The influence of genotypic and paratypic factors on the intestinal microbiome of quails

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Abstract. The article discusses the features of the gastrointestinal tract microbiome of white Texas quails using various probiotic preparations and the relationship between immune genes and its biodiversity. The highest biodiversity was found in the quails of the first group, which were kept on the main feeding ration. Among the experimental groups, the highest biodiversity was demonstrated by the groups that were given the probiotics "Em-Kurunga" and "Yarosil" with water (0.6 ml/kg). Birds with all the studied immune genes have a higher biodiversity of microorganisms in the gastrointestinal tract.

1 Introduction

The gastrointestinal microbiome is a collection of all microorganisms that inhabit the digestive tract of animals and birds. Microorganisms help break down complex carbohydrates, proteins, and fats, promote the development and functioning of the immune system, participate in the metabolism of various substances, and promote the restoration and protection of the intestinal mucosa. Changes in the intestinal microbiome occur under the influence of genetic and paratypic factors. In the first case, immune system genes play a key role in the interaction of the body with the intestinal microbiome. They are responsible for the inflammatory response to the presence of pathogenic microorganisms in the gastrointestinal tract, encode proteins involved in the production of antibodies that neutralize pathogens, as well as antimicrobial peptides and enzymes, thereby participating in the control of the number of different types of intestinal microorganisms. The genes of greatest interest to researchers are interleukin, interferon, tumor necrosis factor, occludin and claudin [1-3]. In the second case, they talk about the influence of conditions of keeping and feeding, the use of various probiotic and prebiotic preparations, which affect not only the microbiome, but also the gain in live weight, biochemical and physiological indicators [4-8]. At the same time, scientists note that in quails, the main share of the microbial community is made up of microorganisms belonging to the types Bacillota, Pseudomonadota, Actinomycetota, Cyanobacteria, Bacteroidota and Deferribacterota [9-10].

Information on the influence of various factors on the gut microbiome of Texas white quail was not found in the available scientific literature, so the aim of our work is to study

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the influence of genetic and paratypic factors on the biodiversity of the gut microbiome of quail.

2 Materials and methods

The objects of the study were four groups of white Texas quail roosters: group 1 - quail kept on the main diet (samples 102-104), group 2 - in addition to the main diet received the probiotic supplement EM-kurunga at a dosage of 0.02 g / kg (samples 105-107), groups 3 and 4 - probiotic Yarosil-0.2 ml / kg and 0.6 ml / kg of live weight (samples 111-113 and 108-110, respectively). Quails were raised in brooders until one month of age, then they were transplanted into cages.

Genomic DNA was isolated from the caecum of birds using the DNA-Extran 2 kit (Synthol, Moscow). The isolated DNA was sent for bioinformatics analysis to Sequence LLC (Moscow) and used to determine the immunity genes in the studied birds using Real-time PCR (Organic Agriculture Competence Center of the Yaroslavl State Agricultural University, Yaroslavl). The polymerase chain reaction was carried out using the BioMaster HS-qPCR SYBR Blue (2x) kit (Biolabmix LLC) in the LightCycler® 96 real-time PCR analyzer. The following oligonucleotides complementary to DNA were used for PCR:

IL-2	F: 5′-GTGCAAAGTACTGATCTTCGCC-3′ R: 5′-CTTGGTGTGTAGAGCTCGAGATG-3′
IL-1β	F: 5′-CTTCCTCCAGCCAGAAAGT-3′ R: 5′-CAGCTTGTAGCCCTTGAT-3′
IL-4	F: 5′-GAGAGCATCCGGATAGTGAAG-3′ R: 5′-TTCGCATAAGAGCTGGGTTC-3′
IL-6	F: 5′-CAACCTCAACCTGCCCAA-3′ R: 5′-GGAGAGCTTCCTCAGGCATT-3′
IL-8	F: 5′-CTGAGGTGCCAGTGCATTAG-3′ R: 5′-AGCACACCTCTCTTCCATCC-3′
IL-10	F: 5′-CACAACTTCTTCACCTGCGAG-3′ R: 5′-CATGGCTTTGTAGATCCCGTTC-3′
IL-12a	F: 5′-AAGACCTGAAAACCTACAAGGC-3′ R: 5′-GGCTTGCATCATGTCATCAA-3′
IL-12b	F: 5′-CACAGCAGCTTTTCATCAGAGA-3′ R: 5′-ATAGGACTTTGGTGTGCTCCAG-3′
IL-13	F: 5′-CTGCAAGAAGGACTATGAGCCC-3′ R: 5′-CAGTGCCGGCAAGAAGTT-3′
IL-18	F: 5′-GCAGCGGAATGTACTTCAAC-3′ R: 5′-CTCTTATCTTCTACCTGGACGCTG-3′
IFN-α	F: 5′-CCTTGCTCCTTCCAACGACA-3′ R: 5′-CGCTGAGGATACTGAAGAGGT-3′
IFN-γ	F: 5′-CAACCTTAATGATGGCACGA-3′ R: 5′-CTTTGCGGTGGATTCTCA-3′
TNF-a	F: 5′-GCCCTTCCTGTAACCAGA TG-3′ R: 5′-ACACGACAGCCAAGTCAA CG-3′
Occludin	F: 5′-ATGAGACCGACTACACCACG-3′ R: 5′-CTGATTGAGGCGGTCGTTGA-3′
Claudin-1	F: 5′-CTGATTGCTTCCAACCAG-3′ R: 5′-CAGGTCAAACAGAGGTACAAG-3′

3 Results and Discussion

Bioinformatics analysis is the processing of data obtained using high-throughput sequencing. The results are visualized in Figure 1.

In the control group, the prevalence of bacteria of the Bacillota, Actinomycetota, and Bacteroidota types was observed. In sample #102, the microbiome was mostly occupied by bifidobacteria, streptococci, and bacteroids. *Bifidobacterium pullorum* occupied the largest share of the microbiome, accounting for 16%. In sample #104, a distinctive feature is the presence of bacteria of the Fusobacteriota (6%) and Pseudomonadota (9%) types. In addition, in sample #103, the first place in the microbiome is occupied by *Megamonas hypermegale* (19%), and in sample #104 - *Micrococcus luteus* (13%).

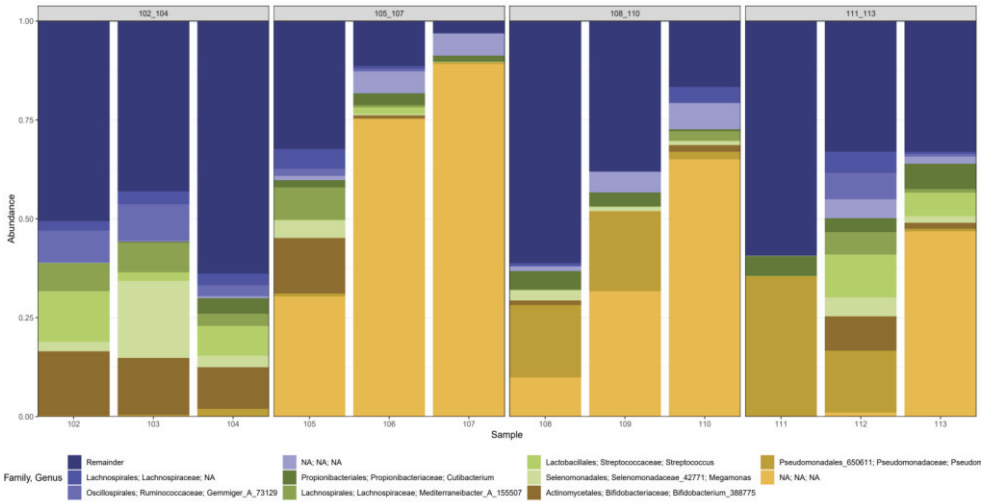

Fig. 1. Results of taxonomic classification of intestinal microorganisms of four groups of quails (No. 102-104 – group I; No. 105-107 – group II, No. 111-113 – group III; No. 108-110 – group IV).

When using the probiotic supplement EM-kurunga (group II), the largest proportion of bacteria in the samples are representatives of the types Bacillota (14-52%), Actinomycetota (20-32%) and Pseudomonadota (4-9%). In sample No. 105, the largest shares in the microbiome are occupied by *B. pullorum* (20%), *Mediterraneibacter_A_155507 cottocaccae* (12%) and *Megamonas hypermegale* (6%), and in the other two - *Cutibacterium acnes* (12-15%).

In groups III and IV, which were characterized by the use of the probiotic preparation "Yarosil" in various dosages, it was noted that the dominant position was occupied by representatives of the Pseudomonadota type (23-63%). The exceptions are samples No. 110 and 113, in which the Bacillota and Actinomycetota types prevail. When studying the species composition, it was noted that in samples No. 108, 109, 111 and 112, the largest share was occupied by the bacterium *Pseudomonas_E_647464* (from 16 to 35%). In addition, in sample No. 112 of group III, about 9% were occupied by bacteria of the species *Streptococcus alactolyticus* and *B. pullorum*. In sample No. 108 of group IV, *Paracoccus carotinifaciens* (20%) and *Enterococcus cecorum* (14%) were found. In sample No. 110, the largest share of the microbiome is occupied by *Mediterraneibacter cottocaccae* (7%), and in sample No. 113 – by *Cutibacterium acnes* (12%).

The analysis of alpha diversity by the Shannon index is presented in Figure 2.

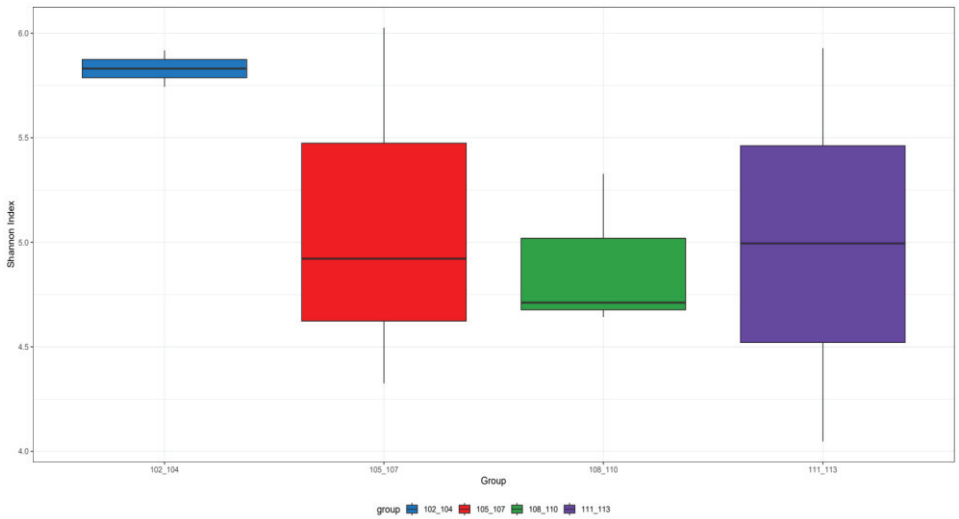

Fig. 2. Biodiversity of the intestinal microbiome of quails according to Shannon (No. 102-104 – group I; No. 105-107 – group II, No. 111-113 – group III; No. 108-110 – group IV).

The biodiversity of intestinal microorganisms is higher in quails of the first group, it fluctuates within 5.8-5.9. In the second group, the Shannon index was 4.6-5.4, in the third - 4.5-5.45, and in the 4th - 4.7-5.1. Thus, among the experimental groups, the highest biodiversity is found in the groups that were given the probiotic "Em-Kurunga" and "Yarosil" (0.6 ml / kg). At the same time, the first and fourth groups are the most uniform.

Table 1 presents the results of the polymerase chain reaction in real time for the detection of immunity genes in the studied birds.

Table 1. Results of qPCR testing for the presence of immunity genes in quails.

No. p/p	Name of the gene being studied	Sample No.											
		105	**107**	**112**	**109**	**110**	**102**	**103**	**104**	**111**	**113**	**108**	**106**
1	*IL-2*	+	+	+	+	+	+	+	+	+	+	+	+
2	*IL-1β*	+	+	+	+	+	+	+	+	+	+	+	+
3	*IL-4*	+	+	+	+	+	-	-	-	-	-	-	-
4	*IL-6*	+	+	+	+	+	+	+	+	+	+	+	+
5	*IL-8*	+	+	+	+	+	+	+	+	+	+	+	+
6	*IL-10*	+	+	+	+	+	+	+	+	+	+	+	-
7	*IL-12a*	+	+	+	+	+	+	+	+	+	+	+	-
8	*IL-12b*	+	+	+	+	+	+	+	+	+	+	+	+
9	*IL-13*	+	+	+	+	+	+	+	+	+	+	+	+
10	*IL-18*	+	+	+	+	+	+	+	+	+	+	+	-
11	*IFN-α*	+	+	+	+	+	+	+	+	+	+	+	+
12	*IFN-γ*	+	+	+	+	+	+	+	+	+	+	+	+
13	TNF-a	+	+	+	+	+	+	+	+	+	+	+	+
14	Occludin	+	+	+	+	+	+	+	+	+	+	+	+
15	Claudin-1	+	+	+	+	+	+	+	+	+	+	-	+
Total, %		100.00					93.33					86.66	73.33

Note: "+" – positive, "-" – negative; No. 102-104 – group I; No. 105-107 – group II, No. 111-113 – group III; No. 108-110 – group IV.

Genes IL-2, IL-1β, IL-6, IL-8, IL-12b, IL-13, IFN-α, IFN-γ, TNF-a and Occludin were found in all bird samples. IL-4 is absent in samples 102-104, 106, 108, 111 and 113.

Sample No. 106 also lacks the genes IL-10, IL-12a and IL-18, and sample No. 108 lacks Claudin-1.

Comparing the results of taxonomic classification of intestinal microorganisms of the studied quails with the PCR results, it was found that samples No. 105, 109, 110 and 112 showed a greater diversity of microorganisms. Bacteria of the Lachnospiraceae family, *Bifidobacterium, Megamonas, Streptococcus, Pseudomonas*, and *Cutibacterium* genera were present. In the birds of the control group, even in the absence of the IL-4 gene, the microbiome was represented by a variety of microorganisms. In sample No. 107, only bacteria of the *Cutibacterium* genus were classified.

In sample #106, in the absence of IL-4, IL-10, IL-12a and IL-18, bacteria of the genera *Megamonas* and *Cutibacterium* have equal shares. In sample #108, in the absence of the IL-4 and Claudin-1 genes, pseudomonads predominate.

A group of American scientists found that greater microbial diversity was observed in the cecum microbiome of quail with high stress levels compared to the microbiome of quail with low stress levels for all three alpha diversity indices. These results are the first evidence that in birds that are hosts of stress sensitivity, and the corticosterone response to acute stress in particular, may be associated with intestinal microbial diversity [12]. At the same time, the Shannon index in the study by Lyte J.M. and co-authors ranged from 2.7 to 3.0, whereas in our study it was significantly higher: from 4.5 to 5.5.

Liu S. et al., studying the interaction between quail genotype and diet, established possible explanations for the different effectiveness of probiotic treatment. Of particular interest from the authors' point of view is the excess of Lactobacillus in the ileum. Using PICRUSt predictions, Liu S. et al. linked gut microbiota with host resistance and susceptibility to diet-induced atherosclerosis.

4 Conclusion

For the first time, the microbiota of the cecum of the gastrointestinal tract of Texas white quail was studied using various probiotic preparations. It is characterized by the presence of bacteria of the main types, such as Bacillota, Actinomycetota and Pseudomonadota. When using the probiotic preparation "Yarosil" in various dosages, bacteria of the Pseudomonadota type prevailed in 67% of samples. Birds that had all the immunity genes had a higher biological diversity of intestinal microorganisms.

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