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

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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 against eimeriosis infestation at a dose of 5.0, 10.0, and 15.0 ml, once daily for 2-week courses without breaks 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 daily for 2-week courses without breaks, resulted in 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 fostered a growing interest in investigating natural, sustainable alternatives for the management of eimeriosis. Within this context, our study addresses the assessment of the effectiveness of conifer-phytogenic feed supplements as a natural treatment and prophylactic agent for protection of the calf from eimeriosis. These supplements are naturally derived from such materials as aspen bark and pine needles, and are thought to have immunomodulatory activities that can provide a safe and effective alternative for conventional treatments. The experimental design, preparation and dosing with the coniferous-phytogenic feed additives, as well as the techniques for monitoring and assessing their efficiency as a prophylaxis or treatment for eimeriosis, are specified in materials and methods. In the results we report the outcomes of our experiments, such as the effect of the feed additives on the prevalence and severity of eimeriosis, as well as their effect on calf health in general. In the Discussion section interprets the results in comparison with the relevant literature, in addition to discussing their relevance in the general context of treatment of eimeriosis as well as 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 experiment was performed on breeding heifers of the black-and-white Holstein breed at the Agrokombinat Krasnogorsky breeding plant Joint Stock Company (JSC) (Pasegovo Kirovo-Chepetsky district branch) between March and May 2023. For the purpose of the experiment, we took breeding heifers of the black-and-white Holstein breed, reared in cold conditions, and placed in single stalls in an unheated calf pen. Colostrum- and milk-fed calves were reared in cold conditions and kept in single stalls in an unheated room in 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

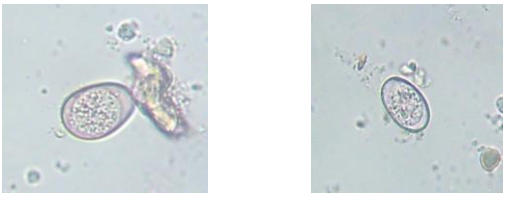
Clinical observations and body temperature measurements were conducted for the animals included in the experiments. To diagnose eimeriosis in the CG and EG calves, fecal samples were taken from the rectum and examined by the Fülleborn's method [21-23] according to the State Standard (GOST) 25383-82 at 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 (extensive and intensive effectiveness (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 infestation intensity (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 symptoms of diarrheal dyspeptic syndrome (DDS).



a b

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 infestation intensity (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 ± 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±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 in the 1st experimental group, 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 microscopic examination yielded negative results for eimeriosis. Therefore, the extensive and intensive effectiveness (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 infestation intensity (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.

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 *E. bovis*, %	0	30	50	50	0	0	20	0
Prevalence of *E. ellipsoidalis*, %	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 *E. bovis*, %	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.

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 CSA feed additive showed a 40% prevalence 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 ± 1.52 oocysts in the Goryaev chamber or 2555.3 oocysts/1 g of feces; symptoms of gastroenteritis were observed. 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. Likewise, the feed additive CSA was highly effective in removing eimeriosis caused by *E. bovis* in calves. Feeding the feed additives was effectively digested in the calves and provided relief from the signs accompanying the eimeriosis, such as DDS and gastroenteritis. This reflects the potential of these supplements for enhancing the general health and well-being of the animals.

1. The administration of the CPI in the diet of 5-7 day old calves in a dose of 5.0, 10.0, and 15.0 ml, once a day for 2-week cycles without breaks was 100% effective against *E. bovis* and *E. ellipsoidalis*. This aligns with recent studies, which highlight the need for early treatment in parasitic infections in young hosts. It has been proven in experiments that the use of immunomodulators in the neonatal calf's earlier stages can remarkably boost the immune system under development in the neonatal calf, thus building immunity towards the disease [25, 26].
2. The addition of the CSA feed additive in the diet of 28-30 day-old calves with a 5.0, 10.0, and 15.0 ml dose, once daily for 2-week treatments with no breaks yielded 100% effectiveness in treating eimeriosis infestation caused by *E. bovis*. Recent studies have reported the advantages of the addition of natural extracts, with fewer side effects than the conventional chemotherapeutic agents, in the management of 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 report's investigation into the consumption of coniferous-phytogenic feed additives, CPI and CSA, has proved considerable promise in the treatment of eimeriosis in calves, a widespread and economically relevant parasite infection in livestock. Successful uptake and assimilation of the additives by the calf, in addition to their effectiveness in treating symptoms of the condition such as diarrheal dyspeptic syndrome and gastroenteritis, highlight their promise in the promotion of animal welfare and health. Additional studies are required to better capture the larger context of these findings. More long-term experiments on the effect of these additives on total animal growth, feed

conversion, immune status, and productivity would be most useful. Examination of the underlying mechanisms for the reported actions of these additives might also give further insights into maximizing their use and efficiency. Discovering how these natural compounds affect the immune system of the animal might open up the field for more precise and effective strategies for disease prevention and animal health management in farm livestock.

Author Contributions

Vasily Korotkiy: Conducted experiments, collected data, and contributed to the design of the study. Olga Skornyakova: 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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