Study objectives were to evaluate the effects of hindgut acidosis (HGA) on production, metabolism, and inflammation in feed-restricted (FR) dairy cows. Twelve rumen-cannulated cows were enrolled in a study with 3 experimental periods (P). During P1 (5 d), baseline data were collected. During P2 (2 d), all cows were FR to 40% of their baseline feed intake. During P3 (4 d), cows remained FR and were assigned to 1 of 2 abomasal infusion treatments: (1) control (FR-CON; 6 L of H2O/d; n = 6) or (2) starch (FR-ST; 4 kg of corn starch + 6 L of H2O/d; n = 6). Respective treatments were partitioned into 4 equal doses (1 kg of corn starch/infusion) and were abomasally infused daily at 0000, 0600, 1200, and 1800 h. All 3 P were analyzed independently and the effects of treatment, time, and treatment × time were assessed using PROC MIXED, and P1 and P2 data were analyzed using the treatments cows were destined to be assigned to during P3. Hallmark production and metabolic responses to feed restriction were observed in both treatments, including decreased milk yield (39%) and energy-corrected milk (32%), circulating glucose (12%), insulin (71%), and increased circulating nonesterified fatty acids (3.2-fold) throughout both P2 and P3, relative to P1. However, despite a marked reduction in fecal pH (0.96 units), the aforementioned metrics were unaltered by HGA. During P3, starch infusions increased circulating β-hydroxybutyrate, with the most pronounced increase occurring on d 2 (81% relative to FR-CON). Further, feed restriction decreased blood urea nitrogen during P2 (17% relative to P1) in both treatments, and this was exacerbated by starch infusions during P3 (31% decrease relative to FR-CON). In contrast to our hypothesis, neither feed restriction nor HGA increased circulating acute-phase proteins (serum amyloid A and lipopolysaccharide binding protein) relative to P1 or FR-CON, respectively. Thus, despite marked reductions in fecal pH, prior feed restriction did not appear to increase the susceptibility to HGA.

Key words: starch infusion, inflammation, leaky gut

INTRODUCTION

Substantial attention has focused on improving periparturient dairy cow health, yet common disorders (e.g., hyperketonemia, subclinical hypocalcemia, and fatty liver) remain prevalent, and they presumably compromise animal welfare and producer profitability (Leblanc et al., 2006; Horst et al., 2021). It has recently been proposed that inflammation is causal and central in the development of the aforementioned transition cow maladies (Horst et al., 2021). Some degree of inflammation is normal even in overtly healthy transition cows (Bionaz et al., 2007; Trevisi et al., 2012). However, it has been postulated that the exposure to a multitude of concurrent or successive stressful events (i.e., “stacked stressors”), potentially starting as early as dry-off (Mezzetti et al., 2020; Goetz et al., 2022), can amplify this inflammation either through direct infection (e.g., uterus, mammary gland, or lung) or via stress’s deleterious effects on gastrointestinal tract (GIT) barrier function (Trevisi and Minuti, 2018; Mayorga et al., 2020; Horst et al., 2021). A compromised gut barrier allows luminal antigens (e.g., LPS) translocation into the basal lamina, local tissues, and systemic circulation, where recognition by toll-like receptor 4–possessing cells activates a nutrient- and energetically expensive immune response (Kumar et al., 2011; Johnson, 2012; Kvidera et al., 2017b). Among the stressors that can perturb GIT barrier function, SARA is one in which the etiological effects on GIT hyperpermeability remain unclear.

The adverse effects of rumen acidosis (e.g., decreased feed intake or milk yield) have historically been attributed to a systemic rise in luminaly derived immune-stimulating compounds (e.g., LPS and histamine), which are presumed to emanate from a compromised rumen epithelial barrier (Stone, 2004; Gozho et al., 2022).
2005). However, postruminual starch flow increases in parallel with dietary starch supply (Sanz-Fernandez et al., 2020), thereby increasing the proportion of starch degraded postruminally. Notably, diarrhea and altered fecal characteristics (e.g., foamy or yellow in color) are used as indicators of SARA in the field (Nordlund et al., 2004; Plaizier et al., 2008) and reflect a pernicious hindgut luminal environment (e.g., decreased pH and increased osmolarity, endotoxin, or biogenic amines; Ametaj et al., 2010; Li et al., 2012; Grauso et al., 2019) with increased starch availability. Relative to the rumen, which has 4 multilayered strata of squamous epithelium (Steele et al., 2016), the hindgut comprises a single layer of columnar epithelia covered in mucous and has substantially less buffering capacity due to a lack of saliva and protozoal populations (Gressley et al., 2011). Thus, the noxious environment resulting from excessive microbial fermentation may damage the hindgut epithelium and contribute to the inflammatory signature of acidic cows (Gozho et al., 2005). In fact, Khafipour and colleagues (2009a,b) observed systemic inflammation in cows with grain- but not alfalfa-pellet-induced SARA, and they hypothesized that these discrepancies were due to the lack of hindgut acidosis (HGA) in the alfalfa-pellet diet. In support, Bissell and Hall (2010) observed a systemic inflammatory response and severe morbidity (Dr. Mary Beth Hall, personal communication) during cornstarch-infused (4 kg/d) HGA in dry cows consuming a high-fiber diet. Considering the marked decrease in postpartum fecal pH (McCarthy et al., 2020) and its association with reduced production and inflammation (Rodriguez-Jimenez et al., 2019), there is reason to speculate that HGA may cause or contribute to periparturient inflammation via its impact on hindgut barrier function.

Although the HGA hypothesis is compelling, our group and others have failed to experimentally recreate the circumstances in which isolated HGA initiated an inflammatory response and immune-activated phenotype in dairy cows or pigs (Mainardi et al., 2011; Mayorga et al., 2021; van Gastelen et al., 2021a,b; Abeyta et al., 2023a,b; Piantoni et al., 2023). Although the reasons are unclear, the lack of stacked stressors in these experiments may explain the apparent tolerance to HGA, as previous work has described an augmented and prolonged inflammatory response with multiple successive or concurrent insults (Horst et al., 2020b). This concept is reinforced by the amplification of stress- or trauma-induced inflammation with multiple chronic or acute stressors (i.e., the “2-hit hypothesis”; see reviews by Lang and Hickman-Davis, 2005; Rohleder, 2019). Thus, for a myriad of reasons (e.g., suboptimal feed intake, psychological stress, parturition, or tissue remodeling), the periparturient cow might be more susceptible to the stress of HGA than a healthy cow in established lactation. In this context, HGA may augment the inflammatory state by increasing luminal endotoxin concentrations (Li et al., 2012) or via direct aggravation of large intestine epithelia. Therefore, experimental objectives were to evaluate the isolated effects of HGA in already stressed (feed-restricted, FR) dairy cows with the hypothesis that prior feed restriction would increase the susceptibility to HGA, resulting in an inflammatory response and a malaise phenotype.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Twelve cannulated, midlactation dairy cows (131 ± 43 DIM; 1.7 ± 0.7 parity; 669 ± 43 kg of BW) were used in an experiment conducted in 2 replications. Cows were moved into individual box stalls (4.57 × 4.57 m) at the Iowa State University Dairy Farm in November 2019 and allowed 3 d to acclimate to experimental settings. On d 2 of acclimation, all cows were implanted with jugular catheters as previously described (Horst et al., 2020c). The trial consisted of 3 experimental periods (P). During P1 (5 d), cows were fed once daily (0800 h), and baseline data were generated to determine ad libitum feed intake for later feed restriction calculations. During P2 (2 d), all cows were FR to 40% of their baseline feed intake. During P3 (4 d), cows continued to be FR (40% of baseline intake) and were balanced by milk yield, DIM, parity, and presumed pregnancy status and assigned to 1 of 2 abomasal infusion treatments: (1) control (FR-CON; 6 L of H2O/d) or (2) starch [FR-ST; 4 kg of corn starch (98.5% DM) + 6 L of H2O/d]. The corn starch dose was chosen based on a previous report (Bissell and Hall, 2010). Respective abomasal infusion treatments were split into 4 equal dosages administered at 0000, 0600, 1200, and 1800 h daily such that FR-ST cows received 1 kg of corn starch and both FR-CON and FR-ST received 1.5 L of H2O per infusion. For the FR-CON treatment, 2 cows were in their first lactation, 3 cows were in their second lactation, and 1 cow was in her third lactation, and in the FR-ST treatment 3 cows were in their first lactation and 3 cows were in their second lactation. During P2 and P3, daily feed allotments were allocated into 3 equal portions and provided at 0800, 1300, and 1800 h. Cows were fed a diet formulated to meet or exceed the predicted requirements of energy, protein, vitamins, and minerals (NRC, 2001; Table 1) and had ad libitum access to drinking water throughout the experiment. Feed samples were collected weekly and
stored at −20°C until they were later composited and submitted for chemical analysis using near-infrared spectroscopy (Dairyland Laboratories). Cows were milked twice daily (0600 and 1800 h) in their stalls, and yield was recorded. Additionally, milk samples were collected at each milking on d 4 and 5 of P1 and during P2 and P3, and stored at 4°C with a preservative (bronopol tablet; D & F Control Systems) until composition analysis by Dairy Lab Services (Dubuque, IA) using AOAC-approved infrared analysis equipment and procedures (AOAC International, 1995). Respiration rate and rectal temperature were collected twice daily following each milking (0600 and 1800 h) during P1 through P3. Respiration rate was collected by measuring flank movements for 15 s and subsequently converted to breaths per minute. Rectal temperature was collected using a digital thermometer (GLA M700 Digital Thermometer).

**Abomasal Infusions**

Infusions were facilitated using approximately 3 m of PVC-reinforced braided vinyl tubing (1/2 in outer diameter, 1/4 in inner diameter; Eastman Chemical Company) fitted with a plastisol flange (approximately 11 cm diameter) to allow for continuous placement. The flange had several drilled holes (approximately 2 cm) to allow for fluid passage. Infusion lines were manually inserted into the reticulo-omasal orifice and threaded through the omasum to ensure postruminal treatment administration. Lines remained in the abomasum between infusions and their positions were manually confirmed before treatment administration. The starch solution was homogenized using a mixer (KitchenAid) and infusions were facilitated using a 600-mL drench syringe (Valley Vey Supply). Approximately 300 mL of water was infused thereafter to flush residual starch out of infusion lines. It took approximately 7 min to infuse and flush each infusion.

**Blood Analysis**

Blood samples were collected via the jugular catheter (0600 h) on d 3 to 5 of P1, daily during P2 and P3 for complete blood count (CBC) analysis, and on d 5 of P1, d 2 of P2, and d 1 to 3 of P3 for plasma analysis (24, 48, and 72 h relative to the first abomasal infusion). Plasma samples were collected in K$_2$EDTA tubes (BD) and were subsequently centrifuged at 1,500 × g for 15 min at 4°C before being aliquoted into micro-centrifuge tubes for storage at −20°C until analysis. For CBC data, a 3-mL blood sample was collected in K$_2$EDTA tubes and stored at 4°C for approximately 4 h before submitting to the Iowa State University’s Department of Veterinary Pathology for analysis. Plasma insulin, nonesterified fatty acids (NEFA), BHB, BUN, glucose, LPS-binding protein (LBP), and serum amyloid A (SAA) concentrations were determined using commercially available kits according to manufacturers’ instructions (insulin, Mercodia AB; NEFA, Wako Chemicals USA; BHB, Pointe Scientific Inc.; BUN, Teco Diagnostics; glucose, Wako Chemicals USA; LBP, Hycult Biotech; SAA, Tridelta Development Ltd.). The inter- and intraassay coefficients of variation for insulin, NEFA, BHB, BUN, glucose, LBP, and SAA were 10.4% and 5.8%, 9.1% and 3.7%, 11.8% and 5.0%, 11.1% and 5.9%, 12.1% and 5.7%, 15.3% and 2.8%, and 16.2% and 5.2%, respectively.

**Fecal Analysis**

Fecal samples (approximately 250 g) were collected rectally twice daily (0600 and 1800 h) on d 4 and 5 of P1 and throughout P2 and P3, and data were averaged by day for fecal pH and score analysis. Additional samples were collected at 0600 and 1800 h on d 2 of P2 and d 2 and 4 of P3 and stored at −20°C until later homogenization by day and were sent to a commercial laboratory (DairyLand Laboratories Inc.) for starch analysis using a YSI biochemistry analyzer (Yellow Springs Instrument Inc.). Following collection, samples were scored using a 1-to-5 manure scoring system (Zaaijer et al., 2001) and subsequently processed for fecal pH collection as previously described (Branstad et
Liver Biopsies

Liver biopsies were collected (0800 h) from all animals on d 1 of P1 and at the end of P3 (d 5) as previously described (Rhoads et al., 2010). Briefly, biopsy sites were shaved, scrubbed with povidone-iodine, and sprayed with 70% alcohol. The area was locally anesthetized using 2% lidocaine (MWI Veterinary Supply Co.) before performing a percutaneous biopsy with a trocar. All tissue samples were snap frozen in liquid nitrogen and stored at −80°C. However, liver tissue was not analyzed due to a lack of inflammatory and phenotypic responses to abomasal starch infusions.

Statistical Analysis

Data were statistically analyzed using repeated measures (repeated effect = time) with an autoregressive covariance structure for milk yield, ECM, DMI and calculated DMI, milk variables, CBC metrics, SAA, LBP, vitals, fecal pH, fecal score, and P3 fecal starch, glucose, NEFA, BHB, and BUN. Effects of treatment, time (represented as day or period), their interaction, and replication were included as fixed effects and assessed using PROC MIXED of SAS version 9.4 (SAS Inst. Inc.). For metrics with a single time point, including P2 and P3 insulin and P2 fecal starch, glucose, NEFA, BHB, and BUN, a variance components structure was used, and the fixed effects of treatment and replication were assessed using PROC MIXED. Except for data presented in tables, P1, P2, and P3 were analyzed separately from each other. To compare with baseline, each animal’s average P1 value for each metric was included with P2 or P3 in 2 additional analyses. Despite identical treatment, P1 and P2 data were analyzed using the treatments cows were destined to be assigned to during P3 (FR-CON or FR-ST). All data are reported as least squares means and considered significant if \( P \leq 0.05 \) and a tendency if \( 0.05 < P \leq 0.10 \).

RESULTS

Feed restriction tended to increase fecal pH for both treatments over time during P2 (\( P = 0.06 \)) such that it was increased on d 2 relative to P1 (0.30 units; \( P < 0.01 \)), after which it was markedly decreased by abomasal starch infusions during P3 (0.96 units relative to FR-CON; \( P < 0.01 \); Figure 1A). Similarly, feed restriction increased fecal score for both treatments during P2 (15% relative to P1; \( P = 0.02 \); Figure 1B) and it was decreased by starch infusions during P3 relative to FR-CON (22%; \( P < 0.01 \)). Starch infusions markedly increased fecal starch concentration for FR-ST relative to FR-CON (27.9% vs. 1.5% DM; \( P < 0.01 \); Figure 1C) throughout P3.

By design, DMI decreased (60% relative to P1; \( P < 0.01 \); Table 2) throughout P2 and P3 but was not altered by starch infusions. Further, starch infusions increased calculated DMI (DMI + 3.94 kg DM from starch infusions) in FR-ST cows relative to FR-CON during P3 (41%; \( P < 0.01 \); Table 2). Feed restriction gradually decreased milk yield and ECM over time such that they were decreased (39% and 32%, respectively; \( P < 0.01 \); Table 2) during P3 relative to baseline, but there were no additional effects of ST on milk yield or ECM during P3 (\( P > 0.80 \)). Milk fat content gradually increased with time (27% relative to P1; \( P < 0.01 \); Table 3) for both treatments by P3. In contrast, milk fat and protein yields, protein content, and lactose content decreased with time similarly for both treatments (22%, 43%, 7%, and 2% during P3 relative to P1, respectively; \( P < 0.01 \); Table 3), but these variables were not affected by starch (\( P > 0.73 \)). Milk urea nitrogen increased during P2 for both treatments (17% relative to P1; \( P < 0.01 \); Table 3) before returning to baseline concentrations during P3. However, there was a tendency for MUN to be decreased in FR-ST cows throughout the experiment (18%; \( P = 0.06 \)), and this was most apparent during P3 (28% relative to FR-CON). Feed restriction increased SCS during P2 and P3 (19% relative to P1; \( P = 0.01 \); Table 3), but there was no effect of starch during P3 (\( P > 0.29 \)).

There were no effects of abomasal starch infusions on circulating glucose, insulin, or NEFA concentrations (\( P > 0.21 \); Figure 2A–C). Relative to baseline, feed restriction decreased circulating glucose (12%) and insulin (71%) on average throughout P2 and P3 (\( P < 0.05 \)). Additionally, feed restriction increased circulating NEFA concentrations for both treatments during P2 and P3 relative to baseline (3.2-fold; \( P < 0.01 \)), and NEFA progressively decreased with time throughout P3 (\( P = 0.04 \)) for both treatments. Starch infusions increased BHB during P3 relative to FR-CON cows, with the most pronounced increase occurring on d 2 (81%; \( P = 0.05 \); Figure 2D). Further, BUN was decreased by feed restriction for both treatments during P2 (17% relative to P1; \( P = 0.03 \); Figure 2E), and this decrease was exacerbated by starch infusions during P3 (31%; \( P = 0.03 \)) relative to FR-CON.

There were no effects of treatment or time on circulating white blood cells or neutrophils (\( P > 0.25 \); Table 4). Monocytes and lymphocytes were decreased or
Figure 1. (A) Fecal pH, (B) fecal score, and (C) fecal starch responses in feed-restricted lactating dairy cows abomasally infused with either water (FR-CON) or 4 kg/d of corn starch (FR-ST). Data were analyzed using PROC MIXED and included fixed effects of treatment (Trt), day, and their interaction, and periods were analyzed separately from each other. Experimental period 1 (P1) represents an average of measurements obtained before feed restriction and the administration of respective abomasal infusion treatments (d 1 to 4 of P1). Experimental period 2 (P2) represents data obtained when both treatments were feed-restricted to 40% of their ad libitum feed intake but before abomasal infusions, which were initiated during experimental period 3 (P3). Cows remained feed-restricted during P3 and were abomasally infused with their respective treatments. In (A), P2 treatment: $P > 0.31$, day: $P = 0.06$, and treatment × day: $P < 0.01$. In (B), P2 treatment: $P > 0.31$, day: $P > 0.81$, and treatment × day: $P > 0.26$. P3 treatment × day: $P > 0.23$. In (C), P2 treatment: $P > 0.44$; P3 day: $P > 0.17$, and treatment × day: $P > 0.13$. Results are expressed as LSM ± SEM and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.
Parameter | P1 $^{a}$ | P2 | P3 | Trt | SEM | Trt × period | P-value |
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<tbody>
<tr>
<td>Fat, %</td>
<td>4.02</td>
<td>3.89</td>
<td>4.75</td>
<td>4.60</td>
<td>5.00</td>
<td>5.04</td>
<td>0.30</td>
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<tr>
<td>Fat yield, kg/d</td>
<td>1.49</td>
<td>1.46</td>
<td>1.35</td>
<td>1.30</td>
<td>1.13</td>
<td>1.15</td>
<td>0.09</td>
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<tr>
<td>Protein, %</td>
<td>2.91</td>
<td>3.10</td>
<td>2.78</td>
<td>3.02</td>
<td>2.68</td>
<td>2.89</td>
<td>0.10</td>
</tr>
<tr>
<td>Protein yield, kg/d</td>
<td>1.13</td>
<td>1.17</td>
<td>0.85</td>
<td>0.87</td>
<td>0.65</td>
<td>0.65</td>
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<td>Lactose, %</td>
<td>4.80</td>
<td>4.79</td>
<td>4.66</td>
<td>4.68</td>
<td>4.70</td>
<td>4.71</td>
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<tr>
<td>MUN, mg/dL</td>
<td>11.6</td>
<td>10.1</td>
<td>13.8</td>
<td>11.9</td>
<td>12.3</td>
<td>8.8</td>
<td>0.8</td>
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<tr>
<td>SCS</td>
<td>1.6</td>
<td>2.5</td>
<td>2.0</td>
<td>3.2</td>
<td>2.1</td>
<td>2.9</td>
<td>0.5</td>
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$^{a}$P1–P3 = experimental periods 1–3. During P1, all cows were fed ad libitum. During P2, all cows were FR to 40% of their ad libitum feed intake. Data from P1 and P2 represent an average of cows destined to be assigned to 1 of 2 abomasal infusion treatments (Trt) during P3: (1) control (FR-CON; FR to 40% of baseline ad libitum feed intake + abomasal infusion of 6 L of H$_2$O/d; n = 6) or (2) starch infused (FR-ST; FR to 40% of baseline ad libitum feed intake + abomasal infusion of 4 kg of corn starch + 6 L of H$_2$O/d; n = 6).

tended to be decreased in FR-ST cows relative to FR-CON throughout the entire experiment (15% and 50%, respectively; $P \leq 0.08$; Table 4), but these metrics were not altered by starch infusions over time ($P > 0.15$). In contrast with our hypothesis, circulating SAA and LBP were unaltered over time or due to starch infusions ($P > 0.11$; Table 4). Rectal temperature was unaffected by either feed restriction or starch infusions ($P > 0.11$; Table 4). Feed restriction mildly decreased respiration rate during P3 (6 breaths per minute relative to P1; $P < 0.01$; Table 4) similarly for both treatments but was not altered by starch infusions ($P > 0.48$).

**DISCUSSION**

The prevalence of transition cow disorders (e.g., ketosis, subclinical hypocalcemia, or fatty liver) appears to be mediated in part by the duration and severity of periparturient inflammation, which is potentially exacerbated by the “stacked” nature of stressors associated with parturition (Trevisi and Minuti, 2018; Horst et al., 2021). The abrupt change to a post-fresh high-concentrate diet may contribute to this via disrupting GIT barrier function (Gozho et al., 2005; Zebeli and Metzler-Zebeli, 2012). However, the etiological origin of SARA-induced inflammation (rumen vs. hindgut) remains uncertain despite the hind gut being presumably less resilient against excessive fermentation and its sequelae (Khatipour et al., 2009a,b; Gressley et al., 2011; Plaizier et al., 2018). Reports isolating the effects of HGA on barrier function and systemic inflammation have been inconsistent in dairy cows, with some reporting adverse effects (Zust et al., 2000; Bissell and Hall, 2010) and others not (Mainardi et al., 2011; van Gastelen et al., 2021a,b; Abeyta et al., 2023a,b; Piantoni et al., 2023). However, cows in the aforementioned studies were in established lactation and presumably healthy and thus experienced HGA in isolation from other insults. Transition cows are exposed to several stressors (e.g., psychological stress or calving) that may compromise GIT barrier function, induce inflammation, or increase susceptibility to subsequent insults (e.g., acidosis). Thus, the lack of stacked stressors in prior experiments may explain the observed apparent resilience against HGA. Therefore, experimental objectives were to evaluate the isolated effects of HGA on metabolism, production, and inflammatory biomarkers in FR dairy cows, as feed restriction induces GIT barrier dysfunction in multiple animals (Pearce et al., 2013; Gilani et al., 2018), including ruminants (Zhang et al., 2013; Kvidera et al., 2017c). Notably, all cows were treated similarly during P1 (baseline) and P2 (feed restriction); however, during those periods, data were analyzed using the treatments cows were destined to be assigned to during P3 (FR-CON or FR-ST).

Production and metabolic changes herein were consistent with hallmarks of feed restriction in ruminants, including reductions in milk yield, ECM, circulating glucose, and insulin, and increased NEFA concentrations (de Boer et al., 1985; Horst et al., 2020a). Additionally, previous reports have confirmed the utility of feed restriction or malnutrition as intestinal permeability models in cows (Kvidera et al., 2017a,c; Pederzolli et al., 2018; Pisoni et al., 2022), pigs (Pearce et al., 2013; Garas et al., 2016), poultry (Yamauchi et al., 1996; Gilani et al., 2018), humans (Welsh et al., 1998), and rodents (Rodriguez et al., 1996; Boza et al., 1999). Although a specific biomarker for intestinal hyperpermeability is still lacking, circulating acute-phase proteins (e.g., LBP, SAA, or haptoglobin) following immune activation have previously been used as indicators of intestinal hyperpermeability and subsequent immune activation in ruminant feed restriction models (Kvidera et al., 2017c; Horst et al., 2020a). Additionally, use of these markers was validated in Horst et al. (2020a), where we reported increased SAA, LBP, and haptoglobin in concurrence with a measured increase in feed-restriction-induced GIT hyperpermeability, as indicated
by the paracellular permeability marker Cr-EDTA. In the current study, however, feed restriction failed to increase circulating acute-phase proteins. Although this discrepancy is mechanistically unclear, LBP and SAA concentrations during baseline (before feed restriction or abomasal infusions) for both treatments were un-
characteristically high for healthy (nonperiparturient) ruminants (Humblet et al., 2006; Cecilian et al., 2012). Anecdotally, it appears that liver biopsies performed during P1 ostensibly caused systemic inflammatory responses resulting in blunted or undetectable changes in acute-phase proteins from feed restriction. The liver biopsies were not molecularly analyzed because neither inflammation nor an immune activation phenotype was seemingly detected during P2. Regardless, the overall aim of our current experiment was achieved, as HGA was induced in the context of a multistressor (i.e., feed restriction and prior inflammation) environment and previous work has shown preceding stress may increase susceptibility to otherwise innocuous insults (Lang and Hickman-Davis, 2005; Rohleder, 2019).

In agreement with our previous trials (Abeyta et al., 2023a,b), abomasal starch infusions successfully induced HGA, as indicated by the marked decrease in fecal pH and score (0.96 and 0.9 units relative to FR-CON) and altered manure characteristics (anecdotal observations: lightened color, bubbles, and mucin casts). Sanz-Fernandez et al. (2020) reported that postprandial starch digestibility was 84% ± 10% of duodenal starch appearance in cows, which agrees with our previous experiments (Abeyta et al., 2023a,b), where approximately 84% to 85% of abomasally infused starch (4 kg/d) disappeared in the small and large intestines. We again calculated approximate starch disappearance using an equation developed by Nennich and colleagues (2005) and found it to be relatively similar (76% of abomasally infused starch disappeared) to our previous works (van Gastelen et al., 2021a,b; Abeyta et al., 2023a,b), clearly indicating HGA was achieved.

In stark contrast to our hypothesis, HGA did not negatively affect production (i.e., DMI, milk yield, ECM, milk variables) or inflammatory metrics (i.e., SAA, LBP, CBC) relative to water-infused controls. Mounting evidence suggests that short-term isolated fermentation in the ruminant hindgut does not alter intestinal barrier function, as indicated by the lack of inflammatory and production responses across experiments (Mainardi et al., 2011; van Gastelen et al., 2021a,b; Abeyta et al., 2023a,b; Piantoni et al., 2023). The significance of hindgut acidosis as a pathological event in monogastrics was also challenged by our previous swine experiment (Mayorga et al., 2021), where ileal infusions of 500 g/d of dextrose clearly shifted hindgut fermentation patterns but did not elicit a meaningful inflammatory response or changes in gut morphology. Interestingly, however, relevant rodent literature describes contradictory patterns such that in some instances, distal carbohydrate (e.g., fructo-oligosaccharide, inulin, or resistant starch) fermentation is beneficial to gut health and barrier function (Videla et al., 2001; Moreau et al., 2003; Carvalho et al., 2021), whereas in others it caused extensive damage to the hindgut mucosal barrier (Genda et al., 2018), increased colonic myeloperoxidase activity (an indicator of neutrophil infiltration; Geier et al., 2007), or increased intestinal permeability (Ten Bruggencate et al., 2003; Genda et al., 2018). There are many potential reasons

### Table 4. Effects of abomasal corn starch infusion on immune metrics and thermal indices in feed-restricted (FR) lactating dairy cows

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<tr>
<td>White blood cells, $\times 10^3/\mu$L</td>
<td>8.2</td>
<td>8.5</td>
<td>7.1</td>
<td>8.4</td>
<td>7.4</td>
<td>8.8</td>
<td>0.6</td>
<td>0.25</td>
<td>0.31</td>
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<tr>
<td>Neutrophils, $\times 10^3/\mu$L</td>
<td>3.5</td>
<td>3.7</td>
<td>3.4</td>
<td>3.5</td>
<td>3.8</td>
<td>3.7</td>
<td>0.4</td>
<td>0.90</td>
<td>0.32</td>
</tr>
<tr>
<td>Lymphocytes, $\times 10^3/\mu$L</td>
<td>7.2</td>
<td>3.4</td>
<td>6.8</td>
<td>3.6</td>
<td>7.1</td>
<td>3.6</td>
<td>1.3</td>
<td>0.08</td>
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<tr>
<td>Monocytes, $\times 10^3/\mu$L</td>
<td>0.60</td>
<td>0.56</td>
<td>0.55</td>
<td>0.45</td>
<td>0.62</td>
<td>0.48</td>
<td>0.04</td>
<td>0.04</td>
<td>0.21</td>
</tr>
<tr>
<td>SAA, $\mu$g/mL</td>
<td>523</td>
<td>555</td>
<td>648</td>
<td>555</td>
<td>770</td>
<td>909</td>
<td>182</td>
<td>0.63</td>
<td>0.75</td>
</tr>
<tr>
<td>LBP, $\mu$g/mL</td>
<td>11.5</td>
<td>9.9</td>
<td>10.9</td>
<td>10.2</td>
<td>11.7</td>
<td>9.7</td>
<td>1.6</td>
<td>0.52</td>
<td>0.91</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>38.4</td>
<td>38.6</td>
<td>38.3</td>
<td>38.4</td>
<td>38.3</td>
<td>38.4</td>
<td>0.1</td>
<td>0.22</td>
<td>0.11</td>
</tr>
<tr>
<td>Respiration rate, bpm</td>
<td>37</td>
<td>33</td>
<td>35</td>
<td>35</td>
<td>30</td>
<td>29</td>
<td>2</td>
<td>0.26</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

1P1–P3 = experimental periods 1–3. During P1, all cows were fed ad libitum. During P2, all cows were FR to 40% of their ad libitum feed intake. Data from P1 and P2 represent an average of cows destined to be assigned to 1 of 2 abomasal infusion treatments (Trt) during P3: (1) control (FR-CON; FR to 40% of baseline ad libitum feed intake + abomasal infusion of 6 L of H2O/d; n = 6) or (2) starch infused (FR-ST; FR to 40% of baseline ad libitum feed intake + abomasal infusion of 4 kg of corn starch + 6 L of H2O/d; n = 6).

2SAA = serum amyloid A.

3LBP = LPS-binding protein.
for discrepancies among studies (with the most obvious being dosage and type of fermentable substrate). However, fructo-oligosaccharide can induce intestinal barrier dysfunction or exacerbate colitis in rats fed a semipurified (i.e., purely refined ingredients including corn starch, casein, and soybean oil; Reeves et al., 1993; Pellizzon and Ricci, 2020) but not a nonpurified (i.e., ingredients such as corn, wheat, or soybean; Pellizzon and Ricci, 2020) basal diet, despite identical fructo-oligosaccharide dosages. Although uncertain, it appears that the basal diet (likely fiber content) and resulting differences in the fermentation profile (e.g., production of lactate, succinate, and butyrate) between experiments may have a large influence on whether excessive fermentation is beneficial, neutral, or harmful to intestinal health in monogastrics (Goto et al., 2010; Genda et al., 2018; Mayorga et al., 2021). Thus, there is potential that the dietary fiber content in dairy cow rations (even high-concentrate ones) is enough to prevent HGA-induced barrier dysfunction, as alluded to in monogastric studies (Goto et al., 2010; Genda et al., 2018). However, this remains to be elucidated, as others have described damage to the colonic epithelium with high-concentrate feeding (not isolated to the hindgut) in goats (Tao et al., 2014, 2017; Wang et al., 2021), and there is potential for several other pragmatic discrepancies between experimental or isolated HGA and nonexperimental digestive metrics (e.g., passage rate, starch source, or dosage).

Starch infusions had no impact on circulating glucose, insulin, or NEFA concentrations, but the latter 2 observations are in contrast with previous postruminal carbohydrate infusion studies (Lemosquet et al., 1997; Knowlton et al., 1998; Abeyta et al., 2023a,b). The lack of a glucose response is not unprecedented considering the strict homeostatic control of glucose, relative insulin sensitivity of midlactation cows (Baumgard et al., 2017), and potential for extensive glucose consumption by the portal-drained viscera (Reynolds, 2002; Trotta et al., 2022). However, circulating insulin typically increases with abomasal starch infusions (Westreicher-Kristen et al., 2018; Abeyta et al., 2023a,b), which ostensibly reduces circulating NEFA due to its antilipolytic effects (Brockman and Laarveld, 1986). The reasons why we did not observe changes in these metrics are unclear. However, it may be explained by prior metabolic adaptation to feed restriction, as fasted cows have a reduced insulin response to a glucose tolerance test (Hove, 1978)—an energetic and metabolic scenario not too dissimilar to our FR model.

Interestingly, circulating BHB increased with abomasal starch infusions, which agrees with our previous HGA experiment (Abeyta et al., 2023b) but not others (van Gastelen et al., 2021a,b; Abeyta et al., 2023a). The reasons for these discrepancies are unclear but may be explained by experimental model, stage of lactation, microbiome, or basal diet. Regardless, the increase in circulating BHB may reflect an increase in postruminal alimentary ketogenesis, as butyrate stimulates ketogenesis in colonocytes of rabbits (Henning and Hird, 1972), pigs (Darcy-Vrilion et al., 1993), rodents (Roediger, 1982), and humans (Jørgensen and Mortensen, 2001). Additionally, Herrick et al. (2018) and Doelman et al. (2019) observed an increase in systemic BHB concentrations following abomasal butyrate or calcium gluconate (a prebiotic that encourages hindgut butyrate production) infusions, respectively, suggesting the increase in butyrate availability prompted hindgut ketogenesis in lactating cows. In support, Xiang and colleagues (2016) detected the rate-limiting enzyme for ketogenesis (3-hydroxy 3-methylglutaryl CoA synthase 2; Hegardt, 1999) in colonic tissues of sheep. Most circulating ketones in an ad libitum–fed ruminant originate from ketogenesis in the rumen epithelium (Pennington, 1952). However, as there were no differences in DMI between treatments, the increase in BHB herein was likely sourced outside the rumen epithelium, especially considering that fecal butyrate concentrations typically increase with increased hindgut fermentation (Mao et al., 2012; Westreicher-Kristen et al., 2018; van Gastelen et al., 2021b). Notably, butyrate apparently possesses anti-inflammatory properties (Hamer et al., 2008) in addition to modulating tight junction expression, enhancing intestinal barrier function, and increasing mucin expression in vivo (Han et al., 2015; Chen et al., 2018; Gonzalez et al., 2019). Thus, increased butyrate production with abomasal starch infusions may be a mechanism by which the barrier is protected against the HGA insult in this and other studies (van Gastelen et al., 2021b; Abeyta et al., 2023a,b). Better characterization of the contribution of the hindgut epithelium to a ruminant’s metabolic economy is needed, as it has practical implications for our understanding of periparturient bioenergetics.

Blood urea nitrogen consistently decreases with postruminal carbohydrate infusions (Gressley and Armentano, 2007; van Gastelen et al., 2021a,b), and this is likely explained by increased N sequestration during microbial proliferation in the hindgut. In general, luminal microbial growth rates (and, by default, N requirements) are highly dependent on fermentable dietary carbohydrate availability (Van Soest, 1982). Ammonia primarily derived from amino acid deamination normally serves as the main N source to support microbial proliferation (Van Soest, 1982). Thus, when dietary energy increases, there is a concomitant decrease in luminal ammonia, ultimately reducing the substrate for hepatic urea synthesis and BUN concen-
trations (Røjen et al., 2012). However, luminal ammonia availability may become limiting for microbial growth with excess energy availability, thus requiring additional N supply in the form of BUN, which can be rapidly converted to ammonia by microbial ureases (Stewart and Smith, 2005). Urea recycling has traditionally been viewed as primarily occurring through the rumen epithelium; however, others have demonstrated the capacity for circulating urea to cross to the luminal side of the hindgut in ruminants (Reynolds and Huntington, 1988; Huntington, 1989), pigs (Leeuwen et al., 1995), rabbits (Xiao et al., 2012), rats (Younes et al., 2001), and humans (Wolpert et al., 1971). Thus, the decreased BUN with abomasal starch infusions is likely explained by both a reduction in ammonia entry into hepatic ureagenesis and the requisitioning of BUN into the hindgut to support microbial synthesis.

The lack of inflammatory responses in the aforementioned HGA studies does not entirely negate the possibility of postruminal inflammation with high-concentrate feeding. In fact, Lai et al. (2022) recently described injury and inflammation in the brush border of the jejunum and ileum in high-grain-fed dairy cows, which was later correlated with increased luminal LPS concentrations in those segments. Interestingly, several metabolomics experiments have identified a plethora of potentially toxic compounds (e.g., ethanol, methylamines, and N-nitrosodimethylamine; Ametaj et al., 2010; Saleem et al., 2012; Zhang et al., 2017), which can cause undesirable consequences in other species (e.g., intestinal barrier dysfunction or carcinogenesis; Souliotis et al., 2002; Bala et al., 2014), in the rumen fluid of cows fed increasing concentrations of barley grain. The concoction of potential immunogenic compounds produced in the rumen fluid of high-concentrate-fed cattle and evidence for postruminal GIT injury during rumen acidosis suggest we are far from elucidating the etiology of how SARA becomes pathological. However, better characterizing the impacts of dietary insults on rumen GIT barrier function is a prerequisite to developing future effective and targeted mitigation strategies.

CONCLUSIONS

Hindgut acidosis failed to exacerbate the systemic inflammatory response in FR and immune-activated lactating dairy cows. The accumulation of literature addressing excessive postruminal fermentation in dairy cows suggests that HGA in isolation from altered rumen function does not alter large intestine barrier function, as indicated by a lack of inflammatory or production responses across studies. Nevertheless, it is apparent that the postruminal GIT can become damaged with dietary carbohydrate insults, but it is less certain whether this can be attributed specifically to pH changes within the lumen. There are many potentially toxic compounds in rumen liquor during acidosis, and there is potential for this noxious fluid (independent of pH) to alter postintestinal barrier function. Thus, further work is warranted to better characterize regional damages associated with rumen acidosis and SARA.

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