestinal microbiome affect the digestibility of amino acids in different ways. The representatives of certain bacterial taxa in the small intestine can be competitors for the macroorganism in terms of consumption of amino acids, using them in the cellular anabolism. At the same time, our experiment has shown that some microorganisms were directly related to the increase in certain amino acids digestibility. Thus, one of the ways to increase the amino acids digestibility and productivity of poultry organism can be a correction of several functionally important small intestine microflora representatives.

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