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Technology Development to Suppress the Campylobacter Jejuni in Intestinal Microflora of The Broilers by Lactobacilli

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Abstract*: This article presents a method for determining the microflora of the blind processes of the intestines of broiler chickens receiving heterologous lactobacilli with anti-C.jejuni activity as a feed additive in comparison with intact chickens. In the course of a scientific and production experiment on the Cobb 500 cross broilers, an experiment was carried out to determine the total number of bacteria in the intestinal contents by quantitative PCR. Sequencing was performed on a MiSeq genomic sequencer (Illumina, Inc., USA) using the MiSeq Reagent Kit v3 kit (Illumina, Inc., USA). Data were obtained on the total number of bacteria and their taxonomic diversity. As a result of the studies, it was shown that the microflora of broilers throughout the entire maintenance period corresponded to the norm. The use of feed additives based on lactobacilli in general had a positive effect on the state of microflora of the blind processes of the intestine..

Keywords*: probiotics, lactobacilli, broilers, Campilobacter jejuni antagonism, NGS, real-time quantitative PCR.

I. INTRODUCTION

The central problem of modern biology, medicine and veterinary medicine is the regulation of innate (non-specific) immunity, aimed at increasing the resistance of the human body and animals to infections by improving immune homeostasis. The intestinal microbiota of the host organism plays a special role as a regulator of the innate immune response. Veterinary medicine faces the problem of increasing the non-specific resistance of animals to various infectious diseases.

Solving this problem will limit the widespread use of antibiotics in agriculture.

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In particular, the intensification of poultry farming under the conditions of industrial technology for keeping highly productive crosses of chickens requires the creation of scientifically based food that ensures the optimal functioning of the intestinal microbiota involved in the digestive system, the development of immunity and the formation of colonization resistance against Campylobacter spp and Salmonella spp, which are pathogens for humans.

The study of the ecology of the microbial community of the gastrointestinal tract of poultry has now acquired great fundamental importance. According to recent microbiota data, the gastrointestinal tract is involved not only in the stimulation of postnatal maturation of the digestive system, the formation of innate immunity and colonization resistance, but also takes part in embryogenesis. The ability of microorganisms to colonize the gastrointestinal tract of birds at the stage of embryonic development inside the egg is shown. It was established that the microflora of the laying layer plays a key role in the formation of pathogenic microbiota of the gastrointestinal tract of the egg embryo. The spectra of microorganisms capable of causing death of embryos are diverse, the main ones are: E. coli, Pseudomona ssp., Staphylococcu ssp., Klebsiella sp. The microorganisms present in the digestive tract of the embryo are the basis that determines the formation of the starting intestinal biocenosis of hatched chickens.

Search and comprehensive study of strains with a wide spectrum of antagonistic activity against Campylobacter spp, Salmonella spp, as well as other pathogens, elucidation of the genetic basis of their biological properties, creation of innovative new generation probiotic preparations based on these strains and their use as a feed additive will limit the wide the use of antibiotics in industrial poultry, will increase the quality and safety of poultry products.

II. MATERIALS AND METHODS

The aim of this study was to analyze the microflora of the blind processes of the intestines of broiler chickens receiving heterologous lactobacilli with anti-C.jejuni activity as a feed additive compared to intact chickens.

To solve this problem, the scientific and industrial experience on broilers of the Kobb 500 cross was laid. The chickens were kept in AviMax cell batteries, 35 goals in each group, in compliance with all technological parameters.

Poultry was fed in two phases (6–21 days and from 22 days until the end of rearing). The first 5 days, chickens of all groups received the same starter feed. Additives were introduced into the feed by the method of stepwise mixing. The scheme of scientific and industrial experience is presented in table 1.

Table- I: Scheme of the experiment on broilers of the cross "Kobb-500"

Group	Features of broiler feeding
1 - control	Complete feed with nutrition corresponding to the recommendations for cross-country (PC)
2 - experienced	PC + feed supplement at a dose of 1 kg g / t feed containing 107 CFU / g Lactobacillus plantarum ATCC 8014
3 - experienced	PC + feed supplement at a dose of 1 kg g / t feed containing 107 CFU / g Lactobacillus fermentum ATCC 9338
4 - experienced	PC + feed supplement at a dose of 1 kg g / t feed containing 107 CFU / g Lactobacillus reuteri ATCC 23272

Samples of the intestinal contents of the chickens of the experimental and control groups were taken on the 7th day, after changing the rations (starter to starter), on the 21st day (after the vaccination) and during slaughter, on the 35th day.

Sampling for the analysis of microflora was taken in five replicates from each group with strict adherence to sterility and immediately frozen.

Total DNA from the samples was isolated using the Genomic DNA Purification Kit (Fermentas, Inc., Lithuania) according to the manufacturer's recommendations. The composition and structure of the bacterial community was determined by NGS sequencing.

Amplification was performed using Verity DNA amplifier (Life Technologies, Inc., USA) with primers amplifying a fragment of the 16S rRNA gene: 343F 5-CTCCTACGGRRSGCAGCAG-3', 806R 5-GGACTACNVGGGTWTCTAAT-3'. Sequencing was performed on a MiSeq genomic sequencer (Illumina, Inc., USA) using the MiSeq Reagent Kit v3 kit (Illumina, Inc., USA). Processing of 2 x 300 nt readings and DNA sequences was carried out using the CLC Bio GW7.0 bioinformatics platform (Qiagen, Netherlands).

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The total number of bacteria was determined by qPCR using the "Set of reagents for PCR-RV in the presence of intercalating dye EVA Green" (ZAO Syntol, Russia) and primers Eub338 5-ACTCCTACGGGAGGCAGCAG-3 ', Eub 5185' ATTACCGCGGCTGCTGg-3 'det DT Lite-4 thermocycler (NPO DNA-Technology LLC, Russia) under conditions: 95 ° C - 3 min (1 cycle); 95 ° C - 13 s, 57 ° C - 13 s, 72 ° C - 30 s (40 cycles).

Summary results of NGS sequencing are presented in Table 2.

Table- II: Results of the analysis of the microbiota of the blind processes by the NGS method

Microorganism group	Control (7 days)	+ *L. reuteri* (7 days)	+ *L. plantarum* (7 days)	+ *L.fermentum* (7 days)	Control (21 days)	+ *L. reuteri*(21 cyr)	+ *L. plantarum* (21 days)	+ *L.fermentum* (21 days)	Control (35 days)	+ *L. reuteri* (35 days)	+ *L. plantarum* (35 days)	+ *L.fermentum* (35 days)
Uncultured bacteria	48,4	48,1	39,8	41,8	37,8	47,6	39,4	48,7	39,3	38,6	29,6	39
Beneficial microorganisms												
Cellulolytic	41,7	42,8	45,7	48,85	48,75	41,6	43,2	35,9	50,2	41,6	40,6	51,6
Bacilli	1,1	1,9	1,8	0,77	0,47	2,35	3,1	1	1,23	1,8	2,1	0,59
Bifidobacteria	0,5	2,1	2,72	0,3	0,55	0,85	0,04	0,97	0,05	0,24	2,2	2,1
Lactoba cyllus	0,7	1,2	3,0	0,9	4,86	0,85	9,7	6,4	0,8	2,2	7,9	1,9
Undesirable microorganisms												
Actinomycetes	5,79	2,6	4,4	5,6	2,95	5,9	3	3	3,8	10,7	7,9	2,9
Entero bacteria	0	0,06	0	0	2,8	0	0	0,03	3,2	0	7,9	0,1
Pathogens												
Staphillococci	0,2	0	0,34	0	0,2	0,06	0,35	0	0	0	0	0
Mycoplasmas	1,19	0,96	0,06	0,18	0,77	0,23	0,36	1,6	0,58	0,43	0,2	0,25
Pasteurella	0,42	0,04	0,49	0	0	0	0	1,6	0	0,43	0	0,21
Fusobacteria	0	0,24	0,69	1,6	0,85	0,56	0,85	0,8	0,57	1,8	1,6	0,25
Campylobacter	0	0	0	0	0	0	0	0	0,27	2,2	0	1,1

III. RESULTS AND DISCUSSION

The analysis of the total number of bacteria in the intestinal contents was carried out by quantitative PCR. The data are presented in Table 3. It was shown that the number of uncultivated bacteria throughout the entire period of broiler growing ranged from 29.6 to 48.7%. These microorganisms cannot be identified and studied using traditional methods of microbiology - cultivation on nutrient media, so the role of these microorganisms in the digestion of broilers remained unclear. The introduction of a feed supplement based on L. plantarum changes the structure of the microbiota, reducing the relative number of uncultured bacterial phylotypes.

Other studied strains of lactobacilli modify the microbial community to a lesser extent. The number of useful cellulolytic microorganisms (eubacteria, ruminococci, bacteroids and others) throughout the entire period of broiler growing was quite high.

The number of these microorganisms in the variants with the use of a feed additive on the 7th day of cultivation, when using bacteria L. fermentum and L. plantarum and on the 35th day of maintenance (against the background of L. fermentum) was significantly higher than in the control variants. It is worth noting that the number of cellulolytics in the variants using L. fermentum and L. plantarum was higher than in the variants with L. reuteri and control.

Table- III: Data on the total number of bacteria (number of genomes / g)

Sampling date	Control	+ *L. reuteri*	+ *L. plantarum*	+ *L.fermentum*
7 days	9,56± 0,34	9,82± 0,34	9,11± 0,56	9,56±0,65
21 days	10,01± 0,67	9,45± 0,67	10,45± 0,45	10,13±0,34
35 days	10,16± 0,45	9,15± 0,44	10,5± 0,68	9,35±0,15

The content of useful bacilli was mainly the highest in the variant with the use of lactobacilli compared with the control variants throughout the entire period of broiler growing and ranged from 1.5 to 2.3%. It should be emphasized that the number of bacilli in variants using L. plantarum was higher than in variants with other lactobacilli. It should be noted that bacilli have significant antimicrobial activity against pathogenic microorganisms.

The number of useful bifidobacteria in the variant with the use of the drug L. plantarum on the 7th day of cultivation, on the 21st day of cultivation and on the 35th day of cultivation was higher than in the control variants without the use of drugs. It is worth noting that bifidobacteria have significant antimicrobial activity against pathogens.

It should be noted that the introduction of feed additives based on the studied strains of lactic acid bacteria affects the group of species of lactic acid bacteria. The input of L. plantarum.L increases to the greatest extent the number of useful microbiota. fermentum significantly increases the relative number of lactobacilli by 21 days.

The content of unwanted actinomycetes, among which pathogens are often found, throughout the growing period was within the normal range in all cases. It should be emphasized that the content of actinomycetes was mainly the highest in the variants with L.reuteri compared with other variants throughout the entire period of broiler keeping.

The number of undesirable enterobacteria, among which pathogens are often found, throughout the entire period of broiler maintenance was either within normal limits or below the limit of reliable determination.

The number of pathogenic microorganisms (staphylococci, mycoplasmas, pasteurellas, fusobacteria) throughout the entire period of detention in almost all cases, regardless of the type of vaccine, was large in the control variants compared to variants using lactobacilli.

IV. CONCLUSION

Thus, as a result of the studies, it was shown that the microflora of broilers throughout the entire maintenance period corresponded to the norm. The use of feed additives based on lactobacilli in general had a positive effect on the state of microflora of the blind processes of the intestine.

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