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# Evaluation of the effectiveness of coniferous-phytogenic feed additives in case of eimeriosis infestation in calves

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**Keywords:**

Calves; Calf Pen; Eimeriosis; Coniferous-Phytogenic Feed Additives; Efficiency

**Abstract**

**Background:** Eimeriosis in calves, often treated with harsh chemicals, leads to significant health and economic burdens. This study explores natural coniferous-phytogenic feed additives as an innovative solution, focusing on their efficacy against *Eimeria bovis* and *Eimeria ellipsoidalis*, two common eimeriosis pathogens in calves.

**Methods:** The authors conducted experiments to analyze the therapeutic and preventive effectiveness of the use of a coniferous-phytogenic immunomodulator in the diet of calves from 5-7 days of age and a coniferous and salicylic acid feed additive from 28-30 days of age in case of eimeriosis infestation at a dose of 5.0, 10.0, and 15.0 ml, once a day, in courses of 2 weeks without intervals with a small amount of warm water.

**Results:** The extensivity of the coniferous and salicylic acid feed additive against the eimeriosis infestation in calves caused by *E. bovis* showed maximum result. Both preparations were willingly consumed by calves and eliminated the signs of diarrheal dyspeptic syndrome and gastroenteritis. The inclusion of coniferous-phytogenic feed additives in the calves' diet from 5-7 days of age once a day, in courses of 2 weeks without intervals, contributed to 100% effectiveness against *E. bovis* and *E. ellipsoidalis*.

**Conclusion:** The study concludes that coniferous-phytogenic feed additives are highly effective in treating and preventing eimeriosis in calves, showing 100% effectiveness against the targeted pathogens. These additives also contribute to the overall health and well-being of the animals by alleviating related symptoms.



## Introduction

Cattle eimeriosis (coccidiosis) has a wide distribution and is registered in livestock farms with various technologies for animal keeping [1-3]. The disease causes high economic damage resulting from a decrease in dairy and meat productivity (from 12 to 30%) and high mortality of animals, mainly young ones (from 10 to 100%). Calves under the age of 1 year are most susceptible to eimeriosis, which affects the gastrointestinal tract, resulting in the development of diarrhea, exhaustion, anemia, and death. The coccidia fauna has more than 15 species of pathogens that multiply in the intestinal epithelial cells and cause mass death. The most pathogenic species are *Eimeria bovis* and *Eimeria zuernii* [3-5].

To control ruminant eimeriosis, anticoccidial agents of two groups are used, namely, chemical antibiotics (chemococcid, pluricoccin, diclazuril, etc.) and ionophoric antibiotics. These medications have a detrimental effect on *Eimeria* spp., but can also harm the animal body [6-8]. The side effects of these medications are reduced to a decrease in appetite, diarrhea, a decrease in water consumption, exhaustion, the development of dysbiosis, and intoxication [9-11]. For example, diclazuril, monenza, and narasin turn out to be the most aggressive anticoccidial agents against the beneficial intestinal microflora of animals [12-15].

Traditional treatment approaches, including chemical and ionophoric antibiotics, while effective, often present challenges such as potential harm to animal health and environmental concerns. This has led to an increasing interest in exploring natural, sustainable alternatives for eimeriosis management. In this context, our study focuses on evaluating the efficacy of coniferous-phytogenic feed additives as a potential natural treatment and preventive measure against eimeriosis in calves. These additives, derived from natural sources like pine needles and aspen bark, are believed to possess immunomodulatory properties that could offer a safer and equally effective alternative to conventional treatments.

In materials and methods section details the experimental design, including the preparation and administration of the coniferous-phytogenic feed additives, and the methods used for monitoring and evaluating their effectiveness against eimeriosis.

In results we present the findings from our experiments, including the impact of the feed additives on eimeriosis prevalence and severity, as well as their influence on overall calf health. In the Discussion part interprets the results, comparing them with existing literature, and discussing their implications in the broader context of eimeriosis treatment and calf health.

The conclusion section summarizes the key findings of the study, highlighting the potential of coniferous-phytogenic feed additives as an effective and sustainable alternative to traditional eimeriosis treatments in calves.

## Methods

### Research Design

The scientific experiment was carried out on breeding heifers of black-and-white Holstein breed at the Agrokombinat Krasnogorsky breeding plant Joint Stock Company (JSC) (Pasegovo Kirovo-Chepetsky district branch) from March to May 2023. We selected breeding heifers of the black-and-white Holstein breed, which were raised in cold conditions and housed in individual stalls within an unheated calf pen. These calves were fed a diet of colostrum and milk. Calves fed with colostrum and milk were raised in cold conditions and kept in individual stalls in an unheated room at a calf pen.

### Experimental Groups and Feed Additives

Two experiments were designed, each involving different groups and feed additives. To conduct the first experiment, we used a coniferous-phytogenic immunomodulator (CPI) for milk-fed calves produced by the "Khiminvest" Scientific and Technical Center LLC (Nizhny Novgorod, Russia). The CPI is an extract of green forest biomass in glycerin, enriched with oligosaccharides and energy, which contains pine needles, glycerin, linseed cake, wheat bran, and sugar [16-18]. The duration of administration of the studied supplement was 42 days in various doses: 5.0, 10.0, and 15.0 ml per head in courses for 14 days without intervals. The preparation was given to the 1st experimental group (EG) of animals from the age of 5 days (n=10) in liquid form with a small volume of water (50-100 ml), once a day, in addition to the main diet.

For comparative analysis, the 1st control group (CG) of animals (n=10) receiving the main diet during the entire experiment was formed according to the analog pair principle. In the second experiment, we used a coniferous and salicylic acid (CSA) feed additive produced by the "Khiminvest" Scientific and Technical Center LLC, which consists of extracts of biological components of pine needles and aspen bark in glycerin [19-21]. Two groups of animals were also formed during the study: the 2nd CG (n=10) which consisted of heifers aged 30-35 days and received the main diet, and the 2nd EG (n=10) which included heifers aged 28-30 days and received the CSA feed additive in addition to the main diet in liquid form with a small amount of warm water (150-200 ml) at a dose of 5.0, 10.0, and 15.0 ml courses for 14 days without intervals. The entire course of administration lasted for 42 days.

### Clinical Observation and Diagnostic Procedures

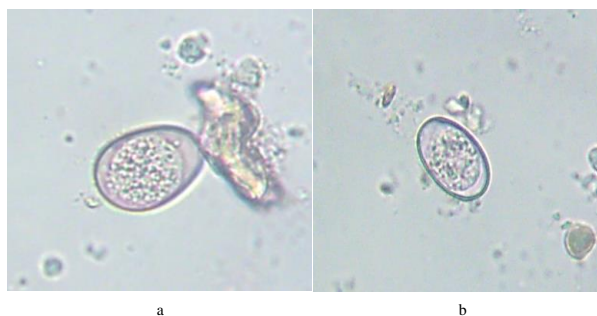
Clinical observation and thermometry were conducted for the animals included in the experiments. To diagnose eimeriosis in the CG and EG calves, fecal samples were taken from the rectum and examined by the Fülleborn's method [21-23] according to the State Standard (GOST) 25383-82 at the veterinary parasitology diagnostic laboratory of the Vyatka State Medical University. Following the GOST, the presence of up to 1,000 oocysts in 1 g of feces (for *E. bovis* and *E. zuernii*) indicates a negligible infestation, up to 5,000 an infestation of moderate severity, and over 5,000 a severe infestation. To differentiate the types of *Eimeria* spp., we used the guide composed by M.V. Krylov (1996). According to the results of scatoscopy, the prevalence and infestation intensity (II) were calculated in three drops of flotation fluid, in 1 g of feces [1-3].

### Efficacy Evaluation

The therapeutic efficacy of the CPI and CSA feed additive against *Eimeria* spp., was analyzed based on the results of fecal cystoscopy before the calves received feed additives and 14, 28, and 42 days after they did. To do this, two indicators were determined, extensive and intensive efficiency (EE and IE). The standard deviation for the group was calculated using Microsoft Excel 2010.

### Results

The microscopic examination of 10 fecal samples taken from calves of the 1st CG showed the presence of unsporulated oocysts *E. bovis* and *E. ellipsoidalis* (Fig. 1a, b) only 14 days after the beginning of the experiment in four samples with the prevalence of *E. bovis* equal to 30% and the prevalence of *E. ellipsoidalis* of 10%. The II of calves aged 19-21 days with *E. bovis* infestation ranged from 1 to 52, and in calves with *E. ellipsoidalis* infestation, it equaled 10 oocysts, which corresponds to a negligible degree of infestation (Table 1). However, one calf showed signs of diarrheal dyspeptic syndrome (DDS).



**Figure 1:** Unsporulated oocysts of *Eimeria* spp., in calves: a: *E. bovis*; b: *E. ellipsoidalis* (x400).

In the scatoscopy of the CG calves after 28 days of the experiment, only *E. bovis* oocysts were found in five samples. The II of calves aged 33-35 days increased and ranged from 2 to 98 oocysts. In three calves, the degree of infestation was negligible, and in two, it had moderate severity with an II equal to  $2.5 \pm 0.71$  oocysts in the Goryaev chamber or 2,777.5 oocysts/1 g of feces; signs of DDS were noted.

Similar dynamics of prevalence and II of *E. bovis* were observed on the 42nd day of the experiment. The percentage of infested calves aged 47-49 days was 50, and the degree of infestation ranged from 1 to 90 oocysts. In three calves, the degree of infestation was weak, and in two, it was average with an II equal to  $2 \pm 0$  oocysts in the Goryaev chamber or 2,222 oocysts/1 g of feces and signs of DDS. One calf with signs of DDS had a mixed infestation caused by *E. bovis* and *E. ellipsoidalis* species with an II equal to 8 and 30 oocysts in three drops of flotation fluid.

The thermometry parameters of the heifers of the 1st CG during the entire experiment ranged from 38.2 to 39.9°C. Elevated indicators were recorded in animals with signs of DDS. In the study of the fecal samples taken from calves of the 1st EG, immature oocysts of *E. bovis* and *E. ellipsoidalis* were detected only in three animals after 28 days of the experiment after the calves had received the CPI at a dose of 5.0 and 10.0 ml (Table 1). The prevalence of *E. bovis* in calves was 20%, and the II ranged from 4 to 9 oocysts. Infestation caused by *E. ellipsoidalis* was found in one calf with an II equal to 22 oocysts. Since the degree of infestation with *E. bovis* and *E. ellipsoidalis* was negligible, there were no signs of diarrhea. Thermometry indicators of heifers of the 1st EG during the entire experiment ranged from 38.1 to 39.8°C. Elevated indicators were recorded in animals at the beginning of the study before the introduction of CPI in calves with signs of alimentary dyspepsia.

When the dose of the CPI was increased to 15.0 ml, the result of microscopy for eimeriosis was negative. Therefore, the EE and IE of the CPI against *Eimeria* species *E. bovis* and *E. ellipsoidalis* was 100%. Microscopic examination of 10 fecal samples obtained from calves of the 2nd CG revealed only unsporulated *E. bovis* oocysts with a prevalence equal to 30% at the beginning and after 14 days of the experiment, 50% after 28 days, and 30% after 42 days. The II of calves aged 30-35 days ranged from 11 to 18, 44-49 days from 1 to 10, and 58-63 days from 2 to 25, and 72-77 days from 1 to 10 oocysts, which corresponds to a negligible degree of infestation (Table 2). The body temperature of all the infested calves ranged from 38.6 to 39.2°C and there were signs of gastroenteritis.

In the study of the fecal samples obtained from calves of the 2nd EG, immature *E. bovis* oocysts were detected at the beginning of the experiment before giving the

Indicators	1st CG (n=10)				1st EG (n=10)			
	Before Treatment	After 14 Days	After 28 Days	After 42 Days	Before Treatment	After 14 Days	After 28 Days	After 42 Days
Prevalence of <i>E. bovis</i> , %	0	30	50	50	0	0	20	0
Prevalence of <i>E. ellipsoidalis</i> , %	0	10	0	10	0	0	10	0
EE, %	-	-	-	-	-	100	70	100
IE, %	-	-	-	-	-	100	-	100

**Table 1:** Performance indicators of the CPI for eimeriosis caused by *E. bovis* and *E. ellipsoidalis* found in calves aged 5-7 days to 1.5 months.

Indicators	2nd CG (n=10)				2nd EG (n=10)			
	Before Treatment	After 14 Days	After 28 Days	After 42 Days	Before Treatment	After 14 Days	After 28 Days	After 42 Days
Prevalence of <i>E. bovis</i> , %	30	30	50	30	40	20	10	0
EE, %	-	-	-	-	-	80	90	100
IE, %	-	-	-	-	-	86.2	55.5	100

**Table 2:** Indicators of the effectiveness of the CSA feed additive against eimeriosis caused by *E. bovis* in calves aged 28-35 days to 2.5 months.

CSA feed additive with a prevalence equal to 40% and II from 6 to 120 oocysts (Table 2). In one calf, the degree of lesion was negligible, and in three, moderate with an II equal to  $2.3 \pm 1.52$  oocysts in the Goryaev chamber or 2555.3 oocysts/1 g of feces; signs of gastroenteritis were noted. After 14 and 28 days of the experiment after giving the CSA feed additive at a dose of 5.0 and 10.0 ml, there was a decreased tendency both in the percentage of infested animals to 20 and 10% and the infestation degree by 86.2 and 55.5%, respectively. The thermometry parameters of the heifers in the 1st EG during the entire experiment ranged from 38.4 to 39.4°C. Elevated indicators were recorded in animals at the beginning of studies before the introduction of the CSA feed additive in calves with signs of catarrhal gastroenteritis.

## Discussion

The results show that the CPI was 100% effective against *E. bovis* and *E. ellipsoidalis*, which indicates its prospects as a therapeutic and prophylactic agent for eimeriosis in milk-fed calves. Similarly, the CSA feed additive showed a high level of effectiveness, eliminating eimeriosis caused by *E. bovis* in calves. Both feed additives were well digested by calves and effectively relieved the symptoms associated with eimeriosis, including DDS and gastroenteritis. This highlights the ability of these supplements to improve the overall health and well-being of animals.

1. The use of the CPI in the diet of calves aged from 5-7 days at a dose of 5.0, 10.0, and 15.0 ml, once a day, in courses of 2 weeks without intervals contributed to 100% effectiveness against *E. bovis* and *E. ellipsoidalis*. This finding aligns with recent research emphasizing the importance of early intervention in managing parasitic infections in young animals. Studies have shown that the early administration of immunomodulatory agents can significantly enhance the developing immune system of

neonatal calves, leading to better disease resistance [25, 26].

2. The use of the CSA feed additive in the diet of calves aged from 28-30 days at a dose of 5.0, 10.0, and 15.0 ml, once a day, in courses of 2 weeks without intervals contributed to 100% effectiveness against eimeriosis infestation caused by *E. bovis*. Recent studies have highlighted the benefits of incorporating natural extracts, which often exhibit fewer side effects compared to traditional chemotherapeutic agents, in managing parasitic infestations in livestock [27].
3. Both preparations were willingly consumed by calves and contributed to the elimination of signs of DDS and gastroenteritis. This aspect is consistent with recent trends in animal nutrition, where the focus has shifted towards natural, animal-friendly feed additives that not only prevent diseases but also promote overall well-being [28, 29].

Given the increasing concerns about antibiotic resistance and the emphasis on sustainable livestock practices, the use of such natural additives could be a significant step forward. However, it is crucial to note that while these results are promising, further long-term studies are needed to assess the broader impacts of these additives on animal health, productivity, and the environment.

This study's exploration into the use of coniferous-phytogenic feed additives, specifically CPI and CSA, has demonstrated significant potential in the management of eimeriosis in calves, a common and economically impactful parasitic disease in livestock. The successful acceptance and digestion of these additives by calves, coupled with their effectiveness in alleviating symptoms associated with eimeriosis such as diarrheal dyspeptic syndrome and gastroenteritis, highlight their potential in improving animal health and welfare.

Further research is necessary to fully understand the broader implications of these findings. Long-term

studies focusing on the impact of these additives on overall animal growth, feed efficiency, immune response, and productivity would be invaluable. Additionally, investigating the mechanisms underlying the observed effects of these additives could provide deeper insights into optimizing their use and efficacy. Understanding how these natural compounds interact with the animal's immune system could pave the way for more targeted and effective approaches to disease prevention and health management in livestock.

## Author Contributions

Vasily Korotkiy: Conducted experiments, collected data, and contributed to the design of the study. Olga Skorniyakova: Designed the experiments, analyzed data, and contributed to the interpretation of results. Victoria Leukhina: Assisted in data collection, conducted clinical observations, and contributed to the diagnostic procedures. Evgeny Sadykov: Contributed to the experimental design, literature review, and manuscript preparation. Viktor Ryzhov: Supervised the study, provided critical guidance, and contributed to the overall research direction. All authors reviewed and approved the final manuscript after revision and final checks.

## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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