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The influence of age and weaning on permeability of the gastrointestinal tract in Holstein bull calves

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ABSTRACT

Fourteen Holstein bull calves were used in a randomized complete block design to investigate the effect of calf age and weaning on permeability of the gastrointestinal tract (GIT). Calves were randomly assigned to 1 of 2 treatments: (1) a weaning protocol that was initiated on d 35; WN; n = 7), or (2) a control treatment where calves were not weaned (CON; n = 7). Calves were bottle-fed milk replacer (150 g/L), in 3 equal portions/d targeting 15% of their body weight (BW) in liquid milk intake [approximately 21.1 g/kg of BW/d, dry matter (DM) basis]. On d 35, the amount of milk replacer offered to WN calves was reduced to 7.5% of BW for 7 d before calves were weaned on d 42. On d 14, 28, and 42, calves were orally dosed with 500 mL of Cr-EDTA (179 m*M* Cr-EDTA solution) and housed in a metabolism crate to enable total urine collection and determination of total urinary Cr recovery as an indicator of total-tract permeability. On d 44, calves were killed and tissues from the rumen, omasum, duodenum, jejunum, ileum, cecum, and proximal and distal colon were collected, rinsed, and transported in buffer solution (pH 7.4 at 38.5°C). Tissues were incubated in Ussing chambers under short-circuit conditions with buffer solutions designed to mimic the mucosal and serosal energy source that would be available in vivo (glucose for tissues from the small intestine and short-chain fatty acids for tissues that would be exposed to fermentation; rumen, omasum, and large intestinal tissues). The serosal to mucosal flux of ^{14}C-mannitol and ^{3}H-inulin was measured for each region. Although we detected treatment × period interactions for BW and starter intake, dietary treatments did not differ within a week. Overall, the time that ruminal pH was <5.5 was less before weaning than after weaning. We observed a differential response for the appearance of Cr in urine for WN and CON calves, where the appearance of Cr (mg/48 h) in urine decreased for both treatments from d 14 to 28, but increased from d 28 to 42 for WN, whereas Cr appearance continued to decrease for CON. The flux of mannitol and inulin did not differ between treatments but did differ among region of the GIT, with rumen, duodenum, and jejunum having the greatest permeability. These data suggest that permeability of the GIT decreases with age but weaning may disrupt this process. The rumen, duodenum, and jejunum appear to be the regions with greatest permeability.
Key words: calf, gastrointestinal tract, permeability, Ussing chamber, weaning

INTRODUCTION

The postnatal period up to and through weaning is critical for dairy calves. For example, a recent study demonstrated that preweaning ADG, driven by greater consumption of milk replacer, was positively associated with first-lactation milk yield (Soberon et al., 2012). This period also represents a time when calves are susceptible to infectious disease. In fact, the leading cause of mortality and morbidity in calves during the first weeks of life in North America is diarrhea (NAHMS, 2007) caused by pathogenic infections of the gastrointestinal tract (**GIT**). As mortality and incidence of health events in calves remain high on dairy farms in North America, an improved understanding of this phase in calf development can lead to opportunities to enhance calf health and lifetime productivity.

Rearing programs for dairy calves have been designed to promote adequate DMI as a mechanism to stimulate development of the ruminal epithelium and minimize the reduction in growth at weaning (NRC, 2001). It should be recognized that weaning also results in a major shift in the site of nutrient digestion, from the intestine to the reticulo-rumen, and correspondingly the primary energy source changes [short-chain fatty acids (**SCFA**) and microbial protein vs. glucose, galactose,

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and milk fat and protein]. It could be expected that these changes may compromise gastrointestinal barrier function (Wijtten et al., 2011) due to low ruminal pH at weaning (Laarman et al., 2012) and that short-term reductions in nutrient provision reduce total-tract barrier function in cattle (Gaebel et al., 1993; Gäbel and Aschenbach, 2002; Zhang et al., 2013). However, only one study known to us has evaluated barrier function in Holstein calves in response to weaning (Malmuthuge et al., 2013). In that study, the mRNA expression of toll-like receptor-2 decreased and the expression of occludin and claudin-4 increased in rumen tissue collected from calves weaned relative to their nonweaned comparatives. It is not clear whether these changes in mRNA expression correspond to changes in innate immune system responsiveness and permeability of the gastrointestinal tract. Moreover, it is not clear how calf age affects intestinal permeability.

Although rumen development is often evaluated to assess weaning programs, it should be acknowledged that rumen development may be associated with developmental changes in the small intestine (Górka et al., 2011, 2013) and thus, weaning may influence more regions than the rumen due to changes in nutrient supply and type of nutrient provided, as discussed above. Mannitol, inulin, and Cr-EDTA are markers that have been used to assess GIT permeability. These compounds are nonnutritive and are suggested to cross the GIT via paracellular movement, thus allowing for their use as markers of tissue permeability (Wijtten et al., 2011; Zhang et al., 2013; Penner et al., 2014). Using this approach, Penner et al. (2014) demonstrated that in 6-mo-old calves, susceptibility for movement of mannitol was greatest in the jejunum, whereas movement of inulin was greatest in the omasum followed by the rumen. This suggests that weaning strategies should consider functional changes associated with the entire GIT.

We hypothesized that weaning will negatively affect permeability of the gastrointestinal tract and will involve an increase in permeability in the rumen, omasum, and jejunum. The main objective was to investigate the influence of weaning and age on the permeability of the GIT in calves using in vivo and ex vivo measures of permeability.

MATERIALS AND METHODS

This experiment was conducted between April and July 2014 at the Livestock Research Building at the University of Saskatchewan (Saskatoon, Saskatchewan, Canada). All procedures used during this experiment were approved by the University of Saskatchewan Research Ethics Board (protocol 20100021).

Animals and Experimental Design

Fourteen Holstein bull calves were used in a randomized complete block design. Bull calves were obtained from 3 local commercial dairy farms (block) and transported to the research barn within 12 h of birth. Calves received a commercially available colostrum replacer (60 g of IgG, HeadStart, Saskatchewan Colostrum Company, Saskatoon, SK, Canada) on farm and a second dose on arrival at the research barn. Upon arrival, calves received an ear tag and were randomly assigned to 1 of 2 dietary treatments: fed milk replacer and starter until 35 d of age at which time they were weaned over 7 d and killed 2 d postweaning (d 44; **WN**; n = 7), or fed milk replacer and starter until d 44 (**CON**; n = 7). Calves were housed in individual pens (1.2 × 2.4 m) with rubber mats on the floor. Trace amounts of wood shavings were provided to absorb moisture associated with fecal and urine excretion.

After receiving their 2 feedings of colostrum, calves were fed a commercially available milk replacer (Nature's Way 26/16, Landmark Feeds, Winnipeg, MB, Canada; contained 26% CP, 16% crude fat, 0.15% crude fiber, 8% ash, 0.95% Ca, 0.7% P, 0.7% Na, 40,000 IU/kg vitamin A, 4,000 IU/kg vitamin D_3, and 150 IU/kg vitamin E) at a rate of 7.5% of BW of liquid milk/d (approximately 10.5 g/kg of BW, DM basis) for 7 d, and were gradually transitioned to 15% of BW (approximately 21.1 g/kg of BW, DM basis) per day over the next 7 d. Each bag of milk replacer was emptied into a large container and mixed before feeding to minimize variation among feedings, and a 500-g sample was obtained for DM and compositional analysis. Milk was prepared according to the manufacturer's instructions [150 g/L; mixed with half the volume of water (45 to 60°C) and whisked for 3 min; then, water was added to bring up to the final volume, resulting in a serving temperature ranging between 38 and 42°C]. Milk was fed by bottle 3 times daily (0700, 1200, and 1700 h) in equal volumes. Calves were given 30 min to consume each meal and if refusal was greater than 50% of the meal allotment, the milk replacer was fed via esophageal tube. This approach was used to minimize variation in milk replacer consumption among treatments. Refusals less than 50% of the meal allotment were removed and the volume was recorded.

Calves were initially offered 100 g/d of a commercially available texturized calf starter ration (Optivia Express Calf Starter, Landmark Feeds, Winnipeg, MB, Canada; contained 21.5% CP, 3% crude fat, 6% crude fiber, 0.99% Ca, 0.53% P, 0.38% Na, 20.4 kIU/kg vitamin A, 3.3 kIU/kg vitamin D, 95 IU/kg vitamin E, and 52 mg/kg sodium monensin). Fresh starter was provided each morning and the provision increased to