

## The Anticoccidial Efficacy of Natural Herb Extracts in Calves

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**Abstract:** The anticoccidial efficacy of natural herb extracts was evaluated in suckling calves with bovine coccidial infection (*Eimeria* sp., including *E. bovis* and *E. zuernii*). The herb extracts were orally administered to 5 calves (treatment group) for 7 weeks and the clinical signs, oocyst excretion and blood parameters, which reflect hepatic and renal functions, as well as nutritional condition and body weight gain of the treatment group were compared with those of untreated 5 calves (control group). During the experimental period, all the 5 calves in the control group had bloody diarrhea accompanied with remarkable oocyst excretion. None of the 5 calves in the treatment group had bloody diarrhea. No significant differences were observed in the blood parameter results between the 2 groups. The results of this study indicate that the daily administration of herb extracts to calves from the suckling to weaning period is effective in preventing bovine coccidial infection.

**Key words:** Coccidiosis, herb extracts, calf

### INTRODUCTION

Coccidiosis is an acute or chronic disease associated with hemorrhagic diarrhea, emaciation, growth retardation and sometimes death; the clinical signs are observed most frequently in young animals. The coccidian parasite that causes disease in cattle is of the genus *Eimeria* and belongs to the same general group of parasites that causes malaria in humans. Therefore, coccidiosis is one of the most economically important diseases to affect livestock. Generally, several drugs such as sulfonamides, the thiamine analogue amprolium and ionophore antibiotics are used for the treatment of clinical coccidiosis<sup>[1,2]</sup>. Although these drugs successfully eradicate clinical coccidiosis, recent consensus among people from the field of veterinary and food science has resulted in the requirement of strict registration of antibiotics, including anticoccidial drugs<sup>[3,4]</sup>. Regarding the clinical application of herb extracts to the veterinary science, although anticoccidial and antiprotozoal efficacies of herb extracts have been reported in chickens and in vitro cultures<sup>[3,5]</sup>, no reports are available regarding not only the application but also their clinical efficacy in

cattle. In the present study, young calves were treated with a natural herb extract product containing plant extracts and essential oils in order to elucidate its anticoccidial efficacy and metabolic effects based on the results of blood examinations.

### MATERIALS AND METHODS

Ten Japanese Black calves, 4-11 days of age, maintained at the Kagoshima University Experimental Farm in Japan were used in the present study. The animals were housed in a barn and were fed their mothers' milk 3 times a day by limited suckling. Before starting the experiment, all calves underwent general and fecal examination and were disinfected for celo- and ecto- (Ivermectin) and ecto- (Flumetrin) parasites. Preliminary clinical monitoring of calf herds revealed that sporadic coccidial infections (*E. bovis* and *E. zuernii*) of the calves could be detected by microscopic examination of their feces and calves with diarrhea or bloody diarrhea have been occasionally treated with sulfonamides. Thus, this suggests that the barn was chronically contaminated with coccidia.

The natural herb extract in powder form (Eimericox; Phytosynthese, France) is a natural complementary food to improve the growth performances for ruminants and contains boldo and eucalyptus extracts along with fenugreek powder. The manufacturer recommends that the herb extract should be administered to calves in their weaning feed from 30 to 45 days at 4 g per 100 kg body weight per day. In the present study, to clarify not only the anticoccidial effect but also its side effects in young calves, the herb extract was administered to the calves at twice the dose indicated in the instructions.

The calves were randomly divided into a treatment group (n = 5) and a control group (n = 5). The calves in the two groups were all housed in the same barn during the experiment; thus, the infection pressure on the two groups might be the same. For successful administration of the herb extract, the calves in the treatment group were administered 5 g herb extract diluted with 500 mL water once a day for 6 to 7 weeks; using a stomach tube, the product was directly administered into the rumen. On the other hand, the calves in the control group received only water. All of the calves were clinically observed and the fecal condition of each calf was recorded every day. Fecal samples were collected directly from the rectum and using the sucrose flotation method, oocyst excretion was determined in each calf once a week on Days 7, 14, 21, 28, 35 and 42 after starting the treatment. In addition to fecal sampling, blood samples were collected from the jugular vein for blood examinations such as complete blood count (CBC; F-820, Sysmex, Japan), serum enzymes (Labospect 7080 autoanalyzer, Hitachi, Japan) and haptoglobin (HP; single-immunodiffusion method; metabolic-eco-system institute, Japan) concentrations in order to monitor their hepatic and renal functions, nutritional status and inflammation status, respectively. Additionally, the body weights of the calves were measured on Day 0, Day 28 and at 3 months after starting the treatment in order to monitor the effect of the herb

extract on the growth performance of the calves. During the experimental period, calves with diarrhea were treated by daily oral fluid therapy. The calves were administered an oral electrolyte solution and commercially available antidiarrheal medicines containing astringent, stomachic and antacid compounds until the feces became normal. However, despite these treatments, if the calves developed bloody diarrhea and an increased OPG count, acute coccidiosis was diagnosed and sulfamonomethoxine was orally administered for 3-5 days.

The results of each group were expressed as means±SD. The results of the total number of days of aquiparous diarrhea, daily gain and blood examinations of each treatment day of the two groups were compared using the unpaired t-test. The OPG values were analyzed by repeated measure ANOVA and post-hoc tests to determine the effects of time course and the herb extract administration, using STATVIEW computer software (Abacus Concept, Inc., Berkeley, CA). P values less than 0.05 were considered to indicate statistical significance.

**RESULTS**

Table 1 shows the clinical data regarding the total number of days of aquiparous diarrhea during the experimental period, the days when bloody diarrhea was observed and the daily gains at 1 and 3 months after commencing treatment. During the experimental period, all calves in both groups showed clinical symptoms of diarrhea and they required occasional treatment with antidiarrheals. Regarding the number of days of aquiparous diarrhea, no significant difference was observed between the treatment group (8±4.2 days) and control group (10.2±4.1 days). However, bloody diarrhea was seen in the control group, but not in the treatment group. Nevertheless, the daily gains at 1 month (treatment, 0.5±0.2 kg; control, 0.6±0.4 kg) and

**Table 1: Clinical data regarding diarrhea and body weight gain in each calf**

Calf No.	Age of commencement	Total days of aquiparous diarrhea	Days of bloody diarrhea	Dairy gain (kg)	
				1 month	3 months
<b>Treatment</b>					
1	Day 6	8	ND	0.6	0.7
2	Day 6	8	ND	0.5	0.7
3	Day 7	2	ND	0.6	0.5
4	Day 11	8	ND	0.7	0.7
5	Day 6	14	ND	0.2	0.4
Mean				0.5±0.2	0.6±0.1
<b>Control</b>					
6	Day 4	8	Day 46	0.3	0.5
7	Day 7	7	Day 9	1.2	0.8
8	Day 4	11	Day 27	0.5	0.7
9	Day 11	8	Day 22	0.5	0.7
10	Day 8	17	Day 42	0.4	0.5
Mean				0.6±0.4	0.6±0.1

3 months (treatment,  $0.6 \pm 0.1$  kg; control,  $0.6 \pm 0.1$  kg) after commencing the experiment were not significantly different.

Figure 1 shows the weekly changes in the OPG count of the treatment and control groups. As in the preliminary study, we did not classify but only identified the oocysts of *E. bovis* and *E. zuernii* based on their morphology during the experimental period in the present study. In the treatment group, although some clinical diarrhea symptoms were observed, oocyst excretion patterns showed no increase or an evanescent increase (maximum OPG count: Calf 1, 27 on Day 28; Calf 2, 2720 on Day 35; Calf 3, 50 on Day 21; Calf 4, 1130 on Day 28; and Calf 5, 4145 on Day 28) during the experimental period. On the other hand, in the control group, oocyst excretion from all 5 calves increased to a maximum (maximum OPG count: Calf 6, 2730 on Day 35; Calf 7, 3470 on Day 28; Calf 8, 12360 on Day 21; Calf 9, > 20000 on Day 21; and Calf 10, 1910 on Day 42) simultaneously with the appearance of bloody diarrhea. Therefore, all the calves were diagnosed with acute coccidiosis at these points and were then treated. Thereafter, the OPG count of each calf in the control group tended to decrease after treatment and the symptoms of bloody diarrhea disappeared. However, no

significant differences in the OPG values ( $p = 0.09$ ) were observed between the treatment and control groups during the experimental period.

Figure 2 shows the results of the weekly blood examinations for monitoring the metabolic effects of the

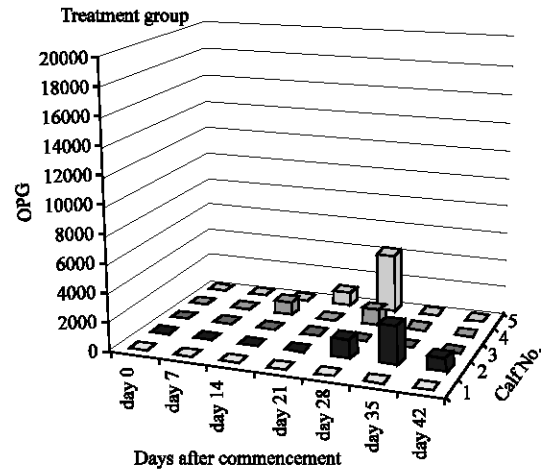


Fig. 1: Weekly changes in the OPG counts of the treatment and control groups

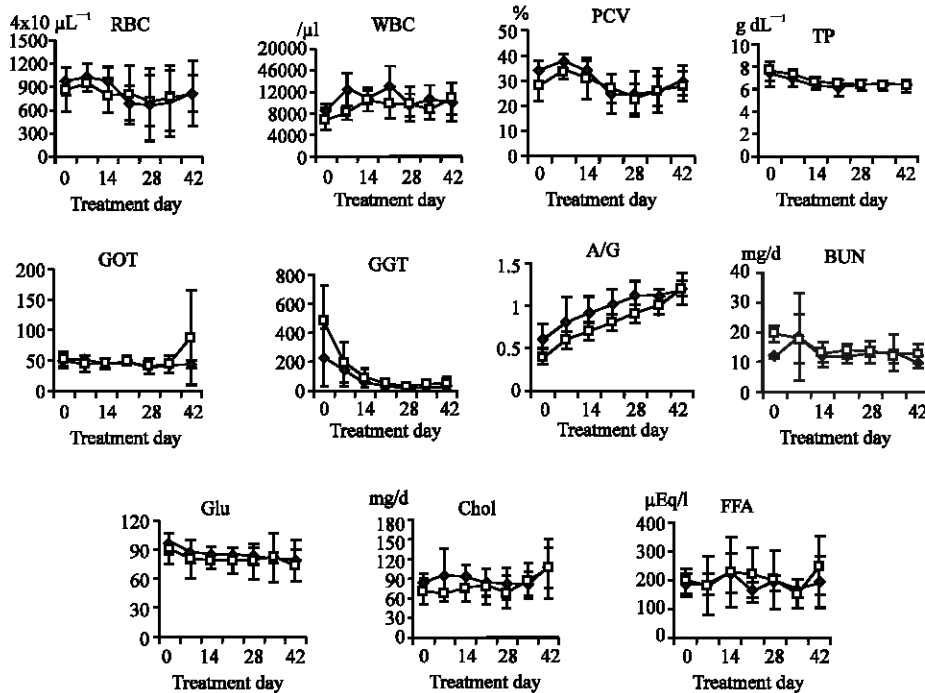


Fig. 2: Weekly changes in each parameter determined for blood examinations that were performed for monitoring the metabolic effects of the herb extract (Eimericox) administration. □, Treatment group; ○, control group

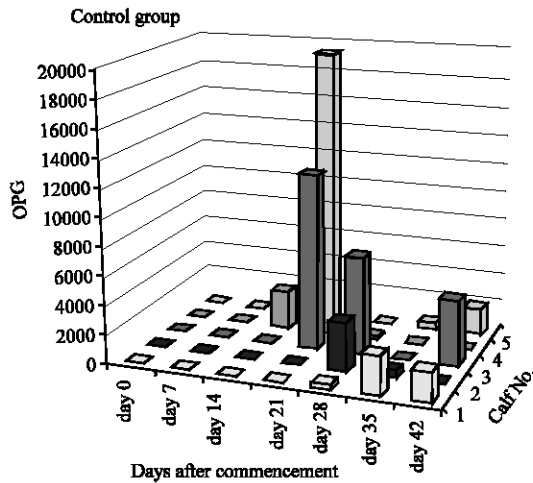


Fig. 3: Weekly changes in the haptoglobin concentrations during the experimental period

herb extract. No significant differences were observed between the treatment and control groups for all that parameters that are representative of metabolic, hepatic and renal functions as well as nutritional status. Additionally, Figure 3 shows the results of the weekly measurements of the HP concentration during the experimental period. In the treatment group, 4 positive points for the serum HP concentrations were detected simultaneously in 4 calves (Calf 1, 245  $\mu\text{g mL}^{-1}$ ; Calf 2, 115  $\mu\text{g mL}^{-1}$ ; Calf 4, 180  $\mu\text{g mL}^{-1}$  and Calf 5, 750  $\mu\text{g mL}^{-1}$ ) from Day 21 to Day 42 after commencing treatment. On the other hand, in the control group, 8 positive points for the serum HP concentrations were detected in 4 calves (Calf 7, 80  $\mu\text{g mL}^{-1}$ , 135  $\mu\text{g mL}^{-1}$  and 245  $\mu\text{g mL}^{-1}$ ; Calf 8, 165  $\mu\text{g mL}^{-1}$  and 460  $\mu\text{g mL}^{-1}$ ; Calf 9, 165  $\mu\text{g mL}^{-1}$  and Calf 10, 195  $\mu\text{g mL}^{-1}$  and 195  $\mu\text{g mL}^{-1}$ ), although Calf 8 showed a positive HP concentration on the day when the experiment was started.

### DISCUSSION

The results of the present study indicate that the herb extract almost entirely prevents coccidial infection when administered by daily oral supplementation from the suckling to weaning period, although it does not appear to exert antidiarrheal effect on calves. To the author's knowledge, this is the first report of successful application of natural herb extract for preventing the coccidial infection in calves without using any antibiotics for treatment of clinical coccidiosis.

Recently, in farm animal practice, considerable attention has been focused on the utilization of antibiotics not only for preventing the emergence of drug resistance

but also from the viewpoint of food safety<sup>[3,4]</sup>. Previous reports indicate that the application of some medicinal herb extracts is useful for the treatment of coccidiosis in avians as an alternative to antibiotics<sup>[3]</sup>. It has been hypothesized that the anticoccidial effect of the natural herb extract on calves occurs through the synergistic effects of both essential oils. These oils may oxidize the cell walls of coccidians as well as those of plants, which may improve the digestibility of nutrients. Thus, our results support the new concept of the application of herb extracts as an alternative supplement for preventing bovine coccidiosis.

In the present study, the OPG count of each identical calf was monitored at weekly intervals due to practical limitations. Increased OPG count in the treatment group may not have been recorded due to the sampling regime. However, previous reports strongly suggested the existence of a relationship between the increase in OPG count and the occurrence of bloody diarrhea under experimental conditions involving artificial infection by *E. bovis*<sup>[2]</sup>; the same tendency was observed in the present study. Therefore, we considered that weekly OPG counts along with daily observation of fecal condition were reasonable.

According to the manufacturer's instructions, the recommended dose of the natural herb extract for calves of 30-45 days of age is 4 g per 100 kg body weight per day. In the present study, the natural herb extract was administered at twice the recommended dose to permit the confirmation of not only the anticoccidial effect but also any clinical side effects. Based on the results of the daily clinical observations, blood examinations and daily gains of body weight, the natural herb extract might have minor side effects, particularly regarding hepatic, renal and general metabolic functions and it is thus a safe daily supplement. HP, which is a hemoglobin-binding protein and an acute inflammatory phase protein, appears in the serum following infection with *Rota*, *Herpes* or foot-and-mouth disease viruses as well as several bacteria and *Trypanosoma*; inflammation, trauma, hepatic lipidosis, or stress; or the administration of toxoid or chemical substances<sup>[6]</sup>. Thus, increased HP concentrations may reflect an inflammatory reaction in the intestinal tissues due to the development of the parasite and our results indicate that the natural herb extract may have anti-inflammatory effects on body tissues. In the present study, however, we obtained no evidence to verify these suppositions and further research, particularly regarding the pathological observations of intestinal tissues, is necessary. On the other hand, the natural herb extract does not appear to exert an antidiarrheal effect on calves. It is well known that diarrhea in calves occurs due to

various reasons and dietary diarrhea is one of the main causes during the neonatal period. Although the pathogens responsible for the diarrhea in the two groups were not examined in the present study, based on the results of the general condition, HP concentration and WBC count of each calf, it may be suggested that one of the causes of diarrhea in the treatment group might be non-infections, for example, indigestion of the mother's milk and not infection by some pathogens during the experimental period.

Recently, it was reported that oocysts of predominant coccidian species, such as *E. bovis* and *E. zuernii*, which have prepatent periods of at least 17 days, could be observed in 3-week-old calves; this indicates that the oocyst infection occurs within the first few days of life. Additionally, the increasing rate of *E. bovis* oocyst excretion in calves is directly correlated with IgM, IgG2 and IgA antibody levels measured at 3 weeks after birth and later; this suggests that these antibodies are synthesized in response to acquired infections<sup>[7]</sup>. Although little information is available regarding the actual mechanisms of immunity against ruminant coccidians, it is well-known that *E. bovis* infection induces immunity in cattle; the immunity rapidly develops within 2 weeks after oocysts appear in the feces and may persist for 3-6 months, a year, or longer<sup>[1]</sup>. Based on the results of our present study, we speculate that feeding of the herb extract, as a daily supplement to young calves, might be an effective method for not only preventing infection but also promoting endogenous protective acquired immunity against *Eimeria* infection in young calves. Further studies are required to clarify the effects of the natural herb extract as a daily supplement on the development of protective immune responses in calves.

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