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< Short Communication >

Effect of feeding the combination with *Lactobacillus plantarum* and *Bacillus subtilis* on fecal microflora and diarrhea incidence of Korean native calves

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Abstract

This study was investigated the effects of feeding the combination with *Lactobacillus plantarum* and *Bacillus subtilis* on the diarrhea incidence and fecal microflora of weaned calves. A total of 12 newly weaned calves were allocated to two dietary treatments in a randomized design based on body weight. The dietary treatments included a commercial basal diet supplemented with: 1) no microbial inoculants (Control); 2) a mixture of *Lactobacillus plantarum* and *Bacillus subtilis* (LB). Calves were fed diets for a 4-week period. At the end of the experiment, the counts of fecal lactic acid bacteria and *Enterobacteriaceae* in LB were significantly improved compared to control (P<0.05). Over the 4-week period, fecal scores and duration of diarrhea in LB were significantly decreased compared with those in control (P<0.05). The present results suggest that LB is a potential feed additive which could be used for the balance of intestinal microflora and the prevention of diarrhea in Korean native calves.

Key words : *Lactobacillus plantarum*, *Bacillus subtilis*, Calf diarrhea, Fecal microflora, Korean native calf

INTRODUCTION

Calf diarrhea is the most frequent disease occurring in cattle farm and causes death and serious economic loss in cattle industry (Radostits, 1975). Because disease resistance of calves is weak in comparison with adult cattle, calves have easily a diarrhea with a little wrong breeding management. As diarrhea in young calves causes growth delay or death, it is very important to prevent and treat diarrhea in calves (Choi et al, 2000). The benefits of antibiotics use in young calves are the decrease of diarrhea incidence, lower calf mortality, and the increase of growth performance (Modi et al, 2011). Generally, only young calves fed milk replacer and calf starter still receives antibiotics such as neomycin to treat diarrhea on the continual basis (Kim et al, 2011). Due to the problems of antibiotics such the emergence of antibiotic-resistant bacteria, many countries in the world have banned or rigorously limited use of these in animal industry since 1996 banned all feed antibiotic by European Union (Carlet et al, 2012). With the tendency to ban antibiotics use in animal feed, the cattle industry cannot but get interested in alternative to antibiotics for growth promotion and maintaining health under commercial conditions. To reduce the side effect of antibiotics, many researchers have been carried out searching for the alternatives to antibiotics using probiotics (Bravo et al, 2008; Collado et al, 2009), herbal medicine (Kong et al, 2007) and propolis (Scazzocchio et al, 2006).

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Among numerous candidates, probiotics are thought to be prospective substitutions of antibiotics (Callaway et al, 2004). Over the years, probiotics have been used as an effective alternative to antibiotics in animal, which have an advantageous effect on the host animal by affecting its gut flora (Gaggìa et al, 2010; Kenny et al, 2011). Many studies reported beneficial effects of probiotics on growth performance, nutrient retention, diarrhea reduction and intestinal microflora for cattle and calves (Aldana et al, 2009; Kawakami et al, 2011).

Lactobacillus spp. can ferment a broad spectrum of plant carbohydrate and is tolerant to bile salts and low pH (Tropcheva et al, 2011). *Lactobacillus plantarum* (*L. plantarum*) has the antagonistic potential against intestinal pathogens through production of lactic acid and/or bactericidal compounds (de Vries et al, 2006). However, the information for the effects of a mixture of *L. plantarum* and *Bacillus subtilis* (*B. subtilis*) (LB) is still limited on the reduction of diarrhea incidence and fecal microflora analysis of calves. Additionally, spore forming *Bacillus* spp. has been regarded as the suitable probiotic due to survival of high temperature, resistance of bile salts in the intestinal tract of the animal and long-term storage at room temperature (Ripamonti et al, 2009).

Therefore, the objective of this study was to evaluate the efficacy of a mixture of *L. plantarum* SY-99 and *B. subtilis* SJ-61 spores, on the reduction of diarrhea duration and fecal scores, and the improvement of fecal microflora in newly weaned Korean native calves.

MATERIALS AND METHODS

Animals and treatment

Twelve newly weaned calves at 78.3±7.2 days of age were allocated to two dietary treatments (equal numbers) based on body weight in a randomized design. The dietary treatments were a commercial basal diet (Milk Gen, CJ Feed Co., Korea) supplemented with: 1) no microbial inoculants (Control); 2) a mixture of *L. plantarum* and *B. subtilis* (LB) 0.2 kg/ton feed. *L. plantarum* SY-99 strain has been isolated from salted and fermented seafood, while *B. subtilis* SJ-61 strain has been isolated from soya bean fermentation. All calves were administered for 4 weeks, and feed and water were freely available.

Fecal microflora bacteria populations

At the end of the experiment, fecal samples were collected by grab sampling from the rectum of calves. About 2 g of fecal sample was diluted in sterile 2.25% peptone water and homogenized to stand at room temperature for 1 h. The samples were diluted for further tenfold series with 2.25% peptone water. The dilution was vigorously shaken by a vortex mixer and spread onto an agar plate. Enumerations of bacteria were performed on MRS agar (LAB M, Bury, UK) for lactic acid bacteria (LAB) and incubated in the anaerobic jar at 30°C for 48 h. Enterobacteriaceae (ENT) enumerations were performed on eosin methylene blue agar (Merck, Germany) and incubated an anaerobic condition at 37°C for 24 h. Numbers of colony-forming unit (CFU) were expressed as $\log_{10}$ CFU/g feces.

Fecal scores and duration of diarrhea

Fecal scoring and duration of diarrhea were conducted daily at the same time in the morning. Fecal scores based on a four-point scale were recorded using the procedure of Larson et al (1977). Scoring was as follows: for fecal fluidity, 1=normal, 2=soft, 3=runny, and 4=watery.

Statistical analysis

Values are presented as the mean±standard deviation (SD). All data were analyzed using one-way ANOVA (SAS Institute, NC, USA), with the calf as the experimental unit, with regard to feed intake and other variables. Duncan's multiple range test was used to compare differences between the treatment groups. Probability values $P<0.05$ were taken to indicate statistical significance.

Table 1. Fecal lactic acid bacteria and *Enterobacteriaceae* population in Korean native calves fed with different dietary treatments for 4 weeks

Items	Treatment*	
	Control	LB
Lactic acid bacteria	7.32±0.51†	8.28±0.47‡
Enterobacteriaceae	7.18±0.32	6.53±0.29‡

Data are expressed as the mean±SD. *Control: no treatment, LB: treated with a mixture of *L. plantarum* and *B. subtilis* 0.2 kg/ton feed. †Data were presented as $\log_{10}$ CFU/g feces. ‡$P<0.05$, significantly difference compared to control.

Table 2. Duration of diarrhea and fecal scores in Korean native calves fed with different dietary treatments for 4 weeks

Items	Treatment*	
	Control	LB
Fecal scores†	2.81±0.26	2.27±0.21‡
Duration of diarrhea (days)	3.84±2.52	1.86±1.36‡

Data are expressed as the mean±SD. *Control: no treatment, LB: treated with a mixture of *L. plantarum* and *B. subtilis* 0.2 kg/ton feed. †Fecal scoring was as follows: for fecal fluidity, 1=normal, 2=soft, 3=runny and 4=watery. ‡$P<0.05$, significantly difference compared to control.

RESULTS AND DISCUSSION

Fecal microflora populations in Korean native calves were shown in Table 1. There was significant difference of probiotics treatment on the numbers of LAB and ENT in the calf fecal samples ($P<0.05$). 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 LB significantly increased the number of fecal LAB of calves suggesting that the bacteria have an ability of probiotics to improve the balance of enteric microbial flora. However, the effect was limited in the early stage of the lactation period (Kawakami et al, 2011). After administration of *Lactobacillus acidophilus* 27SC with twenty-four Holstein bull calves for nine weeks, fecal counts of lactobacilli in the probiotic treatment group were significantly increased compared with those in control ($P<0.05$), but fecal counts of coli forms were not different (Abu-Tarboush et al, 1996). Timmerman et al (2005) reported that a calf-specific probiotic containing six *Lactobacillus* species was administered with one-week-old veal calves for eight weeks, and the probiotic treatment group was not different the fecal counts of lactobacilli and coli forms compared to control. In addition, Jenny et al (1991) reported that the fecal lactobacilli counts in Holstein calves treated with a mixture of *Lactobacillus* (*L.*) *acidophilus*, *L. lactis* and *B. subtilis* were higher than those in control, and those in *B. subtilis* alone treatment group were lower than those in control. The differences in response of fecal microflora by probiotics may be affected by the type, dosage and mixture of bacteria used.

Fecal scores and the duration of diarrhea in Korean native calves were shown in Table 2. In LB-treatment group for 4 weeks, fecal scores and the duration of diarrhea were significantly decreased compared with the control group ($P<0.05$). Kowalski et al (2009) reported that fecal scores in female Holstein calves treated with two *Bacillus* species for eight weeks was not significantly different compared to those in control. Additionally, Abu-Tarbush et al (1996) reported that fecal scores in Holstein bull calves administered with a mixture of *L. acidophilus* and *L. plunturum* for nine weeks were not different with those in control. Reversely, fecal scores in Holstein male calves treated with *L. plantarum* strain Hokkaido for 35 days were significantly decreased compared to control ($P<0.05$) (Nagashima et al, 2010). Furthermore, no effects on frequency or length of diarrhea or fecal score were observed in Holstein calves treated with a mixture of *B. subtilis* and *B. lichenformis* for 56 days (Riddell et al, 2010). However, in the result of the study by Mokhber-Dezfouli et al (2007), new born calves treated with a probiotic mixture of five species including *Lactobacillus* spp. significantly decreased the diarrhea occurrence compared to control ($P<0.01$) and reduced the severity of diarrhea. The inconsistency of the results in above studies might arise from the difference of the used probiotics and the unlike physical conditions of the experimental animals.

In this study, the improvement of diarrhea scores and duration, the positive development of LAB population, and reduction of ENT were obtained in calves administered with LB. The present results suggest that LB

is a potential feed additive that could be used for the balance of intestinal microflora and the prevention of diarrhea in Korean native calves.

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