Modern approach to the problem of bird eimeriosis: diagnosis and treatment

Vladimir Orobets¹, Sergey Larionov², Ludmila Kashkovskaya², Daria Korotova²

¹Stavropol State Agrarian University, Stavropol, Zootechnichesky per. 12, Stavropol, Russia
²Saratov State Vavilov Agrarian University, Saratov, Teatralnaia square, 1, Saratov, Russia

E-mail: kashkovskaya@nita-farm.ru

Abstract. The problem of eimeriosis today is no less relevant than in past years. Eimeriosis is dangerous not only by itself but also by association with other diseases. Current conditions dictate the need to improve preventive measures through the development of new high-performance anticoccidial drugs. In this regard, the effectiveness of some anticoccidial drugs was compared in experimental eimeriosis of broilers. To perform the task, the bird was infected with a mixture of oocyst Eimeria (E. acervulina, E. maxima and E. tenella) at the week-old age. The treatment of the chickens in the experimental groups started at the 12th day after infection using various drugs: toltrazuril, sulfachlorpyridazine and combined products (sulfadimidine and trimethoprim). On the second day after the start of anticoccidial drugs feeding, there was a positive dynamics in experimental groups – decrease of allocated oocyte. Almost complete cessation of their isolation was recorded on 3-4 days of therapy. At the second day of treatment, birds increased activity, appetite, clinically it looked more cheerful, feces acquired a normal consistency and color. It was found that the use of anticoccidial drugs inhibits the development of Eimeria and contributes to high safety of birds, promotes their normal growth and development. Thus, the productivity of chickens increases by 600 g compared to untreated poultry.

1. Introduction

Despite significant successes in the poultry industry and veterinary medicine, not a single poultry farm, regardless of size and form of ownership, can do without the use of anti-coccidia drugs.

The biology and reproduction of pathogens of eimeriosis allow it to adapt quickly to changing environmental conditions and contribute its wide dissemination. In addition, it is known that Eimeria quite quickly form resistance to anticoccidial drugs and transmit it by inheritance [1, 2, 3, 4]. These unique opportunistic mechanisms provoke the outbursts of eimeriosis invasions, which are often recorded in broiler chickens and of rearing animals.

For decades, various coccidiostatics have been used to prevent and treat eimeriosis in farm birds. However, such measures do not exclude the subclinical course of the disease in the enterprise.

In modern conditions, the damage from eimeriosis of infestation is reducing the productive qualities of chickens, increased feed conversion and the huge costs of preventive measures, which do not bring the expected result [5].
In addition, by multiplying in the intestinal wall, coccidias destroy the epithelial layer and open the
gates of infection for opportunistic microflora. In general, eimeriosis is considered one of the most
serious diseases for poultry health, which causes huge economic losses of poultry producers around the
world [1, 5, 6].

Current conditions dictate the need to improve preventive measures through the development of new
highly effective anticoccidial drugs and mapping rotation of coccidiostatic given the sensitivity of
Eimeria.

The above issues have necessitated an evaluation of the effectiveness of some anticoccidial drugs
from different groups during eimeriosis in broiler chickens.

It is known that the fight against endogenous stages of development of Eimeria has two directions:
prevention (containment of the pathogen) and therapy (destruction of the pathogen at different stages of
development). For prevention use coccidiostatics (synthesized by chemical means and polyester
ionophore antibiotics) that are used from the first day of life of the bird throughout cultivation. As a
means of therapy using medications of different classes of compounds (sulfonamide analogs of thiamin,
triazinetrione, etc.), the drugs are assigned in short courses and, if necessary, repeat.

The aim of this work is to assess the effectiveness of certain sulfonamides and derivatives
triazinetrione in the treatment of experimental eimeriosis in broiler chickens.

2. Materials and methods
Research work was carried out at the Department of “Animal diseases and veterinary-sanitary
examination”, as well as in vivarium “Faculty of veterinary medicine of food and biotechnology” of
Saratov state agrarian university in the period from April to August 2017, in 2 stages.

At the first stage, the oocysts of Eimeria species were isolated and sporulated: E. acervulina, E.
maxima and E. tenella, which were later cultured in the body of day-old chickens.

Oocysts were isolated from the litter by a modified method including flotation using a saturated
sucrose solution [7] followed by centrifugation for 7 minutes at 3000 revolutions. After that, they were
subjected to sporulation in wet sand at a temperature of 26-28°C, with periodic stirring and moistening
(do not allow drying of the substrate). In order to determine the stage of sporulation, part of the Eimeria
oocyst was daily examined under a microscope. Sporulated concentrate oocysts washed from sand by
flotation method, centrifuged, collected the upper (supernatant) layer and diluted it with water. 1 ml of
the resulting suspension contained 200-250 thousand sporulated oocysts.

For the next stage of the study, broiler chickens Cross Cobb 500 were involved in the amount of 140
heads under the age of 35 days. The experimental bird was kept in groups in cages of 35 animals each.

Pure water was used for drinking chickens in accordance with Russian State Standard SanPiN
2.1.4.1074-01. For the period of the experiment, in accordance with the research scheme, drinking water
was replaced with solutions of anti-acid drugs. Nipple drinkers were used.

Feeding of birds was carried out by compound feeds according to age requirements, by means of
group feeders of bunker type.

To prevent bacterial infection, which may affect the course of the experiment, chickens, starting from
the second day of life, drank a solution of antibacterial drug, containing as an active substance (AS)
15% levofloxacin for 3 days at a dose of 0.5 ml per one liter of drinking water.

At the weekly age, the bird was infected with a mixture of sporulated Eimeria oocysts, types: E.
acervulina, E. maxima and E. tenella in equal parts at a dose of 20,000 pieces per head. The oral infection
conducted individually.

After infection, the birds were formed into groups of 35 heads each (control and three experimental).
Every day from the beginning of the experiment until its end, the bird was observed. At the same
time, the clinical condition of chickens, their consumption of food and water were evaluated, in case of
death, a pathological autopsy was performed to assess the degree of intestinal damage.

Starting from 6 days after infection in all groups, a laboratory study of litter for the presence of
Eimeria oocysts was performed daily. Litter samples were collected by a group method, depersonalized,
from a pallet. The presence of oocysts in the samples was determined by the Fulleborn flotation method, and their number was counted in 1 g using the Goryaev counting chamber [7].

From the moment of registration of clinical signs of the disease and detection of oocysts in the litter, the treatment of eimeriosis was started in accordance with the scheme presented in table 1.

**Table 1.** The scheme of treatment of eimeriosis of broiler chickens in the experimental groups.

| Group         | Drug (AS)                      | Dose of the drug                          | Period of feeding |
|---------------|--------------------------------|-------------------------------------------|-------------------|
| 1st experimental | toltrazuril                  | 1 ml of 2.5 % solution / 1 liter of drinking water | 2 days           |
| 2nd experimental | sulfadimidine + trimethoprim | 1 ml/1 liter of drinking water            | 5 days           |
| 3rd experimental | sulfachlorpyridazine         | 1 g of 0.03% solution /1 liter of drinking water | 3 days           |
| Control       | -                             | -                                         | -                |

Every day we prepared new solutions of drugs.

The species composition of Eimeria was determined taking into account intestinal lesions described by A. E. Khovanskyh [8, 9] and oocyst morphology determined by M.V. Krylov [10] after preliminary sporulation in a thermostat at t = 18 – 22 °C.

Anticoccidial efficiency of the drugs was assessed in terms of the intensity of infestation according to the average increase, taking into account the degree of intestinal injury, data loss and security of birds in groups at the end of the experiment.

3. **Results of the research**

After oral infection of broiler chickens at 7 days of age with a mixture of oocyst of Eimeria (E. acervulina, E. maxima and E. tenella) at a dose of 20 000 pieces per head, the prepatent period was 6-7 days.

The autopsy of the murdered forced birds on day 6 after infection in some individuals noted changes in the duodenum: small petechial hemorrhages, vascular bowel (figure 1).

![Figure 1. Petechial hemorrhages in the duodenum when cimeriosis.](image)

In mucosal scrapings of the affected areas when microscopy detected oocysts. At this stage of development of the disease, the secretion of E. acervulina oocysts was mainly recorded, which is apparently due to the peculiarities of its development (it has the shortest period of development).
The intensity of the invasion during the experiment increased gradually. Thus, the first oocysts in the litter were recorded on the 7th day after infection, and the greatest number of it was observed in chickens of all groups on the 11th-12th day after infection.

At the same time, the first clinical signs of eimeriosis appeared in chickens (figure 2): oppression, the sick chickens stand with drooping wings, ruffled feather them, scallops are pale, liquid litter, mixed with large amounts of mucus and blood.

![Chicken, with eimeriosis sick.](image)

With the development of the disease, clinical signs increased and at the same time increased the rate of invasion intensity (table 2). Against the backdrop of mass reproduction of eimeria, it was noted a rejection of feed and water by some individuals, as well as intense diarrhea. It was found that the most pronounced clinical signs of Eimeria invasion were registered during the intensive development of Eimeria tenella.

| Group             | Extent of invasion, % / Intensity of invasion, thousand copies in 1 g of litter | extensibility **, % |
|-------------------|----------------------------------------------------------------------------------|---------------------|
| Before treatment  | 1st day since treatment | 5th day since treatment | 10th day since treatment | ** |
| 1st experimental  | 30/ 100/24.9±3.4        | 1.3/*               | 0/*                   | 100 |
| 2nd experimental  | 10.4±2.1                | 2/*                 | 2/*                   | 98  |
| 3rd experimental  | 20/ 100/26.5±2.7        | 2.5/*               | 1.5/*                 | 97  |
| Control           | 9.8±3.8                 | 100/28.1±4.6        | 100/26.4±3.5          | 100 |

* - it is impossible to calculate the number of oocysts in 1 g (single oocysts in the sample)
** - extensibility

After the appearance of clinical signs of eimeriosis, the treatment of chickens in experimental groups has begun.

Since the beginning of the feeding anticoccidial drugs have observed a positive dynamic – a sharp reduce in the number of secreted oocysts and improvement in the clinical condition of chickens.

So, at the second day of treatment, birds increased activity, appetite, clinically she looked more cheerful, feces acquired a normal consistency and color. The mortality stopped with a decrease in invasive intensity for 3-4 days of treatment.

In the group of untreated control during the experiment, the number of oocysts in the litter was at a high level, not falling below 27 thousand pieces in 1 g of litter. This high intensity of invasion led to the mass development of eimeriosis and the death of birds in this group.
The safety of chickens as a result of therapy in experimental eimeriosis of broiler chickens is presented in table 3.

**Table 3. Efficiency of therapy in experimental coccidiosis of broiler chickens.**

| Group          | Period of single therapy course, days | Number of chickens, heads | mortality, heads | Safety, % |
|----------------|---------------------------------------|---------------------------|------------------|-----------|
| 1st experimental | 2                                     | 35                        | 2                | 94.3      |
| 2nd experimental | 5                                     | 35                        | 4                | 88.6      |
| 3rd experimental | 3                                     | 35                        | 3                | 91.4      |
| 4th control     | -                                     | 35                        | 15               | 42.9      |

The table shows that the greatest safety was observed in the first experimental group, its index is 2.9-5.7% higher than in the 3rd and 2nd experimental groups, respectively. The lowest safety was recorded in the untreated control group.

It is known that indicators of extensiveness and intensity of eimeriosis invasive are indirect and do not represent the full invasive process.

To provide a complete picture of the disease and the impact of invasion on the production performance of birds, the livestock was weighed and autopsied forcibly killed and fallen individuals in order to determine the degree of intestinal damage.

The results of the autopsy confirmed the results of coprological studies. We noted that the species dynamics of the oocysts of Eimeria excreted in the litter and the nature of intestinal damage during the experiment were different. Thus, on the 6th day after infection and during the first 3-4 days after detection of oocysts in the litter recorded the development of the simplest species of E. acervulina. At the same time, the main changes were found in the duodenum at the autopsy of the bird. Depending on the intensity of the invasion, the changes were as follows: hemorrhagic duodenitis – more than 5 petechiae per 1 cm², thickening of the intestinal wall. Then, as the process increased, a large amount of bloody contents was found in the lumen of the intestine, the intestinal wall noticeably thickened. These changes indicate a significant degree of invasion.

Later, the results of the study of litter and autopsy data in all groups established the development of the species E. tenella (figure 3). In this case, there were changes in the blind processes of the intestine: a large amount of blood and growths on the intestinal wall, later in some individuals the cecal cavity was filled with blood clots (figure 4).
To assess the productive indicators during the experiment, the chickens were weighed, and the data of the average daily growth were calculated (table 4). The obtained results confirm that the use of anticoccidial drugs in experimental eimeriosis largely suppress the multiplication of the pathogen in the intestine of chickens, which contributes to their normal growth, development and productivity.
Table 4. Dynamics of live weight gain of broiler chickens in the experiment.

| №  | № of group   | Weight (g) during the (days) | Average daily gain for 30 days, g |
|----|--------------|------------------------------|----------------------------------|
| 1. | 1-st experimental | 42±3.7 473±13.1 1585±86.5 2189±56.9 | 71.6±4.4 |
| 2. | 2nd experimental   | 43.3±2.1 464±8.9 1595±51.6 2176.3±61.2 | 68.5±3.2 |
| 3. | 3rd experimental    | 42.8±4.2 474±9.9 1589±46.2 2187.5±51.4 | 71.5±2.9 |
| 4. | Not treated control | 42.5±3.1 472.3±16.3 964±37.6 1569±51.9 | 50.2±3.2 |

Table 4 shows that average daily gain in body weight in the birds not receiving coccidiostatic, on average 20 g less than chickens, which was treated with anticoccidial drugs. At the output, this difference was more than 600 g.

4. Discussion

The studies revealed that after oral infection of 7-day-old chickens with a mixture of sporulated oocysts (Eimeria tenella, Eimeria maxima, and Eimeria acervulina) in equal parts, the prepatency period was as follows: Eimeria tenella - 9 days, Eimeria maxima – 7 days, Eimeria acervulina – 7 days. The most pronounced clinical signs of eimeriosis invasion were registered during the intensive development of Eimeria tenella, which is explained by its high degree of virulence.

Thus, the E. tenella localizes mainly in physiologically important segment of the gastrointestinal tract of a bird – blind processes, strongly affects it. The endogenous stages, especially the second generation meronts, developing in large numbers in the intestinal wall, destroy the mucous membrane, causing extensive hemorrhages. The submucosal layer is exposed, the lumen of the intestine is filled with necrotic epithelial cells, shaped blood elements and parasites at different stages of development. This fact is confirmed by many researchers [6, 12, 13]. Due to the defeat of large areas of the intestine, the function of digestion is impaired. At the background of the developed pathological changes in some individuals noted strong oppression, refusal of food and water. Subsequently, these chickens are almost not consumed solutions of drugs that affect the dynamics of the intensity of invasion and the safety of poultry, in particular recorded a second peak of intensity of invasion and mortality.

It should be noted that the rate of intensity of invasion in the groups before treatment differed, but at the autopsy, the degree of lesion was the same. This fact confirms the need for a comprehensive approach to the diagnosis of eimeriosis. In some cases, the accumulation oocyst of Eimeria by kind of Eimeria tenella in blind processes was noted in the absence of their release into the external environment. Perhaps later such a bird is able to invade the environment for a long time.

Therapy of experimental eimeriosis of chickens showed that the proposed schemes are highly effective. Thus, toltrazuril treatment has a shorter course at the specified dose and leads to complete cessation of oocyst secretion on the third day from the beginning of feeding (extensibility – 100%). The General condition of chickens normalizes on the second day.

The use of sulfachlorpyridazine also demonstrates the high effectiveness of therapy – 88.6%, but requires an increase in the duration of treatment for one day in comparison with toltrazuril.

The longest course of treatment is a combination drug containing as AS the sulfadimezin and trimethoprim (5 days), but the effectiveness of therapy is quite high – 97%.

It should be noted that the re-growth of intensity of invasion and after treatment and the mortality of chickens in all experimental groups is due to the presence of weak individuals who did not take treatment due to their condition (refuse from feed and water). This fact is confirmed by laboratory and autopsy results.

However, analyzing numerous data in the literature, many authors recommend a double courses of eimeriosis therapy with an interval of 2-3 days. Such treatment regimens show the highest and most stable results [10, 14, 15].
5. Conclusions
Therapy of experimental eimeriosis of chickens showed that all the proposed schemes are highly effective. Thus, toltrazuril treatment provides 100% efficiency and high (94.3%) safety of chickens. Sulfachlorpyridazine demonstrates the effectiveness of therapy – 98%, while the safety of the birds is 88.6%.

The therapy is a combined drug containing, as AS sulfadimidine and trimethoprim, provided a sufficiently high effect 97%, at safety 91.4%.

It was found that the best result was obtained from double courses of treatment.

References:
[1] Kirillov A 2008 Coccidiosis of birds (Moscow: Russian Agricultural Academy) p 229
[2] Kornishina M, Grigorieva N 1985 Coccidiosis chickens and the organization of treatment and preventive measures in the farms. Methodical instruction (Kazan) p 17
[3] Mishin V, Rozbickiy V, Kalinin A 2007 The III international veterinary poultry Congress: The Russian poultry Union pp 221-224
[4] Titova T, Rozbickiy V 2016 Effective farming 8 129 pp 53-55
[5] Mishin V, Rozbickiy V, Dikovskaya V 2006 Farming in Russia 4 pp 25-26
[6] Kolabskiy N, Pashkin P 1974 The coccidiosis of farm animals (Leningrad: Kolos) p 159
[7] Russian Federation Standard GOST 25383-82 Methods of laboratory diagnostics of coccidiosis
[8] Khovansky A 1984 Biochemistry of coccidia and coccidiosis (Leningrad: Nauka) p 192
[9] Khovansky A, Ilyushechkin Y, Kirillov A 1990 Coccidiosis of poultry (Leningrad: Agropromizdat) p 151
[10] Krylov M 1996 The Determinant of parasitic protozoa (St. Petersburg: Nauka) p 601
[11] Akhmedov E 2014 Bulletin Belorussian State University. Series 2, Chemistry. Biology. Geography 1 pp 35-39
[12] Muzyka V, Stetsko T, Kalinina O, Murskaya S 2012 Notes of the institution of education “Vitebsk order “badge of honor” state Academy of veterinary medicine” 2 48 pp 9-13
[13] Sandul A 2004 Scientific notes of the educational institution “Vitebsk order “badge of honor” state Academy of veterinary medicine” 1 40 pp 292-293
[14] Khaziev G, Sagitova A 2006 Theory and practice of parasitic diseases of animals 7 pp 113-115
[15] Tcheprasova O, Daeva T 2010 News of the lower Volga agro technical University complex 4 pp 129-133