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Prophylactic use of a standardized botanical extract for the prevention of naturally occurring diarrhea in newborn Holstein calves

A. G. V. Teixeira, B. L. Ribeiro, P. R. M. Junior, H. C. Korzec, and R. C. Bicalho[1]
Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401

ABSTRACT

The objectives of this study were to evaluate the prophylactic use of SB-300 (Jaguar Animal Health, San Francisco, CA), a standardized botanical extract isolated from the bark latex of *Croton lechleri*, on reducing fecal water losses and diarrhea events in Holstein bull calves individually housed under a restricted whole-milk feeding regimen (6 L/d) from 1 to 25 d of life. Fluid therapy administration due to dehydration, average weight gain, and the fecal microbiome were also evaluated. Bull calves used in this study were born from normal parturition, fed 4 L of pooled pasteurized colostrum by esophageal feeder, and moved to a research facility at Cornell University (Ithaca, NY). A double-blinded randomized clinical trial was designed to allocate a total of 40 newborn calves into 1 of 2 treatment groups: calves receiving (twice daily) a solution containing 500 mg of SB-300 added to the whole milk for the first 15 d of life (SB-300, n = 20) or a control group receiving sterile water added to whole milk for the same period (CTR, n = 20). Treatment solutions had a total volume of 10 mL per treatment. Data regarding fecal dry matter were collected to precisely measure water content in fecal samples and to define diarrhea events; the SB-300 group had significantly increased fecal dry matter during the study period. Additionally, significantly fewer events of diarrhea were observed for calves in the SB-300 group (16.9%) compared with calves in the CTR group (46.5%). Dehydration status was evaluated and treated accordingly; calves with moderate dehydration were offered oral electrolytes, and calves with severe dehydration were rescued with intravenous fluid therapy. Calves in the SB-300 group had fewer intravenous fluid therapies administered during the study period (1.6%) compared with the CTR group (3.1%). Overall fluid therapy administered (oral electrolytes plus intravenous fluids) was significantly higher for the CTR group (9.2%) compared with the SB-300 group (6.1%) during the study period. No differences in milk consumption, calf starter intake, or weight gain were observed between treatment groups. A single time increase in *Bifidobacterium* was observed on d 20 of life for the SB-300 group; otherwise, no differences in fecal microbiome profile were detected between treatment groups. These results suggest that 500 mg of SB-300 added to the milk for 15 d can reduce the incidence of diarrhea and reduce severe dehydration in milk-fed calves.
Key words: calves, diarrhea, microbiome, SB-300 botanical extract

INTRODUCTION

Neonatal calf diarrhea is a multifactorial disease that can be caused by infectious and noninfectious factors (Walker et al., 1998; O'Handley et al., 1999). In a report from the 2010 National Animal Health Monitoring System study, diarrhea was the most common disorder affecting preweaned dairy heifers, with a nationwide incidence of almost 19%, and was the leading cause of death in preweaned heifers (USDA, 2012). Enteropathogens such as viruses, bacteria, and protozoa are often identified as etiological agents in calf diarrhea (Cho and Yoon, 2014).

Depending on the pathogen, calf diarrhea can have different pathophysiological mechanisms. These mechanisms have been well explained for *Escherichia coli* enterotoxin-mediated secretory diarrhea (Thiagarajah and Verkman, 2013), villus atrophy-mediated malabsorptive diarrhea caused by *Cryptosporidium parvum* (Heine et al., 1984), and coronavirus (Lewis and Phillips, 1978). Additionally, rotavirus can cause villus atrophy-mediated and chloride secretion-mediated diarrhea (Thiagarajah and Verkman, 2003). Briefly, the mechanisms by which loose stools are produced could be categorized as secretory, malabsorptive, or both.

Mixed infections are often reported in calves suffering from naturally occurring diarrhea (Reynolds et al., 1986). The prevalence of pathogens capable of producing secretory-induced neonatal diarrhea is reported to range between 2.0 and 45.0% for enterotoxigenic *Escherichia coli* (Frank and Kaneene, 1993; Luginbühl et al., 2005; Gulliksen et al., 2009) and between 17.0 and

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[1]Corresponding author: rcb28@cornell.edu

80.0% for rotavirus (Bartels et al., 2010; Smith, 2012; Klein-Jöbstl et al., 2014). These pathogens can induce secretory diarrhea by increasing chloride secretion in the intestinal lumen, consequently leading to intestinal fluid hyper-secretion (Thiagarajah and Verkman, 2003).

Extensive review on prevention and treatment of neonatal calf diarrhea is available (Constable, 2009). Fluid therapy remains the standard treatment for undifferentiated naturally occurring neonatal calf diarrhea. However, approximately 55% of calf operations facilities in the United States make use of medicated milk replacers to control neonatal calf diarrhea, and about 54% of operations treat their scouring calves with antibiotics (Walker et al., 2012). Five antimicrobials are approved, labeled, and allowed by the US Food and Drug Administration to be used in medicated milk replacer for the control of bacteria; however, maximum effort should be made to avoid routine administration of antibiotics because of the risk of antimicrobial resistance (van den Bogaard and Stobberingh, 2000). Therefore, alternatives to antimicrobials for control and prevention of neonatal calf diarrhea are needed.

Recently, a natural product with antisecretory properties have shown efficacy in reducing water losses measured in fecal samples of neonatal Holstein bull calves experimentally challenged with enterotoxigenic *E. coli* (Teixeira et al., 2015). The natural product comprises proanthocyanidin oligomers, which are polyphenolic molecules extracted from the bark latex of the plant species *Croton lecheri*. This botanical extract has been studied for its antisecretory actions that involve the inhibition of 2 distinct chloride channels on the luminal membrane of the intestine: cystic fibrosis transmembrane conductance regulator (CFTR) and calcium-activated chloride channel (CaCC) (Fischer et al., 2004; Tradtrantip et al., 2010).

The objective of this study was to evaluate the effect of daily doses of a botanical extract on fecal water content, diarrhea incidence, and use of fluid therapy in Holstein bull calves affected with naturally occurring undifferentiated diarrhea. Additionally, we used a metagenomic approach to describe the effect of the botanical extract on the fecal microbiome. The study hypothesis was that fecal dry matter, diarrhea incidence, and use of fluid therapy are affected by the use of an anti-secretory botanical extract when given in milk.

MATERIALS AND METHODS

Experimental Design, Animals, and Facility

This experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Cornell University (protocol number 2013-0075). Sample size was calculated based on a fecal DM difference previously evaluated by a pilot study. Based on an expected mean difference of fecal DM percentage between treatment groups of 1.4, a treatment group standard deviation of 1.2, and a control group standard deviation of 1.7, with a treatment group ratio of 1, and assuming a type I error rate of 5% and a power of 80%, a sample size of 18 calves per group was calculated. As 10% mortality was anticipated, 20 calves per group were enrolled in this study.

The study design was a double-blinded randomized clinical trial. Randomization was performed a priori using the Excel random function (Microsoft Corp., Redmond, WA) to create a balanced number of calves per treatment group. A total of 40 Holstein bull calves from one commercial dairy farm (Scipio Center, NY) were enrolled in the study. Newborn Holstein bull calves were monitored during parturition by on farm staff. Calves were eligible to be enrolled in the study if no assistance was required during parturition, no twins, and no birth defects detected by physical exam after parturition. To mimic farm conditions, 4 L of pasteurized (60°C for 60 min) pooled colostrum (T-300 GoodNature Products Inc. Orchard Park, NY) with Brix reading averaging 22.8% (ranging from 20.6 to 24.8%) was administered by esophageal tube to all calves within 45 min of birth (Oral Calf Feeder Bag with Probe, Jorvet, Loveland, CO). All calves were transported from the farm to the study site within 4 h after birth. Briefly, calves were transported using an adapted vehicle for animal transportation; calves were kept inside individual crates with proper ventilation. Cleaning and sanitization of the vehicle was performed before and immediately after transportation.

The study site was a research barn with controlled temperature (20.6°C, ranging from 19.4 to 21.8°C) and humidity (50%, ranging from 45 to 55%) containing 20 individual stalls (2.1 m^2 each) isolated by concrete walls, where calves were unable to have any contact with other calves or outside areas. A 3-step cleaning procedure was used for water buckets, feed buckets, and bottles after each feeding, consisting of rinsing all the equipment with lukewarm water, scrubbing with a mixture of hot water and alkaline detergent solution, and finally rinsing in chlorinated water. The research barn used to conduct this trial was used twice because limited space was available (20 stalls). The first run was performed from January to March 2016, and the second run from March to May 2016. The 2 runs were performed using the same procedures for enrollment, data collection, cleaning, and laboratory procedures.

Calves were fed saleable whole milk purchased from the Cornell Teaching Dairy (Cornell University, Ithaca,