Efficacy of the coadministration of coccidiocide and prebiotic to broiler chickens infected with Eimeria tenella oocysts

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Abstract. Coccidiostatics are used in poultry farms to prevent and treat eimeriosis, and antibiotics are used to reduce pathogenic microflora, which negatively affects the gut microbiome of birds. In some poultry farms, probiotics are used to correct the gut microbiome of birds with eimeriosis. The use of prebiotics for this purpose has been little studied, although they can also serve as an alternative to antibiotic therapy. The work is devoted to studying the effect of combined and separate administration of chemical coccidiocide and lactulose-containing prebiotic to broilers on the intensity of eimeriosis invasion caused by Eimeria tenella, as well as on the number of mesophilic aerobic and facultative anaerobic microorganisms, including bacteria of the genus Bifidobacterium, Lactobacterium, Clostridium and Escherichia in cecum of 28-day-old broilers. The results showed that on average, for the entire period of the experiment, the lowest intensity of eimeriosis invasion was in the groups where coccidiocide and prebiotic were used: 17.50 ± 5.91 and 17.32 ± 5.67 thousand oocysts/g respectively. In the group where their combined purpose was, the intensity was 27.41 ± 8.13, which was slightly lower than in the infected control - 29.21 ± 8.70 thousand oocysts/g. The introduction of a prebiotic into the broiler diet allowed increasing the number of native bacteria of the genera Bifidobacterium and Lactobacterium, which reduced the number of opportunistic microorganisms such as Escherichia and Clostridium.

1. Introduction
In poultry farms with the system of floor-management, eimeriosis is a significant part of all parasitic diseases [1]. It is known that the gut microbiome of birds is associated with the severity of eimeriosis. It was found that Eimeria sporozoites and merozoites are contaminants through which pathogenic bacteria penetrate the gut-associated tissues [2]. Under the influence of eimeria, the lining of intestines is damaged, which leads to a defect of the mucin layer formed by the parietal microflora. For this reason, favorable conditions are created for the development and introduction of secondary microflora, dysbacteriosis occurs [3, 4]. In gastrointestinal tract of chickens there is a characteristic composition of microorganisms that changes with age. The formation of the gut microbiome occurs during the first week of life [5]. The gut microbiome of healthy chickens is relatively stable and is represented by the
genera *Bifidobacterium, Lactobacterium, Enterobacterium* and *Enterococcus*, as well as transient and saprophytic microflora. The largest number of microorganisms is contained in cecum - $10^{10}$–$10^{11}$/g of content and lactobacilli and bifidobacteria are dominant [6, 7]. Intestinal microbiota dysbiosis in cecum leads to serious consequences for the whole organism. Hence, the infection of chickens with eimeriosis caused by the species *Eimeria tenella*, whose endogenous stages are localized in the epithelial cells of the cecum of the large intestine, is especially dangerous [8]. With this type of eimeriosis, the number of bacteria of the genera *Lactobacterium* and *Enterococcus* in the cecum decreases, while the number of anaerobes and coliforms increases which complicates the course of eimeriosis [9].

Scientists have been trying to solve the problem of correcting the gastrointestinal microflora of birds with eimeriosis for a long time both in our country and abroad [10]. The use of coccidiostatics or coccidiocides for the prevention and treatment of eimeriosis invasion is an indispensable condition in industrial poultry farms with floor management, and antibiotics are used to reduce conditionally pathogenic microflora in most poultry farms, which negatively affects the normal gastrointestinal microflora of birds [11]. To solve this problem, scientists have proposed using probiotics as an addition to broiler diets, which have proved to be a good alternative to antibiotics in the treatment of eimeriosis in farm birds [12, 13]. At present, preparations of non-microbial origin that can have a positive effect are gradually beginning to be used to treat microflora in the gastrointestinal tract [14]. These drugs also include prebiotics. Prebiotics are drugs that supply the body with substances that are poorly digested in the small intestine and are a substrate for the development of microbiota in the cecum [15]. The use of prebiotics for the correction of microflora in broiler eimeriosis is little studied, but it is known from the literature that the use of prebiotics in the broiler diet in general contributes to a favorable change in the composition of the intestinal microflora, which has an improvement in growth and economic indicators [16]. Therefore, the possibility of using a prebiotic for the correction of the intestinal microflora of broilers with eimeriosis invasion is relevant and requires testing in practice.

The purpose of the work was to study the effect of combined and separate administration of chemical coccidiocide and lactulose-containing prebiotic to broilers on the intensity of eimeriosis invasion caused by *E. tenella*, as well as on the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM), including on the number of bacteria of the genus *Bifidobacterium, Lactobacterium, Enterococcus, Clostridium, Escherichia* and *Salmonella* in the cecum of 28-day-old broiler chickens.

2. Materials and methods

The experiment was carried out on 30 9-day-old broilers of the cross Hubbard at the experimental base Kurilovo ARSRIP. Chickens from 9-days-old to 28-days-old were kept in metal bunk cages and were given the same diet — growth feed PK-5 without coccidiostatics (Ramensky Grain Processing Plant PJSC). Drinking water was supplied separately to each cage in nipple drinkers.

At 10 days of age, feces of chickens were examined for the presence of *Eimeria* oocysts. Then, five groups of six animals each were formed from analog chickens: experimental group No. 1, which received toltrazuril coccidiocide 2.5% and a lactulose-containing prebiotic; experimental group No. 2 - toltrazuril 2.5%; experimental group No. 3 - prebiotic; control group No. 1 served as non-infected control; control group No. 2 – an infected control.

At 11 days of age, chickens of all experimental groups and control group No. 2 were individually given 1 ml of diluted *E. tenella* with 2 thousand oocysts. Chickens of the control group No. 1 received 1 ml of buffer solution. The chickens of the experimental groups 1 and 3 from the age of 15 days were given an individually lactulose-containing prebiotic at a recommended dose of 0.1 ml/kg of weight daily for five days, diluting with a small amount of water. Prebiotic contains lactulose (at least 50%) as an active substance, as well as concomitant sugars: lactose, galactose and purified water. The chickens of the experimental groups 1 and 2 at the age of 21–22 days old were given an individual oral coccidiocide toltrazuril 2.5% at the recommended dose of 7 mg/kg body weight for 48 hours, diluting with a small amount of water. Toltrazuril belongs to the group of triazintrions and is a drug that does not interfere with the formation of immunity against *Eimeria*. During the experiment, all chickens were in the same
conditions and in isolation. Daily monitoring of the general condition of the chickens, their safety, and productivity was conducted and evaluated by indicators of weight gain in groups.

At the first stage, the effectiveness of the use of drugs against *Eimeria* was determined by calculating the intensity of invasion for each group, which was determined as the Oocysts number Per Gram of excreta (OPG). For this, all poultry litter from 17 to 24-days-old chickens was collected (that is, 6-13 days after infection) separately for each group, homogenized; samples were taken from the total litter of each group in an amount of 25 g for further research [17]. OPG was determined by Zajíček [18]. Coproscopic studies were performed in an ARSRIP laboratory.

At the second stage, MAFAM, the number of bacteria of the genera *Bifidobacterium, Lactobacterium, Enterococcus, Clostridium, Escherichia, and Salmonella* in the cecum of 28-day-old broilers were determined. To do this, 28-days-old chickens were slaughtered and samples of cecums from each chicken in sterile disposable plastic bags were taken, labeled and sent to the laboratory to assess the composition of microflora.

Isolation and identification of microorganisms was carried out according to relevant regulatory documents: GOST R 50396.1-2010 “Poultry meat, offal and semi-finished products from poultry meat. Method for determining the number of mesophilic aerobic and facultative anaerobic microorganisms”; GOST 28566-90 “Food products. A method for identifying and determining the number of enterococci”; GOST 56139-2014 “Functional food products. Methods for determining and counting probiotic microorganisms”; GOST 26503-85 "Agricultural animals. Methods of laboratory diagnosis of clostridiosis" and other relevant documents [19, 20]. First, the material was prepared for sowing and diluted with saline 1:10. Next, the subsequent ten-fold dilutions were made to a titer of 10-9. To isolate the above bacterial genera, different dilutions were inoculated on solid media: Endo, Ploskireva, Wilson Blair, Blaurock, MRS, bismuth sulfite agar (BSA), enterococci-agar (ECA), 0.5% blood meat-and-peptone agar and liquid enrichment media: selenite broth and Rapoport-Vassiliadis (Rappeort Vassiliadis Medium).

Next, cultivation of seeds was carried out at 37 °C from 18 hours to 5 days (depending on the medium). On the second day after cultivation, Petri dishes were examined; grown colonies were studied; suspicious colonies were transferred from Endo and Ploskirev media onto media for the primary biochemical identification of microorganisms (Kligler); colonies were quantified; plated from liquid accumulation media on solid nutrient media; Wilson Blair media was examined; suspicious colonies were transplanted onto blood meat-and-peptone agar to determine hemolytic activity. On the third day after cultivation, cups of blood meat-and-peptone agar medium were examined; the results of primary biochemical of blood were taken into account; seeding on the medium of the differentiating "motley" row; serological identification (with agglutinating and adhesive sera) was performed. On the fourth day, the results of biochemical tests for the bacteria of the genera *Enterococcus, Bifidobacterium, Lactobacterium, Enterococcus, Clostridium, Escherichia and Salmonella* were taken into account; looked through cups with ICA; removed suspicious colonies; put tests to determine antibiotic sensitivity; looked through tubes with Blaurock medium and Petri dishes with medium MRS. On the fifth day after cultivation, the results of additional biochemical tests were recorded: the test for determining the antibiotic sensitivity of crops was carried out; selected cultures were identified. Culture inoculation and seeding on microtest systems for identification were performed on microtest systems for the differentiation of entero bacteria (PBDE) (Gorky RIEM). The number of microorganisms in solid nutrient media was determined in colony forming units (CFU)/g and expressed in decimal logarithms. Microbiological research was carried out in the laboratory of microbiology Institute of Veterinary Medicine, Veterinary Sanitary Expertise and Agro-Security.

The data obtained during the experiment were subjected to statistical analysis according to the method of N.A. Pluhinsky [21] and using the SAS/Stat software, version 9 of the SAS system for Windows (SAS Institute Inc., USA). Differences were considered significant for p <0.05.
3. Results
A study of the litter of 10-day-old broilers before the start of the experiment showed the absence of Eimeria oocysts. The results of the first stage of research are presented in table 1.

Table 1. The intensity of eimeriosis invasion in chickens by groups, thousand oocysts/g feces.

| Age | Experienced No. 1 | Experienced No. 2 | Experienced No. 3 | Control No. 2 | Control No. 1 |
|-----|-------------------|-------------------|-------------------|---------------|---------------|
| Day |                   |                   |                   |               |               |
| 17  | 51.32±12.21       | 22.21±6.33        | 16.11±4.18        | 38.51±11.30   | -             |
| 18  | 53.23±16.71       | 21.13±2.42        | 22.34±8.71        | 45.61±14.25   | -             |
| 19  | 53.56±14.52       | 24.50±6.16        | 25.52±7.15        | 56.92±17.19   | -             |
| 20  | 50.41±15.81       | 36.11±10.17       | 43.13±14.13       | 57.41±14.60   | -             |
| 21  | 49.12±12.55       | 38.22±11.31       | 27.61±6.70        | 35.15±11.42   | -             |
| 22  | 37.23±10.42       | 35.24±8.45        | 25.20±7.72        | 34.60±11.51   | -             |
| 23  | 25.42±8.13        | 19.32±4.56        | 23.47±6.82        | 26.71±6.94    | -             |
| 24  | 2.33±0.71         | 3.32±0.94         | 6.40±2.11         | 12.81±3.26    | -             |
| 25  | 2.11±0.50         | 2.91±0.40         | 6.20±1.84         | 12.46±3.91    | -             |
| 26  | 1.72±0.51         | 2.52±0.61         | 5.52±1.61         | 11.93±3.14    | -             |
| 27  | 1.10±0.26         | 2.33±0.61         | 4.20±1.22         | 9.50±2.32     | -             |
| 28  | 0.94±0.12         | 1.80±0.41         | 2.11±0.62         | 9.16±1.24     | -             |
| Average | 27.41±8.13    | 17.50±5.91        | 17.32±5.67        | 29.21±8.70    | -             |

Control group No. 1 throughout the experiment was free from Eimeria. The OPG of 28-days-old chickens of the experimental groups compared with the control infected group differed significantly (p < 0.05). On average, over the entire period of the experiment, the lowest intensity of eimeriosis invasion was in the experimental groups No. 2 and No. 3: 17.5 ± 13.9 and 17.3 ± 12.1 thousand oocysts/g respectively. The average values for all groups did not differ significantly (p > 0.05).

The results of individual weighing of chickens in groups were as follows. At 9 days of age, the mass of chickens in the experimental group No. 1, No. 2, No. 3 and control groups No. 1 and No. 2 was 201.67 ± 16.89; 190.83 ± 11.61; 222.86 ± 9.19; 192.5 ± 10.07; 220.83 ± 11.61; at the age of 15 days - 355 ± 30.33; 369.17 ± 17.74; 432.14 ± 20.54; 400.83 ± 27.58; 405.83 ± 18.62; at the age of 20 days - 639.16 ± 37.11; 676.67 ± 34.02; 763.57 ± 33.22; 654.17 ± 43.29; 709.67 ± 38.15; at 28 days of age - 1179.17 ± 55.34; 1166.67 ± 97.95; 1337.86 ± 68.26; 1182.46 ± 70.81; 1270.83 ± 33.98 g, respectively (p > 0.05).

Weight gain at 20 days of age (after a prebiotic course) in experimental group No. 1, No. 2, No. 3 and control groups No. 1 and No. 2 amounted to 437.49; 485.84; 540.71; 517.17; 433.34 g, and over the entire period of the experiment - 977.5; 975.84; 1115.0; 1078.33; 961.63 g, respectively. The maximum gain for the entire period was observed in the experimental group 3, the chickens of which were given a prebiotic, and the smallest weight gain was in the control group 2.

The results of the second stage of the study of the microflora of cecum of 28-day-old chickens are presented in table 2.

In all infected groups, MAFAM was lower compared with the uninfected control group No. 1. Moreover, in experimental groups No. 1 and No. 2, this indicator was 10 times lower, and in experimental group No. 3 and infected control group No. 2 - 100 times (p < 0.05). The number of bacteria of the genus Enterococcus in the experimental groups No. 1, 2 and the control group No. 2 was 10 times lower compared to the uninfected group, while in the experimental group No. 3 their number was 1000 times lower (p < 0.05). The number of bacteria of the genera Bifidobacterium and Lactobacterium in all infected groups was lower compared to the control uninfected group. So, in experimental groups No. 1-3, these indicators were 10 times lower, and in the infected control group, 100 times lower (p <0.05). The number of bacteria of the genera Clostridium and Escherichia, on the contrary, was greater in the infected groups. Moreover, bacteria of the genus Clostridium in the experimental group No. 1, 2 and in the control infected group were 100 times larger than the uninfected group (p <0.05), while the number of bacteria of this kind in the experimental group No. 3 was...
practically the same from those in pure control (p > 0.05). The number of bacteria of the genus *Escherichia* in the experimental groups No. 1, 3 and the control group No. 2 differed 10 times from this parameter in the uninfected group (p < 0.05), while in the experimental group No. 2 this indicator practically did not differ from the pure control (p > 0.05). Bacteria of the genus *Salmonella* were not found in any group. The smallest MAFAM - 8.58 ± 1.15; the number of bacteria of the genus *Bifidobacterium* - 5.47 ± 0.07 and *Lactobacterium* - 7.61 ± 0.87 log CFU/g and the largest number of bacteria of the genus *Escherichia* - 8.14 ± 0.20 and *Clostridium* - 4.98 ± 1.02 log CFU/g was in the infected control group No. 2. And the uninfected control group No. 1, on the contrary, had the highest MAFAM - 10.93 ± 2.05 log CFU/g; the number of bacteria of the genus *Bifidobacterium* - 7.93 ± 0.05 and *Lactobacterium* - 9.34 ± 1.98 and the smallest number of bacteria of the genera *Escherichia* and *Clostridium* - 7.41 ± 0.34 and 2.39 ± 0.05, log CFU/g respectively. In addition, the uninfected group had the highest number of bacteria of the genus *Enterococcus* - 6.33 ± 1.25 log CFU/g.

**Table 2.** The number of bacteria of the determined genera in the cecum of broilers in groups, log CFU/g.

| Defined indicator | Groups              |
|-------------------|---------------------|
|                   | Experienced No. 1   | Experienced No. 2 | Experienced No. 3 | Control No. 2 | Control No. 1 |
| MAFAM             | 9.68±1.24           | 9.41±1.47         | 8.83±1.21         | 8.58±1.15     | 10.93±2.05    |
| *Enterococcus*    | 5.23±1.01           | 5.86±1.15         | 3.97±0.78         | 5.06±0.98     | 6.33±1.25     |
| *Bifidobacterium* | 6.34±0.06           | 6.28±0.07         | 6.94±0.08         | 5.47±0.07     | 7.93±0.05     |
| *Lactobacterium*  | 8.49±0.48           | 8.46±1.24         | 8.65±1.14         | 7.61±0.87     | 9.34±1.98     |
| *Clostridium*     | 4.32±0.07           | 4.43±0.08         | 2.73±0.04         | 4.98±1.02     | 2.39±0.05     |
| *Escherichia*     | 8.02±1.02           | 7.59±1.12         | 8.06±1.14         | 8.14±0.20     | 7.41±0.34     |
| *Salmonella*      | -                   | -                 | -                 | -             | -             |

4. Conclusion

The current studies showed for the first time how the combined and separate administration of a chemical coccidiocide and a lactulose-containing prebiotic to broilers affects the intensity of the eimeriosis invasion caused by *E. tenella*. As can be seen from the results of the study, the lowest average invasion intensity for the entire period of the studies was in experimental group No. 2, where toltrazuril 2.5% was given and No. 3, where the prebiotic was given to chickens. Toltrazuril is a chemical coccidiocide that has proved to be an effective tool in the fight against broiler eimeriosis [22]. The prebiotic vetelact does not have anticoccidic activity. A decrease in the intensity of eimeriosis invasion in chickens that received a prebiotic suggests an indirect effect of this drug on *Eimeria*. The results of the studies confirmed the data of other authors that the introduction of a prebiotic into the broiler diet can increase the number of native bacteria of the genera *Bifidobacterium* and *Lactobacterium*, which reduces the number of opportunistic microorganisms such as *Escherichia* and *Clostridium* [23, 24]. In addition, as a result of applying the prebiotic, it was possible to obtain an increase in broiler productivity, as evidenced by the increase in body weight of chickens over the entire observation period. In connection with the foregoing, the use of a prebiotic to correct intestinal microflora in eimeriosis is a good way to increase broiler productivity.

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### References

[1] Dalloul R A and Lillehoj H S 2006 Poultry coccidiosis: recent advancements in control measures
and vaccine development Expert Rev Vaccines 5 143-63
[2] Fanthan H B 1910 The morphology and life-history of Eimeria (Coccidium) avium: a Sporozoö causing a fatal disease among young grouse Proc.of the Zool. Society of London 80 (Oxford,UK: Blackwell Publishing Ltd) pp 672-91
[3] Ohe O and Akarawa A 1975 Effect of feed additive antibiotics on chickens infected with Eimeria tenella Poultry science 54 1008-18
[4] Turk D E and Littlejohn V P 1987 Coccidial infections and gut microflora Poultry science 66 1466-9
[5] Pervova A M 2003 The effectiveness of using probiotics in industrial poultry farming Agricultural biology 4 26-30
[6] Georgievskiy V I 1990 Livestock Physiology (Moscow:Agropromizdat) p 510
[7] Bakhareva O P and Sarazhakova I M 2009 The composition of the microflora of the cecum of the broilers from 1 to 63 days old Vestnik of Krasnoyarsk Agrarian University 80 672-91
[8] Conway D P, McKenzie M T and Dayton A D 1990 Relationship of coccidial lesion scores and weight gain in infections of Eimeria acervulina, E.maxima and E.tenella in broilers Avian Pathology 19 489-96
[9] Johansson K R and Sarles W B 1948 Bacterial population changes in the cecum of young chickens infected with Eimeria tenella J.of bacteriology 56 635-47
[10] Burlakov V P 2006 The effect of eimeriosis invasion on the intestinal biocenosis of chickens (St.Petersburg) p 149
[11] Nigoev O, Skvortsova L and Skoblikov N 2007 Intestivitis corrects intestinal biocenosis of broilers Livestock in Russia 12 19-20
[12] Burlakov V P, Shustrova M V and Kirzhaev F S 2005 Control and correction methods of normal gut microflora of chickens with eimeriosis invasion Veterinary practice 4 4-5
[13] Labir S M 2009 The role of probiotics in the poultry industry Int.J.of Molecular Sciences 10 3531-46
[14] Yang Y, Iji P A and Choñt M 2009 Dietary modulation of gut microflora in broiler chickens: a review of the role of six linds of alternatives to in-feed antibiotics World’s Poultry Sci.J.65 97-114
[15] Suray P F, Kochisch I I, Fisinin V I, Grozina A A and Shatskikh E V 2018 Molecular mechanisms for maintaining the health of poultry intestines: the role of microbiota (Moscow: Agricultural technology) p 342
[16] Patterson J A and Burkholder K M 2003 Application of prebiotics and probiotics in poultry production Poultry science 82 627-31
[17] Murzakov P P 2013 Epizootic situation on emeriosis of chickens with different management-systems in the Central zone of Russia and improvement of control measures (Moscow) p 223
[18] Zajiček D 1978 Comparison of the efficiency of two quarantative ovoscopic methods Vet medicina 23 275-80
[19] Guidelines for the bacteriological diagnosis of colibacteriosis (Esherichiosis) in animals 2000 (Moscow:Minselkhoz) p 17
[20] Labinskaya A S, Blinkova L P and Eschina A S 2019 General and sanitary microbiology with the technique of microbiological research (St.Petersburg:Lan) p 592
[21] Plokhinskiy N A 1978 Mathematical methods in biology (Moscow:MSU) p 265
[22] Bondarenko L A, Safiullin R T, Kachanova E O and Tashbulatov A A 2018 Coccidiosis control in a poultry farm with floor-management system Int.Sci.Conf. Theory and practice of controlling parasitic diseases 19 93-6
[23] Kuznetsov V V 2006 The effect of some eimeriostatics and eimeriosis prevention regimens on the clinical status and enterobiocenosis of broiler chickens (Tyumen) p 122
[24] Skvortsova L N 2015 Improvement of the gut microflora of poultry with the use of lactolose-containing prebiotic in feeding Poultry and poultry products 3 33-5