

DEVELOPMENT OF PREVENTING MEANS FOR RABBITS' COCCIDIOSIS

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Abstract

The great problem of rabbits breeding today is coccidiosis. An agent of the disease spreads not only through ill animals and is preserved well in the external environment. Oocysts of coccidia live in cells for a long time, so even temporal vacation of an accommodation between placing of rabbits doesn't prevent infection. It was experimentally proved, that there is a very limited spectrum of means that are coccidiostatics. For preventing and controlling rabbits' eimeriosis, coccidiostatic means were tested. It is very difficult to annihilate coccidia in vivo and in vitro because of peculiarities of their construction. The aim of the first experiment was to determine the influence of an acidifier Cronocyde L and its components on oocysts of coccidia that allows to determine an optimal composition and concentration of the preparation. At conducting the research, there was used the flotation method of Fulleborn and McMaster one for calculating oocysts. The aim of the second experiment was to determine the influence of the acidifier Cronocyde L standard and Cronocyde L concentrate (1 ml/1l of water) on the intensity of the living mass increment in rabbits. For that there were used clinical, hematological, zootechnical and microscopic methods. Optimal results in the experiment were obtained at using Cronocyde L concentrate and Cronocyde L standard in concentration 0,1 %: maximal growth and health status of rabbits, biochemical indices of blood serum were within the physiological norm. It testifies to the effectiveness of this preparation at eimeriosis of rabbits and to harmlessness for them. The studies, realized under laboratory conditions, prove the effectiveness of the offered means.

Keywords: coccidia, oocysts, eimeria, rabbits, coccidiostatics, water acidifier.

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1. Introduction

Coccidiosis are spread in all countries of the world and cause essential losses to animal husbandry. Climate conditions don't influence coccidian. It occurs in animals not depending on their age or race. Ill animals excrete oocysts that are a source of invasion with excrements, and transmission factors are forages, water, bedding, equipment of animal husbandry accommodations, servicing subjects and so on, polluted by them. Inflammation by coccidia is realized by the alimentary way as a result of swallowing oocysts with water or forage [1, 2].

The high vitality of cysts and oocysts of protozoa, eggs and larva of helminthes, ticks and insects and also their ability to survive after the contact with chemical substances in concentrations and expositions, destructive for pathogenic microorganisms, is a serious problem in the fight against invasive diseases of animals and humans. The stabledness degree of oocysts of eimeria of pigs, ruminants, rabbits and poultry relates to the group of high-stable agents of parasitosis [3, 4].

Coccidiosis is one of oldest diseases in hares and wild rabbits, and although young individuals suffer more from it, adult rabbits that survived after it are permanent carriers of this disease all life long. The maximal risk zone includes rabbits that have already stopped to consume maternal milk, but have not achieved the age of four months yet. The danger of inflammation increases because of reconstruction of the not yet strong intestine for assimilating new forage [5, 6].

There are several factors that provoke the disease development; owners of animals must know about them:

- inobservance of quarantine at purchasing new rabbits that results in presence of ill animals in common cages;
- infected forage, purchased in fishy suppliers;
- appearance of other animals and insects that are spreaders of protozoa at the territory of cages;
- carelessness of the staff and careless construction of cages and enclosures;
- milk of a feeding female rabbit, infected by protozoa.

Such set of factors, provoking the epidemic, is explained very simply – coccidia must undergo two forms for the full-value development, one of which is an intrauterine stage that takes place

in the intestine of an infected animal, and the other one – in results of animal life activity that is in excrements. If they are not removed in time, the possibility of inflammation of other individuals grows in several times [7, 8].

Clinical symptoms of intestinal and hepatic coccidiosis are very similar, it is difficult to distinguish them. Healthy rabbits often can be carriers of protozoa, but don't manifest symptoms of the disease. Its severity depends on a number of oocysts that get into the organism of a rabbit. Penetration of eimeria oocysts in the intestine and liver causes destruction of cells. Erosion and ulcer take place in the epithelial tunic of the intestine that results in bad absorption of nutritive substances, disturbance of electrolytic imbalance, anemia, dehydration of cells. General clinical signs include the appetite decrease, depression, pains in the belly, cachexy, diarrhea and anemia of mucous membranes. Faeces may contain blood or slime. Any one or all these signs may be absent in older rabbits [9, 10].

The aim of the first experiment was to determine the influence of an acidifier Cronocyde L and its components on oocysts of coccidia, that allows to determine an optimal composition and concentration of the preparation for annihilating oocysts of coccidia. At conducting the second experiment, the aim was to determine the influence of the acidifier Cronocyde L standard and Cronocyde L concentrate of the intensity on the dynamics of eimeria invasion, living mass increment in rabbits and biochemical indices of blood.

2. Materials and Methods

Experiment 1. Acids (components of the acidifier) were added in sterilized running water for getting pH 3,0–4,5. There were studied: orthophosphoric, formic, propionic, acetic, butyric, lemon, benzoic, sorbic acids. They were added in water at continuous measuring of pH. pH was brought to the necessary level. The solution of acids with oocysts was incubated for 0,5 hours and 1 hour at t 25 °C, taking into account the minimal time, in which the preparation can probably contact with oocysts of coccidia in the intestine of a rabbit. After that oocysts were fivefold washed, and samples were set for sporulation. Then the status of oocysts was studied. The analogous experiment was conducted with the ready acidifier Cronocyde L concentrate, produced by PE “Cronos Agro”, Ukraine. Cronocyde L concentrate contains chelate of iron, manganese and copper, and also benzoic, acetic, propionic acids and marjoram extract. The preparation Cronocyde L standard contains thymol, formic, orthophosphoric, sorbic, lemon acids.

The diagnosis of eimeriosis was set according to results of the laboratory examination of rabbits' excrements by Fulleborn's method. The number of oocysts for a gram of excrements was determined by the McMaster method) [11, 12], and Eimeria times were identified using morphological criteria.

10–15 samples of oocysts were put in Petri dishes, and the working solution of the acidifier was added. At that there were gotten 11 experimental variants with different variants of pH 3.5–4.5 and one control with water (**Table 1**). The Petri dishes with oocysts and 5 cm³ of distilled water were control.

Experiment 2. It was conducted on the vivarium of the Sumy National Agrarian University (city Sumy, Ukraine) from April to May of 2019. 5 laboratorially proved invaded repair females, treated for coccidiosis, were chosen for the studies. Young rabbits of these females were kept separately from other rabbits and were divided in three groups, ten individuals in each one: two depending on treatment scheme, one control. All rabbits were kept in equal conditions and get equal nutrition. All young rabbits during the experimental period were placed in three-floor cage batteries with sizes 3000×650×1800 mm, 5 rabbits in each one, males and females separately.

The area of the floor of a cage for one rabbit was 0.25 m², place for feeding – 60 mm. Feeding took place two times a day – in morning and in evening. Rabbits were supplied with water by brooder water bowls with the preparation according to the treatment scheme.

Parameters of temperature, humidity and illumination corresponded to all norms and rules of rabbits keeping and were stable during the whole research period. The chosen coccistatics were used for the first time on the husbandry base. The treatment of young rabbits

was started at clinical manifestations of coccidiosis – emaciation, disorder of the esophagus, depression, after the laboratorial verification of invasion. Clinical observations were conducted from 1 to 21 day of the treatment. The level of eimeria invasion was determined microscopically by calculating the number of oocysts in the preparation before the treatment and for 7, 14 and 21 day respectively. The treatment of rabbits was realized in the first group as Cronocyde L standard, in the second one (young rabbits) – Cronocyde L concentrate, third group – phyto-preparation Artemisia (tarragon extract – *Artemisia annua*). This phyto-preparation is a dry extract of granulated tarragon, easily soluble in water with bactericidal and coccidiostatic properties. In the experiment for rabbits it was dissolved in water and given as the solution of 0.05 %, 0.1 %, 0.2 % concentration. The control group didn't receive any coccidiosis treatment till the end of the research. Young rabbits didn't receive any prophylactic treatment, eimeriosis infection took place by the faecal-oral way because of the impossibility to provide hundred per cent sterility after invaded animals and high concentration of livestock at the farm. Clinical, hematological, zootechnical methods were used [1, 5].

3. Results

The study (experiment 1) for determining the influence of the acidifier Cronocyde L and its components on oocysts of rabbits' eimeria was conducted under laboratory conditions. At conducting the study, the condition of oocysts was estimated by the morphological signs (form, size, color, localization of the embryonic layer, presence of the polar granule and microfield). The research results demonstrated that oocysts of coccidia were influenced by all presented preparations: Cronocyde L standard and Cronocyde L concentrate and acids. But the effectiveness of their effect was different. The effectiveness of the preparations, namely death of oocysts, was expressed in per cents (**Table 1**).

Table 1
Effectiveness of coccidiostatics on oocysts of rabbits' eimeria

No.	Active substance	pH	Exposition time	% of oocysts' death
1	2	3	4	5
1	Cronocyde L standard 1 ml/1l of water	4.5	30 min	15
			60 min	35
2	Cronocyde L standard 2 ml/1l of water	3.5	30 min	20
			60 min	60
3	Cronocyde L concentrate 1 ml/1l of water	4.5	30 min	60
			60 min	97
4	Cronocyde L concentrate 1 ml/1l of water	3.5	30 min	78
			60 min	99
5	Benzoic acid	3.5	30 min	85
			60 min	98
		4.0	30 min	60
			60 min	95
		4.5	30 min	50
			60 min	90
6	Formic acid	3.5	30 min	55
			60 min	80
		4.0	30 min	30
			60 min	65
4.5	30 min	10		
	60 min	24		

1	2	3	4	5
		3.5	30 min	25
			60 min	50
7	Sorbic acid	4.0	30 min	35
			60 min	50
		4.5	30 min	10
			60 min	20
		3.5	30 min	60
			60 min	95
8	Orthophosphoric acid	4.0	30 min	30
			60 min	60
		4.5	30 min	35
			60 min	45
		3.5	30 min	80
			60 min	100
9	Propionic acid	4.0	30 min	60
			60 min	98
		4.5	30 min	50
			60 min	90
		3.5	30 min	50
			60 min	90
10	Lemon acid	4.0	30 min	30
			60 min	45
		4.5	30 min	20
			60 min	30
		3.5	30 min	80
			60 min	95
11	Acetic acid	4.0	30 min	40
			60 min	60
		4.5	30 min	15
			60 min	45
12	Control	(distilled water)	30 min	0
			60 min	0

Thus, the highest results of oocysts' death was in the preparation Cronocyde 1 concentrate pH 3.5, comparing to Cronocyde L standard. Such data were obtained due to the composition of the preparations. Cronocyde L concentrate includes benzoic, acetic and propionic acids that at pH 3.5 and exposition 60 minutes caused death of 80–98 % of oocysts. The effect of acids on the oocysts' tunic is conditioned by not only their concentration, but chemical construction that gives a possibility to penetrate the tunic of coccidia and to wrinkle a cytoplasm or to break it. The preparation Cronocyde L standard included formic, orthophosphoric, sorbic, lemon acids that at pH 3.5 and exposition 60 minutes caused death of 50–90 % of oocysts. Normal pH of water for animal consumption is 3.5–4.5 and doesn't cause destruction of epithelium of the mucosa. The impossibility to increase or decrease pH of the preparations, because of using acidifier in rabbits, limits the effect. From the other side, it is necessary to create conditions, at which the ovocidal or coccidiostatic effect for coccidia is manifested.

It is impossible to annihilate coccidia totally, that is an acidifier must be given every day with water, because at decreasing the therapeutic concentration of the preparation rabbits become ill. At the subclinical form of coccidiosis rabbits look completely healthy, but they consume less forage, become apathetic. Their weight and forage conversion decrease.

The obtained data are proved by the microscopic photos (Fig. 1–11).

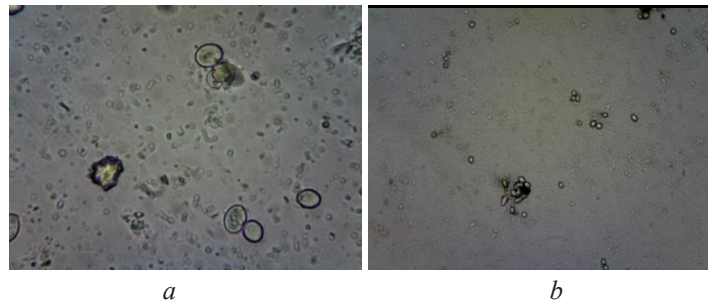


Fig. 1. Coccidia without adding a disinfectant: *a* – magnification $\times 1000$; *b* – magnification $\times 400$;

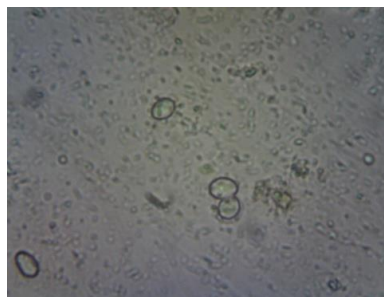


Fig. 2. Cronocycle L standard pH 3.5 – wrinkling of the cytoplasm of coccidia at exposition 60 minutes



Fig. 3. Cronocycle L concentrate pH 4.5 – wrinkling of the cytoplasm of coccidia at exposition 30 minutes

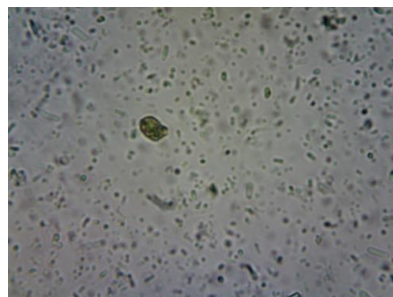


Fig. 4. Cronocycle L concentrate pH 3.5 – wrinkling of the cytoplasm and break of the tunic of coccidia at exposition 60 minutes

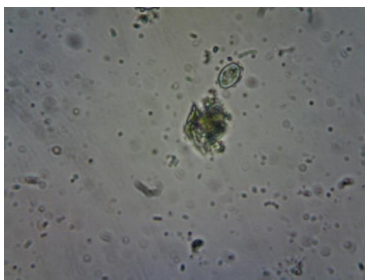


Fig. 5. Benzoic acid pH 4.5 – wrinkling and break of the cytoplasm of coccidia at exposition 30 minutes

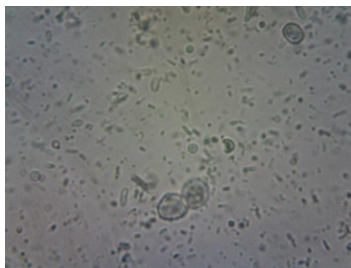


Fig. 6. Formic acid pH 3.5 – wrinkling of the cytoplasm of coccidia at exposition 60 minutes

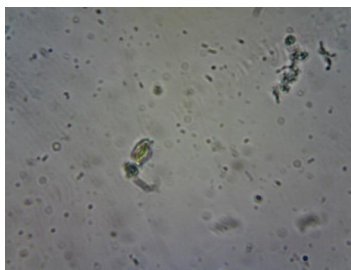


Fig. 7. Orthophosphoric acid pH 3.5 – wrinkling and break of the cytoplasm of coccidia at exposition 60 minutes

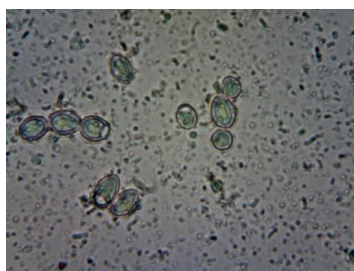


Fig. 8. Sorbic acid pH 3.5 – wrinkling of the cytoplasm of coccidia at exposition 60 minutes



Fig. 9. Propionic acid pH 4.5 – wrinkling and break of the cytoplasm of coccidia at exposition 60 minutes

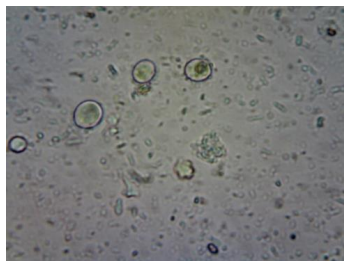


Fig. 10. Lemon acid pH 3.5 – wrinkling of the cytoplasm of coccidia at exposition 60 minutes



Fig. 11. Acetic acid pH 4.5 – wrinkling and break of the cytoplasm of coccidia at exposition 60 minutes

According to the results of the conducted studies, oocysts *Eimeria stiedae*, *E. Perforans*, *E. Magna*, *E. Media* were detected in rabbits' faeces. The research results (invasion extensive-ness IE, preparation extensive effectiveness EE and lethality in the treatment period) are grouped in **Table 2**.

Table 2

Dynamics of eimeria invasion in rabbits

		First experiment (0,2 %)							
Group	Number of rabbits	IE, % of eimeria				EE, % of preparation			Death
		Day				day			
		0	7	14	21	7	14	21	
1	10	100	89	16	3	11	84	97	0
2	10	100	91	15	2	9	85	98	0
3	10	100	95	89	88	5	12	20	1
Control	10	100	95	102	97	–	–	–	2
		Second experiment (0.1 %)							
Group	Number of rabbits	IE, % of eimeria				EE, % of preparation			Death
		Day				day			
		0	7	14	21	7	14	21	
1	10	100	84	21	9	16	79	91	0
2	10	100	89	15	4	11	85	96	0
3	10	100	93	85	90	7	15	10	2
Control	10	100	103	98	97	–	–	–	5
		Third experiment (0,05 %)							
Group	Number of rabbits	IE, % of eimeria				EE, % of preparation			Death
		Day				day			
		0	7	14	21	7	14	21	
1	10	100	86	22	16	14	78	84	0
2	10	100	87	14	3	13	86	97	0
3	10	100	99	97	95	1	3	5	3
Control	10	100	96	101	99	–	–	–	8

The research results testify that the extensive effectiveness of the preparation in the groups of rabbits decreased depending on concentration of the preparation and its properties. In rabbits of the third group, where the phyto-preparation Artemisia was used, the extensive effectiveness was very low during 21 days, possibly because of weak coccidiostatic properties of the preparation and high invasion extensiveness. Probably at increasing the concentration of Artemisia, the result can be improved, but then there appears a problem of toxicity and save term of usage. Frequent consumption of coccidiostatic, anti-helminth and bactericidal preparations results in allergic and immune-suppressive reactions. The hepatobiliary system is destructed at the unsystematic use of chemotherapeutic preparations for rabbits, especially in young individuals less 60 weeks old. At the beginning of the second experiment rabbits' invasion degree was high and reliably decreased to the 21 day of the study in the first group of rabbits, where Cronocyde L concentrate as 0.1–0.2 % was used. There was revealed the maximal coccidiostatic effect due to effective components of this preparation, comparing with the control and Cronocyde L standard. It proves the previous laboratory studies in the first experiment.

For determining the effectiveness of the preparations Cronocyde L standard, Cronocyde L concentrate and phyto-preparation Artemisia as to coccidiosis and safety for rabbits, zootechnical parameters were studied.

10 animals-rabbit analogues with the initial weight 1,0 kg were used for the studies in each group. Rabbits were treated in the first group by Cronocyde L concentrate, young rabbits of the second group – by Cronocyde L standard, third group – by the phyto-preparation Artemisia, concentration 0,1 %. The results are presented in **Table 3**.

Table 3
Comparative data of rabbits breeding

Parameters	Control	Experimental groups		
		1	2	3
Number of animals, ind.	10		10	
Initial living mass, kg	1.0±0.13	1.0±0.10	1.0±0.10	1.0±0.10
Final living mass, kg	1.5±0.45	1.9±0.84	1.8±0.72	1.6±0.50
Absolute increment, kg	0.5±0.23	0.9±0.95	0.8±0.18	0.6±0.18
Average daily increment, g	16.4±1.34	30.5±2.10*	27.6±2.53	20.5±1.50
Forage consumption, kg	120.6	123.1	134.0	125.5
Forage consumption for 1 kg of the living mass increment, kg	1.57	1.66	1.64	1.67
% to the control	97.5	100.5	98.8	102.5

Note: * – $p \leq 0,05$ comparing with the control

As a result of the conducted studies, it has been established, that the average daily increment is more in the first experimental group by 18.5 %, in the second – by 15.6 %, and in the third – by 8 %. At conducting the experiment, there was determined the clinical status of rabbits. At that attention was paid to the forage conversion. The animals in the control consumed less forage than in the experimental groups.

The rabbits of the control group also demonstrated signs of coccidiosis, such as: exhaustion, swelling of the belly, apathy, absence of appetite, delay in growth and development, diarrhea and mucous extractions from the asshole. Most of these rabbits die even at treatment because of dehydration of the organism or injury of the liver. The results of the biochemical blood analysis of the experimental rabbits are presented in **Table 4**.

Table 4
Biochemical analysis of blood serum at rabbits' eimeriosis

Parameters	1 experimental group	2 experimental group	3 experimental group	control
Total protein, g/l	57.3±1.15	66.9±0.92	71.4±0.99	41.6±0.81
Albumin, g/l	39.1±0.72	33.6±1.01	29.9±1.52	21.7±0.67
Thymol test, un.	2.3±0.12	3.3±0.28	3.9±0.31	2.4±0.27
Total bilirubin, mcmol/l	7.4±0.24	10.5 ±1.19	13.3 ±1.32	31.2 ±0.63
Direct bilirubin, mcmol/l	2.4±0.15	4.2±0.83	4.0±0.56	14.7±0.16
Indirectbilirubin, mcmol/l	5.1±0.35	6.4±0.42	9.1±0.81	17.1±0.66
Urea, mmol/l	3.8±0.35	7.5±0.51	4.0±0.29	24.4±0.56

At appearing clinical manifestations there was observed the decrease of albumin and total protein in blood serum of rabbits from the second and third experimental groups and in the control. At injuring the liver by coccidia, bilious tracts are congested, mechanical jaundice appears. Auto-intoxication of the liver and hepatitis develop in an ill rabbit. The insufficient amount of bilious acids forms; and an animal can't digest food. That is why the level of albumins is low in blood serum of animals with coccidiosis. Bilirubin in blood of ill rabbits rises because of the same.

The level of direct bilirubin in the first experimental group is less by 6 % in the second – by 1.8 %, in the third – by 1,6 %, comparing with the control group. There was observed the reduction of globulins in rabbits of the second and third experimental groups and in the control that is confirmed by the thymol test. The urea level in blood serum was less in the first experimental group comparing with the control one by 6,4 %, in the second – in 3,3 times, in the second – by 6.1 %. It testifies to the hepatorenal syndrome and renal function disorder.

4. Conclusion

The obtained results of annihilating coccidia at pH of the preparation within 3.5–4.5 in vitro under laboratory conditions gives a possibility to use this composition as a coccidiostatic for rabbits. It has been experimentally proved, that the preparation Cronocyde L concentrate reduces eimeria invasion, favors the rabbits' living mass increase and forage conversion. This composition essentially differs from the effect mechanism of most coccidiostatics and is not toxic that is proved by the results of the biochemical study of rabbits' serum. The preparation also improves digestion processes and inhibits the development of the conventionally pathogenic microflora, is an additional source of microelements.

At using the coccidiostatic for rabbits there was the short term of the experiment, so in further it is possible to conduct a more detail experiment. The special attention is planned to be paid to the creation term of coccidia forms, resistant to Cronocyde L.

The practical importance of the conducted experiments is in getting the effective and safe means – coccidiostatic and at the same time stimulator of metabolic processes for rabbits.

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