

Effect of Probiotics on Bacterial Flora of Various Gastrointestinal Regions in Holstein Calves

Shin-Ichi Kawakami, Tomoya Yamada, Naoto Nakanishi and Yimin Cai

Research Team for Functional Feed, Nasu Research Station,

National Institute of Livestock and Grassland Science, Nasushiobara, Tochigi, 329-2793, Japan

Abstract: Calves are often suffering from diarrhea. The previous researches showed that addition of two selected strains, *Lactobacillus plantarum* Chikuso-1 and *Candida* sp. CO119 to milk replacer significantly suppressed calf diarrhea and increased the number of fecal Lactic Acid Bacteria (LAB). It remains unknown however, whether feeding of the microbe could affect bacterial flora in the specific areas of gastrointestinal tract in host animals. In the present study, the effects of feeding of Chikuso-1 and CO119 (microbe-fed) on bacterial flora of various gastrointestinal regions were examined in Holstein calves. Gastrointestinal contents of control and microbe-fed calves were collected from rumen, duodenum, jejunum, ileum, cecum and colon, respectively and immediately inoculated on the selective agar media for LAB, coliform, aerobic bacteria, bacilli and clostridia for bacterial enumeration. Feeding of the microbe significantly increased the number of LAB and LAB/coliform ratio in rumen, duodenum and jejunum. The present results suggest that feeding of Chikuso-1 and CO119 improves bacterial flora on rumen and upper small intestine in the gastrointestinal tract of Holstein calves.

Key words: Lactic acid bacteria, yeast, probiotics, diarrhea, gastrointestinal tract, bacterial flora, Holstein calves

INTRODUCTION

Calves are often suffering from diarrhea which is one of the main causes of calf mortality and morbidity and also of economic loss in the cattle industry. Antibiotics are commonly the first choice for treatment and prevention of calf diarrhea (Braidwood and Henry, 1990). But development of new technologies alternative to antibiotics is strongly needed because of the increasing risk of emergence of antibiotic-resistant bacteria from cattle industry (Fey *et al.*, 2000). Among numerous candidate technologies, probiotics are expected to serve as one of the alternatives to antibiotics (Callaway *et al.*, 2004).

It shown that addition of two selected Lactic Acid Bacteria (LAB) and yeast strains, *Lactobacillus plantarum* Chikuso-1 (Cai *et al.*, 2003) and *Candida* sp. CO119 to milk replacer significantly promotes growth and suppresses diarrhea in Holstein calves (Kawakami *et al.*, 2010a). Feeding of the microbe is also shown to increase the number of fecal LAB of the calves (Kawakami *et al.*, 2010b), it is likely that Chikuso-1 and CO119 would function by modifying intestinal bacterial flora of host animals. However, it has been known that bacterial flora in feces do not represent those in gastrointestinal contents such as in ileum (Hartman *et al.*, 1966;

Smiricky-Tjardes *et al.*, 2003). These suggest that bacterial enumerations from various gastrointestinal contents should be needed to clarify in part the mode of action of probiotics to host animals.

In the present study therefore, the effects of feeding of Chikuso-1 and CO119 on bacterial flora of contents collected from various regions of gastrointestinal tract were examined in Holstein calves. Gastrointestinal contents were collected from rumen, duodenum, jejunum, ileum, cecum and colon, respectively. The results suggest that the microbe treatment significantly increased the number of LAB and LAB/coliform ratio in upper regions of gastrointestinal tract of Holstein calves.

MATERIALS AND METHODS

Animals, feeding and treatment: All animal experiments in the present study were conducted according to the animal care and use guidelines of the National Institute of Livestock and Grassland Science of Japan.

Animals, feeding and treatment were described before (Kawakami *et al.*, 2010a). Briefly, eight Holstein calves at 6.3 ± 1.5 days of age were divided into 2 groups, control ($n = 4$) and microbe-fed ($n = 4$). Microbe-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 microbe treatment in control group. On 29th day from the beginning of the experiment, calves were sacrificed by exsanguination under anesthesia. Gastrointestinal contents were collected from rumen, duodenum, jejunum, ileum, cecum and colon, respectively and immediately used for bacterial enumerations.

Bacterial enumerations from gastrointestinal contents:

Ten gram of the gastrointestinal contents 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 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 institute (2001). Differences of the CFUs of the gastrointestinal bacteria were evaluated by repeated measurements ANOVA using the MIXED procedure of SAS. The statistical model included fixed effects for treatment, gastrointestinal region and treatment x region 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 of contents from various gastrointestinal regions were shown in Table 1. There

were significant effects of microbe treatment on the number of LAB and LAB/coliform ratio (Table 1, $p < 0.01$). Significant effects of region were observed in the number of LAB, coliform, aerobic bacteria, bacilli and LAB/coliform ratio (Table 1, $p < 0.01$). Because significant interactions of treatment x region were observed on the number of LAB and LAB/coliform ratio (Table 1, $p < 0.01$), simple effects were calculated by using the slice option for the LSMEAN statement of SAS (Fig. 1).

The number of LAB and LAB/coliform ratio in microbe-fed group were significantly increased in rumen, duodenum and jejunum compared with those in control group (Fig. 1a and c, $p < 0.05$ and 0.01). The present results suggest that feeding of Chikuso-1 and CO119 increased the number of LAB and LAB/coliform ratio in rumen and upper small intestine not in ileum and large intestine in Holstein calves. The data about large intestine was expected because we previously observed that the microbe treatment increased the number of fecal LAB of the calves in their early stage of lactation period not in their late stage (Kawakami *et al.*, 2010b).

No differences in ileal bacterial flora might account for the previous results (Kawakami *et al.*, 2010c) showing that feeding of Chikuso-1 and CO119 did not increase leukocyte phagocytic activities in Holstein calves because ileum are known to contain peyer's patches which are organized lymphoid tissue and play important roles in gut immunity (Yasuda *et al.*, 2006).

As shown in Fig. 1b, the number of coliform in microbe-fed group seems to be smaller than that in control group in upper gastrointestinal tract such as rumen, duodenum and jejunum.

It has been previously reported that coloform number and LAB/coliform ratio in calves could be used as indices for estimating intestinal microbial flora associated with diarrhea (Abu-Tarboush *et al.*, 1996). Thus, feeding of Chikuso-1 and CO119 might suppress diarrhea by increasing LAB/coliform ratio and decreasing coliform number in upper gastrointestinal tract of Holstein calves.

Table 1: Effects of addition of two selected Lactic Acid Bacteria (LAB) and yeast strains, *Lactobacillus plantarum* Chikuso-1 and *Candida* sp. CO119 to milk replacer on bacterial flora of various gastrointestinal regions in Holstein calves

Bacteria	Treatments			p-value		
	Control	Microbe-fed	SE	Treatment	Region	Treatment x Region
LAB	5.11	6.66	0.23	**	**	**
Coliform	5.23	4.60	0.51	NS	**	NS
LAB/Coliform	0.99	1.73	0.13	**	**	**
Aerobic bacteria	5.90	5.25	0.46	NS	**	NS
Bacilli	4.44	4.42	0.12	NS	**	NS
Clostridia	3.04	2.12	0.43	NS	NS	NS

Data was presented as a logarithmic conversion of Colony Forming Units (CFUs) except LAB/Coliform ratio. **Significant difference ($p < 0.01$). SE: Standard Error of the mean; NS: Not Significant

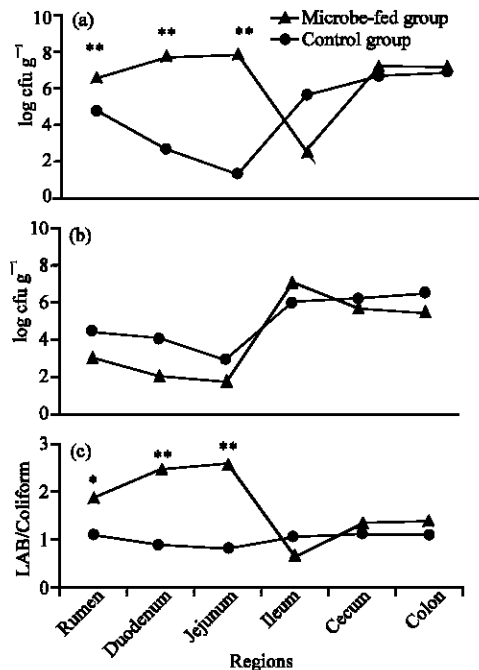


Fig. 1: The number of LAB (a), coliform (b) and LAB/coliform ratio (c) in various gastrointestinal regions of Holstein calves. *Significant difference between the treatment groups ($p<0.05$); **Significant difference between the treatment groups ($p<0.01$)

Previous report shows that calf rumen starts to work as early as 1 week of age and the function become similar to the adult by 6 weeks of age (Huber, 1969). Ages of the calves used in the present study were about 5 weeks old suggesting that function of the rumen of the calves in the present study was nearly identical to adult at slaughter. Because orally administrated microbes are reported to affect ruminal environment in adult ruminants (Oeztuerk and Sagmanligil, 2009), the main target of orally added Chikuso-1 and CO119 might be a rumen in preweaning Holstein calves.

In the knowledge, only 1 previous report of Agarwal *et al.* (2002) investigated the effects of microbial feeding on calf ruminal environment: feeding of LAB or yeast strain suppressed calf diarrhea and decreased the number of coliform in feces, not in ruminal contents. Further studies are needed because the information about the change of calf ruminal environment by microbe feeding is limited at present.

CONCLUSION

The present results showed that feeding of Chikuso-1 and CO119 significantly increased the number of LAB and

LAB/coliform ratio in rumen, duodenum and jejunum. These suggest that feeding of the microbe improves bacterial flora in upper regions of gastrointestinal tract of Holstein calves.

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