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Feeding of Lactic Acid Bacteria and Yeast Affects Fecal Flora of Holstein Calves

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Abstract: Neonatal calves are frequently affected by diarrhea which is the main cause of mortality and of economic loss in the cattle industry. The previous report showed that addition of *Lactobacillus plantarum* Chikuso-1 and *Candida* sp. CO119 to milk replacer significantly suppressed diarrhea of holstein calves but the mechanisms remain unknown. In the study, the effect of feeding of Chikuso-1 and CO119 (bacteria-fed) on fecal bacterial flora of holstein calves was examined. Fecal samples were collected on days 0, 3, 8, 15 and 22 from control and bacteria-fed calves and inoculated on the selective agar media for Lactic Acid Bacteria (LAB), coliform, aerobic bacteria, bacilli and clostridia, respectively for bacterial enumeration. Feeding of Chikuso-1 and CO119 significantly increased the number of fecal LAB on day 3 and tended to increase on day 8. The present results suggest that the addition of Chikuso-1 and CO119 improves fecal bacterial flora of holstein calves in early stage of lactation period when diarrhea is severer.

Key words: Lactic acid bacteria, yeast, probiotics, diarrhea, fecal flora, holstein calves

INTRODUCTION

Neonatal diarrhea is the main cause of calf death and economically serious problem in the cattle industry. Antibiotics are generally used as feed additives for milk replacer to prevent calf diarrhea (Braidwood and Henry, 1990), a need for establishment of new technologies alternative to antibiotics has been increasing because of the risk of the emergence of antibiotic-resistant bacteria from cattle industry (Fey *et al*., 2000). Among numerous candidate technologies, probiotics are thought to be prospective substitutions of antibiotics (Callaway *et al*., 2004).

The research by Kawakami *et al*. (2010) has shown that addition of Lactic Acid Bacteria (LAB) and yeast strains, *Lactobacillus plantarum* Chikuso-1 (Cai *et al*., 2003) and *Candida* sp. CO119 (Kawakami *et al*., 2010) to milk replacer significantly decreases fecal scoring of holstein calves suggesting that the strains have a probiotic ability to suppress calf diarrhea. But the mechanisms remain unknown. Probiotics are generally defined as microbials to affect beneficially the host animal by improving its intestinal microbial balance (Fuller, 1989), it must be necessary to verify whether feeding of Chikuso-1 and CO119 could affect calf enteric environment.

In the present study therefore, the effects of feeding of Chikuso-1 and CO119 on fecal bacterial flora of holstein calves were examined. Bacterial enumerations from calf fecal samples were performed for the detection of aerobic bacteria, LAB, coliform, bacilli and clostridia, respectively. The results suggest that Chikuso-1 and CO119 could function as probiotics by improving bacterial flora, especially the number of LAB of holstein calves in early stage of lactation period when diarrhea is severer.

MATERIALS AND METHODS

Animals, feeding and treatment: Animals, feeding and treatment were described previously (Kawakami *et al*., 2010). Eight holstein calves at 6.3±1.5 days of age were divided into 2 groups, control (n = 4) and bacteria-fed (n = 4).

Bacteria-fed group received milk replacer containing Chikuso-1 (3.7×10^{11} Colony Forming Unit (CFU) head^{-1}) and CO119 (2.6×10^{9} CFU head^{-1}) in the every morning for 28 days whereas no bacterial treatment in control group.

Bacterial enumerations from fecal samples: In the morning of days 0 (a day before beginning of the bacterial feeding), 3, 8, 15 and 22 fecal samples were removed by grab sampling from the rectums.

About 10 g of the samples was blended with 90 mL of sterilized distilled water and serial dilutions from 10^{-1}-10^{-8} were made. From each dilution, 0.05 mL of

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suspension was spread on Nutrient Agar (Eiken Chemical, Tokyo, Japan), de Man-Rogosa Sharpe Agar (Becton, Dickinson and Company, Maryland, USA) and Blue Light Agar (Eiken Chemical) for enumeration of aerobic bacteria, LAB and coliform, respectively. Aliquot of the dilution was heated in 75°C for 15 min and spread on Nutrient Agar and Clostridia Count Agar (Eiken Chemical) for enumeration of bacilli and clostridia, respectively. Colonies were enumerated after 48 h aerobical incubation in an incubator (Sanyo Electric Co. Ltd. Tokyo, Japan) at 30°C for aerobic bacteria, coliform and bacilli or in an anaerobical glove box (Hirasawa Co. Ltd., Tokyo, Japan) at 30°C for LAB and clostridia, respectively. Results were presented as a logarithmic conversion of CFUs of the bacteria.

Statistical analysis: Statistical analyses were performed using SAS (2001). Differences of the CFUs of the fecal bacteria were evaluated by repeated measurements ANOVA using the Mixed procedure of SAS. The statistical model included fixed effects for treatment day and treatment x day interaction with calf as random effect. If the interaction was significant, simple effects were calculated by using the slice option for the LSMEAN statement. The level of significance was set at $p<0.05$ and at $p<0.1$ for a trend.

RESULTS AND DISCUSSION

Bacterial enumerations in calf fecal samples were shown in Table 1. There were no significant effects of bacterial treatment on the numbers of aerobic bacteria, LAB, coliform, bacilli and clostridia and on the LAB/coliform ratio in the calf fecal samples. The effect of day was also non-significant. Because a significant interaction of treatment x day was observed on the number of fecal LAB (Table 1, $p<0.01$), simple effects were calculated by using the slice option for the LSMEAN statement of SAS (Fig. 1). Feeding of Chikuso-1 and CO119 significantly increased the number of fecal LAB on day 3 (Fig. 1, $p<0.01$) and tended to increase on day 8 (Fig. 1, $p<0.1$). The number of LAB in feces is a widely-used index for estimation of intestinal microbial balance of host animals (Fuller, 1989). The present results showed that feeding of Chikuso-1 and CO119 significantly increased the number of fecal LAB of holstein calves suggesting that the bacteria have a probiotic ability to improve intestinal microbial flora. However, the effect was limited in the early stage of lactation period.

The previous report also showed that addition of Chikuso-1 and CO119 to milk replacer significantly decreased calf fecal scoring only in the early lactation period (Kawakami *et al*., 2010). In addition, it is known that calves are affected by diarrhea more severely at early stage of lactation period (Virtala *et al*., 1996). These suggest that probiotics fulfill their functions more effectively when disease condition of host is worse. It is necessary, therefore to apply probiotics to host animals at the stage of higher risk of diseases.

In the present study, the numbers of aerobic bacteria, coliform, bacilli and clostridia in addition to LAB/coliform ratio in the calf feces were not affected by feeding of Chikuso-1 and CO119. Coliform include the genera *Citrobacter*, *Escherichia*, *Enterobacter* etc. which are opportunistic pathogens associated with diarrhea (Plews *et al*., 1985).

Abu-Tarbush *et al*. (1996) reported that the number of coliform was higher than that of LAB in the calves suffering from diarrhea but lower in healthy calves, suggesting that coloform number and LAB/coliform ratio could be used as indices for estimating intestinal microbial flora associated with diarrhea. However, the results of previous reports were controversial about the effect of probiotics on the number of fecal coliform of calves. Agarwal *et al*. (2002) reported that feeding of *Lactobacillus acidophilus*-15 strain significantly decreased fecal coloform number and suppressed diarrhea but Ellinger *et al*. (1980) showed that feeding of *Lactobacillus acidophilus* Fagro 606 strain did not significantly affect fecal coliform number. Both

Table 1: Effects of addition of Lactic Acid Bacteria (LAB) and yeast to milk replacer on fecal bacterial flora of holstein calves. Data was presented as a logarithmic conversion of Colony Forming Units (CFUs) except LAB/Coliform ratio

	Treatment			p value		
Bacteria	Control	Bacteria-fed	SE	Treatment	Day	Treatment x Day
LAB	7.91	8.12	0.17	NS	NS	**
Coliform	8.01	7.97	0.23	NS	NS	NS
LAB/Coliform	1.00	1.03	0.04	NS	NS	NS
Aerobic bacteria	8.13	8.09	0.20	NS	NS	NS
Bacilli	4.23	4.40	0.27	NS	NS	NS
Clostridia	4.34	4.14	0.38	NS	NS	NS

**: Significant difference ($p<0.01$). SE: Standard Error of the mean; NS: Not Significant

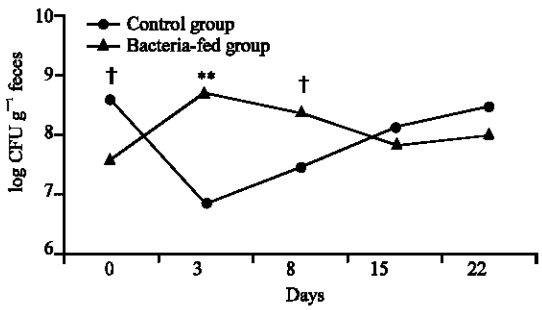

Fig. 1: The number of LAB in the feces of Holstein calves. **: Significant difference between the treatment groups ($p<0.01$); †: Difference between the treatment groups ($p<0.1$)

previous and present reports examined naturally occurring diarrhea in calves without experimental infection of specific pathogens causing the disease. The inconsistency of the results therefore, might arise from the difference of pathogenesis of diarrhea between reports. Further study is required to clarify whether the difference of pathogen causing diarrhea contribute to the change of intestinal bacterial flora in the holstein calves.

CONCLUSION

Addition of *Lactobacillus plantarum* Chikuso-1 and *Candida* sp. CO119 to milk replacer significantly increased the number of fecal LAB on day 3 and tended to increase on day 8.

The present results suggest that the feeding of Chikuso-1 and CO119 improves fecal bacterial flora of holstein calves in early stage of lactation period when diarrhea is severer.

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