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EFFECTS OF DIETARY FIBER ON MINERAL METABOLISM AND CAECAL MICROBIAL DIVERSITY IN BROILER CHICKENS (*Gallus gallus* L.) FED A SEMI-SYNTHETIC DIET

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Abstract

Various additives used in poultry diets can change the mineral status of the body. Dietary fiber has long been considered an anti-nutritional factor due to adverse effects on feed intake and nutrient absorption. However, with increasing evidence, it has been found that dietary fiber has a positive effect on nutrient digestion, fermentation, and absorption processes in poultry. In this work, for the first time, data were obtained on the influence of dietary fibers, the microcrystalline cellulose, lactulose and chitosan on mineral metabolism and caecal microbiocenosis of broiler chickens fed a semi-synthetic diet. A decrease in the accuulation of toxic microelements in the body of a bird was demonstrated, as well as a change in the microbial community of the caecum. Experiments on the Arbor Acres cross broiler chickens (*Gallus gallus* L.) were carried out in the vivarium (the FSC BSA RAS). A total of 150 of week-old broiler chickens were divided into 5 groups of analogues ($n = 30$ each). The duration of the experiment was 35 days. The first control group C_1 was fed with a semi-synthetic diet (SS). The second control group C_2 received a semi-synthetic diet deficient in trace elements (DSS). For dietary fibers, test group I was fed with dietary microcrystalline cellulose (E460, 0.25 g/kg feed), test group II with dietary lactulose (1 g/kg feed), and test group III with dietary chitosan (0.5 g/kg feed). In feed and biomaterial of broilers, 25 chemical elements were assayed: Ca, Cu, Fe, Li, Mg, Mn, Ni, As, Cr, K, Na, P, Zn, I, V, Co, Se, Ti, Al, Be, Cd, Pb, Hg, Sn, Sr by atomic emission spectrometry and mass spectrometry techniques. Microbial biodiversity of the caecum was assessed on day 42. NGS sequencing was performed using a MiSeq platform (Illumina, Inc., USA). In test group I, the dietary fiber led to a statistically significant increase in the calcium (by 23,4 %. $p \le 0.05$) vs. C_2. In test group III, there was a 1.5-forl decrease in the indicator ($p \le 0.05$) vs. C_1 and a 26.3 % decrease ($p \le 0.05$) vs. C_2. The lithium content increased 1.7 times ($p \le 0.05$) vs. C_1 when chitosan was added to a semi-synthetic diet deficient in trace elements. The concentration of manganese and cobalt significantly ($p \le 0.05$) decreased in all test groups vs. C_1. In group I, the amount of selenium increased 2.35 times ($p \le 0.05$) vs. C_1 it decreased 1.74 times ($p \le 0.05$) vs, C_2. In the same group, the iodine level increased 1.74 times and 1.5 times ($p \le 0.05$) vs. control groups. In test groups II and III, selenium decrease 4.64 times and 4.55 times ($p \le 0.05$) vs. C_2. The concentration of arsenic in group II exceeded C_1 1.63 times ($p \le 0.05$), and in group III, its concentration, on the contrary, decreased 1.58 times and 2.0 times ($p \le 0, 05$) vs. C_1 and C_2. The dietary fiber scontributed to the removal of toxic elements. In test group I and group III, the concetration of strontium decreased ($p \le 0.05$) by 25.7 and 45.9 %, respectively, vs. C_1. For C_2, a decrease in the amount of strontium by 22.2 and 43.4 % was similarly revealed ($p \le 0.05$). In group I, the counts of *Rikenellaceae* increased 6.3 and 6.8 times, *Lachnospiraceae* 12 and 4.9 times, *Ruminococcaceae* 2.1 times and 3. 9 times compared to C_1 and C_2, respectively. In group II, the abundance of *Lactobicallaceae* decreased 6 times, the number of *Rikenellaceae* increased 6.2 times, *Lachnospiraceae* 9.57 times, *Ruminococcaceae* 3.1 times compared to C_1. In group III, there was a decrease in the content of *Lactobicallaceae* by 13.3 and 1.55 times compared to C_1 and C_2. The number of *Rikenellaceae* increased 5.5 times, *Lachnospiraceae* 11.8 times, *Ruminococcaceae* 3.5 times compared to C^1. Thus, dietary fibers added to a semi-synthetic diet led to a decrease in the content of macroelements in the body of Arbor Aikres cross

broiler chickens, the elimination of toxic elements, and increased the counts of *Rikenellaceae* and *Lachnospiraceae* taxa with a simultaneous decrease in the number of *Lactobacillaceae* in the intestine.

Keywords: semi-synthetic diet, dietary fiber, metabolism, mineral metabolism, microbiome, caecum

Over the past few decades, poultry feeding concepts have undergone significant changes, driven by the transition from domestic to industrial feed production [1]. This became possible due to assessment of nutritional needs and the metabolic role of nutrients in birds.

Since the 1950s, numerous experiments have been conducted to determine the protein and essential amino acid requirements of poultry [2, 3] and the ideal protein ratio in diets [4]. With the five synthesized essential amino acids now available, it is possible to formulate a balanced semi-synthetic diet in which the crude protein content is provided by the most limiting amino acid sourced from feed proteins [5]. In addition, a semi-synthetic diet may ensure balanced feeding by compensating for deficiencies in certain nutrients [6]. The use of a semi-synthetic diet will allow a more complete assessment of the effects of feed additives or other dietary components on the mineral metabolism and microbial diversity of the bird's gut, and will facilitate the study of genetic and environmental variations in the population.

Compiling semi-synthetic diets must consider the microelement status of the body that depends on the exogenous intake of microelements from feed during normalization of the intestinal chyme composition [7]. As a result, and due to the body's desire for a constant internal environment, absorption processes alter which leads either to normalization of the content of certain elements, or to microelement deficiency [8]. In turn, the intensity of absorption depends on many factors, particularly on normal functioning of the intestinal microbiota which can modify the bioavailability of microelements through their accumulation in microbial cells and changes in intestinal pH [9].

Various dietary additives used in poultry can alter the mineral status of the body. For example, dietary fiber has long been considered antinutritional due to its adverse effects on feed intake and nutrient digestibility. However, scresearchers later discovered that dietary fiber has a positive effect on the digestion, fermentation, and absorption [10]. Moderate amounts of fiber in diets also modify growth performance and improve gut health by modulating beneficial microbiota in the colon and enhancing immune function [11].

This paper is the first to reveal a decrease in the content of toxic microelements and a change in the microbial community of the cecum of broiler chickens fed a semi-synthetic diet supplemented with microcrystalline cellulose, lactulose and chitosan.

Our goal was to study the effect of dietary fiber on mineral metabolism and microbiocenosis of the cecum in broiler chickens fed a semi-synthetic diet.

Materials and methods. For experiment, 150 Arbor Acres cross broiler chickens (*Gallus gallus* L.) of 1-week age were divided into 5 groups, $n = 30$ each (the vivarium of the Federal Scientific Center BST RAS, https://цкп-бст.рф/).

During the experiment, all birds were kept under the same conditions and all manupulations were in accordance with the instructions and recommendations of Russian Regulations, 1987 (Order No. 755 on 08/12/1977 the USSR Ministry of Health) and The Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996). All efforts were made to minimize bird suffering and reduce the number of samples used (Protocol No. 1 of 05/21/2021).

Of 35-day experiment, the preparatory and test periods were 7 and 28 days, respectively. During the test period, the first control group (C_1) was fed a semi-synthetic diet (SS), the second control group (C_2) was fed a micronutrient-

deficient diet (SSD), in treatments, the birds were fed SDD added with 0.25 g microcrystalline cellulose (E460) per 1 kg feed (group I), 1 g/kg lactulose (group II), and 0.5 g/kg food-grade chitosan (group III). The chickens can drink distilled water without restriction. A semi-synthetic diet (C1) was as recommended by M.L. Scott et al. [12] and a semi-synthetic diet deficient in microelements (C2) was modified by us. Feed samples were prepared by stepwise mixing.

The bird was decapitated under nembutal ether on day 42. Carcasses were ground whole, and bulk samples were subjected to analysis for 25 chemical elements: Ca, Cu, Fe, Li, Mg, Mn, Ni, As, Cr, K, Na, P, Zn, I, V, Co, Se, Ti, Al, Be, Cd, Pb, Hg, Sn, Sr using atomic emission and mass spectral methods. The biomaterial was ashed (a microwave decomposition system MD-2000, PerkinElmer, Inc., USA) and the content of elements in the ash was measured (an Elan 9000 mass spectrometer and an Optima 2000 V atomic emission spectrometer, PerkinElmer, Inc., USA).

The microbial biodiversity of the bird's cecum was assessed on day 42 at the Institute of Cellular and Intracellular Symbiosis, Ural Branch of the Russian Academy of Sciences, Orenburg (https://ikvs.info/tskp/). For DNA extraction, samples were incubated at 37 °C for 30 min in 300 μl of sterile lysis buffer (20 mM EDTA, 1400 mM NaCl, 100 mM Tris-HCl, pH 7.5; lysozyme solution of 100 mg/ml concentration, 50 μl). The purity of the DNA preparations was assessed by electrophoresis in a 1.5% agarose gel with photometry (NanoDrop 8000, Thermo Fisher Scientific, Inc., USA). DNA concentration was measured fluorometrically (a Qubit 2.0 device with high sensitivity for dsDNA determination, Life Technologies, USA).

DNA libraries for sequencing were created using the Illumina protocol (Illumina, Inc., USA) with primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 to the variable region V3-V4 of the 16S rRNA gene [24]. NGS sequencing was performed using a MiSeq platform (Illumina, Inc., USA) with the MiSeq Reagent Kit V3 PE600 (Illumina, Inc., USA) at the Center for Shared Use of Scientific Equipment "Persistence of Microorganisms" (Institute of Cellular and Intracellular symbiosis Ural Branch RAS, Orenburg). The resulting operational taxonomic units (OTUs) were classified with a VAMPS online tool and the RDP database (http://rdp.cme.msu.edu). Some OTUs were aligned using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the databases for nucleotide sequence nr/nt (NCBI, https://www.ncbi.nlm.nih.gov/) and aligned ribosomal RNA gene sequences SILVA (https://www.arb-silva.de).

Statistical processing was carried out using the Statistica 10.0 program (StatSoft, Inc., USA). Results are submitted as arithmetic means (M) and standard errors of the mean (±SEM). Differences were considered statistically significant at $p \leq 0.05$ (Student's t-test). The USEARCH v8.0.1623_win32 software package (https://www.drive5.com/usearch/download.html) was used for bioinformatic processing of sequencing data. Processing included merging of paired reads in operational taxonomic units, filtering of reads by quality and length (minimum size of 300 bp), removal of chimeras, doubletons and singletons, clustering of reads in OTUs at a similarity level 97% [26].

Results. Table 1 shows the composition of the diets for the broilers.

1. Composition (g/100 g of feed) **of a semi-synthetic diet** (SS) **and a semi-synthetic microelement deficient diet** (SSD) **of Arbor Acres cross broiler chickens (*Gallus gallus* L.)** (vivarium of the Federal Scientific Center for Biological Systems and Agrotechnologies RAS)

Ingredient	SS	SSD
Casein	20	20
Gelatin	5	5
Cellulose	3	3

Continued Table 1

Vegetable oil	3	3
Choline chloride	0.2	0,2
Glucose	1.25	1,25
Rice	61.38	61,38
Methionine	0.1	0,1
Cystine	0.2	0,2
$CaHPO_4 \cdot H_2O$	1.8	1,8
$CaCO_3$	1.45	1,45
KH_2PO_4	1.013	1,013
KCl	0.21	0,21
Na_2CO_3	0.555	0,555
$MnCl \cdot 4H_2O$	0.04	–
$FeSO_4 \cdot 7H_2O$	0.05	–
$MgSO_4 \cdot 7H_2O$	0.615	0,615
KJ	0.001	0,001
$CuSO_4 \cdot 5H_2O$	0.001	–
$ZnCl_2$	0.016	–
$CoCl_2$	0.0002	–
$NaMoO_4 \cdot 2H_2O$	0.0008	–
Na_2SeO_3	0.000015	–
Vitamin mixture	0.052	0,052

N o t e. Composition of the vitamin mixture (mg/100 g of feed): B_1 — 2.5, B_2 — 1.5, B_6 — 0.6, B_{12} — 0.002, Ca-pantothenate — 2.0, biotin — 0.06, folic acid — 0.4, K_3 — 0.5, C — 25.0, PP — 15.0, A — 1000 IU, D_3 - 360 IU, E — 0.5 IU. Dashes mean that the ingredient was not added.

When identifying chemicals in biological substrates, it was first necessary to study their accumulation in the body of chickens in order to draw conclusions about the accumulation of macro- and microelements.

The dietary fiber in the broilers' diet led to a statistically significant increase in calcium content in experimental group I by 23.4% ($p \le 0.05$) vs. C_2. In group III, on the contrary, we noted its decrease by 1.5 times ($p \le 0.05$) compared to C_1 and by 26.3% ($p \le 0.05$) vs. C_2. In general, there was a tendency towards a decrease in the content of all macroelements in the group that additionally received chitosan, including a statistically significant decrease in the amount of phosphorus by 17.5% ($p \le 0.05$) vs. C_1 (Table 2).

2. Content (g/bird) **of macroelements, essential, conditionally essential microelements and toxic elements in the body of Arbor Acres cross broiler chickens *(Gallus gallus* L.) fed a semi-synthetic diet added with various dietary fibers** (n = 30, M±SEM; vivarium of the Federal Scientific Center for Biological Systems and Agricultural Technologies RAS)

Element	Group				
	C_1	C_2	I test	II test	III test
			Macronutrients		
Na	12.9±1.03	12.7±1.01	13.4±1.21	13.3±1.23	11.7±1.07
P	63.3±3.12	60.6±2.86	69.6±3.21	68.5±3.68	52.2±2.69[a]
K	35.2±1.89	35.8±2.11	35.3±1.58	35.3±2.11	32.8±3.11
Ca	91.9±5.42	80.7±2.31	99.6±3.14[b]	89.3±2.89	59.5±3.11[ab]
Mg	4.1±0.65	3.9±0.72	4.1±0.81	4.3±0.78	3.6±0.98
			Microelements		
Li	0.2±0.02	0.2±0.01	0.2±0.03	0.1±0.02	0.3±0.01[a]
B	0.9±0.03	1.3±0.04	1.1±0.02	0.9±0.01	0.4±0.03[ab]
Si	488.3±23.12	458.9±31.83	454.1±34.17	545.8±24.61	398.6±31.64
V	0.7±0.03	0.7±0.04	0.7±0.02	0.9±0.02	0.5±0.03
Cr	4.4±1.11	4.4±1.09	4.6±1.23	6.0±2.11	5.3±3.12
Mn	16.9±1.32	8.8±2.11	9.1±2.36[a]	11.2±3.12[a]	9.5±4.17[a]
Fe	916.6±30.10	883.4±29.86	960.5±35.44	956.2±41.2	852.6±34.97
Co	4.1±1.45	0.4±0.02	0.5±0.03[a]	0.3±0.03[a]	0.2±0.04[a]
Ni	6.1±1.21	5.6±1.45	5.2±1.24	4.5±2.11	5.1±2.45
Cu	24.6±2.36	21.5±2.45	20.5±3.11	29.2±2.58	17.8±3.12
Zn	383.4±25.64	339.9±18.95	376.7±21.37	377.5±31.20	283.6±29.34
As	0.19±0.00	0.24±0.001	0.20±0.001	0.31±0.002[a]	0.12±0.001[ab]
Se	4.7±1.24	19.5±2.36	11.2±3.12[ab]	4.2±2.86[b]	4.3±3.14[b]
I	3.3±1.32	3.9±1.45	5.8±2.11[ab]	4.7±3.11	3.7±2.87
			Toxic elements		
Sr	29.2±1.31	27.9±1.87	21.7±2.23[ab]	23.9±3.11	15.8±2.89[ab]
Cd	0.12±0.001	0.14±0.001	0.13±0.001	0.13±0.001	0.12±0.001

Continued Table 2

Sn	0.03±0.001	0.12±0.001	0.03±0.001[b]	0.03±0.001[b]	0.03±0.001[b]
Hg	0.03±0.001	0.03±0.001	0.03±0.001	0.03±0.001	0.03±0.001
Pb	1.2±0.63	0.6±0.03	0.6±0.02[a]	0.6±0.03[a]	0.6±0.04[a]
Al	1.6±0.74	1.1±0.68	0.5±0.02[ab]	0.7±0.01[ab]	0.7±0.03[ab]

N o t e. For a description of the groups, see the Materials and methods section.
[a, b] Differences from C1 and V2 are statistically significant at $p \leq 0.05$.

Adding chitosan into the SSD contributed to an increase in the amount of lithium by 1.7 times ($p \leq 0.05$) vs. C1. The boron content in test group III decreased by 2.19 times ($p \leq 0.05$) and 3.15 times ($p \leq 0.05$), respectively, compared to the two control groups (see Table 2). The manganese content decreased in all experimental groups ($p \leq 0.05$) vs. C1, in group I 1.86-fold, in roup II 1.50-fold, and in group III by 1.77-fold. A similar trend was for cobalt in the test groups, its amount statistically significantly decreased 7.98 times, 12.70 times and 16.90 times, respectively, compared to C1.

In group I, we recorded an increase in the amount of selenium ($p \leq 0.05$) by 2.35 times compared to C1 and a decrease ($p \leq 0.05$) by 1.74 times compared to C2. In the same group, a significant ($p \leq 0.05$) increase in iodine content was revealed (1.74 times and 1.50 times vs. both control groups). In groups II and III, the amount of selenium decreased by 4.64 and 4.55 times ($p \leq 0.05$) compared to C2. In the As accumulation group II exceeded C1 by 1.63 times ($p \leq 0.05$), and in group III, on the contrary, it is 1.58 times and 2.00 times ($p \leq 0.05$) less vs. C1 and C2.

Absorption, distribution and toxicity of heavy metal compounds depend both on the biological features of the digestive organs and the physicochemical properties of the absorbed substances, their interaction with feed components and on the presence of various additives in feed. As is known, the protein content in the diet affects the absorption of toxic elements in the body. In our case, the addition of dietary fiber contributed to the active elimination of toxic elements (see Table 2). The strontium content in groups I and III decreased by 25.7 and 45.9%, respectively ($p \leq 0.05$) vs. C1. Compared to C2, the parameter decreased by 22.2 and 43.4% ($p \leq 0.05$). The content of tin in birds from the test groups was 4.0 times less ($p \leq 0.05$) vs. C2, of lead 2.0 times less ($p \leq 0.05$) compared to C1. Data for aluminum were similar. A statistically significant ($p \leq 0.05$) decrease in its content was noted in all experimental groups, e.g., in group I by 3.19 and 2.22 times ($p \leq 0.05$) compared to C1 and C2, respectively, in group II by 2.51 and 1.74 times ($p \leq 0.05$), in III by 2.23 and 1.55 times ($p \leq 0.05$).

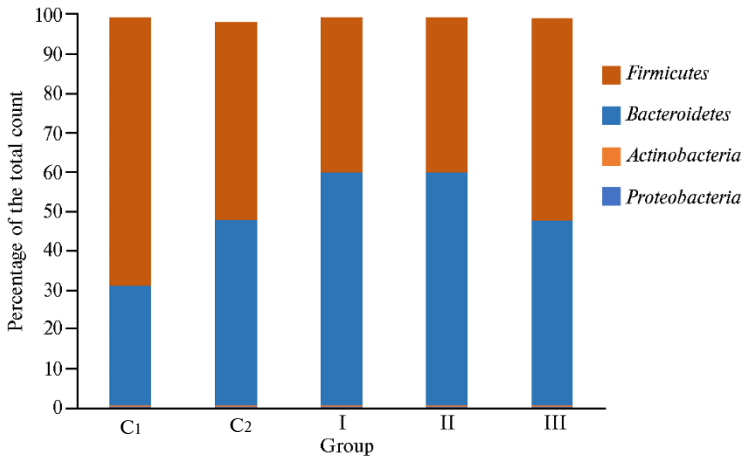

Fig. 1. Microbial profile of the cecum in the Arbor Acres cross broiler chickens (*Gallus gallus* L.) of fed a semi-synthetic diet added with various dietary fibers (n = 30; vivarium of the Federal Scientific Center for Biological Systems and Agricultural Technologies RAS). For a description of the groups, see the Materials and methods section.

In the microbial profile of the cecum contents in groups C_1 and III, we revealed the dominance of the phylum *Firmicutes*, while in groups I and II, the *Firmicutes* abundance was 39.7 and 39.8%, respectively. The number of *Bacteroidetes* in C_1 was 30.5%, or 16.8, 28.9, 28.9 and 16.6% less than in C_2, I, II and III groups, respectively. The abundance of other taxa did not exceed 3% (Fig. 1).

At a lower taxonomic level, C_1 group was dominated by the family *Lactobicallaceae* (61.6%); *Bacterodaceae* (26.2%), *Ruminococcaceae* (4.5%) and *Rikenellaceae* (4.2%) were also represented. In C_2 group, the bacteria of the families *Rikenellaceae* (26.5%), *Lactobicallaceae* (22.7%), *Bacterodaceae* (20.6%) and *Lachnospiraceae* (16.8%) had the greatest abundance. In group C_2 compared to group C_1, the number of *Lactobicallaceae* decreased 2.71 times, while the *Rikenellaceae* increased 6.30 times, *Lachnospiraceae* 12 times, *Ruminococcaceae* 2.10 times, and *Enterobacteriaceae* 5.60 times.

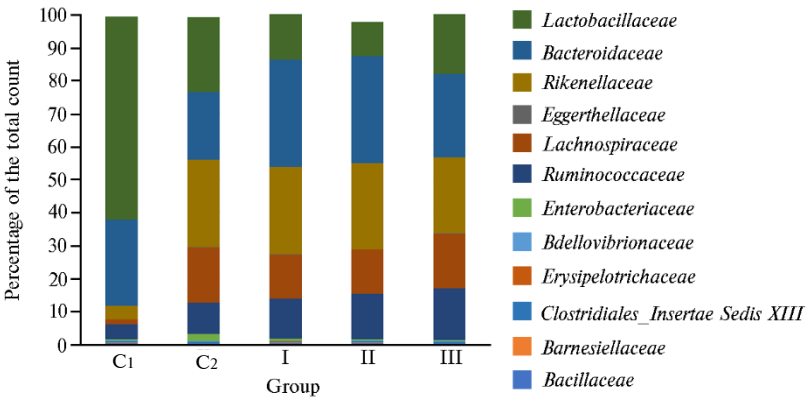

Fig. 2. Bacterial families found in the cecum of Arbor Acres cross broiler chickens (*Gallus gallus* L.) fed a semi-synthetic diet added with various dietary fibers (n = 30, vivarium of the Federal Scientific Center for Biological Systems and Agricultural Technologies RAS). For a description of the groups, see the Materials and methods section.

In group I, the number of *Lactobicallaceae* decreased 4.5 times, *Bacterodaceae* increased by 3.8 and 9.4%, *Rikenellaceae* 6.3 and 6.8 times, *Lachnospiraceae* 12 and 4.9 times, *Ruminococcaceae* 2.1 and 3.9 times compared to C_1 and C_2. In group II, when lactulose was added to the diet, the abundance of *Lactobicallaceae* decreased 6 times, and the number of representatives of *Bacterodaceae* increased by 6.5 and 12.1% compared to C_1 and C_2. The number of *Rikenellaceae* increased 6.20 times, *Lachnospiraceae* 9.57 times, and *Ruminococcaceae* 3.10 times vs. C_1. In group III, when chitosan was added, a decrease in the abundance of *Lactobicallaceae* was 13.30-fold and 1.55-fold, respectively, compared to C_1 and C_2. The abundance of *Rikenellaceae* increased 5.5-fold, *Lachnospiraceae* 11.8-fold, and *Ruminococcaceae* 3.5-fold vs. C_1 (Fig. 2).

The NGS sequencing showed that at genera level, in the cecum contents of the broilers from the group receiving SS diet, on day 42 the majority were represented by *Lactobacillus* (59.8%), *Bacteroidetes* (25.9%), *Alistipes* (4.2%) (Fig. 3, A). In group C_2, fed SSD deficient in minerals, representatives of the genus *Alistipes* dominated (26.5%, or 22.3% higher than for C_1). The number of *Lactobacillus* was lower by 39.5%, *Bacteroidetes* by 8.5% vs. C_1. Unclassified representatives of the microbial community accounted for 11.2%. Also genera *Mediterraneibacter* (7%), *Merdimonas* (5.6%), *Limasilactobacillus* (2.1%), and *Intestinomonas* (1.9%) were present in group C_2 (see Fig. 3, B).

In group I, according to the metagenomic sequencing data, representatives of the genus *Alistipes* dominated in the cecum contents (28.9%, or 24.7 and 2.4% highercompared to C_1 and C_2, respectively). Bacteria of the genus *Bacteroidetes*

accounted for 27.5%, which is 10.1% higher than in C_2, unclassified microorganisms accounted for 9.4%. The proportion of *Lactobacillus* was 47.6 and 8.1% lower than in groups C_1 and C_2, respectively. The number of bacteria of the genus Intestinomonas (10%) turned out to be 9.8 and 8.1% higher than in C_1 and C_2 (see Fig. 3, C).

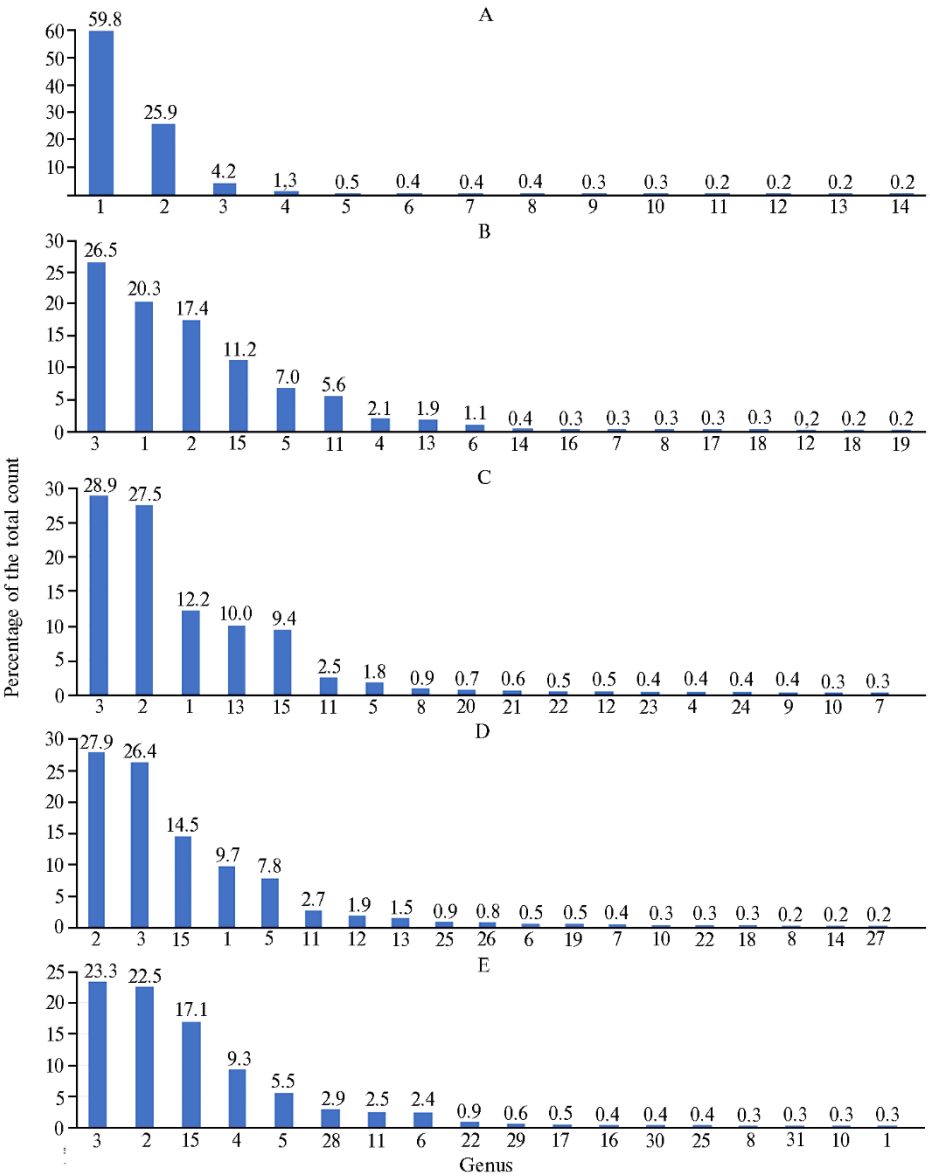

Fig. 3. Cecum microbiome genera composition in Arbor Acres cross broiler chickens (*Gallus gallus* L.) fed a semi-synthetic diet added with the of various dietary fibers: 1 — *Lactobacillus*, 2 — *Bacteroides*, 3 — *Alistipes*, 4 — *Limosilactobacillus*, 5 — *Mediterraneibacter*, 6 — *Faecalibacterium*, 7 — *Pseudoflavonifractor*, 8 — *Ligilactobacillus*, 9 — *Enterobacter*, 10 — *Rubneribacter*, 11 — *Merdimonas*, 12 — *Subdoligranulum*, 13 — *Intestinimonas*, 14 — *Neglecta*, 15 — unclassified, 16 — *Frisingicoccus*, 17 — *Eisenbergiella*, 18 — *Lachnospiraceae incertae sedis*, 19 — *Monoglobus*, 20 — *Fournierella*, 21 — *Ruthenibacterium*, 22 — *Coprobacter*, 23 — *Dysosmobacter*, 24 — *Catabacter*, 25 — *Anaerotignum*, 26 — *Clostridium XVIII*, 27 — *Anaeromasillibacillus*, 28 — *Weisella*, 29 — *Blautia*, 30 — *Bacillus*, 31 — *Ihubacter*; A — first control group (K_1), B — second control group (K_2), C — I test group, D — II test group, E — III test group ($n = 30$, vivarium of the Federal Scientific Center for Biological Systems and Agricultural Technologies RAS). For a description of the groups, see the Materials and methods section.

In group II, the most numerous taxa were *Bacteroidetes* and *Alistipes*. The number of *Bacteroidetes* was 10.5% higher than in C_2. The proportion of *Alistipes* bacteria was 22.2% higher compared to C_1. Unclassified microorganisms accounted for 14.5% of the total number. The abundance of *Lactobacillus* was 50.1 and 10.6% lower than in groups C_1 and C_2, respectively, of *Mediterraneibacter* was 7.3% higher than in C_1. Bacteria of the genus *Merdimonas* accounted for 2.7%, *Subdoligranulum* for 1.9%, *Intestinmonas* for 1.5%. Bacteria of other genera accounted for no more than 1% of the total number (see Fig. 3, D).

In group III, the most numerous bacteria were the genus *Alistipes* (23.3%, or 19.1% higher than in group C_1) and *Bacteroidetes* (22.5%, or 5.1% higher than in C_2). In this group, we revealed 17.1% of unidentified representatives of the bacterial community. The proportion of *Limosilactobacillus* bacteria was 9.3%, which is 8.0 and 7.2% higher compared to groups C_1 and C_2. In this group additionally fed chitosan, representatives of the genera *Weisella* (2.9%), *Merdimonas* (2.5%), *Faecalibacterium* (2.4%) were identified. Bacteria of other genera accounted for less than 1% (see Fig. 3, E).

Overall, we showed that the addition of dietary fiber led to a decrease in the accumulation of heavy metals in broiler chicken tissues, which may also be due to the excretion from the body with dietary fiber and a decrease in absorption in the intestine. This observation makes it promising to develop dietary fiber-based supplements for improving the health of farm animals under constantly increasing anthropogenic load on the environment.

Fiber is known to reduce mineral absorption. Metals bound by indigestible substances, mainly fiber, remain unavailable for absorption. Fiber can be hydrolyzed by colonic bacteria to release metals, but absorption will not occur and the metals will be excreted in the feces. Therefore, it is the fiber content of feed that can largely ensures the availability of minerals [13]. Due to the rise in cost of traditional feed components, new ingredients are needed that will reduce the cost of poultry feed. The use of dietary fiber is being considered as a solution [14].

The effectiveness of new feed substrates must not only be assessed according to generally accepted parameters. Special attention should be paied to the metabolism of chemical elements. Dietary fiber has a significant impact on mineral metabolism, impare of which can lead to various disorders [1], and, conversely, balance in chemical elements ensures increased productivity of animals and poultry [15]. Fermented dietary fiber helps reduce intoxication because it strengthens the intestinal barrier wall, normalizes its motor activity, and restores microbiota [6]. When analyzing the microbial profile of the cecum contents, it is noteworthy that the number of *Lactobacillaceae* decreased significantly in group C2 and in the test groups, which may be due to a lack of minerals necessary for growth. Importantly, no significant increase in the abundance of opportunistic microflora was recorded. Note, the abundance of cellulolytic bacteria from the taxa *Rikenellaceae* and *Lachnospiraceae* increased. This is due to an increase in the content of difficult to decompose components in the diet of broilers.

Note that dietary fibers, as anti-nutrients, have not been considered for a long time as an additive to the diet of animals and poultry. There are works [16, 17] that show a strong negative correlation between the fiber content in the diet and the digestibility of proteins and fats. Dietary fiber is not hydrolyzed by digestive enzymes of the small intestine, but can be partially fermented by the microflora of the gastrointestinal tract [16, 17]. The end products of microbial fermentation are various gases (H_2, CO_2, CH_4), lactic acid and short-chain fatty acids. Dietary fiber remains almost completely undigested, but when it is fermented into short-chain fatty acids, the energy produced can be used by host animals [18]. A

number of studies have shown that adding moderate amounts of various sources of fiber to the diet is beneficial. Diets high in fiber, especially insoluble fiber, have been shown to reduce disease incidence in poultry [19]. Dietary fiber improved the functions of the digestive organs, especially the stomach [20], increased the secretion of bile acids and enzymes [21], and changed the intestinal microflora [22]. This led to more efficient use of nutrients and an increase in animal growth rates [23]. Additionally, fiber in poultry diets may have a positive effect on gut health by preventing the adhesion of pathogenic bacteria to the epithelial mucosa [24], which is consistent with our findings.

Our data are consistent with reports supporting the trend toward the use of fiber as an alternative to antibiotics as growth promoters. Previously, antibiotics were widely used in poultry feed for the prevention and treatment of diseases. However, the indiscriminate use of antibiotics can lead to their residual content in meat and the selection of antibiotic-resistant forms of microorganisms. With the ban or strict regulation of the use of antibiotics in feed as growth promoters in the global poultry industry, an increased incidence of intestinal disorders in poultry has been documented [25]. Therefore, alternatives to antibiotics are being sought, feed formulations with easily digestible ingredients and enzyme additives are being developed, and the use of various feed processing methods is being considered in order to ultimately improve the growth performance of poultry.

Moderate amounts of fiber in the diet have been considered as one alternative to improve nutrient absorption and growth performance. Y.P. Li et al. [26] found that low-fiber diets do not provide full utilization of feed proteins and birds receive less energy than from high-fiber diets. In addition to feed additives, e.g., probiotics, prebiotics, and plant extracts, feed ingredients or feed components, e.g., fiber, hold promise for developing nutritional strategies to reduce gastrointestinal morbidity and improve poultry productivity [23]. However, we note that the available data on improved nutrient absorption when feeding dietary fiber are contradictory. For example, M. Houshmand et al. [27] examined the ability of fiber to compensate for calcium deficiency in poultry diets. There was no deficiency in the second group when using the low-calcium diet and the fiber-supplemented diet. That is, fiber is beneficial as a nutritional supplement to improve poultry growth performance and nutrient utilization. However, adding fiber does not always improve growth performance and nutrient absorption. In experiments of A. Sadeghi et al. [28], the authors found that intestinal villi length decreased in birds fed dietary fiber. This caused a decrease in the absorption of nutrients in the jejunum and an increase in their excretion, which is consistent with our findings.

Based on our data, methods can be developed to modulate the microbial profile of poultry intestines in order to use inexpensive feeds containing difficult-to-degrade fiber. It is important to note that when introducing dietary fiber into a diet deficient in minerals, we did not notice any severe dysbiotic processes in poultry.

Thus, dietary fibers (microcrystalline cellulose, lactulose, edible chitosan) added to the semi-synthetic diet of Arbor Acres cross broiler chickens leads to a decrease in the accumulation of macroelements in the bird's body, promotes the elimination of toxic chemical elements and an increase in the number of taxa *Rikenellaceae* and *Lachnospiraceae* with a simultaneous decrease of *Lactobacillaceae* in the intestines. The strontium content in poultry consuming cellulose and edible chitosan decreased by 25.7 and 45.9%, respectively ($p \leq 0.05$) vs. C_1 control (a semi-synthetic diet). A decrease by 22.2 and 43.4% ($p \leq 0.05$) was detected compared to C_2 control (a semi-synthetic diet deficient in microelements). In test groups, the comtent of tin reduced by 4.0 times ($p \leq 0.05$) vs. C_2, of lead by 2.0

times ($p \leq 0.05$) vs. C_1. In all test groups, the aluminum content decreased statistically significantly ($p \leq 0.05$). In group I fed cellulose, the number of *Lactobicallaceae* decreased by 4.5%, while the number of *Bacterodaceae* increased by 3.8 and 9.4%, *Rikenellaceae* by 6.3 and 6.8 times, *Lachnospiraceae* by 12.0 and 4.9 times, *Ruminococcaceae* by 2.1 and 3.9 times compared to C_1 and C_2. In group II, when lactulose was added to the feed, the number of *Lactobicallaceae* decreased by 6 times, and the number of *Bacterodaceae* increased by 6.5 and 12.1% compared to C_1 and C_2. The number of *Rikenellaceae* increased by 6.2 times, *Lachnospiraceae* by 9.57 times, and *Ruminococcaceae* by 3.1 times vs. C_1. In group III, when broiler chickens were fed chitosan, the number of *Rikenellaceae* increased by 5.5 times, *Lachnospiraceae* by 11.8 times, and *Ruminococcaceae* by 3.5 times vs. C_1. In general, the ability of dietary fiber to influence the cecum microbiome composition in broilers has been revealed. We believe that based on the biotic relationships between bacteria, targeted improvements in poultry productivity will be possible.

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