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# Feeding of Lactic Acid Bacteria and Yeast on Growth and Diarrhea of Holstein Calves

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**Abstract:** Neonatal diarrhea is the main cause of calf death and economically serious problem in the cattle industry. Probiotics are thought to be one of the technologies to solve the problem. The objective was to examine the effects of feeding of Lactic Acid Bacteria (LAB) and yeast as probiotics for calves. *Lactobacillus plantarum* Chikuso-1, an inoculant for whole crop rice silage was used for LAB and *Candida* sp. CO119, isolated from bovine rectal content, was used for yeast. Eight six days old Holstein calves were divided into 2 groups, control and bacteria-fed. Bacteria-fed group received milk replacer containing Chikuso-1 and CO119 for 4 weeks, whereas no bacterial treatment in control group. Daily Gain (DG), dry matter intake, feed efficiency and fecal scoring were evaluated during experimental period. Inclusion of both bacteria significantly increased DG and feed efficiency and decreased fecal scoring. The present results suggest that the bacteria have probiotic abilities, which promote growth and suppress diarrhea in Holstein calves.

Key words: Lactic acid bacteria, yeast, probiotics, daily gain, feed efficiency, diarrhea, Holstein calves

# INTRODUCTION

Neonatal calves are often affected by diarrhea, which is the main cause of mortality and also of economic loss in the cattle industry. The cause of calf diarrhea is mainly the infections of pathogenic bacteria in the calf intestine, antibiotics and/or other antimicrobials are commonly added to milk replacers for prevention of calf diarrhea (Braidwood and Henry, 1990). But use of antibiotics in animal production may contribute to emergence of antibiotic-resistant bacteria from cattle industry (Fey et al., 2000), the establishment of new technologies alternative to antimicrobial agents is strongly needed. Probiotics, which are defined by Fuller (1989) as follow: a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance are thought to be strong candidates alternative to antibiotics (Callaway et al., 2004).

In the present study, the effects of feeding of Lactic Acid Bacteria (LAB) and yeast on Daily Gain (DG), Dry Matter Intake (DMI), feed efficiency and fecal scoring of Holstein calves were examined. *Lactobacillus plantarum* Chikuso-1, an inoculant for whole crop rice silage (Cai et al., 2003) was used for LAB and *Candida* sp. CO119, isolated from bovine rectal content and deposited with National Institute of Technology and

Evaluation Patent Microorganisms Depositary (accession number: NITE P-375) was used for yeast. The results suggest that the bacteria could be used as probiotics for calves.

### MATERIALS AND METHODS

**Bacterial preparations:** Lactobacillus plantarum Chikuso-1 was purchased from Snow Bland Seed Co., Ltd., Hokkaido, Japan. Candida sp. CO119 was supplied from Dr. Cai Yimin, National Institute Livestock and Grassland Science. Chikuso-1 was harvested in de Man Rogosa-Sharpe broth (Becton, Dickinson and Company, Maryland, USA) for 8 h at 30°C. CO119 was harvested in Yeast Mold broth (Becton, Dickinson and Company) for 8 h at 30°C.

Animals, feeding and treatment: All animal experiments in the present research were conducted according to the animal care and use guidelines of the National Institute of Livestock and Grassland Science of Japan. Eight male Holstein calves at 6.3±1.5 days of age were fed non-medicated milk replacer containing 26.8% of Crude Protein (CP) and 24.3% of crude fat in a Dry Matter (DM) basis. The milk replacer was provided twice a day (08:30 and 16:00) at 8.5% of birth weight. Calf starter (in a DM basis:

CP: 22.6%; crude fat: 4.0%; Neutral Detergent Fiber (NDF): 12.9%; Acid Detergent Fiber (ADF): 6.7%; ash: 5.8%; Non-Structural Carbohydrate (NSC): 63.9%) and alfalfa hay (in a DM basis: CP: 18.2%; crude fat: 1.7%; NDF: 42.4%; ADF: 34.9%; ash: 14.7%; NSC: 38.2%) were offered *ad libitum* and feed intake was measured every day. Eight Holstein calves were divided into 2 groups, control and bacteria-fed. Bacteria-fed group received milk replacer containing Chikuso-1 (3.7×10<sup>11</sup> cfu head<sup>-1</sup>) and CO119 (2.6×10<sup>9</sup> cfu head<sup>-1</sup>) in the every morning for 28 days, whereas no bacterial treatment in control group. Body weight was measured at the starting day and once a week during experimental period. DG, DMI, feed efficiency and fecal scoring were evaluated in the present study.

**Fecal scoring:** Fecal scoring for estimation of fecal fluidity was conducted according to the procedure of Larson *et al.* (1977). The fluidity of calf feces was classified as follow: 1 = normal, 2 = soft, 3 = runny, 4 = watery. Fecal scoring was recorded three times a day at 09:00, 13:00 and 17:00 every day during experimental period. The data was averaged per week.

**Statistical analysis:** Statistical analyses were performed using SAS Institute (2001). Differences of body weight, DG, DMI and feed efficiency between experimental groups were evaluated using t-test. Difference of fecal scoring was evaluated by repeated measurements ANOVA using the MIXED procedure of SAS.

#### RESULTS AND DISCUSSION

Initial and final body weight, DG, DMI and feed efficiency were shown in Table 1. DG was significantly higher in bacteria-fed group than in control (p<0.05). There were no significant differences observed for DMI between treatments. Feed efficiency was significantly higher in bacteria-fed group than in control (p<0.01).

Fecal scoring was shown in Fig. 1. Fecal scoring was significantly lower in bacteria-fed group than in control (p<0.001). Especially, significant differences between groups were observed on the early part of the experimental period: fecal scoring of bacteria-fed group was significantly lower than that of control after 1 week from the beginning of the experiment (Fig. 1, p<0.05) and tended to be lower after 2 weeks (Fig. 1, p<0.1).

DG, DMI and feed efficiency were evaluated in the present study because growth promotion of host animals is one of the most essential indices for probiotics (Fuller, 1989). The results showed that addition of 2 kinds of bacterial strains, Chikuso-1 and CO119, to milk replacer significantly increased DG and feed efficiency of Holstein

Table 1: Effects of addition of Lactic Acid Bacteria (LAB) and yeast to milk replacer on body weight, Daily Gain (DG), Dry Matter Intake (DMI) and feed efficiency in Holstein calves. Data was expressed as mean±SD

Body weight	Control	Bacteria-fed
Initial (kg)	47.05±8.86	43.13±4.32
Final (kg)	48.28±5.62	48.43±3.82
DG (kg day <sup>-1</sup> )	$0.04\pm0.02$	$0.18\pm0.09*$
DMI (kg day <sup>-1</sup> )	$0.57\pm0.01$	$0.53\pm0.04$
Feed efficiency (DG/DMI)	$0.06\pm0.04$	0.33±0.15**

<sup>\*</sup>Significant difference between the treatment groups (p<0.05); \*\*Significant difference between the treatment groups (p<0.01)

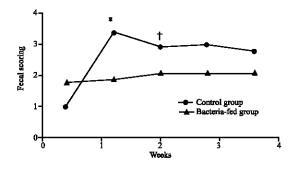


Fig. 1: Effects of addition of LAB and yeast to milk replacer on fecal scoring of Holstein calves. The fecal scoring was classified as follow: 1 = normal, 2 = soft, 3 = runny, 4 = watery. \*: Significant difference between the treatment groups (p<0.05); † : Difference between the treatment groups (p<0.1)

calves, suggesting that the bacteria have an ability to promote calf growth as probiotics. DMI did not differ between experimental groups, it is apparent that the increase of DG mainly contributes to that of feed efficiency in the present study. Such characteristics of the bacteria are of great advantage from a farm management point of view because feed cost could be reduced by using probiotics, which improve DG and feed efficiency, rather than DMI. Previous report of Abe et al. (1995) also showed that addition of Bifidobacterium pseudolongum M-602 or Lactobacillus acidophilus LAC-300 to milk replacer improved DG and feed efficiency without affecting DMI of Holstein calves. Such bacteria are considered to be actually available as probiotics for cattle industry.

In the present study, addition of Chikuso-1 and CO119 to milk replacer suppressed calf diarrhea. This is also an important ability for probiotics (Schrezenmeir and de Vrese, 2001). The present results also showed that the significant suppression of diarrhea was observed after 1 week from the beginning of the experiment. Virtala *et al.* (1996) reported that calves had a higher risk of diarrhea at

early stage of lactation period. These suggest that probiotics could act more effectively to suppress calf diarrhea at early lactation period, when the illness is severer.

However, the mechanisms of suppression of calf diarrhea by Chikuso-1 and CO119 remain unknown. Previous papers reported that feeding of LAB or yeast in calves increased the number of LAB (Abu-Tarboush *et al.*, 1996) or decreased the number of coliform bacteria (Agarwal *et al.*, 2002) in calf feces, when diarrhea was suppressed by the probiotics. These suggest that addition of probiotics would modify the microbial status in the intestine of host animals to ameliorate diarrhea. Further studies are required about the effects of Chikuso-1 and CO119 on the intestinal microbial flora of calves.

# CONCLUSION

Addition of *Lactobacillus plantarum* Chikuso-1 and *Candida* sp. CO119 to milk replacer significantly increased DG and feed efficiency and decreased fecal scoring of Holstein calves. The present results suggest that the bacteria could be used as probiotics for promoting growth and suppressing diarrhea of Holstein calves.

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