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Supplementing a yeast probiotic to pre-weaning Holstein calves: Feed intake, growth and fecal biomarkers of gut health

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ABSTRACT

Diarrhea, resulting from gastrointestinal infection by pathogens, is a common cause of the high mortality and morbidity of neonatal calves. The objective of this study was to evaluate the effects of supplementing a yeast product in milk replacer (MR) on growth and health of calves, and on fecal populations of some targeted microorganisms related to calf health and growth (*i.e.*, total bacteria, *Escherichia coli*, *Clostridium* cluster XIVa, *Faecalibacterium prausnitzii* and *Bifidobacterium* spp.). We hypothesized that feeding a *Saccharomyces cerevisiae* var *boulardii* (SCB) product would improve gastrointestinal health and growth performance of calves. Forty-two Holstein bull calves (42.6 ± 0.77 kg at birth) were randomly assigned on day 2 of age to either a control or SCB treatment. The SCB was supplemented in MR and fed at 5 g/d per head to supply 10 billion colony-forming units per day. All calves received high quality colostrum (> 50 mg/mL of immunoglobulin G) during the first 24 h of life, and were fed with 8 L MR (150 g/L mixed with 40 °C water) daily from day 2–35, and 4 L daily from day 35–42. Calves were also fed calf starter *ad libitum* from day 7–56. Daily MR and starter offered and refused, daily fecal scores, nasal scores, ear scores, and weekly body weight of calves were recorded. Fecal samples were collected on day 7, 35 and 56 after the first feeding of that day for microbial targets analysis. Overall, there is no serious disease challenge for all the calves during the entire experimental period. No differences were observed in MR intake, starter intake, metabolizable energy (ME) intake, average daily gain, ME intake to gain ratio, fecal score, nasal score, eye score or any targeted microorganisms between treatments throughout the experiment. These results suggest that supplementing SCB in MR has no additive effects on animal growth or fecal biomarkers of gut health when calves do not show deteriorated health status.

1. Introduction

The high rate of neonatal calf mortality – approximately 7.8% of pre-weaned heifers across North America (USDA, 2010) – remains a long standing challenge for the dairy industry. Gastrointestinal infections and subsequent diarrhea and dehydration account for the majority of mortality and morbidity of neonatal calves (USDA, 2010). Common practices employed by producers to treat diarrhea include electrolyte therapy to replace lost fluids, or the administration of antibiotics to combat invading pathogens

Abbreviations: ADG, average daily gain; BW, body weight; DM, dry matter; *E. coli*, *Escherichia coli*; ME, metabolizable energy; MR, milk replacer; PCR, polymerase chain reaction; SC, *Saccharomyces cerevisiae*; SCB, *Saccharomyces cerevisiae* var *boulardii*; STP, serum total protein; xfp, xylulose-5-phosphate/fructose-6-phosphate phosphoketolase

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(Lorenz et al., 2011). However, the extensive use of antibiotics may result in antibiotic resistant pathogens, posing a potential risk of exposure for calves and humans (Friedman et al., 2007). As such, alternatives to antibiotics for preventing and treating diarrhea are desirable.

Probiotics, including live bacteria and yeasts, are microbial food supplements that may beneficially affect the host by improving its intestinal microbial balance and have been widely studied as production enhancers (Hume, 2011). Recent studies in monogastrics have demonstrated that feeding *Saccharomyces cerevisiae* var *boulardii* (SCB), a subtype of *Saccharomyces cerevisiae* (SC) species, could reduce the risk of enteric diseases by blocking pathogenic toxin receptors sites, destructing pathogenic toxin receptors, inhibiting the growth of some pathogens within the intestinal lumen, and/or regulating immune responses (McFarland, 2010; Kelesidis and Pothoulakis, 2012). At birth, the digestive system of a calf functions similarly to that of a monogastric animal (Drackley, 2008). Because of this similarity, we hypothesized that feeding SCB would have similar effects in calves, thereby improving their gastrointestinal health and growth. The objective of this study was to evaluate the effects of supplementing SCB in milk replacer (MR) during the pre-weaning period on intake, growth and health of calves.

Moreover, because of the growing concept that establishing a proper microflora population in the gut can defend against pathogenic infections (Williams, 2010) and early bacterial colonization may impact the growth and development of animals later in life (Malmuthuge et al., 2015), our second objective was to evaluate if the gut microflora was influenced by SCB supplementation. Five general microbial markers associated with animal growth and health, including *Escherichia coli* (*E. coli*; Muktar et al., 2015), *Clostridium* cluster XIVa (Lopetuso et al., 2013), *Faecalibacterium prausnitzii* (Uyeno et al., 2010) and *Bifidobacterium* spp. (Picard et al., 2005) were evaluated by quantifying their populations in feces.

2. Materials and methods

2.1. Animals and diets

This study was approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta, Edmonton, Canada, and was conducted in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2009) at Eckerlea Acres Dairy Farm in Seaforth, Ontario, Canada. Forty-two male Holstein calves, averaging 42.6 ± 0.77 kg at birth, were used. The calves were removed from the dam within 2 h of birth, ear tagged, weighed, and housed in individual pens with straw bedding. The calves were given 4 L of quality colostrum (> 50 mg/mL of immunoglobulin G) within 3 h of birth and another 3 L of fresh colostrum at the next set feeding time. After the second colostrum feeding, calves were randomly assigned to 1 of 2 dietary treatments: 1) Control: a treatment fed commercial MR; or 2) Treat: a treatment fed commercial MR additionally supplemented with 5 g of live SCB product per day which was expected to supply 10 billion colony-forming units of SCB to each calf daily. The SCB product (ProTernative® Milk; Levucell SB20 containing the Pasteur Institute CNCM I-1079 strain of SCB; Lallemand Animal Nutrition, Montreal, Quebec, Canada) was mixed with MR in a bucket for each calf daily before morning feeding and given to them at the first feeding of the day. All calves were fed the MR solution by a nipple bottle (Super Calf Nipple; Merrick's, Middleton, Wisconsin, USA) during the first week of life, and then transitioned at 7 ± 3 d to a gate-mounted artificial teat (Peach Teats; Skellerup Industries Ltd., Woolston, New Zealand). The artificial teat feeding setup consisted of the teat mounted at the front of the pen, attached to a tube fitted with a one-way valve, running into a 8-L bucket placed outside the pen. The MR used in this study contained 260 g/kg crude protein, 160 g/kg crude fat, and 4.58 Mcal/kg metabolizable energy (ME) on a dry matter (DM) basis (MapleviewAgri, Palmerston, Ontario, Canada), and were mixed to 150 g/L with 40 °C water. For the first 2 days, MR was fed 3 times per day (0700, 1600 and 2100 h) with 3 L given at each of the first 2 feedings and 2 L at the third feeding. From day 3 until weaning at day 42, MR was fed in 2 equal volumes daily at 0700 and 1600 h, with 8 L offered daily from day 3 to 34, and 4 L daily from day 35 to 42. Calves also received *ad libitum* calf starter containing 220 g/kg crude protein, 37 g/kg crude fat, 57 g/kg crude fibre and 2.63 Mcal/kg ME on a DM basis (Nieuwland Feeds Elora Ltd., Elora, Ontario, Canada) from day 7 of age. They also had free access to water throughout the experiment.

Calves were given First Defense bolus (Immucell, Portland, Maine, USA) and Dystosel vitamin E/selenium (Zoetis, Kirkland, Quebec, Canada) to protect against *E. coli* K99+ and coronavirus and prevent white muscle disease after birth, respectively. Calves were vaccinated against Bovine Respiratory Syncytial Virus on day 1 of age using Inforce 3 (Zoetis, Kirkland, Quebec, Canada). Draxxin (tulathromycin; Zoetis, Kirkland, Quebec, Canada) was administered as a preventative for bovine respiratory disease on day 5 of age. Calves with diarrhea that required treatment received Metacam (Boehringer Ingelheim Ltd., Burlington, Ontario, Canada) subcutaneously and 2–4 L of oral electrolytes fluids in the evening after 9 pm. Any calves with digestive and respiratory problems were also treated and remained in the study for its duration, which was 56 days.

2.2. Feed intake, animal growth, health measurements, and sample collection

Calf starter offered and orts were weighed and recorded daily until 56 days of age to determine daily feed intakes. The calves were weighed at birth, and weekly thereafter until week 8, to determine weekly average daily gain (ADG). Fecal scores, nasal scores, and ear scores were recorded daily before the morning feeding using a 0–3 (fecal score and nasal score) or 0–4 (ear score) scale developed by University of Wisconsin-Madison (McGuirk, 2008). Fecal scores were 0: normal, 1: semi-formed, 2: loose, and 3: watery, with fecal score ≥ 2 considered as diarrhea. Nasal scores were categorized as 0: no discharge, 1: small amount of cloudy discharge from one nostril, 2: cloudy discharge from both nostrils, and 3: excessive thick cloudy discharge from both nostrils. Ear scores were categorized as 0: normal, 1: one ear droopy, 2: both ears slightly droopy, 3: both ears straight downward, and 4: head tilt.

Blood samples were collected via jugular venipuncture from the calves into vacutainer tubes 24 h after colostrum administration.