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Changes in the intestinal bacterial community, short-chain fatty acid profile, and intestinal development of preweaned Holstein calves. 1. Effects of prebiotic supplementation depend on site and age

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ABSTRACT

Digestive disorders are common during the first few weeks of life of newborn calves. Prebiotics are nondigestible but fermentable oligosaccharides that modulate growth and activity of beneficial microbial populations, which can result in enhanced gut health and function. Galactooligosaccharides (GOS) have demonstrated such prebiotic potential. In this study, the effect of GOS supplementation on intestinal bacterial community composition and fermentation profiles; intestinal health, development, and function; and growth was evaluated in dairy calves fed for high rates of growth. Eighty male Holstein calves were assigned either to a control treatment consisting of commercial milk replacer or to a GOS-rich (i.e., 3.4% of dry matter) milk replacer treatment. After 2 and 4 wk, 8 calves per treatment were slaughtered at each age. Samples of intestinal digesta and tissue were collected for assessment of bacterial communities, short-chain fatty acid concentrations, in vitro measurement of nutrient transport and permeability, histomorphology, and gastrointestinal organ size. The remaining 48 calves continued to wk 8 to measure body growth, nutrient intake, and fecal and respiratory scores. Calves fed GOS displayed greater *Lactobacillus* and *Bifidobacterium* relative abundance and more developed intestinal epithelial structures, but also had greater fecal scores presumably related to greater colonic water secretion. Control calves showed slightly better growth and milk dry matter intake. Size of intestinal organs, intestinal nutrient transport, and epithelium paracellular resistance were not affected by treatment. Excessive GOS supplementation had both prebiotic and laxative effects, which led to slightly lower growth performance while promoting commensal bacteria population and greater intestinal epithelium growth.

Key words: calf, gut health, prebiotic, permeability

INTRODUCTION

Neonatal calves are faced with a variety of stressors. Promoting an early balance among the animal, its environment, and etiological agents is necessary to minimize the probability of illness episodes (Davis and Drackley, 1998). At this stage, the gut has a central role not only in nutrient assimilation, but also in preventing direct contact between pathogens and the internal body as mediated by commensal microbial colonization (Martin et al., 2010).

Intestinal illness is common in newborn calves. Diarrhea is one of the most frequent health problems in young dairy calves (Svensson et al., 2003). The last National Animal Health Monitoring System survey (NAHMS, 2010), which represented ~80% of US dairy operations, reported a death rate for preweaning heifers of 7.8%. Scours (diarrhea) or other digestive problems accounted for 56.5% of this mortality. Although somewhat better than the 60.5% found in the previous survey (NAHMS, 1996), the incidence of intestinal illness is still too high.

A potential alternative to promote gut health in newborns is to prevent infection by fostering growth of lactic acid bacteria (**LAB**) to inhabit the gastrointestinal (**GI**) tract. To achieve this, different types of prebiotics have been developed and their potential health benefits have been evaluated experimentally in different species. Prebiotics have been defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the GI microbiota that confers benefits upon host wellbeing and health" (Gibson et al., 2004). Usually, *Bifidobacterium* and *Lactobacillus* are the bacterial genera targeted by prebiotics such as fructooligosaccharides and galactooligosaccharides (**GOS**; Macfarlane et al.,

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2008). These microbes can potentially displace pathogenic bacteria by, for instance, competing for nutrients and attachment sites in the gut epithelium, producing bacteriocins, increasing production of short-chain fatty acids (**SCFA**) that lower intestinal pH, and stimulating the immune system (Van Loo and Vancraeynest, 2008).

The main source of GOS is whey permeate, which is a byproduct of cheese manufacturing. Briefly, the residual whey left after cheesemaking is rich in whey proteins (e.g., β-LG and α-LA) and lactose. This whey can be ultrafiltered by membrane technology to isolate the protein fraction, which is of high nutritional value to humans. A subsequent byproduct from this filtration is whey permeate, which is nearly devoid of whey proteins and very rich in lactose (Torres et al., 2010). Lactose in this whey permeate can then be crystallized and subjected to enzymatic digestion with β-galactosidases that attack the o-glucosyl group of lactose. Through a sequence of reactions between galactose and multiple di- and oligosaccharides other than lactose, β-galactosidase activity yields a variety of compounds collectively named GOS. Lactose hydrolysis to form GOS also results in an increase in free sugars (Torres et al., 2010). Commercially available GOS are generally mixtures of lactose, glucose, galactose, and oligosaccharides. The oligosaccharide profile of GOS is extremely variable and depends on the specific β-galactosidase used in the manufacturing process, among other processing conditions (Angus et al., 2005). Galactooligosaccharides have the chemical structure G-Gal_n with G = glucosyl moiety, Gal = galactosyl moiety, and n = number of galactosyl units linked together (Frank, 2008; Torres et al., 2010). Bound to the Gal-Glu unit in the reducing end of the oligosaccharide can be additional galactose units but also *N*-acetyl-glucosamine, fucose, or sialic acid derivatives (Urashima et al., 2009). The degree of GOS polymerization ranges from 2 to 8 residues, with an average of about 3 (Frank, 2008).

The objective of this experiment was to evaluate the prebiotic potential of a β-galactosidase treated whey permeate as a source of GOS in milk replacer (**MR**) on fore and distal gut bacterial communities, fermentation profiles, health, intestinal development and function, and growth of preweaned calves fed for high rates of growth. The milk-derived oligosaccharides reported here fit within the definition of GOS as described in the previous paragraph.

MATERIALS AND METHODS

Animals, Feeding, and Treatment Allotment

All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee (protocol #122109) of the University of Illinois at Urbana-Champaign. Eighty newborn male Holstein calves were purchased from sale barns. Upon arrival to the University of Illinois research facilities, calves received an electrolyte solution (Electrolyte System Base plus Add Pack, Land O'Lakes Animal Milk Co., Arden Hills, MN), as well as vaccines and prophylactic antibiotic treatment (2 mL of BoSe, Shering-Plough, Madison, NJ; 1 mL of vitamin A and D; 1.1 mL of Draxxin, Zoetis, Kalamazoo, MI; 50 mL of antibody serum (Bovisera), Colorado Serum, Denver, CO; 20 mL of C and D antitoxin, Boehringer Ingelheim, St. Joseph, MO; and 2 mL of INFORCE-3, Zoetis). Navels were treated with povidone iodine once daily for the first 3 d. Calves were weighed and a sample of blood from a jugular vein was used to estimate plasma protein score by refractometer. Arrival BW and plasma protein scores ranged from 36.3 to 51.3 kg and 4.6 to 7.2 g/dL, respectively. Individual age of calves was not known but was estimated to be between 1 and 4 d of age. Because colostrum intake also was unknown, calves were sorted by arrival BW and plasma protein score, then blocked by BW, and within block, treatments were assigned randomly to obtain treatment groups of calves as homogeneous as possible.

Treatments were (1) a commercial MR (**CON**; Excelerate, Milk Specialties Global Animal Nutrition, Eden Prairie, MN) and (2) a similar MR containing whey permeate rich in GOS (Milk Specialties Global Animal Nutrition). The production practices and conditions for GOS manufacture and incorporation were proprietary but generally followed the procedures described in the Introduction. Final measured concentration of oligosaccharides for the GOS treatment was 3.35% of MR DM and 0.06% of MR DM for CON (Table 1). The amount of GOS in the treatment MR was the maximum that could be produced by the technique and incorporated into the MR. This dosage was consistent with previous studies with pigs where ~4.8% of the diet DM as GOS supplementation resulted in a clear prebiotic effect (Smiricky-Tjardes et al., 2003). Analysis showed that CON contained ~44% of DM as lactose versus ~20% in GOS (Table 1), which reflects the treatment with β-galactosidase that converted lactose into a variety of oligo- and free monosaccharides.

Milk replacers were formulated to be isoenergetic and isonitrogenous and to meet or exceed NRC daily nutrient allowance recommendations (NRC, 2001). Protein was provided exclusively by dried whey and whey protein concentrate. Fat was a proprietary blend of tallow, lard, and coconut oil. Milk replacers were reconstituted with water to achieve 13% solids. Although higher intakes of nondigestible oligosaccharides cause changes in the osmotic gradient within the large intestine (Binder,