

# Probiotic therapy as an alternative to antibiotics in calves with moderate diarrhea

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## Introduction

Neonatal calf diarrhea, which mainly affects animals before weaning, is easy to diagnose and is characterized by frequent passing of soft feces (less than 10% dry content). Long-term utilization of broad-spectrum antibiotic therapy is being followed in order to control these infections. The use of probiotics for diarrhea treatment may have value as an alternative therapy that prevents the use of antibiotics, thereby reducing the emergence and spread of antibiotic-resistant bacteria and residual antibiotics in dairy foods, meat, and milk. We aimed to assess the utility of the probiotic products containing 3 probiotics (*Enterococcus faecalis* T-110, *Clostridium butyricum* TO-A, and *Bacillus mesentericus* TO-A) in shortening the duration of diarrhea symptoms and normalizing the consistency of the feces. In addition, we assessed whether probiotics therapy could be an alternative to antibiotic therapy in calves with moderate diarrhea.

## Materials and Methods

Ten Japanese black-breed calves with moderate diarrhea,  $10.0 \pm 4.1$  days of age, were enrolled in this study. Calves with moderate diarrhea were divided into the probiotic group (n=5) that received 100 g of probiotics (BIO-THREE for animals, Toa Pharmaceutical CO., Ltd., Tokyo, Japan) per day for 8 days from the initial examination, and the control group (n=5) that received traditional antibiotic therapy. If improvement of fecal status was observed, administration of antibiotics was terminated. The fecal characteristics were recorded as 4 scores (1=normal, 2=loose, 3=muddy, and 4=watery) on every day until the final examination (d 8). Venous blood samples were collected for measuring diamond oxidase (DAO) activity using a specific ELISA system at day 1, 3, and 8. T-cho, BUN, TP, Alb, AST, GGT, and TG were also measured using an automatic analyzer; the data are shown as means  $\pm$  SD. Regarding normally distributed data, the mean values for each dependent variable were compared between groups using the Student's t-test, and were compared among each date of treatment in each group using Dunnett's test after ANOVA with the F test. Regarding non-normally distributed data, the Mann-Whitney

U-test was employed for comparisons between groups. The significance level was set at  $P < 0.05$ .

## Results

There was no significant difference in the treatment period and cure rate of up to 8 (each 4/5) between the control and the probiotic group. Fecal scores in the control and probiotics groups on d 8 were  $1.2 \pm 0.4$  and  $1.2 \pm 0.4$ , respectively, which were significantly lower than those in the control ( $3.6 \pm 0.5$ ,  $P < 0.01$ ) and probiotics groups ( $3.4 \pm 0.5$ ,  $P < 0.01$ ) on d 1. In comparisons across treatments, such as probiotics and traditional antibiotic therapy, significant differences in DAO activity and T-Cho concentration in serum were observed, but there were no significant differences in the other parameters measured in this study. Although there were no time-course changes in plasma DAO activity in the control group, the DAO in the probiotics group on d 3 ( $6.80 \pm 0.53$ ) and d 8 ( $7.34 \pm 0.54$ ) was significantly higher than that on d 1 ( $5.39 \pm 0.53$  IU/L). As with DAO, the T-Cho concentration in the probiotics group on d 8 ( $126.8 \pm 15.8$ ) was significantly higher than that on d 1 ( $87.4 \pm 20.7$  mg/dL,  $P < 0.05$ ), whereas there were no time-course changes in plasma T-Cho concentration in the control group.

## Significance

In this study, the treatment days and improvement in fecal characteristics of calves that received probiotics were not different from those of traditional antibiotic therapy. However, calves treated with probiotics had a significantly higher plasma DAO concentration, which is an indicator of intestinal villi repair, than calves receiving traditional antibiotic treatment. In the probiotic group, absorption of nutrient components, such as T-Cho, in the intestinal tract may be improved by repairing the intestinal mucosa and villi. Treatment of calf diarrhea using probiotics as an alternative to antibiotics is extremely promising because it not only solves the problem of residual antibiotics and resistance, but also improves nutrition due to repair of the intestinal mucosa.