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Evaluation of probiotic therapy for calf diarrhea with serum diamine oxidase activity as an indicator

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Abstract

The objectives of study were to examine whether probiotic therapy is alternative to antibiotic therapy in diarrheic calves, and to examine whether the serum diamine oxidase (DAO) activity in calves are related to diarrhea. Twenty-two diarrheic Japanese black calves were received probiotics (n=11) or antibiotics (n=11) therapy for up to 8 days from the initial examination, respectively. There was no significant difference between treatments in the variations of fecal score and serum biochemical value. Serum DAO activity increased significantly in only probiotic treatment, from 64.4 ± 7.2 on day 1 to 76.3 ± 5.1 IU/ml on day 8. Our results suggested that probiotics therapy could be alternative to antibiotic therapy, and could be affecting serum DAO activity in diarrheic calves.

Key Words: calf, diamine oxidase, probiotics

Calf diarrhea remains the most common cause of death in beef and dairy calves, and continues to be a major cause of economic loss for the cattle industry. The long-term utilization of broad-spectrum antibiotic therapy is employed as a strategy to control this disease. Constable[4)] noted that use of antibiotics is justified even in nonbacterial diarrhea as the possibility of overgrowth of *Escherichia coli* in the small intestine may occur. Walker et al.[21)] also reported antibiotic therapy is commonly recommended regardless of the aetiological agent. However, if the cause of diarrhea is not bacteria, the antibiotic is not sensitive. Furthermore, because of concerns that antibiotic use in food animal production has the potential to increase antibiotic resistance in human pathogens, it has been demanded strategies to reduce antibiotic use are needed. In this way, the efficacy of antibiotic agents in treating calf diarrhea is controversial.

This situation is has prompted increasing interest in the development of veterinary probiotics[8)]. Rolf[18)] suggested that there are many potential advantages to probiotics over conventional therapy, including relatively low cost, the fact that probiotics are unlikely to

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increase the incidence of antibiotic resistance, and the multiple mechanisms by which probiotics presumably inhibit pathogens. Indeed, several researchers showed the usefulness of probiotics in gastrointestinal diseases in calves[7,8,20]. Renaud et al.[17] demonstrates a multispecies probiotic product, commercially available probiotics product in Canada, contained *Pediococcus acidilactici, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum* bolus administered to calves at the onset of diarrhea reduced the duration of diarrhea. Likewise, Hayakawa et al.[11] clarified that commercially available probiotics product in Japan containing *Bacillus mesentericus* strain TO-A, *Clostridium butyricum* strain TO-A and *Enterococcus faecalis* strain T-110 induced the enlargement of villous height and prevented muscle layer thinning in the small intestine of weaning piglets. Therefore, it seems utility the probiotic therapy as an alternative to antibiotic therapy for calves with diarrhea, especially non-bacterial infection. Unfortunately, the effect of Japanese probiotics product on calf diarrhea has not been studied.

Several studies related to the treatment of calves with diarrhea have been previously reported in which indirect indicators, such as mortality, treatment period, and fecal character, were used to evaluate the therapeutic effects[5,6,7]. However, evaluating the treatment of diarrhea according to the states of intestinal mucosa seems to be more meaningful. Serum Diamine oxidase (**DAO**) activity was initially reported as a histamine oxidoreductase in 1930[2]. Mucosal epithelial cells in the small intestine, particularly villi, were subsequently shown to be very active and involved in the control of cell proliferation[22]. Since DAO produced in the villi of the small intestine is metabolized in the liver soon after its release into the blood, serum DAO activity in the blood reflects the state of the villi of the small intestine in real time[3]. Therefore, in recent years, various clinical studies have reported indices reflecting the state of intestinal villi[12]. Several researchers showed that serum or plasma DAO activity could be a useful predictor of intestinal mucosal damage in human[15,16]. However, to our knowledge, no report has clarified the relationship between bovine intestinal condition and serum or plasma DAO activity.

The objectives of this study were (1) to examine whether probiotic containing three kinds of bacterial species, commercially available probiotics product in Japan, and approved for the treatment of simple diarrhea is alternative to empiric antibiotic therapy in non-bacterial infectious diarrheic calves, and (2) to examine whether the serum DAO activity in calves are related to diarrhea.

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the School of Veterinary Medicine, Rakuno Gakuen University (Approval#: VH15C21). This study was conducted on calves with diarrhea that were requested for treatment by farmers at large animal hospitals in Miyagi prefecture between June 2015 and November 2017.

Twenty-two Japanese black calves from 15 different farms, aged 10.2 ± 2.7 (mean ± SD) days old and 45.3 ± 10.6 kg of body weight measured by weight estimation measure (Fujihira Industry Co., Ltd., Tokyo, Japan) at first day, were enrolled in this study. The veterinary practitioner classified fecal characteristics into four scores every day until day 8, as shown below 1: normal, 2: loose, 3: muddy, and 4: watery. Calves with diarrhea were examined for four pathogens (rotavirus, coronavirus, *Cryptosporidium parvum*, and K99 *E. coli*) using the fecal strip test (Tetrastrips, Bio-X Diagnostics S.A., Rochefort, Belgium) on day1. Regardless of the results of the strip fecal test, twenty-two calves with diarrhea were alternately divided into two groups of empiric therapy using antibiotics (antibiotic group, n=11) or probiotic therapy using the product containing three kinds of bacterial species (probiotic group, n=11). Both groups consisted of calves, who belonged to eight different farms, respectively.

All calves were raised with their dams after birth and were allowed to suckle freely in a

nursing barn and had ad libitum access to hay and water. In the present study, calves with the diarrhea were clinically normal except for the presence of diarrhea, without clinical signs such as fever (more than 39.5°C), decline in suckle reflex and eyes deeply sunkens. Therefore, the veterinarian determined that parenteral fluid therapy was not necessary. Indeed, no calves have been received fluid therapy during treatment period.

In addition, as control group, ten healthy Japanese black calves without diarrhea, aged 11.5 ± 0.8 days old, were also enrolled in the study to obtain reference values for serum DAO activity and blood biochemical test values. All calves in control group were clinically normal, as determined on the basis of vital signs, attrition, food and water intake, and urine and feces production. These calves were also kept on farms in the same area.

The regimen of the antibiotic group was as follows: 10 mg/kg of ampicillin (Kyoritsu Seiyaku, Tokyo, Japan) was intramuscularly administered on until day 5 from day1 as the first medication, and then 10 mg/kg of kanamycin sulfate (Meiji Seika, Co., Tokyo, Japan) was thereafter administered until day 8. The probiotic group received 100 g of the probiotic product (Bio-Three for animal, Toa Pharmaceutical Co., Ltd., Tokyo, Japan) including *Bacillus mesentericus* strain TO-A, *Clostridium butyricum* strain TO-A and *Enterococcus faecalis* strain T-110. The probiotic was dissolved in 500 ml of warm water and then orally administered using a catheter every day. In addition to antibiotics or probiotics administration, 10 mg/kg of menbutone (Boehringer Ingelheim Japan, Inc., Tokyo, Japan) were administered into both groups. There was no other administration and except for administration of probiotics or antibiotics all treated the same. Calves with diarrhea were determined to be cure when fecal characteristics became score 1. All treatments were terminated at the time and were observed untreated until day 8.

On days 1, 3 and 8, the veterinary practitioner collected blood samples. They recorded general physical findings and fecal scores every day until day 8. The number of days for recovery and the cure rates of both groups were investigated. Blood samples were collected from the jugular vein using a sterile plastic disposable syringe and needle and 10 ml was immediately stored in a vacuum blood collection tube on days 1, 3 and 8 after the initial examination. Blood samples were then centrifuged at 3,000×g at 4ºC for 10 min in order to harvest serum. Serum samples were stored at -20°C until assayed. In the serum biochemical analysis, total cholesterol **(T-Cho)**, blood urea nitrogen (**BUN**), aspartate transaminase (**AST**), gamma-glutamyl transferase (**GGT**), and triglycerides (**TG**) were measured using enzyme methods with the Discrete Method Clinical chemistry automatic analyzer (Dade Behring, Inc., Deerfield, IL, U.S.A.). Serum total protein (**TP**) and serum albumin (**Alb**) levels were measured using the Biuret and Bromocresol Green methods, respectively. Serum DAO activity was assessed by a specific ELISA system (Bovine DAO ELISA kit, My BioSource Inc., San Diego, CA, U.S.A).

Normally distributed data are reported as mean ± standard deviation (**SD**), and non-normally distributed data are expressed as median and ranges. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics[13]. We processed serum biochemical values for each dependent variable (treatments and times) with two-way repeated measures ANOVA. If interaction was found, measured dependent variables were compared between and within groups for each sample collection period using the student's *t*-test or Mann-Whitney *U*-test after the *F*-test and Dunnett's test, respectively. The significance level was $P < 0.05$.

Table. The mean values of blood biochemical test in antibiotic, probiotic and control groups.
Antibiotic group is received empiric antibiotic therapy (n=11). Probiotic group is received probiotic therapy (n=11). Control group is clinically normal (n=10).

Parameter		Day1 (Initial examination)	Day3	Day8	Control
T-cho (mg/dl)	Antibiotic	90.7±33.0	91.7±40.6	122.1±38.5	128.5±46.6
	Probiotic	99.7±18.8	111.8±28.5	142.2±25.8	
TG (mg/dl)	Antibiotic	18.2±19.6	19.3±14.1	30.1±15.9	27.2±20.5
	Probiotic	19.9±9.8	33.9±17.2	43.6±20.4	
BUN (mg/dl)	Antibiotic	18.4±4.6	17.6±8.2	10.7±2.0	18.7±12.5
	Probiotic	14.4±2.8	12.4±3.9	10.4±1.7	
TP (g/dl)	Antibiotic	6.4±0.8	6.2±0.8	6.0±0.6	6.7±0.6
	Probiotic	6.0±0.3	5.9±0.4	5.8±0.4	
Alb (g/dl)	Antibiotic	3.0±0.2	3.0±0.3	3.2±0.4	3.2±0.3
	Probiotic	3.0±0.2	3.0±0.3	3.1±0.2	
AST (IU/l)	Antibiotic	44.0±7.8	50.7±9.3	49.8±6.1	58.8±6.1
	Probiotic	45.2±6.7	54.1±7.7	51.0±7.5	
GGT (IU/l)	Antibiotic	116.6±45.6	86.7±26.5	56.8±16.9	187.5±119.9
	Probiotic	156.5±96.3	118.5±65.2	77.5±40.7	

In each groups, rotavirus and *C. parvum* were detected, respectively: two of 11 calves (18.2%) in probiotic group and five of 11 (45.5%) in antibiotic group. Rotavirus, coronavirus, *C. parvum*, and K99 *E. coli* were not detected in the other calves. Rotavirus and *C. parvum* damages the small intestinal villi, which results in failure of electrolyte and water absorption (malabsorptive diarrhea)[9]. It is widely accepted that damage to the intestinal mucosa is more severe in malabsorptive diarrhea than secretory diarrhea. These pathogens could cause prolonged diarrhea in calves. However, in probiotic group, diarrhea with Rotavirus (n=1) and *C. parvum* (n=1) detected was cured in 3 and 7 days, respectively. Similarly, in antibiotic group, diarrhea with Rotavirus (n=4) and *C. parvum* (n=1) detected was cured in 5 (min-max, 2–7 days) and 5 days, respectively. Number of treatment day in the probiotic and antibiotic groups were 4 (min-max, 3–8) and 5 (min-max, 2–8), respectively. Similarly, cure rates were 81.8% (9/11) in the probiotics group and 90.9% (10/11) in the antibiotic group, respectively. Fecal scores in the probiotic and antibiotic groups were 3.0 (min-max, 3.0–4.0) and 4.0 (min-max, 3.0–4.0), respectively, on day 1. No significant differences were observed in fecal scores between the probiotic and antibiotic groups on day 1. In addition, there was no significant difference in fecal score between both groups during examination. As a result, fecal scores in the probiotic and antibiotic groups were 1 (min-max, 1–2) and 1 (min-max, 1–4), respectively, on day 8. Cure rate and fecal score on day 8 has not significant differences between both groups. In addition, no there any died and/or exacerbated clinical findings for calves with a diarrhea during an observation period. The variations of the blood biochemical analysis are summarized in Table. The variations of serum concentrations of T-Cho, TG, BUN, TP, Alb, AST and GGT statistics by repeated measures ANOVA revealed no significant difference in interaction between treatment and time, respectively. Generally, variations of T-Cho, TG, TP and Alb concentrations were attributed to improvements in nutritional states. However, our results revealed that probiotics supplementation, at most 8 days of supplementations, did not affects these parameter compared with antibiotics administration. But, Maldonado et al.[14] demonstrated that the calves fed with probiotics, including 4 species of lactic acid bacteria, for 60 days evidenced an improvement in nutritional parameters, body condition and weight gain. The

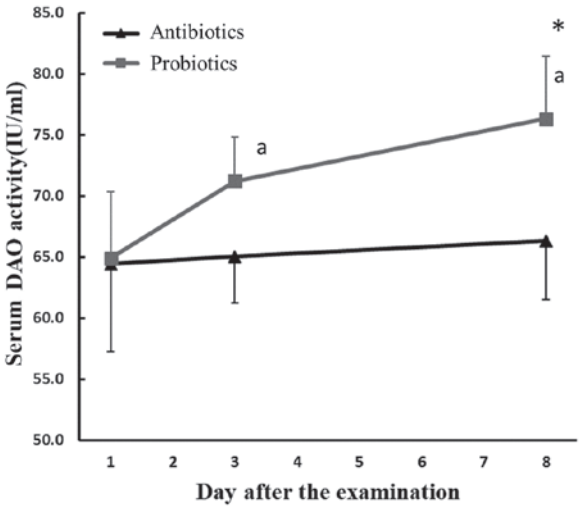

Fig. Changes in serum DAO activity of antibiotic group and probiotic group and comparison with control group at initial examination.
Antibiotic group is received empiric antibiotic therapy (n=11). Probiotic group is received probiotic therapy (n=11). Control group is clinically normal (n=10). The initial examination was set as day 1.
*: $P < 0.001$ Significant difference was observed in interaction between treatment and time by two-way repeated mesures ANOVA.
a: $P < 0.01$ vs the same day antibiotic group by Student's t-test.

usefulness of probiotic is also reported by further studies[1,10,19]. Possibly, the effect of probiotics therapy may be influenced by the species of bacteria and the supplementation period. In this study, probiotics therapy seems to have the same ability to resolution of diarrhea as antibiotics therapy. In the first place, because non-bacterial diarrhea was not targets for antibiotics, diarrhea may have improved despite the administration of antibiotics in this study. Although our suggestion has limitations due to differences in pathogen detection, probiotic product containing *Bacillus mesentericus* strain TO-A, *Clostridium butyricum* strain TO-A and *Enterococcus faecalis* strain T-110 could be alternative to empiric antibiotic therapy in non-bacterial infectious diarrheic calves.

It is really mystifying that significant variation in serum DAO activity was observed in only probiotic group (Fig). Serum DAO activity on day 1 in the probiotic and antibiotic groups were 64.4 ± 7.2 and 64.9 ± 5.4 IU/ml, respectively. Serum DAO activity of all calves with diarrhea on the initiation of treatment (64.7 ± 7.7 IU/ml, n=22) was significantly lower than that of reference value of 153.9 ± 72.0 IU/ml, obtained from control group. Based on the repeated measures ANOVA, there were significant difference in interaction between treatment and time ($P < 0.001$). Sequential change in serum DAO activity of the antibiotic group was not observed (66.3 ± 4.9 IU/ml on day 8). Although the serum DAO activity of the calves in the probiotic group did not return to reference values obtained from control group, serum DAO activity on day 8 (76.3 ± 5.1 IU/ml) was significantly higher than that of the initiation of treatment ($P < 0.01$). Further, serum DAO activity of the probiotic group on day 8 was significantly higher than that of antibiotic group ($P < 0.01$). In this study, the serum DAO activity of the probiotic group was significantly elevated compared to the antibiotics group, but it was not reflected in the recovery of fecal score or the treatment period. However, because the serum DAO activity in diarrheic calves were significantly lower than that in healthy calves, we could not concluded that there was no relationship between diarrhea and serum DAO activity in calves.

Our results suggested that probiotic therapy containing *Bacillus mesentericus* strain TO-A, *Clostridium butyricum* strain TO-A and *Enterococcus faecalis* strain T-110 could be alternative to empiric antibiotic therapy in non-bacterial infectious diarrheic calves. In addition, we have shown that serum DAO activity decreased in calves with diarrhea and that probiotic therapy could affect recovery of serum DAO activity. Further study is needed to clarify whether serum DAO activity in diarrheic calves reflect the intestinal damage or systemic nutritional state, as in human.

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