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Effects of cellooligosaccharide or a combination of cellooligosaccharide and live *Clostridium butyricum* culture on performance and intestinal ecology in Holstein calves fed milk or milk replacer

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ABSTRACT

The effects of oral administration of a prebiotic (cellooligosaccharide [CE]) and a combination of a probiotic (a commercial *Clostridium butyricum* strain) and prebiotics (referred to as symbiotics [SB]) on performance and intestinal ecology in Holstein calves fed milk replacer (MR) or whole milk were evaluated. Forty female calves (experiment 1) and 14 male and female calves (experiment 2) were used in this study. Calves were fed MR (experiment 1) or whole milk (experiment 2) necessary for daily weight gain of 0.3 kg based on birth weight in two daily feedings and weaned at 46 days. Calves were divided into a CE feeding group, SB feeding group (only in experiment 1), and control group. The CE and SB groups were fed CE at 5 g/day before weaning and 10 g/day postweaning. Only the SB group received 10^8 colony-forming units (CFU) of *C. butyricum* culture per day. Commercial calf starter was offered for ad libitum intake. Health and feed intake of the animals were monitored daily, and body weight was measured weekly. Fecal samples were analyzed for determination of bacterial community composition by an RNA-based method (sequence-specific SSU rRNA cleavage method) and for organic acid profiling. In 49-day experiments, feed intake, daily gain, and occurrence of diarrhea of the calves were unaffected by either CE supplementation or SB supplementation, and all calves were healthy during each experiment. The fecal bacterial community compositions and the organic acid profiles were not different among groups in experiment 1. In experiment 2, the level of the *Clostridium coccoides–Eubacterium rectale* group was higher in the feces of CE group than controls at 4 weeks of age and fecal butyric acid concentration was

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higher (8.0 vs. 12.2 [mmol/kg feces], $P < 0.05$) at that time. There were no differences in prebiotic bacteria (the genera *Lactobacillus* and *Bifidobacterium*) between groups at this time point. These results suggested that CE and *C. butyricum* supplementation have less effect on the performance of healthy calves fed MR. However, prebiotic supplementation seems effective for modulation of the intestinal bacterial community of calves when administered with whole milk.

1. Introduction

Diarrhea is regarded as a major problem in preweaned dairy calves (Cowles et al., 2006; Hill et al., 2005), and prevention of diarrhea is important to promote the growth of calves. Antibiotics have been widely used in milk replacer (MR) in the USA and Japan (Kobashi et al., 2005; Sawant et al., 2005) to improve performance and reduce scours in dairy calves. However, as the use of antibiotics in animal feed was prohibited in the European Union in 2006 as part of an initiative to promote the prudent use of antibiotics, there is increasing interest in alternatives (Berge et al., 2009).

A detailed understanding of indigenous intestinal microflora is a way to address diarrhea, as the microflora is involved in host nutrition, mucosal defense, and host immunity, and therefore influences the performance of the animals (Gibson et al., 2004; Zoetendal et al., 2004). The intestines of newborn animals and humans are sterile, but microbial colonization of the gastrointestinal tract begins immediately at birth (Favier et al., 2002). Thereafter, a complex and dynamic microbial ecosystem with high densities of living bacteria is established in the large intestine as animals grow to maturity. Molecular-based monitoring of the intestinal bacterial communities of calves has revealed that the community undergoes dynamic changes during the first 12 weeks of life, including reduction of health-promoting bacteria such as lactobacilli and bifidobacteria from the community in the early stage of life in cattle (Uyeno et al., 2010a). It is considered effective for healthy calf rearing to optimize the enteric flora by increasing the number of potentially beneficial microorganisms.

One potential measure to enhance the impact of these beneficial bacteria is the use of oligosaccharides. Non-digestible oligosaccharides have been used in calf diets to eliminate harmful bacteria from the intestine and help improve the health of the animals (Heinrichs et al., 2003; Quigley et al., 1997). It is possible that these compounds affect the viability of this beneficial bacterial group, and as a result strengthen gastrointestinal function. Cellooligosaccharide (CE), consisting of glucose with beta-1–4 linkages, is a commercially available oligosaccharide. We have reported that CE feeding in milk-fed calves resulted in improvements in daily gain (DG) and feed efficiency during the postweaning period (Hasunuma et al., 2011). However, little information is available and further investigations are required to determine how the commensal bacteria composition of calves changes with the administration of CE, particularly in the preweaning period at which probiotic bacteria decrease the populations.

The objective of this study was to evaluate the effects of oral administration of CE or *Clostridium butyricum* cells and CE (referred to as symbiotics [SB]) in the preweaning period on feed intake, DG, fecal bacterial community compositions, and organic acid profiles of Holstein calves fed MR or whole milk, both of which are practical liquid feed for calves.

2. Materials and methods

2.1. Animals and diets

The animal study was conducted in the same way at the research institutes of six prefectures (Toyama, Chiba, Aichi, Ishikawa, Ibaraki, and Kanagawa) in Japan. The calves were cared for according to the Guide for the Care and Use of Agricultural Animals in Agricultural Research of the National Institute of Livestock and Grassland Science. We performed two experiments, one of which used 40 female calves (referred to as experiment 1) and the other used seven male and seven female calves (experiment 2). Calves obtained from each institute were randomly assigned to one of three groups in the order of birth: CE feeding group ($n=13$), SB feeding group ($n=14$), and control group ($n=13$) in experiment 1, CE feeding group ($n=7$), and control group ($n=7$) in experiment 2. They remained at institutes where they were born through experiments. All calves were given colostrum within 30 min of birth and housed individually in calf hutches or pens bedded with sawdust. Ambient temperatures during experiments ranged from 12 °C to 28 °C in experiment 1 and from 21 °C to 32 °C in experiment 2. The calves were fed mother's milk for 1–3 days after birth and then abruptly switched to MR without antibiotics (24% crude protein [CP] and 21% crude fat) dissolved in four times (w/w) of warm water (experiment 1), or mother's milk without pasteurization (experiment 2). Major ingredients of MR were skim milk powder, whey powder, vegetable oil, hydrolyzed soy protein, and feed additives (vitamins, minerals, and amino acids). The liquid feed necessary for a daily body weight gain of 300 g (corresponding to 370–500 g of MR powder and 3900–5200 g of mother milk [as fed]) based on birth weight (NARO, 2006) was provided in two daily feedings at 07:30 and 17:00 using a feeding bottle or a bucket with a nipple. The animals were weaned at 46 days. The CE group and the SB group were fed CE (Nippon Paper Chemicals Co., Ltd, Tokyo, Japan) at 5 g/day dissolved in the liquid feed. CE was mixed in starter at 10 g/day postweaning. The SB group was fed 0.1 g (corresponding to 1.0×10^8 colony forming units [CFU]) of a commercial

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