Fecal bacterial community succession of Holstein calves and its modulation by providing prebiotics fed with milk replacer

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ABSTRACT

The objectives of this study were: i) to quantitatively analyze the fecal bacterial communities of Holstein calves and track their succession up to 12 weeks of age (Experiment 1), and ii) to investigate the effects of prebiotics fed with milk replacer on health and the fecal bacterial community composition of Holstein calves (Experiment 2). In experiment 1, fecal samples obtained from four female Holstein calves were analyzed by the RNA-based sequence-specific rRNA cleavage method. At one week of age, 16S rRNAs from members of the *Bacteroides-Prevotella* group, *Faecalibacterium*, the *Clostridium coccoides-Eubacterium rectale* group and the *Atopobium* cluster were detected at high levels. Throughout the 12-week period, rRNAs of the *Bacteroides-Prevotella* and *Cl. coccoides-Eu. rectale* groups constituted the major fraction of microbiota (approximately 50 to 70% of the total). The relative abundances of the *Atopobium* cluster, *Faecalibacterium*, and some probiotic bacteria (such as those of the genera *Lactobacillus* and *Bifidobacterium*) decreased as the animal aged. Instead, an uncultivated rumen bacterial group, *Ruminococcus flavefaciens* and *Fibrobacter* emerged at detectable levels (1-2 %) in the feces sampled at a postweaning age. In experiment 2, 24 male calves fed a commercial milk replacer were used to evaluate the effects of prebiotics administration on the growth performance and intestinal condition of the calves. Calves were divided into the cello-oligosaccharide [CO] feeding group, isomalto-oligosaccharide [IO] feeding group and control group in this experiment. The prebiotic groups were fed CE at 5 g/day or IO at 15 g/day, respectively, by dissolving in liquid feed. Commercial calf starter and chopped timothy hay were offered preweaning and postweaning. The animals' health and feed intake were monitored daily and body weight was measured weekly. Fecal samples were analyzed for the determination of bacterial community composition. Feeding CO decreased the incidence of diarrhea compared to the IO group and control group. The *Cl. coccoides-Eu. rectale* group was higher in the feces of CO group than IO group and control group at seven weeks of age, while no difference was observed in viable lactobacilli counts among the three groups at that time. Results of this study demonstrate that young calves undergo dynamic changes in their intestinal bacterial community during the first 12 weeks of life. Since young ruminants undergo metabolic and physiological development in their digestive tracts in their transition from a monogastric to a ruminant animal at an early age, the intestinal bacterial community may reflect such development. In addition, prebiotics supplementation seems effective for modulation of the intestinal bacterial community of calves when it is administered with liquid feed.

Key Words: Bacterial community, Gastrointestinal tract, Oligosaccharides, Diarrhea

INTRODUCTION

The period from birth to complete weaning at 6-8 weeks of age represents the period of greatest stress and metabolic challenge to young calves. During this critical stage, calves

make the metabolic, nutritional, and behavioral changes to become a functional ruminant. At the same time, a complex and dynamic microbial ecosystem with a high density of living bacteria is established in the large intestine. Beneficial microbiota play an important role in the health status of the animal host thanks to their involvement in nutritional, immunologic and physiological functions. Characterizing the community structure of the gastrointestinal tract (GI) microbiota of calves is an important step in studying this ecosystem, especially tracking changes from the beginning of their life including the period of weaning.

Diarrhea is regarded as a major problem in preweaned dairy calves, and antibiotics have been widely used in milk replacers (MR) in many countries to improve performance and reduce scours in dairy calves. However, antibiotic treatment for diarrhea at an early stage of life may have an adverse effect on the establishment of a desirable bacteria community, and interest in alternatives has become stronger than ever. In recent years, probiotics or prebiotics that improve the host microflora have received a lot of attention for this purpose. There is increasing evidence that lactobacilli and bifidobacteria, which inhabit the gastrointestinal microbiota, develop antimicrobial activities that participate in the host's gastrointestinal system of defense. Non-digestible oligosaccharides have been used in calf diets to eliminate harmful bacteria from the intestine and help improve the animals' health. In livestock, probiotic and prebiotic supplemented feed have been evaluated and exploited to decrease diarrheal disease, reduce odors, and improve growth.

The objectives of this study were to monitor the succession of the intestinal bacterial community of calves from birth to three months and to investigate the effects of the administration of oligosaccharides on growth performance and the fecal bacterial community composition.

MATERIALS AND METHODS

Experiment 1. Four female Holstein calves obtained from a single dairy farm were used. Calves were removed from their dams and fed colostrum within 1 h of birth. From three days of age, a commercial MR (28% crude protein and 15% crude fat) reconstituted with warm water to 16% dry matter was fed in two daily feedings. MR was fed according to an intensive feeding program at 600, 800, 1200 and 800 g per day in weeks 1 to 2, 3, 4 to 6 and 7, respectively, and they were weaned at 8 weeks of age. Water was available at all times. A commercial calf starter (18% CP and 72% TDN) and chopped timothy hay were offered for ad libitum intake. Health status, body weight, and feed intake were routinely recorded throughout the trial. Fecal samples were collected on the first day of week 1, 3, 5, 7, 9 and 12. Fecal samples were collected from calves by rectal stimulation and the sample (2.0 to 2.5 g) was suspended in 20 ml PBS buffer. RNA extraction and subsequent bacteria determination by an RNA-based method (sequence-specific SSU rRNA cleavage method) was according to previous studies (Uyeno et al., 2008; Uyeno et al., 2010).

Experiment 2. Twenty-four male Holstein calves obtained from 13 dairy farms were randomly assigned to three groups (isomalto-oligosaccharide [IO] group [n = 8], cello-oligosaccharide [CO] group [n = 8] and control group [n = 8]) in the order of birth. A commercial MR (24% CP and 21% crude fat, fed daily at 400g up to 2 weeks of age and thereafter 500g) was reconstituted with warm water and was fed in two daily feedings. Calf starter and timothy hay were offered as in experiment 1. The IO and CO groups were fed a commercial IO (15 g/day) and CO (5 g/day) by dissolving in reconstituted milk. Calves were weaned with reference to the daily intake of calf starter. The animals' health and feed intake were monitored daily and body weight was measured weekly before the morning feeding. Experimental period was 49 days (0 to 49 day). Fecal samples were collected from calves on one day in week 1 and 3, the weaning day, and one week postweaning. Measurements were analyzed using a randomized

block design by Fisher's protected least significant difference test. All measurements were analyzed using Stat View 5.0J (SAS Institute, Cary, NC, USA).

RESULTS

Experiment 1. All calves consumed all the milk replacer provided in the experimental period. Average starter consumption was 1700 g at weaning and 2500 g at 12 weeks of age. Average body weight was 44 kg at birth, 93 kg at weaning, and 127 kg at 12 weeks of age. Throughout the experimental period, all the calves were healthy. At one week of age, 16S rRNAs from members of the *Bacteroides-Prevotella* group (40.0% of the total 16S rRNAs), *Faecalibacterium* (21.7%), the *Clostridium coccoides-Eubacterium rectale* group (16.7%) and the *Atopobium* cluster (10.9%) were detected at high levels. *Lactobacillus–Enterococcus* group and *Bifidobacterium* have been shown to constitute approx. 1% each of the total rRNA at four weeks of age. *Bacteroides-Prevotella* and the *Cl. coccoides-Eu. rectale* groups constituted the major fraction of microbiota (approximately 50 to 70% of the total) throughout the 12-week period. The relative abundances of the *Atopobium* cluster, *Faecalibacterium*, *Lactobacillus-Enterococcus*, and *Bifidobacterium* decreased as the animal aged, and these groups had a low proportion in the samples at 7 and 13 weeks of age. Instead, fibrolytic bacteria such as *Ruminococcus flavefaciens* and *Fibrobacter* emerged at detectable levels (1-2%) in the feces sampled at a postweaning age (12 weeks). These observations indicated changes in the fecal bacterial population in response to animal growth at the group level.
Experiment 2. All calves suffered diarrhea on at least one day during the experimental period. Rates of diarrhea incidence per head (as a proportion of diarrhea days per experiment days) were 0.12, 0.19, and 0.08 in the control, IO, and CO groups, respectively. In particular, the rates in the first 21 days were 0.29, 0.30, and 0.20 respectively. This result suggested that feeding CO decreased the incidence of diarrhea ($P<0.05$). No other performance measure (feed intake, daily gain and feed efficiency) was affected by prebiotics supplementation. *Cl. coccoides-Eu. rectale* was higher in the feces of the CO group than IO group and control group one week postweaning. CE feeding also increased viable lactobacilli at three weeks but no difference was observed in the counts among the three groups one week postweaning.

DISCUSSION

This study demonstrated that young calves undergo marked changes in their intestinal bacterial community during the first three months of life. The majority of the calf fecal bacterial community is affiliated with two phyla, *Bacteroidetes* and *Firmicutes*. Other group have a certain niche in the community, while major groups changed in response to the growth stage of calves. The microflora of the gastrointestinal tract of young ruminants at the age of 1–4 weeks differs greatly from that at more advanced ages. This is possibly linked to metabolic and physiological development in the GI system, which develops so that it can function as a ruminant with age. A change in the major source of the feed materials after weaning (i.e. milk or milk replacer to solid feed) may also have a significant effect on the transition of GI microbiota. For instance, uncultured rumen bacteria belonging to the phylum *Firmicutes* and major ruminal fibrolytic bacteria (*Fibrobacter* and *R. flavefaciens*) increased with the calves aged, possibly as a result of postnatal development of the digestive tract and subsequent improvement in the digestibility of fiber.

Findings on the calf fecal bacterial community transition with age may also have important implications for the prevention of diseases and maintenance of the health of calves. It may be crucial for animal health to address the dissipation of health-promoting bacteria (i.e. lactobacilli and bifidobacteria) from the community in an early stage of life. In experiment 1,

lactic acid bacteria were shown to constitute less than 1% of the total bacterial community and decreased with the passage of time. Feeding CO increased fecal lactobacilli, but the effect was limited in the preweaning period. CO and IO supplementation seemed to have little influence on the lactic acid bacterial count postweaning. It is therefore suggested that such oligosaccharides have no more effect when the population of probiotics reaches a low level in the large intestine of ruminants; however, the addition of CO improved fecal scores, while this increase was not shown in BW measurements. Addition of CO to milk replacer therefore appeared to benefit calf health and reduce scours, as has been observed by feeding other kinds of oligosaccharides, such as mannan-oligosaccharides and fructo-oligosaccharides, both of which are involved in selective stimulation of probiotic bacteria growth (Gibson et al., 2004). On the other hand, IO supplementation may not have an obvious effect on a calf's intestinal flora, in which limited bacterial species are influenced by the introduction of this oligosaccharide.

A previous *in vivo* test (Hasunuma et al., 2011) indicated that CO feeding in calves improved DG and feed efficiency during the postweaning period, mainly due to the enhancement of rumen VFA production by affecting specific groups of rumen microbes. In this study, we found that CO also has the potential to change bacterial flora in the large intestine, resulting in enhancing butyric acid-producing bacteria belonging to *Cl. coccoides-Eu. rectale*. Butyric acid is involved in the growth and differentiation of intestinal cells, thereby improving digestion and absorption. This may also contribute to the improvement of growth performance at an older age. Because young calves possess a specific flora composition, applying oligosaccharides still advantageous; it is possible to promote bacterial groups which are specifically recognized in the ruminant's community, for instance, fibrolytic bacteria and lactic acid-utilizing bacteria, other than lactobacilli or bifidobacteria.

In conclusion, the bacterial community inhabiting a calf's large intestine undergoes marked changes in the period between preweaning and postweaning. This change possibly reflects growth and postnatal development, as well as metabolic, nutritional and behavioral changes during the period. In addition, unlike monogastric animals, the use of prebiotic oligosaccharides seems to have a minor effect on vitalizing probiotic bacteria inhabiting the large intestine of weaned ruminants. Further extension of our current knowledge on metabolic, bacterial and immunological interactions in the calf GI tract will certainly benefit the development of functional 'health-improving' feed additives.

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