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Intestinal permeability and incidence of diarrhea in newborn calves

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ABSTRACT

Seventy-six newborn Holstein calves (44.4 ± 6.15 kg of body weight) were involved in this study from birth until 21 d of age. Within 2 h after birth, calves received 4 L of maternal colostrum via an esophageal tube. The following 3 meals consisted of 2 L of late colostrum (or transition milk). After that, calves were fed 1.5 L of milk replacer (22.9% CP, 20.1% fat) twice daily. Calves were considered diarrheic when they showed fecal scores ≥3 for 3 consecutive days. Then, data from a random subset of 30 calves (45.9 ± 5.47 kg of body weight), 15 that never had diarrhea and 15 that had diarrhea, were used to assess potential associations between intestinal permeability and incidence of diarrhea. On d 0, 7, 14, and 21 of life, intestinal permeability of calves was measured by dosing 2 markers (lactulose and D-mannitol) and assessing their concentration in serum by ultra-HPLC-mass spectrometry. Plasma IgG concentration was measured at birth and at 6 h, 24 h, and 12 d after first colostrum intake, and efficiency of IgG absorption was calculated. Plasma and colostrum IgG contents were determined by radial immunodiffusion and bacterial load in colostrum samples by colony counting. All diarrhea incidences occurred between 7 to 14 d of life. Overall colostrum quality was good, with an IgG content >100 mg/mL, but total bacterial load was slightly high (>100,000 cfu/mL). However, there were no differences in these 2 parameters between colostrums consumed by calves that did and those that did not incur diarrhea later in life, and efficiency of IgG transfer from colostrum to bloodstream was similar for all calves. Diarrheic calves had greater serum lactulose concentrations than healthy calves throughout the first 21 d of life. Furthermore, diarrheic calves tended to have a greater serum lactulose-to-D-mannitol ratio from birth until 21 d of life compared with healthy calves. In conclusion, calves that incur diarrhea show an altered intestinal permeability within the first 2 h of life compared with those that do not suffer scours.

Key words: calf, colostrum, diarrhea, intestinal integrity

INTRODUCTION

The intestinal epithelium is the first protective barrier from exogenous pathogens (Deitch and Berg, 1987). Thus, the integrity of the intestinal mucosa ensures proper nutrient absorption while avoiding translocation of pathogens into the lamina propria. Intestinal permeability can be transcellular (through epithelial cells by active or passive transport), which is mainly used for absorption of small molecules, or paracellular (through tight junctions that connect epithelial cells), which is mainly used for absorption of macromolecules (Bjarnason et al., 1995; Hall, 1999). Intestinal permeability is high in newborn calves during the first 24 to 36 h of life (Bush and Stanley, 1980; Besser and Gay, 1994), which is crucial to facilitate transfer of Ig from the colostrum into the calf bloodstream via a nonselective macromolecular transport system across the small intestinal epithelium (Staley and Bush, 1985). However, on the other hand, this increased permeability during the first few hours after birth renders the intestinal wall of newborn calves highly susceptible to bacterial translocation and increases the susceptibility to infections (Berg, 1995; Uil et al., 1997).

Our hypothesis was that calves that suffer diarrhea would have an altered intestinal permeability Thus, the objective of this study was to evaluate potential changes in intestinal permeability before and after an incidence of diarrhea in newborn calves.

MATERIALS AND METHODS

Animals and Treatments

Seventy-six singleton newborn Holstein calves (44.4 ± 6.15 kg of BW) born to cows that needed no as-

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sistance for calving were involved in this study from birth until 21 d of age. Within 2 h after birth, calves were fed 4 L of colostrum either frozen, refrigerated, or fresh (either from their dam or from another cow) using an esophageal tube, followed by 3 meals of 2 L of late colostrum or transition milk in a bucket. The following 3 wk, calves were fed 1.5 L of a commercial (Naturmilk Super 60, Ouest Elevage, France) milk replacer (**MR**) containing, on a DM basis, 22.9% CP and 20.1% fat twice daily (0830 and 1700 h) at a 15% DM concentration. Animals were raised in individual hutches (3.1 × 1.2 m) and had ad libitum access to water and starter feed (containing 19.7% CP and 3.9% fat on a DM basis) throughout the study.

Measurements and Sample Collection

Individual BW was recorded at 0, 7, 14, and 21 d of age. Fecal consistency of calves was evaluated on a daily basis following a 5-point-scale fecal score (Lesmeister and Heinrichs, 2004). Also, a permeability test using lactulose and D-mannitol as markers (Hall, 1999) was performed in all 76 calves at birth (d 0; coinciding with the first colostrum feeding) and 7, 14, and 21 d of age (while offering the morning MR allowance). The permeability tests consisted of administering (via colostrum or MR) at birth 20 g of lactulose (Duphalac, Madrid, Spain) and 4 g of D-Mannitol (Sigma-Aldrich Corp., St. Louis, MO), at 7 and 14 d of age 21 g of lactulose and 4.2 g of D-mannitol, and at 21 d of age 22 g of lactulose and 4.4 g of D-mannitol dissolved either in the colostrum or MR. In all permeability tests, 60 min after dosing the markers via colostrum or MR, blood samples were collected (BD Vacutainer clot activator, Belliver Industrial Estate, Plymouth, Devon, UK) for subsequent determination of lactulose and D-mannitol serum concentrations and estimated intestinal permeability based on these concentrations.

Two replicates of first- and late-colostrum (or transition milk) samples were taken in sterile 50-mL collection tubes and immediately frozen at −20°C for later IgG and bacterial load determinations. At birth, a blood sample was collected from the jugular vein (BD Vacutainer spray-coated K_2EDTA 4 mL Tubes, Belliver Industrial Estate) from all calves to later determine basal plasma IgG concentrations. Additional blood samples for IgG determination were taken 6 h, 24 h, and 12 d after first colostrum consumption. All blood samples were kept on ice for a minimum of 20 min and then centrifuged at 3,500 × *g* for 10 min at 4°C. Serum and plasma samples were stored at −20°C until subsequent analysis.

Chemical Analyses

Samples of MR were analyzed for DM (24 h at 103°C), ash (4 h at 550°C), and N content according to the method of the Association of Official Analytical Chemists (method 988.05; AOAC, 1990) adapted for an automatic distiller Kjeldahl (Kjeltec Auto 1030 Analyzer, Tecator, FOSS, Hillerød, Denmark) with copper sulfate/selenium as a catalyst instead of copper sulfate/titanium dioxide and for ether extract following method 920.39 of AOAC (1990) with petroleum ether used for distillation instead of diethyl ether (AOAC, 1990).

Determination of serum lactulose and D-mannitol concentrations was performed by ultra-HPLC-mass spectrometry (Xevo G2 Tof, Waters, Milford, MA) with an electrospray ionization source operating in negative mode. Serum extract was injected (5 μL) onto a BEH amide column (2.1 mm × 100 mm, 1.7 μm, Waters). The mobile phases were water + 0.1% NH_4OH, and methanol + 0.1% NH_4OH. Elution conditions, at a flow rate of 0.3 mL/min, were as follows: 90% methanol + 0.1% NH_4OH maintained for 2 min, linear gradient from 90 to 60% in 4 min, and equilibration to initial conditions over 4 min. Column and auto-sampler chamber temperatures were maintained at 45 and 4°C, respectively. The operating conditions were as follows: source temperature = 120°C; desolvation temperature = 350°C; desolvation gas = 900 L/h; cone gas = 10 L/h; capillary voltage = 0.5 kV; cone voltage = 30 V; and extraction cone = 4 V. Leucine enkephalin at a concentration of 2 μg/mL was used as a lock mass for mass accuracy and infused at a flow of 5 μL/min. Chromatograms were processed using Quanlynx software (v 4.1, Waters).

Concentrations of IgG in plasma and colostrum were measured by radial immunodiffusion (Triple J Farms, Bellingham, WA). Determination of colostrum bacterial loads were performed by colony counting using 3 dilutions (1:10, 1:100, and 1:1000) of each specimen. For total bacterial counts, 100 μL of the dilutions 1:10 and 1:100 were plated onto trypticase soy agar (Difco, Detroit, MI) with 5% bovine blood and the plates incubated at 37°C in an atmosphere of 5% CO_2 for 24 h. For total enterobacterial counts, 100 μL of dilutions 1:100 and 1:1000 were plated onto MacConkey agar plates and incubated at 37°C for 24 h.

Calculations and Statistical Analysis

Whenever an animal presented a fecal score ≥3 for 3 consecutive days, they were considered diarrheic. That allowed classifying calves as healthy or diarrheic. After