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Microbiota in The Intestines of Cross Chick Lohmann Brown in Ontogeny.

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ABSTRACT

This paper presents the results of a molecular genetic analysis of the changes in the composition of the microbiota of the blind processes of the intestine of the hens of the industrial loam "Lohmann Brown" during ontogeny. According to the results of the analysis of taxonomic affiliation it is established that over 70% of the phylotypes belong to the three phylums - Firmicutes, Bacteroidetes and Proteobacteria, less represented were Actinobacteria, Tenericutes and Fusobacteria, and a significant number of unidentified bacteria was detected. During ontogenesis, birds exhibited marked changes in the ratio of the number of phylotypes and taxonomic groups of the intestinal microbiota. At the age of 20-40 weeks, the birds showed a significant increase in the representatives of the Clostridia class involved in the metabolism of carbohydrates, acid-utilizing bacteria of the order Negativicutes and bacteria with high antagonistic properties (Bifidobacteriales, Bacillus), as well as a significant decrease in the content of a number of opportunistic and pathogenic taxa - family Enterobacteriaceae, the order of Pseudomonadales, phylum Tenericutes. The greatest homogeneity of the bacterial community of the blind processes of the gastrointestinal tract in laying hens was revealed at the age of 20 weeks, which is confirmed by the estimation of biodiversity by means of ecological indices.

Keywords: bacterial community, microbiota, blind intestinal processes, chickens, Lohmann Brown cross, T-RFLP analysis.

List of abbreviations: PCR - polymerase chain reaction, T-RFLP-analysis (length polymorphism of terminal restriction fragments).

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INTRODUCTION

Poultry farming is one of the leading branches of agriculture in our country due to the high production of poultry meat and eggs. However, today in this area, experts note a number of problems associated with the intensification of poultry production.

Microorganisms inhabiting the gastrointestinal tract (GIT) provide the host organism with nutrients through the use of its own cellulolytic and amylolytic enzymes, due to their complete absence in agricultural birds, as well as vitamins, antibiotics, proteins, hormones and other compounds [1, 2].

The bacterial community of the digestive tract during the life of the bird undergoes a number of consecutive changes associated with a number of factors, the main of which are the growth and development of the intestinal tract, the feeding regimen and the composition of the feed. In this case, the microorganisms of the intestine act as a highly sensitive indicator system, which reacts in response to changes occurring. It should be noted that a change in the ecological relationship between obligate species of microorganisms in the digestive tract does not always have a positive effect on metabolic processes and the state of bird health [3]. In this regard, the actual issue is the study of the qualitative and quantitative composition of the gastrointestinal microbiota in the process of ontogenesis of birds.

The development and application of modern technologies aimed at achieving maximum productivity, such as frequent vaccinations, widespread use of antibiotics and chemical antibacterials, often lead to deterioration of poultry health associated with the development of uncontrolled secondary infections - salmonellosis, campylobacteriosis, staphylococcosis, clostridiosis, and polymicrobial diseases [4, 5]. Pathogenic microorganisms cause a disturbance in the composition of the intestinal microbiota, changes in thickness, appearance, muscle tone, strength and increased paracellular permeability of intestinal walls for toxic metabolites, which ultimately affects the health and productivity of the herd.

Reduction of the risk of development of infectious pathologies in birds is widely associated with the formation of a healthy microbiota of the digestive tract, which is able to provide high resistance to colonization of the intestine by pathogens [6-8] due to the synthesis of volatile fatty acids (PLFA), bacteriocins and others that inhibit the growth and development of pathogenic species , compounds [9-11].

Until the 1990s, studies of microorganisms in different ecosystems were limited to the study of cultivated strains on artificial nutrient media. To significantly expand the understanding of the microbiota composition, the development of metagenomic methods for the study of microorganisms, an important feature of which can be considered the absence of the need for cultivation of microorganisms [12-13]. This point is important in understanding the existing biodiversity, since up to 99% of the microorganisms of the biosphere can not be cultivated on artificial nutrient media, but can play an important ecological role [14].

In a number of works, a multifaceted characterization of the microbiota of the intestines of broiler chickens was given, which made it possible to characterize in detail a number of important regularities in the functioning of this complex microbioecosystem [15-16].

However, such studies are practically absent in laying hens, despite the high demand for such data.

The aim of this work is to study the succession of the bacterial community of the gastrointestinal tract of the birds of the industrial cross-country "Lohmann Brown" in the process of ontogenesis using the molecular genetic method of T-RFLP.

MATERIAL AND METHODS

The subject of the study were hens of the egg cross "Lohmann Brown" of different ages: 4, 20, 40 and 60 weeks. The investigations were carried out in the molecular-genetic laboratory of BIOTROF + LLC and the International Laboratory of Molecular Genetics and Genomics of Poultry at the Moscow State Academy of Veterinary Medicine and Biotechnology - MVA named after KI. Scriabin (Moscow) with observance of all technological parameters. Feeding of the poultry was carried out manually, with dry full-fat mixed fodders in accordance with the norms of VNITIP [17].

The selection of the contents of the blind intestinal processes for molecular genetic studies was conducted from 5 heads from each age group of birds at slaughter with strict observance of sterility according to established requirements [18].

The composition of the microbial community of the gastrointestinal tract was examined by the T-RFLP assay [19].

T-RFLP analysis of the bacterial community

Total DNA from the samples was isolated using the Genomic DNA Purification Kit (Fermentas, Inc., Lithuania) according to the manufacturer's instructions. DNA amplification was performed using a Verity DNA amplifier ("Life Technologies, Inc.", USA) using eubacterial primers: 63F (CAGGCCTAACACATGCAAGTC) labeled at the 5'-end (D4 WellFed fluorophore) and 1492R (TACGGHTACCTTGTTACGACTT), which allow the fragment of the 16S rRNA gene to be amplified with positions from 63 to 1492 (numbering is indicated for the Escherichia coli 16S rRNA gene) in the regime: 95 ° C - 3 min (1 cycle); 95 ° C - 30 s, 55 ° C - 40 s, 72 ° C - 60 s (35 cycles), 72 ° C - 5 min.

The fluorescently labeled amplitudes of the 16S rRNA gene were purified using a 3M guanidine isothiocyanate solution according to the standard procedure [20]. The final concentration of total DNA in the solution was determined using a Qubit fluorometer ("Invitrogen, Inc.", USA) using "Qubit dsDNA BR Assay Kit" ("Invitrogen, Inc.", USA), as recommended by the manufacturer.

The restriction of 30-50 ng DNA was carried out with restriction enzymes HaeIII, HhaI and MspI following the manufacturer's recommendation (Fermentas, Lithuania) for 2 hours at 37 ° C. The restriction products were precipitated with ethanol, then 0.2 µl of the Size Standard-600 molecular weight marker ("Beckman Coulter", USA) and 10 µl of Sample Loading Solution formamide ("Beckman Coulter", USA) were added. The analysis was carried out using CEQ 8000 (Beckman Coulter, USA) according to the manufacturer's recommendations. The error of the CEQ 8000 device was not more than 5%. The calculation of peak sizes and their areas was carried out in the Fragment Analysis program ("Beckman Coulter", USA), on the basis of which subtypes (filotypes) were distinguished with a 1-nucleotide error in the study and their relative content in the microbial community was determined.

Processing Results

The affiliation of bacteria to a specific taxonomic group was determined using the Fragment Sorter program (http://www.oardc.ohiostate.edu/trflpfragsort/index.php).

Mathematical and statistical processing of the results, calculation of the environmental indices of Simpson dominance and Shannon's biodiversity were carried out in the program Past (http://folk.uio.no/ohammer/past/).

RESULTS AND DISCUSSION

In this study, using the molecular genetic method of T-RFLP, we first analyzed the composition of the bacterial community of the gastrointestinal tract of chickens during ontogeny.

In the presented study, we studied the features of the composition of the microbiota of the blind processes of the intestine. According to the testimony of the majority of authors, the microbiota of this particular part of the digestive tract of birds is of greatest interest, where the contents are retained for the longest period and significant processes of microbial fermentation of fodders take place, including. cleavage of cellulose. In addition, in this section of the intestine the birds show the greatest biodiversity and the number of bacteria (more than 1011 cfu / g) compared with other parts of the digestive tract, where their numbers rarely exceed 108 cfu / g [21-27].

Studies were conducted on one of the most common industrial crosses of egg birds - "Lomann Brown" at the age of 4, 20, 40 and 60 weeks. Nesushki and broilers have a number of important genetic and

physiological features associated with differences in the directions of their selection - improving reproductive properties in laying hens and increasing the live weight of broiler chickens [28, 29]. This affects not only the significant differences in the rate of growth, but also the difference in metabolic needs, feed intake, nutritional assimilation efficiency [30]. This also involves significant differences in the conditions of growing, feeding, keeping, vaccinating broiler chickens and laying hens.

Interest in the study of the intestinal microbiota in these periods of ontogenesis is associated with the physiological characteristics of laying hens, and their differences from broiler chickens. At the age of 4 weeks, laying hens, on average, have a 2-4 times lower live weight than broilers, for which the indicated period is a period of physiological maturity when slaughter is carried out [28, 29]. By the 20-week period, the laying hens reach puberty, i. E. the age of the beginning of oviposition, which on the average is about 1.5 years, gradually decreasing to 60% and lower in comparison with the period of peak egg production (95-100%).

T-RFLP analysis of the bacterial community of the blind processes of the intestine made it possible to establish the presence of a significant number of microorganism philotypes, the total number of which was depending on the ontogeny period from 94.10 ± 5.05 to 142.00 ± 7.20 (Table 1).

Table 1: Biodiversity indices of the bacterial community of chicken blind guts

Biodiversity index	Age of bird (weeks)			
	4	20	40	60
Total number of philotypes	142.00±7.20	94.10±5.05	124.54±4.90	121.65±6.01
Share of unidentifiable types,%	15.09 ±0.42	14.05 ±0.64	10.23 ±0.38	24.09 ±0.92
The Shannon Index	4.09 ± 0.18	3.35 ± 0.18*	4.12 ± 0.18	4.12 ± 0.21
The Simpson Index	0.96 ± 0.03	0.89 ± 0.02	0.97 ± 0.03	0.98 ± 0.04

*P < 0.05

According to the results of the assessment of taxonomic affiliation, over 70% of the identified types of phylotypes were classified into three phylums - Firmicutes, Bacteroidetes and Proteobacteria (Figure 1). To a lesser extent, the chickens contained the phylum Actinobacteria bacteria, in a minor amount were found representatives of phylums Tenericutes and Fusobacteria.

Some of the philotypes could not be identified and attributed to a specific taxon. The presence of unidentified microorganisms in the digestive tract indicates the constant presence of unknown taxa in the gastrointestinal tract of the bird and leads to the need for additional studies of their role.

We note that the results obtained generally correspond to modern concepts of the intestinal microbiota of birds [3, 21, 31, 32].

A comparative analysis of the bacterial community of the contents of the blind processes of the intestine of the bird made it possible to establish statistically significant differences in the composition of microbiota associated with the period of ontogenesis.

During the whole period of maintenance, the birds had a change in the total number of taxonomic units. The greatest number of phylotypes in birds was observed at the age of 4 weeks - 142 ± 7.2 ($P < 0.05$).

In the process of ontogeny, the number of bacterial phylotypes in a bird decreases. The minimum number of philotypes was observed at the age of 20 weeks.

At the age of 60 weeks, which is characterized by a significant decrease in egg production in birds of this cross, an increase in the number of filotypes was observed, but their number averaged 1.2 times lower than at 4 weeks of age.

The change in the number of taxonomic units during ontogeny in blind processes of birds can be characterized using ecological tools for biodiversity assessment.

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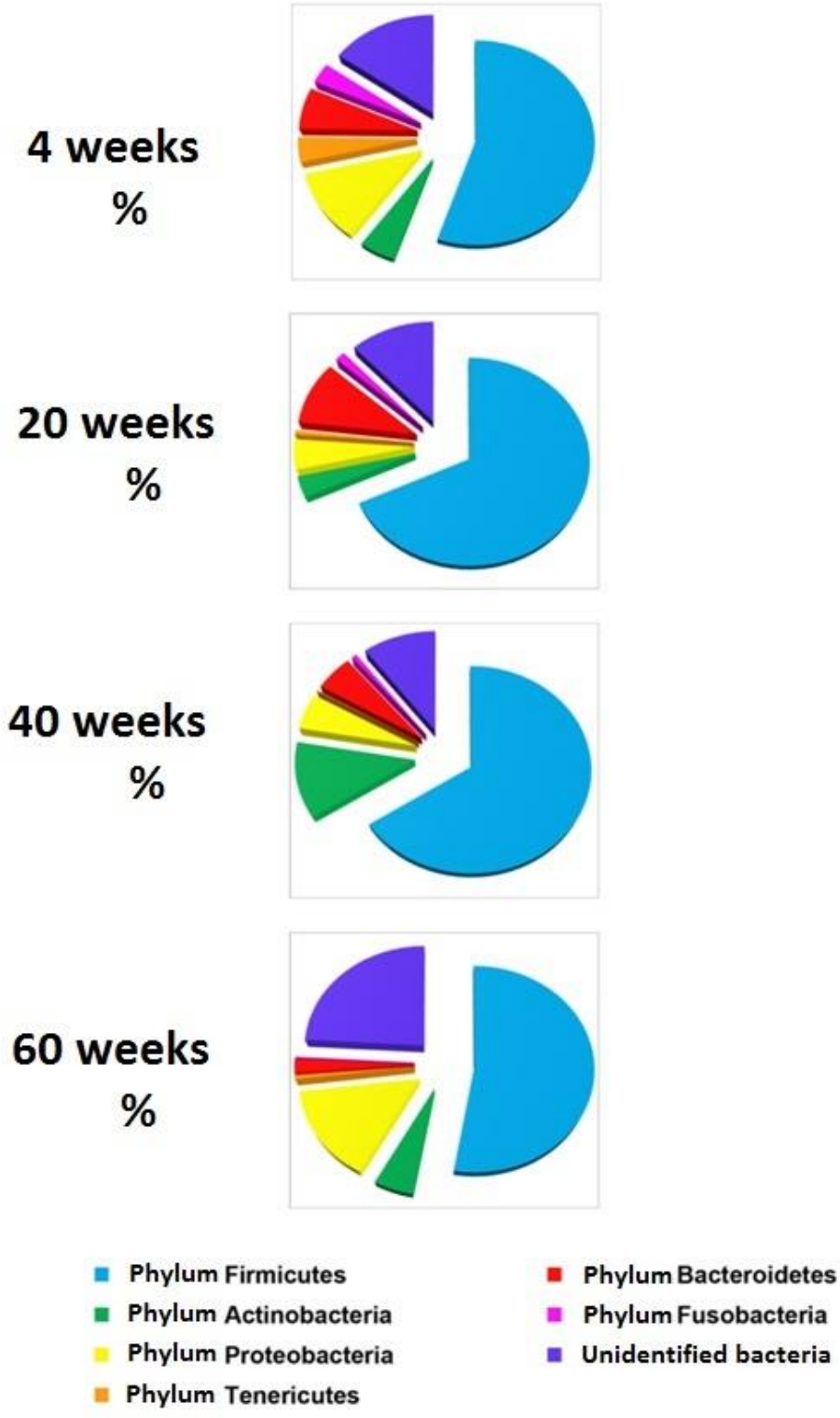

Figure 1: Composition of phylums the bacterial community of the blind processes intestines of chickens in ontogenesis

Comparison of ecological indices of biodiversity indicates the greatest homogeneity of the composition of the intestinal microbiota of layers at the 20-week age. During this period, the lowest indicators of the Shannon biodiversity index and the Simpson dominance index were observed.

The results of estimating the number of taxonomic units, as well as the analysis of the biodiversity of microbiocenosis based on environmental indices in the process of poultry ontogeny, suggest a certain periodicity in the development of the microbial community of the intestine.

It was reported that such a pattern existed in the development of gastrointestinal microbiocenosis in broilers, which depended on a number of factors, including ration, stress, antibiotic therapy [34, 35]. Similar changes in the composition of the microbial community of the blind processes of the intestine were observed in turkeys during the 3-4 and 11-18 weeks of growth [36].

Analyzing the composition of the microbiocenosis of the blind processes of the intestines of the Lohmann Brown crochets, significant changes in the composition of the Phylum Firmicutes were revealed, the total share of which in the community was more than half, from 51.63 ± 2.48 to 65.80 ± 2.99%.

From the data presented in Figure 2A, it can be seen that the total proportion of members of the Clostridia class, which are members of the phylum Firmicutes, possessing the ability to hydrolyse the carbohydrates of plant foods with the formation of volatile fatty acids (FLV), reached the maximum values in the poultry - by the age of 40 weeks ($P < 0.05$), gradually decreasing to 60 weeks ($P < 0.05$).

In birds, there was a significant increase in cellulolytic microorganisms of the families Clostridiaceae, Ruminococcaceae and Eubacteriaceae ($P < 0.05$) by the age of 20-40 weeks, while the proportion of cellulozolitic bacteria of the Lachnospiraceae family was the smallest ($P < 0.05$) in this period.

The level of representation of microorganisms of the phylum Bacteroidetes (including genera Bacteroides, Prevotella), including bacteria with similar properties - the ability to ferment starch, fiber and some other carbohydrates, proteins and deaminate amino acids, increased from 4 weeks of age, reaching a maximum of 20-week-old age ($P < 0.05$), significantly decreasing to 60 weeks ($P < 0.05$).

The content of acid-utilizing bacteria of the Negativicutes class, associated with the assimilation of volatile fatty acids (FLV), incl. acetic, propionic, butyric, valeric and isovaleric acids formed during the splitting of monosaccharides, oligo- and polysaccharides of fodder, reached a maximum in birds at 40 weeks of age.

Interesting age changes were noted for obligate representatives of the intestine of the bird (Figure 2B) - lactobacillus bacteria of the genera Lactobacillus and bifidobacteria of the order Bifidobacteriales, which due to the synthesis of various organic acids and bacteriocins are capable of antagonizing the intestinal pathogens, including salmonella, proteins, staphylococci, Escherichia coli , pseudomonas, streptococci [24-26]. The level of lactobacilli was the highest at 4 weeks of age - 11.24 ± 0.42% ($P < 0.05$), and by the 40-week period of cultivation it decreased as much as possible to the level of 2.37 ± 0.12% ($P < 0.05$). The inverse relationship was observed in the case of bacteria of the genus Bacillus, which had similar properties and were not considered obligate inhabitants of the intestine, capable of colonization of the digestive tract of a bird according to the information of a number of authors [37]. The highest proportion of bacteria of the genus Bacillus was detected in birds aged 40 weeks - 14.40 ± 0.65% ($P < 0.05$). The content in the community of representatives of the order of Bifidobacteriales in poultry also reached the maximum values by the age of 20-40 weeks - to 3.32 ± 0.12% ($P < 0.05$).

The share in the community of other lactobacilli of the genus Enterococcus, Leuconostoc in the community was minor, and did not change significantly during ontogeny.

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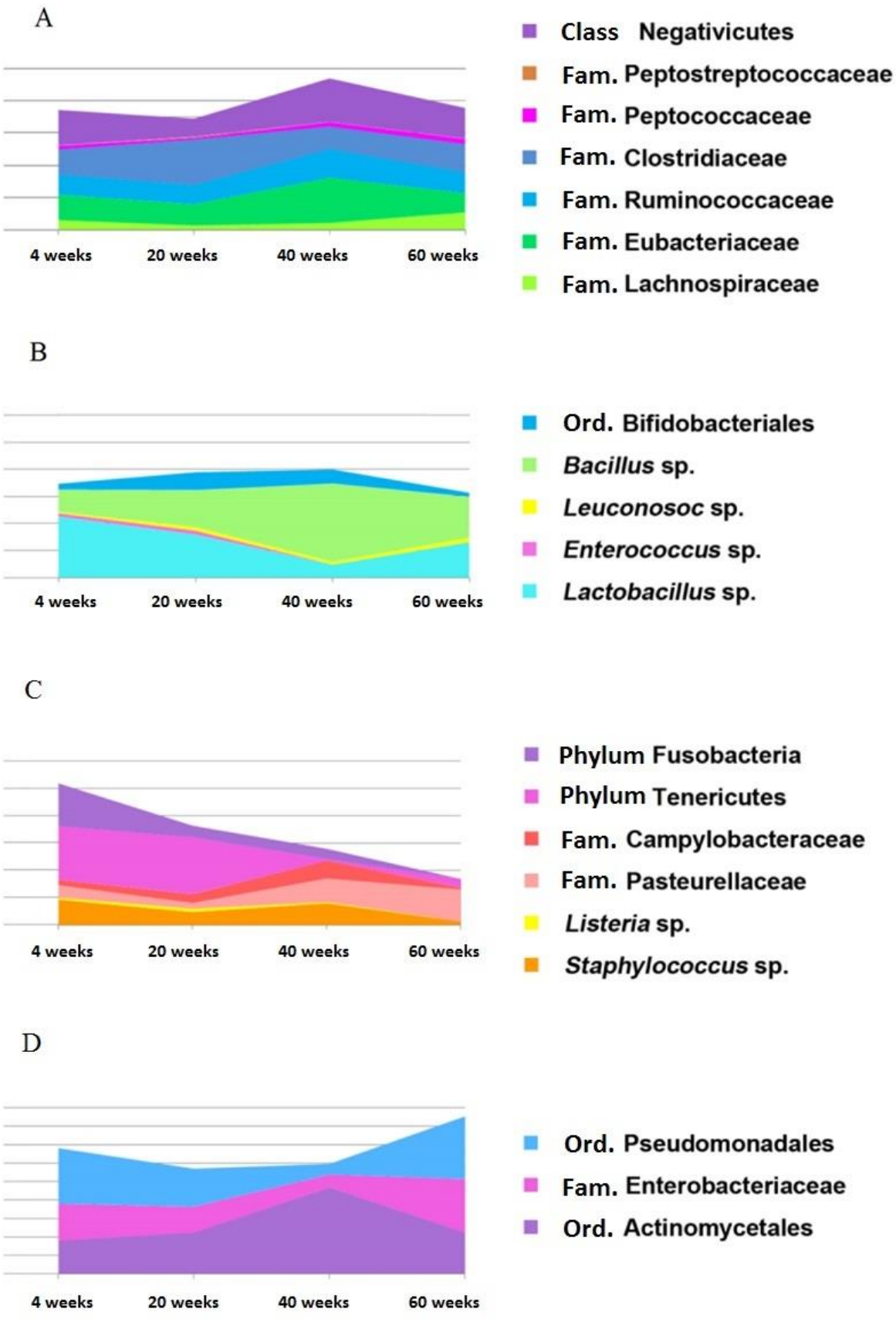

Figure 2: Changes in the microbiota of the blind processes of the intestines of chickens

The proportion of causative agents of various diseases varied depending on the period of maintenance of layers (Fig. 2C and 2D). Their minimum total share in laying hens was revealed in the period of 40-60 weeks (P <0.05). Among the bacteria that cause infectious diseases, the causative agents of campylobacteriosis (Campylobacteraceae family - Arcobacter, Campylobacter), pasteurellosis (Pasteurellaceae-Pasteurella, Haemophilus family), listeria (Listeria genus), mycoplasmosis (Mycoblasma phylum Tenericutes), necrotic enteritis (Fusobacteria phylum), purulent-necrotic infections (genus Staphylococcus). The content of most of the listed microorganisms in the intestinal community of birds was minor, with the exception of pasteurellus, mycoplasmas and fusobacteria.

From the data presented in Fig. 2D, it can be seen that the maximum content of opportunistic bacteria of the families Enterobacteriaceae, Pseudomonadaceae and order Actinomycetales was detected in birds at the age of 60 weeks (P <0.05), which indicates some imbalance of microbial bird communities during these ontogeny periods.

At the age of 20-40 weeks, laying revealed a significant decrease in the content of a number of opportunistic and pathogenic taxa - the family Enterobacteriaceae, the order Pseudomonadales, the phylum Tenericutes.

The revealed regularities testify to the presence of a negative correlation of the percentage of a number of pathogenic microorganisms with the proportion of representatives of normal intestinal microbiota. The validity of the existence of such regularities is confirmed by published data on bacteriostatic properties (due to the action of the synthesized spectrum of metabolites, including volatile fatty acids, bacteriocins) of representatives of normal intestinal microbiota [6-11, 38] with respect to a number of pathogens, incl. enterobacteria, pseudomonads.

CONCLUSION

In this study, using the molecular genetic method of T-RFLP, the composition of the bacterial community of the blind processes of the gastrointestinal tract of the Lohmann Brown crochet for the first time was analyzed during ontogeny. The results of the conducted studies testify to the presence in the hens of significant changes in the bacterial community of the blind processes of the intestine during ontogeny.

Poultry microbiocenosis at 20 weeks of age was characterized by the greatest homogeneity of the composition, as evidenced by data from the analysis of biodiversity based on environmental indices.

In the 20-40-week period, a significant shift in the composition of the microbiocenosis of the blind processes of the gastrointestinal tract is observed in birds.

An increase in representatives of the Clostridia class, involved in the metabolism of carbohydrates, acid-utilizing bacteria of the Negativicutes class and bacteria with high antagonistic properties (Bifidobacteriales, Bacillus), as well as a significant decrease in the content of a number of opportunistic and pathogenic taxa.

The obtained results of the analysis of the composition of the intestinal microbiota are quite natural and have a visual relationship with the physiological state of birds. At the age of 4 weeks, the digestive tract of laying hens is underdeveloped, in connection with which there is a certain destabilization of the microbiocoenal system of the intestine, characterized by a high content of opportunistic and pathogenic microorganisms. At the age of 20 weeks, laying hens reach a period of physiological maturity, they have a high level of egg production, gradually decreasing to 60 weeks of age.

In general, the data obtained from the work on changes in the composition of the intestinal microbiota of the bird during ontogeny can be considered in terms of their possible application for the development of the industry of growing young animals and keeping adult birds, obtaining a more productive healthy herd and ecologically clean poultry products.

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REFERENCES

[1] Tarakanov B.V. Methods for studying the microflora of the digestive tract of agricultural animals and poultry. M .: "Scientific world". 2006. 188 p.

[2] Stanley D., Hughes R.J., Moore R.J. Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease. Appl. Microbiol. Biotechnol., 2014, 98: 4301-4309 (doi: 10.1007/s00253-014-5646-2).

[3] Torok V.A., Hughes R.J., Mikkelsen L.L., Perez-Maldonado R., Balding K., MacAlpine R., Percy N.J., Ophel-Keller K. // Appl. Environ. Microbiol. 2011. V. 77. № 17. P. 5868-5878.

[4] Dzhavadov E.D., Dmitrieva M.E., Trefilov B.B., Novikova O.B., Titova T.G. // Veterinary Medicine and Feeding. 2016. № 2. P. 24-27.

[5] Collier C.T., Hofacre C.L., Payne A.M., Anderson D.B., Kaiser P., Mackie R.I., Gaskins H.R. Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting *Clostridium perfringens* growth // Vet. Immunol. Immunopathol. 2008. V. 122. P. 104–115.

[6] Yeoman C.J., Chia N., Jeraldo P., Sipos M., Goldenfeld N.D., White B.A. The microbiome of the chicken gastrointestinal tract // Anim. Health Res. Rev. 2012. V. 13. P. 89–99.

[7] Callaway T.R., Edrington T.S., Anderson R.C., Harvey R.B., Genovese K.J., Kennedy C.N., Venn D.W., Nisbet D.J. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease // Anim. Health Res. Rev. 2008. V. 9. P. 217–225.

[8] Kerr A.K., Farrar A.M., Waddell L.A., Wilkins W., Wilhelm B.J., Bucher O., Wills, R.W., Bailey R.H., Varga C., McEwe, S.A., et al. A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on *Salmonella* spp. prevalence and concentration in broiler chickens // Prev. Vet. Med. 2013. V. 111. P. 112–125.

[9] Brisbin J.T., Gong J., Orouji S., Esufali J., Mallick A.I., Parvizi P., Shewen P.E., Sharif S. Oral treatment of chickens with lactobacilli influences elicitation of immune responses // Clin. Vaccine Immunol. 2011. V. 18. P. 1447–1455.

[10] Dobson A., Cotter P.D., Ross R.P., Hill C. Bacteriocin production: a probiotic trait? // Appl. Environ. Microbiol. 2012. V. 78. P. 1–6.

[11] Messaoudi S., Kergourlay G., Dalgalarrondo M., Choiset Y., Ferchichi M., Prévost H., Pilet M.F., Chobert J.M., Manai M., Dousset X. Purification and characterization of a new bacteriocin active against *Campylobacter* produced by *Lactobacillus salivarius* SMXD51 // Food Microbiol. 2012. V. 32. P. 129–134.

[12] Park S.H., Lee S.I., Ricke S.C. Microbial populations in naked neck chicken ceca raised on pasture flock fed with commercial yeast cell wall prebiotics via an Illumina MiSeq Platform. PLoS One. 11(3): e0151944 (doi: 10.1371/journal.pone.0151944). Appl. Environ. Microbiol., 2008, 74(3): 783-791.

[13] Amann R.I., Ludwig W., Schleifer K.H. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev., 1995, 59: 143–169.

[14] Dibner J.J., Richards J.D., Knight C.D. Microbial imprinting in gut development and health. J. Appl. Poult. Res., 2008, 17: 174–188.

[15] Mohd Shaufi M.A., Sieo C.C., Chong C.W., Gan H.M., Ho Y.W. Deciphering chicken gut microbial dynamics based on high-throughput 16S rRNA metagenomics analyses // Gut Pathogens. 2015. V. 7. P. 4.

[16] Wei S., Lilburn M., Yu Z. The bacteriomes of ileal mucosa and cecal content of broiler chickens and turkeys as revealed by metagenomic analysis // International Journal of Microbiology. 2016. V. 2016. Article ID 4320412. P. 1-12.

[17] Kalashnikov A.P., Fisinin V.I., Scheglov V.V., Kleimenov N.I. Norms and rations of feeding of farm animals. Reference manual. M., 2003. 456 p.

[18] Instructions for the sanitary-microbiological control of carcasses, poultry meat, poultry products, eggs and egg products at poultry and processing enterprises. M., ut. The State Committee for Trade and Commerce of the USSR on 30.08.1990.

[19] Fisinin V.I. Method of carrying out scientific and industrial research on feeding of agricultural poultry. Molecular genetic methods for determining intestinal microflora. Sergiev Posad, 2013. 51 p.
[20] Bryukhanov A.L., Rybak K.V., Netrusov A.I. Molecular biology. Moscow: Izd-vo MU, 2012. 480 c.
[21] Wei S., Morrison M., Yu Z. Bacterial census of poultry intestinal microbiome // Poult. Sci. 2013. V. 92. № 3. P. 671-683.
[22] Stanley D., Denman S.E., Hughes R.J., Geier M.S., Crowley T.M., Chen H., Haring V.R., Moore R.J. Intestinal microbiota associated with differential feed conversion efficiency in chickens // Appl. Microbiol. Biotechnol. 2012. V. 96. P. 1361–1369.
[23] Stanley D., Geier M.S., Denman S.E., Haring V.R., Crowley T.M., Hughes R.J., Moore R.J. Identification of chicken intestinal microbiota correlated with the efficiency of energy extraction from feed // Vet. Microbiol. 2013. V. 164. P. 85–92.
[24] LeBlanc J.G., Milani C., de Giori G.S., Sesma F., van Sinderen D., Ventura M. Bacteria as vitamin suppliers to their host: a gut microbiota perspective // Curr. Opin. Biotechnol. 2013. V. 24. № 2. P. 160-168.
[25] Fasina Y.O., Hoerr F.J., McKee S.R., Conner D.E. Influence of Salmonella enterica serovar Typhimurium infection on intestinal goblet cells and villous morphology in broiler chicks // Avian Dis. 2010. V. 54. P. 841–847.
[26] Chae B., Ingale S., Kim J., Kim K., Sen S., Lee S., Khong C., Kim E.K., Kwon I.K. Effect of dietary supplementation of probiotics on performance, caecal microbiology and small intestinal morphology of broiler chickens // Animal Nutrition and Feed Technology. 2012. V. 12. P. 1–12.
[27] Danzeisen J.L., Kim H.B., Isaacson R.E., Tu Z.J., Johnson T.J. Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment // PLoS One. 2011. V. 6. № 11. e27949.
[28] Nangsuay A., Molenaar R., Meijerhof R., van den Anker I., Heetkamp M.J., Kemp B., van den Brand H. Differences in egg nutrient availability, development, and nutrient metabolism of broiler and layer embryos // Poult. Sci. 2015. V. 94. № 3. P. 415-423.
[29] Buzała M., Janicki B., Czarnecki R. Consequences of different growth rates in broiler breeder and layer hens on embryogenesis, metabolism and metabolic rate: A review // Poult. Sci. 2015. V. 94. № 4. P. 728-733.
[30] Sawicka D., Samek K., Chojnacka-Puchta L., Witkowski A., Knaga S., Dębowska M., Bednarczyk M. Changes in quail blastodermal cell status as a result of selection // Folia Biol (Krakow). 2015. V. 63. № 1. P. 63-67.
[31] Diaz-Sanchez S., Hanning I., Pendleton S., D'Souza D. Next-generation sequencing: the future of molecular genetics in poultry production and food safety // Poult. Sci. 2013. V. 92. P. 562–572.
[32] Choi J.H., Kim G.B., Cha C.J. Spatial heterogeneity and stability of bacterial community in the gastrointestinal tracts of broiler chickens // Poult. Sci. 2014. V. 93. № 8. P. 1942-1950.
[33] Sun H., Tang J.W., Yao X.H., Wu Y.F., Wang X., Feng J. Effects of dietary inclusion of fermented cottonseed meal on growth, cecal microbial population, small intestinal morphology, and digestive enzyme activity of broilers // Trop. Anim. Health Prod. 2013. V. 45. P. 987–993.
[34] Lu J., Idris U., Harmon B., Hofacre C., Maurer J.J., Lee M.D. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken // Appl. Environ. Microbiol. 2003. V. 69. № 11. P. 6816-6924.
[35] Ranjitkar S., Lawley B., Tannock G., Engberg R.M. Bacterial succession in the broiler gastrointestinal tract // Appl. Environ. Microbiol. 2016. V. 82. № 8. P. 2399-2410.
[36] Scupham A.J. *Campylobacter* colonization of the Turkey intestine in the context of microbial community development // Appl. Environ. Microbiol. 2009. V. 75. № 11. P. 3564-3571.
[37] Mazza P. The use of *Bacillus subtilis* as an antidiarrhoeal microorganism // Boll. Chim. Farm. 1994. V. 133. P. 3-18.
[38] van der Wielen P.W.J.J., Biesterveld S., Notermans S., Hofstra H., Urlings B.A.P., van Knapen F. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth // Appl. Environ. Microbiol. 2000. V. 66. № 6. P. 2536–2540.