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Intentionally induced intestinal barrier dysfunction causes inflammation, affects metabolism, and reduces productivity in lactating Holstein cows

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ABSTRACT

Study objectives were to evaluate the effects of intentionally reduced intestinal barrier function on productivity, metabolism, and inflammatory indices in otherwise healthy dairy cows. Fourteen lactating Holstein cows (parity 2.6 ± 0.3; 117 ± 18 d in milk) were enrolled in 2 experimental periods. Period 1 (5 d) served as the baseline for period 2 (7 d), during which cows received 1 of 2 i.v. treatments twice per day: sterile saline or a gamma-secretase inhibitor (GSI; 1.5 mg/kg of body weight). Gamma-secretase inhibitors reduce intestinal barrier function by inhibiting crypt cell differentiation into absorptive enterocytes. During period 2, control cows receiving sterile saline were pair-fed (PF) to the GSI-treated cows, and all cows were killed at the end of period 2. Administering GSI increased goblet cell area 218, 70, and 28% in jejunum, ileum, and colon, respectively. In the jejunum, GSI-treated cows had increased crypt depth and reduced villus height, villus height-to-crypt depth ratio, cell proliferation, and mucosal surface area. Plasma lipopolysaccharide binding protein increased with time, and tended to be increased 42% in GSI-treated cows relative to PF controls on d 5 to 7. Circulating haptoglobin and serum amyloid A concentrations increased (585- and 4.4-fold, respectively) similarly in both treatments. Administering GSI progressively reduced dry matter intake (66%) and, by design, the pattern and magnitude of decreased nutrient intake was similar in PF controls. A similar progressive decrease (42%) in milk yield occurred in both treatments, but we observed no treatment effects on milk components. Cows treated with GSI tended to have increased plasma insulin (68%) and decreased circulating nonesterified fatty acids (29%) compared with PF cows. For both treatments, plasma glucose decreased with time while β-hydroxybutyrate progressively increased. Liver triglycerides increased 221% from period 1 to sacrifice in both treatments. No differences were detected in liver weight, liver moisture, or body weight change. Intentionally compromising intestinal barrier function caused inflammation, altered metabolism, and markedly reduced feed intake and milk yield. Further, we demonstrated that progressive feed reduction appeared to cause leaky gut and inflammation.
Key words: inflammation, insulin, intestinal integrity, lipopolysaccharide

INTRODUCTION

Appreciation is growing for the importance of proper intestinal barrier function in domestic farm animals. The luminal content of the gastrointestinal tract technically remains extrinsic to the animal, and thus serves the dual role of absorbing valuable nutrients while preventing infiltration of unwanted compounds and molecules (Mani et al., 2012). The human gastrointestinal tract has a surface area of ∼400 m^2, which is 200 times greater than that of the skin (Murphy, 2012), and it is continuously subjected to potentially pathogenic microorganisms and toxins (Mani et al., 2012). Barrier importance is heightened in cattle, because both the size of the gastrointestinal tract and potential toxin exposure are more extensive in ruminants due to pregastric fermentation compartments. It is not surprising that a large majority of the immune system resides in the splanchnic bed (van der Heijden et al., 1987).

A variety of diseases, albeit with etiological differences, have a common dominant pathology of impaired intestinal barrier function (i.e., leaky gut), including Crohn's disease, inflammatory bowel syndrome, celiac disease, and alcoholism (Draper et al., 1983; Bargiggia et al., 2003; Ludvigsson et al., 2007; McGowan et al., 2012). Recognized circumstances in animal agriculture in which gastrointestinal tract barrier function is compromised include weaning (Boudry et al., 2004; Moeser

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et al., 2007), heat stress (Baumgard and Rhoads, 2013; Pearce et al., 2013), and rumen acidosis (Emmanuel et al., 2007; Khafipour et al., 2009; Minuti et al., 2014). Additionally, reduced feed intake decreases barrier integrity in humans (Welsh et al., 1998) and farm animals (Pearce et al., 2013; Zhang et al., 2013; Stoakes et al., 2015a). Further, we have preliminary evidence strongly implicating leaky gut as the etiological origin of ketosis in transitioning dairy cows (Abuajamieh et al., 2016). Accordingly, multiple situations experienced by farm animals have the potential to induce leaky gut.

Presumably, an impaired intestinal barrier will negatively affect economically important phenotypes. However, directly studying post-absorptive and production consequences of leaky gut is difficult, because the conditions thought to be responsible for reducing intestinal barrier integrity also affect multiple tissues and systems. Obvious examples of biologically confounding situations include the periparturient period and heat stress, both of which are accompanied by marked homeorhetic adaptations to support a new dominant physiological state (Bauman and Currie, 1980; Baumgard and Rhoads, 2013). Evaluating the metabolic, endocrine, inflammatory, and production consequences of leaky gut in isolation would provide insight into its direct impact on the pathophysiology of common on-farm disorders.

We hypothesized that intestinal tract barrier dysfunction (in apparently otherwise healthy animals) would detrimentally affect production parameters, metabolic variables, and inflammatory indices, and that these post-absorptive consequences would resemble characteristic biomarkers in the aforementioned disorders. To test this, we used gamma-secretase inhibitor (**GSI**) to decrease intestinal barrier integrity. Administrating GSI causes intestinal metaplasia of mucus-secreting goblet cells from crypt cells via Notch pathway interference (Milano et al., 2004; van Es et al., 2005), which is necessary for normal absorptive enterocyte maturation and proliferation (Okamoto et al., 2009). Disrupting ordinary crypt cell differentiation using GSI severely damages intestinal structures (Wong et al., 2004) and inhibiting the Notch pathway decreases epithelial cell turnover and increases intestinal permeability (Obata et al., 2012).

MATERIALS AND METHODS

Animals and Sampling

The Institutional Animal Care and Use Committee at Iowa State University approved all procedures involving animals. Fourteen lactating Holstein cows (117 ± 18 DIM; 666 ± 14 kg BW; parity 2.6 ± 0.3) were housed at the Iowa State University Dairy Farm and enrolled in 2 experimental periods. Period 1 (**P1**) lasted 4 to 5 d and served as the baseline (data generated for covariate analysis) for period 2 (**P2**). Period 2 lasted 7 d, during which cows received 1 of 2 i.v. treatments twice daily at 0600 and 1800 h: control (1 L sterile saline; n = 7) or GSI (1.5 mg/kg of BW semagacestat dissolved in 1 L of sterile saline; LY-450139; Eli Lilly and Company, Indianapolis, IN; n = 7). The GSI dose was selected from a preliminary dose-response trial, where 1 mg/kg/d BW did not induce overt phenotypic responses and 6 mg/kg day caused a severe and rapid decrease in feed intake (data not shown). Control animals were pair-fed (**PF**) to the GSI-treated cows to eliminate the confounding effects of dissimilar nutrient intake, as we have described (Wheelock et al., 2010).

All cows were individually fed a TMR once daily at 0800 h, and orts were recorded daily before feeding. The TMR was formulated by Nutrition Professionals Inc. (Neenah, WI) to meet or exceed the predicted requirements of energy, protein, minerals, and vitamins (NRC, 2001; Table 1). Reductions in daily feed intake by GSI-treated cows in P2 were determined as a percentage of their mean daily ad libitum intake during P1. Initiation of P2 for the PF cows occurred 1 d later to allow for pair-feeding calculations and implementation. For tissue-collection consistency, PF and GSI cows were euthanized after morning blood samples on the same

Table 1. Ingredients and composition of diet[1]

Composition	% of DM[2]
Ingredient	
Corn silage	33.6
Alfalfa hay	19.8
Rolled corn	17.1
Ground corn	13.7
Whole cotton	8.6
Soy Plus[3]	4.2
High-protein soybean meal[4]	3.0
Chemical analysis	
CP	15.7
NDF	31.6
ADF	22.7
NE_L (Mcal/kg DM)	1.6

[1]Values represent an average of samples collected and composited throughout the trial. Dry matter averaged 53%.

[2]Average nutrient levels: 5.74% fat, 0.84% Ca, 0.34% P, 0.37% Mg, 0.19% S, 1.1% K, 0.44% Na, 0.47% Cl, 56.30 mg/kg Zn, 60.08 mg/kg Mn, 95.76 mg/kg Fe, 17.28 mg/kg Cu, 0.19 mg/kg Co, 0.28 mg/kg Se, 43.68 mg/kg I, 4,475.9 IU/kg vitamin A, 1,438.8 IU/kg vitamin D, and 26.95 IU/kg vitamin E.

[3]Cooker-expeller processed soybean meal produced by West Central Cooperative (Ralston, IA), containing 46.6% CP, 60% RUP (% CP); DM basis.

[4]Solvent-extracted soybean meal containing 54.5% CP, 35% RUP (% CP); DM basis.