ABSTRACT

Postruminal intestinal barrier dysfunction caused by excessive hindgut fermentation may be a source of peripheral inflammation in dairy cattle. Therefore, the study objectives were to evaluate the effects of isolated hindgut acidosis on metabolism, inflammation, and production in lactating dairy cows. Five rumen-cannulated lactating Holstein cows (32.6 ± 7.2 kg/d of milk yield, 242 ± 108 d in milk; 642 ± 99 kg of body weight; 1.8 ± 1.0 parity) were enrolled in a study with 2 experimental periods (P). During P1 (4 d), cows were fed ad libitum a standard lactating cow diet (26% starch dry matter) and baseline data were collected. During P2 (7 d), all cows were fed the same diet ad libitum and abomasally infused with 4 kg/d of pure corn starch (1 kg of corn starch + 1.25 L of H2O/infusion at 0600, 1200, 1800, and 0000 h). Effects of time (hour relative to the first infusion or day) relative to P1 were evaluated using PROC MIXED in SAS (version 9.4; SAS Institute Inc.). Infusing starch markedly reduced fecal pH (5.84 vs. 6.76) and increased fecal starch (2.2 to 9.6% of dry matter) relative to baseline. During P2, milk yield, milk components, energy-corrected milk yield, and voluntary dry matter intake remained unchanged. At 14 h, plasma insulin and β-hydroxybutyrate increased (2.4-fold and 53%, respectively), whereas circulating glucose concentrations remained unaltered. Furthermore, blood urea nitrogen increased at 2 h (23%) before promptly decreasing below baseline at 14 h (13%). Nonesterified fatty acids tended to decrease from 2 to 26 h (40%). Circulating white blood cells and neutrophils increased on d 4 (36 and 73%, respectively) and somatic cell count increased on d 5 (4.8-fold). However, circulating serum amyloid A and lipopolysaccharide-binding protein concentrations were unaffected by starch infusions. Despite minor changes in postabsorptive energetics and leukocyte dynamics, abomasal starch infusions and the subsequent hindgut acidosis had little or no meaningful effects on biomarkers of immune activation or production variables.

Key words: leaky gut, starch, abomasal infusion

INTRODUCTION

Inflammation is a rapidly growing area of interest in animal agriculture because of its connection to animal welfare and economically important phenotypes (Lochmiller and Deerenberg, 2000; Kvidera et al., 2017a; Horst et al., 2021). All transition dairy cows experience some degree of immunoactivation and, although a basal level of inflammation is necessary during parturition, it may be augmented by concurrent physiological, psychological, and environmental stressors associated with the peripartum period (Bionaz et al., 2007; Bertoni et al., 2008; Trevisi and Minuti, 2018). Aside from uterine tissue damage and mastitis, several of these stressors seemingly initiate immunoactivation in the absence of obvious external immunogen exposure (i.e., heat stress, feed restriction, and SARA). The centrality of these etiologies is attributed to antigen and endotoxin (i.e., LPS) translocation across the gut epithelium (Gozho et al., 2005; Kvidera et al., 2017b; Al-Qaisi et al., 2020a). However, regardless of origin, inflammation is nutrient and energetically expensive (Johnson, 2012; Kvidera et al., 2017a); thus, it is important to identify circumstances under which alimentary tract barrier function is compromised to effectively develop strategies to ameliorate it.

Periparturient TMR fed dairy cows are transitioned to an energy-dense diet (mostly characterized by increased starch) to support the onset of lactation. Feeding highly fermentable carbohydrates leads to the accumulation of VFA and microbial fermentation by-product in the rumen liquor (Van Soest, 1982a), and VFA accumulation can cause SARA and its associated sequelae (i.e., inflammation, hypophagia, decreased production, laminitis; Nocek, 1997; Khafipour et al.,...
2009a; Aschenbach et al., 2011). Traditionally, these consequences were presumed to emanate from rumen epithelial damage and subsequent infiltration of LPS and other immunogenic compounds into circulation (Gozho et al., 2005; Emmanuel et al., 2008); however, the hindgut may be more susceptible to direct and indirect dietary insults than once thought (Gressley et al., 2011; Sanz Fernandez et al., 2020). We previously demonstrated a marked decrease in fecal pH (0.6 units) following parturition and the switch to a higher concentrate diet (McCarthy et al., 2020). Interestingly, the severity of reduction in fecal pH was negatively associated with milk yield and the acute phase protein (APP) response (Rodriguez-Jimenez et al., 2019), which supports previous hypotheses suggesting that inflammation can originate from excessive hindgut fermentation. For instance, Khafipour et al. (2009a,b) induced SARA using either grain- or alfalfa pellet-based dietary challenges; although both treatments resulted in similar rumen conditions (decreased pH, increased luminal LPS), systemic inflammation was observed only in the grain-challenged cows. This suggests that increased postprandial starch flow caused excessive hindgut fermentation that disrupted barrier function and allowed for LPS translocation (Khafipour et al., 2009a; Gressley et al., 2011). In support, others have observed inflammation and severe morbidity when hindgut acidosis (HGA) was induced in nonlactating cows abomasally infused with 4 kg/d of corn starch (Zust et al., 2000; Bissell and Hall, 2010). However, other HGA models ostensibly failed to cause immunoinactivation and had minimal effects on production in cows and pigs (Mainardi et al., 2011; Piantoni et al., 2018; Mayorga et al., 2021; van Gastelen et al., 2021a,b). Variations in experimental design, species, life stage, basal diets, or methods may explain inconsistencies observed between studies. Consequently, it is of interest to better characterize the effects of isolated HGA (in the absence of rumen acidosis) in a lactating dairy cow model. Therefore, our objectives were to determine the effects of HGA on production, metabolism, and inflammation in lactating dairy cows. We hypothesized that experimentally induced HGA would initiate peripheral inflammation and negatively affect economically important phenotypes (i.e., milk yield).

**MATERIALS AND METHODS**

**Animals, Diets, and Experimental Design**

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Five lactating rumen-cannulated Holstein cows (32.6 ± 7.2 kg/d of milk; 242 ± 108 DIM; 642 ± 99 kg of BW; 1.8 ± 1.0 parity) were housed in individual box-stalls (4.57 × 4.57 m) and allowed 3 d to acclimate to housing conditions, during which all cows were jugular catheterized as previously described (Horst et al., 2020a). The trial included 2 experimental periods (P) and took place in March 2018. Period 1 lasted 4 d, during which baseline data were collected. During P2 (7 d), all cows were abomasally infused with 4 kg of pure corn starch/d at 0600, 1200, 1800, and 0000 h (1 kg of corn starch + 1.25 L of lukewarm H2O/infusion; Premium Corn Starch, Kari-Out Co.). Throughout the experiment, cows were allowed ad libitum access to feed and water, and diets were formulated to meet or exceed requirements for energy, protein, minerals, and vitamins for lactating dairy cows (Table 1; NRC, 2001). 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, Arcadia, WI). Cows were fed once (0700 h) and milked twice (0600 and 1800 h) daily in their stalls. Additionally, milk samples were collected at each milking on d 3 and 4 of P1 and daily during P2 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). Rectal temperature and respiration rate were obtained twice daily (0600 and 1800 h) and condensed into a daily average. Rectal temperature was monitored using a digital thermometer (GLA M700 Digital Thermometer, Agricultural Electronics). Respiration rate was measured as flank movements for 15-s intervals and later converted to breaths/min.

**Abomasal Infusions**

Starch infusions were administered using an abomasal weight (Deutsche Edelstahlwerke GmbH) as previously described (Westreicher-Kristen and Susenbeth, 2017). Each weight was attached to approximately 3 m of polyvinyl chloride (PVC)-reinforced braided vinyl tubing (1.3-cm outer diameter, 0.6-cm inner diameter; Eastman Chemical Company) and were manually inserted into the reticulo-omasal orifice and threaded into the abomasum on d 3 of P1. Weights remained in the abomasum between infusions, and their positions were manually confirmed before each infusion. At each infusion, 1 kg of corn starch (98.5% DM) was mixed with 1.25 L of lukewarm tap water using a mixer (KitchenAid) to ensure solution homogeneity. The starch/water mixture was then infused into each cow using a 600-mL drench syringe (Valley Vet Supply). Lukewarm water (1.5 L) was used to flush any residual starch out...
of the infusion line following each administration. It took approximately 7 min/cow to complete each abomasal infusion.

**Blood Analysis**

Blood samples were collected via the jugular catheter from all cows at −24, 2, 14, 26, 48, 96, and 168 h relative to the first infusion (RFI) for plasma and at −48, −36, −24, −12, 0, 2, 8, 14, 20, 26, 32, 38, 44, 48, 72, 96, 120, 144, and 168 h RFI for complete blood cell count (CBC) analysis. For clarity, CBC samples were then averaged by day. Except for CBC, blood samples were collected into tubes containing K<sub>2</sub>EDTA (BD Vacutainer) and centrifuged at 1,500 × g for 15 min at 4°C before being transferred to microcentrifuge tubes for storage at −20°C. For CBC samples, a 3-mL blood sample was collected (K<sub>2</sub>EDTA; BD Vacutainer) and stored at 4°C for ~12 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 Inc.; LBP, Hycult Biotech; SAA, Tridelta Development Ltd.). The intra-assay coefficients of variation for insulin, NEFA, BHB, BUN, glucose, LBP, and SAA were 5.1, 4.8, 4.3, 4.9, 2.4, 6.5, and 5.7%, respectively. The interassay coefficients of variation for BUN and SAA were 7.5 and 19%, respectively.

**Fecal Analysis**

Fecal samples (~250 g wet basis) were collected rectally twice (0600 and 1800 h) on d 4 of P1, 4 times daily on d 1 and 2 of P2 (2 h after each infusion), and twice daily (0600 and 1800 h) on d 3 to 7 of P2. All time points were averaged by day for fecal pH and score analysis. Fecal pH was determined using a 1:1 ratio of distilled water to fecal material as described by Branstad et al. (2017). In brief, fecal pH was measured immediately following collection using a portable pH meter (Oakton Instruments) by placing the probe directly into a homogenized (1:1) solution of 50 g of fecal matter with 50 g of distilled water. The solution was homogenized for 1 min using a blender (Lab-Blender Stomacher 80, Seward Ltd.). Fecal scores were obtained following sample collection using a 1 to 5 manure scoring system. The remaining intact fecal samples were immediately stored at −20°C. Samples obtained on d 4 of P1 and d 3 and 7 of P2 were later homogenized by day (a.m. and p.m.) and analyzed for fecal starch content using a YSI biochemistry analyzer (Yellow Springs Instrument Inc.; DairyLand Laboratories, Arcadia, WI).

**Statistical Analysis and Calculations**

Data were analyzed using the MIXED procedure of SAS version 9.4 (SAS Institute Inc.). The model included the fixed effect of time (hour RFI or day) and the random effect of cow. Data from P1 were condensed into an average by cow and included in the analysis with data from P2. The covariance structures used to analyze the repeated-measures data were autoregressive for milk yield, milk composition, voluntary and calculated DMI, feed efficiency, CBC, fecal pH, fecal score, and vitals, and spatial power for fecal starch, metabolites, insulin, and circulating SAA and LBP. All data are reported as least squares means and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.15$. Calculated DMI and feed efficiency included voluntary TMR consumption plus 4 kg (DM) from starch infusions.

**RESULTS**

Starch infusions markedly decreased fecal pH throughout P2, with the nadir occurring on d 3 (pH = 5.84; 0.91-unit decrease relative to P1; $P < 0.01$;
Figure 1A) and tended to decrease fecal score on d 1 of infusions (0.45 units; *P* = 0.14; Figure 1B). Additionally, abomasal starch infusions markedly increased fecal starch content throughout P2 (2.2 vs. 9.6% DM; *P* = 0.03; Figure 1C). In contrast to our hypothesis, starch infusions did not affect milk yield, ECM yield, or voluntary feed intake throughout P2 (*P* > 0.43; Table 2). By design, calculated DMI (voluntary DMI + 4 kg/d DM from daily starch infusions) increased throughout P2 relative to P1 (*P* < 0.01; Table 2). Because the infusions had no effect on milk yield, calculated feed efficiency decreased in P2 relative to P1 (16%; *P* < 0.01; Table 2). Milk composition remained largely unchanged throughout P2, as there were no time effects for milk fat or protein contents, their yields, lactose content, or MUN (*P* > 0.31; Table 3). However, SCC increased on d 5 relative to P1 (4.8-fold; *P* < 0.01; Table 3).

Starch infusions did not alter circulating glucose (*P* > 0.32; Figure 2A) but increased circulating insulin concentrations at 14 h (2.4-fold relative to P1; *P* = 0.05; Figure 2B). Circulating NEFA concentrations tended to decrease from 2 to 26 h (41%; *P* = 0.11; Figure 3A)
relative to baseline. Circulating BHB increased at 14 h (53% relative to baseline; \( P < 0.01 \); Figure 3B) before decreasing again through 48 h. Circulating BUN followed a similar trend and increased at 2 h (23% relative to \( P_1 \); \( P < 0.01 \); Figure 3C) before decreasing below baseline values at 14 h (13% relative to \( P_1 \); \( P = 0.05 \)) and remaining numerically decreased from 26 to 168 h relative to baseline concentrations (10%; \( P > 0.12 \)).

Circulating white blood cells and neutrophils increased on d 4 of infusions relative to baseline (36 and 73%, respectively; \( P < 0.01 \); Table 4). Starch infusions tended to decrease circulating lymphocytes on d 6 relative to both d 5 and 7 of \( P_2 \) (\( P = 0.09 \); Table 4); however, concentrations remained similar to those of \( P_1 \) throughout \( P_2 \). No changes in circulating monocytes due to starch infusions were observed. Both rectal temperature and respiration rate were unaltered over time throughout \( P_2 \) (\( P > 0.22 \); Table 4). Similarly, and in contrast to our hypothesis, circulating SAA and LBP were unaffected by starch infusions (\( P > 0.63 \); Table 5).

### DISCUSSION

Gastrointestinal tract (GIT) barrier hyperpermeability allows luminal antigens to infiltrate into local and systemic circulation, which negatively affects production, ostensibly because immune activation has a large nutrient and energy demand (Johnson, 2012; Kvidera et al., 2017a) but is accompanied by inappetence (Kuhla, 2020). High grain feeding can induce intestinal barrier dysfunction (especially abrupt dietary changes), but it is unclear in which segment(s) antigen translocation is occurring (Gressley et al., 2011; Plaizier et al., 2018; Sanz Fernandez et al., 2020). The traditional dogma suggests that grain-induced hyperpermeability occurs predominantly in the rumen epithelium; however, evidence indicates the large intestine may contribute to LPS translocation during SARA (Khafipour et al., 2009a,b; Gressley et al., 2011). There are stark and pragmatic anatomical (4 strata of multilayered squamous epithelia vs. 1 layer of columnar epithelium + 2 mucous layers; Steele et al., 2016) and buffering capacity (lack of saliva and protozoa; Gressley et al., 2011) differences between the rumen and hindgut, suggesting that the large intestine may be less resilient against luminal insults (i.e., pH changes). Further, some have observed adverse events (i.e., inflammation, severe morbidity) with isolated HGA (Zust et al., 2000; Bissell and Hall, 2010), whereas others have not, in pigs (Mayorga et al., 2021), steers (Mainardi et al., 2011), pigs (Abeyta et al., 2023).

### Table 2. Effect of abomasal corn starch infusion on production

| Variable               | \( \text{P1} \) | \( \text{P2} \) | \( \text{P3} \) | \( \text{P4} \) | \( \text{P5} \) | \( \text{P6} \) | \( \text{P7} \) | SEM | \( P \)-value |
|------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----|--------------|
| Milk yield (kg/d)      | 33.2           | 32.7           | 32.8           | 32.6           | 31.7           | 31.4           | 30.9           | 30.7 | 3.5          | 0.96          |
| ECM yield (kg/d)       | 33.4           | 34.1           | 33.1           | 32.8           | 31.9           | 32.4           | 32.4           | 31.8 | 2.5          | 0.92          |
| Voluntary DMI (kg/d)   | 18.4           | 17.4           | 17.4           | 17.4           | 16.8           | 17.1           | 17.8           | 17.9 | 0.7          | 0.43          |
| Calculated DMI\(^a\) (kg/d) | 21.5\(^a\) | 21.4\(^a\) | 21.4\(^a\) | 20.8\(^a\) | 21.7\(^a\) | 21.8\(^a\) | 21.9\(^a\) | 0.7  | <0.01        |
| Calculated FE\(^b\) | 1.83\(^b\) | 1.59\(^b\) | 1.59\(^b\) | 1.58\(^b\) | 1.57\(^b\) | 1.57\(^b\) | 1.49\(^b\) | 1.48\(^b\) | 0.14 | <0.01        |

\(^a\)Values within a row with differing superscripts denote differences (\( P < 0.05 \)) among days.
\(^b\)P (period) 1 = average of baseline (preinfusion data); d 1–7 = daily average of each metric during \( P_2 \) (abomasal infusion of 4 kg/d corn starch DM).

### Table 3. Effect of abomasal corn starch infusion on milk variables

| Variable               | \( \text{P1} \) | \( \text{P2} \) | \( \text{P3} \) | \( \text{P4} \) | \( \text{P5} \) | \( \text{P6} \) | \( \text{P7} \) | SEM | \( P \)-value |
|------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----|--------------|
| Fat (%)                | 3.81           | 3.83           | 3.60           | 3.56           | 3.60           | 3.78           | 3.89           | 3.85 | 0.20         | 0.31          |
| Fat yield (kg/d)       | 1.20           | 1.22           | 1.16           | 1.15           | 1.12           | 1.16           | 1.18           | 1.15 | 0.08         | 0.83          |
| Protein (%)            | 3.18           | 3.24           | 3.15           | 3.19           | 3.17           | 3.22           | 3.24           | 3.23 | 0.19         | 0.66          |
| Protein yield (kg/d)   | 1.00           | 1.05           | 1.02           | 1.01           | 0.98           | 0.98           | 0.98           | 0.96 | 0.07         | 0.89          |
| Lactose (%)            | 4.92           | 4.92           | 4.94           | 4.94           | 4.93           | 4.95           | 4.94           | 4.90 | 0.08         | 0.98          |
| MUN (mg/dL)            | 9.41           | 8.78           | 8.59           | 8.52           | 7.99           | 8.42           | 8.31           | 7.95 | 0.56         | 0.57          |
| SCC \( (x10^3 \text{cells/mL}) \) | 308\(^b\) | 268\(^b\) | 112\(^b\) | 123\(^b\) | 582\(^b\) | 1,490\(^b\) | 870\(^b\) | 528\(^b\) | 395 | <0.01        |

\(^a\)Values within a row with differing superscripts denote differences (\( P < 0.05 \)) among days.
\(^b\)P (period) 1 = average of baseline (preinfusion data); d 1–7 = daily average of each metric during \( P_2 \) (abomasal infusion of 4 kg/d corn starch DM).
and lactating Holstein cows (Piantoni et al., 2018; van Gastelen et al., 2021a,b). Reasons for the aforementioned inconsistencies are not clear, but differences in experimental design, species, and physiological state are potential contributors. Regardless, a thorough understanding of the consequences of postruminal acidosis is lacking; thus, our objectives were to evaluate the effects of HGA on production, metabolism, and inflammatory biomarkers in lactating dairy cows.

Although thresholds are ill-defined, HGA is characterized by increased hindgut fermentation resulting in an excess of VFA, decreased luminal and fecal pH, and damage to the intestinal epithelium (Gressley et al., 2011; Plaizier et al., 2018). Herein, starch infusions appeared to induce HGA, as indicated by a marked reduction in fecal pH (pH = 5.84 at nadir) which, while already striking, was likely even lower in the proximal hindgut (cecum/proximal colon) where most of the fermentation presumably occurred (Macfarlane et al., 1992; Pederzolli et al., 2018). Additionally, we anecdotally observed altered fecal characteristics (i.e., foamy appearance, yellow starchy color, gas bubbles in manure) and decreased fecal score during P2, which is consistent with other reports (as reviewed by Plaizier et al., 2008). Mucin casts were intermittently identified (anecdotal observations) in the feces following starch infusions, which is used as a marker for SARA in the field (Nordlund et al., 2004) and has been observed in previous postruminal fermentation studies (Mainardi et al., 2011). Fecal starch markedly increased during P2 (2.2 vs. 9.6% DM), indicating that the threshold for enzymatic hydrolysis in the small intestine and fermentative capacity in the hindgut had been exceeded. Utilizing an equation developed by Nennich et al. (2005): fecal DM output = [DMI × 0.356 (±0.011)] + 0.80 (±0.34), and our calculated DMI and fecal starch data, we estimated total starch excretion to be approximately 0.81 kg/d during P2. After accounting for fecal starch from the diet (using P1 as a proxy; 0.17 kg/d), total fecal starch excretion from starch infusions was approximately 0.64 kg/d, or 16% of the originally infused starch. In the current experiment, it was not possible to determine the amount of starch hydrolyzed in small intestine versus fermented in the hindgut; however, the decrease in fecal pH observed herein (0.9 units) clearly indicates marked HGA (Gressley et al., 2011).

In stark contrast to our hypothesis, the development of HGA failed to elicit a systemic immune response, as indicated by unaltered APP, but this corroborates previous reports (Mainardi et al., 2011; Piantoni et al., 2018; van Gastelen et al., 2021a,b). However, there were mild alterations in circulating neutrophils that also corresponded with an increase in SCC on d 5 of infusions. Interestingly, mammary epithelial barrier function appears to be impaired with exposure to both intramammary (Wellnitz et al., 2013) and nonmammary LPS (from i.v. infusion; Kvidera et al., 2017a; Horst et al., 2020a), resulting in increased SCC. Further, mammary

Figure 2. Effects of abomasal infusion of 4 kg/d of corn starch DM on circulating (A) glucose and (B) insulin concentrations in lactating dairy cows. The −24 h sample was obtained before the initiation of abomasal infusions. Results are expressed as LSM ± SEM and considered significant if \( P \leq 0.05 \) and a tendency if 0.05 < \( P \leq 0.15 \). Values with differing superscripts denote differences (a, b = \( P \leq 0.05 \)) among hours.
Immune cell infiltration may be a result of GIT-derived endotoxin, as has been shown following the intentional induction of intestinal hyperpermeability (Kvidera et al., 2017a) or other intestinal insults such as heat stress (Safa et al., 2019; Al-Qaisi et al., 2020b), feed restriction (Kvidera et al., 2017b; Horst et al., 2020b), or rumen acidosis (Zhang et al., 2016; Hu et al., 2022). In support, Hu et al. (2022) observed a marked inflammatory response (i.e., increased APP, cytokines, and LPS in milk, architectural damage, among others) in the mammary glands of dairy cows induced with SARA, and others have described negative relationships between ruminal pH and SCC (Villot et al., 2018; Antanaitis et al., 2020). The increased SCC suggests that changes to hindgut luminal pH may have contributed to the leaky mammary phenotype sometimes observed with

**Figure 3.** Effects of abomasal infusion of 4 kg/d of corn starch DM on circulating (A) nonesterified fatty acid (NEFA), (B) BHB, and (C) BUN concentrations in lactating dairy cows. The −24 h sample was obtained before the initiation of abomasal infusions. Results are expressed as LSM ± SEM and considered significant if \( P \leq 0.05 \) and a tendency if \( 0.05 < P \leq 0.15 \). Values with differing superscripts denote differences \( (\text{a–c} = P \leq 0.05; \text{x–z} = 0.05 < P \leq 0.15) \) among days.
high-concentrate feeding. However, other indicators of intestinal barrier hyperpermeability were not observed herein; thus, the ostensible relationship between HGA and mammary barrier function remains to be fully elucidated.

Starch infusions did not alter plasma glucose concentrations over time, which agrees with other postruminal starch infusion studies (Knowlton et al., 1998; Westreicher-Kristen et al., 2018) and is not surprising considering the strict homeostatic regulation of circulating glucose and the relative insulin sensitivity associated with the late lactation stage of the cows used herein (Baumgard et al., 2017). Other explanations may include marked glucose utilization by the portal-drained viscera (PDV; Reynolds, 2002; Trotta et al., 2022) and decreased hepatic gluconeogenesis from hyperinsulinemia (Eisemann et al., 1994). Notably, ruminant splanchnic metabolism is not completely understood as it varies considerably between diets, stage of lactation, and so on (see reviews by Reynolds, 2002; Trotta et al., 2022). However, in general, net PDV glucose flux is often zero or negative in ruminants because of the substantial utilization of both luminal and arterial glucose supplies by PDV tissues (Reynolds, 2002). As such, the lack of alterations in circulating glucose concentrations herein does not necessarily reflect a complete absence of alimentary glucose absorption but instead indicates the potential for glucose metabolism by PDV tissues (i.e., GIT, spleen, pancreas, omental and mesenteric fat; Reynolds, 2002) from both arterial and luminal sources to match the quantity of glucose being absorbed from starch infusions. Thus, transient increases in portal glucose and, to a lesser degree, absorption of VFA (mainly propionate) from the hindgut may have stimulated hyperinsulinemia at 14 h (Manns and Boda, 1967; Harmon, 1992; Subiyatno et al., 1996). Because insulin has strong antilipolytic effects (Brockman and Laarveld, 1986), hyperinsulinemia is likely responsible for the reduction in NEFA concentrations from 2 to 26 h. These findings agree with other studies that also demonstrated increased insulin and decreased NEFA concentrations with abomasal starch or glucose infusions (Lemosquet et al., 1997; Knowlton et al., 1998). Notably, a true control was not used in the current study, and it is thus difficult to distinguish between the effects of HGA and normal circadian patterns in the measured metabolites and hormones (especially up to the 26th hour).

Interestingly, circulating BHB transiently increased at 14 h, which may be explained by increased ketone production by large intestine epithelium. Literature addressing the relationship between luminal VFA (i.e., butyrate) and hindgut ketone synthesis in ruminants is scarce; however, butyrate induced ketone synthesis in colonocytes of other species such as rabbits (Henning and Hird, 1972), pigs (Darcy-Vrillon et al., 1993), rodents (Roediger, 1982), and humans (Jørgensen and Mortensen, 2001). Further, Xiang et al. (2016) detected

| Variable                        | P1         | 1   | 2   | 3   | 4   | 5   | 6   | 7   | SEM | P-value |
|---------------------------------|------------|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| White blood cells (×10⁵/µL)     | 5.98b      | 6.45b| 6.71b| 6.93b| 8.14b| 6.44b| 6.14b| 6.81b| 0.63 | <0.01   |
| Neutrophils (×10⁵/µL)           | 2.59b      | 2.74b| 2.92b| 3.21b| 4.47b| 2.63b| 2.44b| 2.97b| 0.39 | <0.01   |
| Lymphocytes (×10⁵/µL)           | 2.60b      | 2.78b| 2.82b| 2.75b| 2.77b| 2.84b| 2.49b| 2.85b| 0.38 | 0.09    |
| Monocytes (×10⁵/µL)             | 0.40       | 0.40| 0.44| 0.41| 0.38| 0.39| 0.42| 0.36| 0.07 | 0.98    |
| Rectal temperature (°C)         | 38.2       | 35.2| 38.0| 38.2| 38.3| 38.2| 38.1| 38.1| 0.1  | 0.59    |
| Respiration rate (breaths/min)  | 37         | 36  | 33  | 32  | 28  | 30  | 32  | 34  | 3    | 0.22    |

a,bValues within a row with differing superscripts denote differences (P < 0.05) among days.

x,yValues within a row with differing superscripts denote a tendency to differ (0.05 < P ≤ 0.15) among days.

1P (period) 1 = average of baseline (preinfusion data); d 1–7 = daily average of each metric during P2 (abomasal infusion of 4 kg/d corn starch DM).
3-hydroxy 3-methylglutaryl CoA synthase 2 (the rate-limiting enzyme of ketogenesis) in sheep colonic tissue, which suggests the capacity for hindgut ketogenesis in ruminants. Insulin was concurrently increased at 14 h and it suppresses liver ketogenesis (Brockman and Laarveld, 1986), and the simultaneous reduction in NEFA suggests a decrease in substrate availability for hepatic ketogenesis (Brockman and Laarveld, 1986). Additionally, most ketones in an ad libitum–fed ruminant originate from the rumen epithelium (Pennington, 1952) and, considering that voluntary DMI was unaffected, increased BHB herein was likely sourced nonhepatically and outside of the rumen epithelium. Thus, the hindgut epithelium is potentially a meaningful contributor to systemic ketone concentrations in ruminants, and having a better understanding of its role in whole-body ketogenesis would have practical implications for our interpretation of periparturient bioenergetics.

Other than in the first 2 h, BUN concentrations were decreased by abomasal starch infusions, which agrees with findings from other hindgut fermentation studies (Gressley and Armentano, 2007; van Gastelen et al., 2021a,b). This is likely reflective of increased N usage by the hindgut microflora following starch infusions, as energy availability is the primary determinant of microbial growth rates and, by consequence, N requirements (Van Soest, 1982b). Typically, ammonia (primarily from amino acid deamination) serves as a major N source for both rumen and hindgut microflora (Van Soest, 1982b); however, in situations where ammonia is rapidly scavenged for microbial growth, it is reasonable to posit that ammonia absorption by the GIT and ultimate entry into the urea cycle would be lessened and therefore BUN concentrations would be decreased. In fact, Røjen et al. (2012) observed this phenomenon with abomasal oligofructose infusions (1,500 g/d) and attributed the reduction of BUN to decreased urea output by the liver in lactating dairy cows. However, others have also demonstrated the capacity for blood urea to cross to the luminal side of the lower GIT in ruminants (Reynolds and Huntington, 1988; Huntington, 1989), pigs (Van Leeuwen et al., 1995), humans (Bergen and Wu, 2009), rabbits (Xiao et al., 2012), and rats (Younes et al., 2001), and luminal microflora are equipped with ureases that enable the utilization of urea for microbial growth purposes (Stewart and Smith, 2005). Thus, BUN should not be discounted as a potential N source to support hindgut microbial proliferation. Therefore, reductions in BUN herein are likely explained by the combination of a reduction of plasma ammonia entrance into the hepatic urea cycle and BUN entry into the hindgut to support microbial growth. This scenario also has implications for our understanding of periparturient energetic and protein metabolism.

In contrast to Bissell and Hall (2010), who observed inflammation in nonlactating cows following 3 d of 4 kg/d abomasal infusions of corn starch, it is unclear why we did not observe inflammation despite clear evidence of excessive hindgut fermentation. However, the severity of HGA developed herein was less than that in the Bissell and Hall (2010) study (fecal pH: 5.8 vs. 4.6). A potential explanation might be differences in the basal diet; the cows in the Bissell and Hall (2010) study were consuming a high-forage diet before starch infusions, whereas cows in our study were acclimated to a high-starch lactating TMR (26% starch DM). The ruminant microbiome closely reflects the diet consumed to maximize substrate utilization (Van Soest, 1982b), and animals previously acclimated to relatively higher starch diets elicit blunted inflammatory responses following SARA induction (Danscher et al., 2011). Thus, it is important to consider the probable differences in hindgut microbial populations between the 2 experiments, particularly because of potential implications of postruminal hyperpermeability during the transition period when postpartum cows are switched to a higher energy diet. Transition dairy cows experience a multitude of concurrent psychological, physiological, and environmental stressors, and a disproportionate number of periparturient animals develop metabolic disorders or are culled (Trevisi and Minuti, 2018; Horst et al., 2021). It is thus of interest to further elucidate the potential contribution of HGA in these transition maldies by investigating the relationship between hindgut fermentation and postruminal barrier function. A potential limitation of the current model is that the use of a higher starch TMR may not appropriately reflect the dietary adjustments that periparturient cows experience postpartum, a scenario that potentially blunts the severity of HGA and prevents barrier dysfunction. Thus, future work investigating the role of HGA in postruminal hyperpermeability is warranted in a model that mimics traditional transition cow dietary changes.

**CONCLUSIONS**

Abomasal corn starch infusion caused extensive hindgut fermentation, as indicated by a marked decrease in fecal pH. Despite altered postabsorptive metabolism metrics, HGA did not initiate systemic inflammation, affect appetite, cause a febrile response, or decrease milk yield. Cows used herein were consuming a high starch (26% DM) TMR before abomasal infusion, which likely influenced microbial populations and severity of response to starch infusions. Hindgut acidosis...
and its downstream consequences may be exacerbated in a model that more closely reflects the low- to high-concentrate dietary transition experienced by periparturient dairy cows. Therefore, future work is warranted to investigate the role of HGA on postuminal intestinal barrier dysfunction in a model that better reflects dietary challenges faced by transition dairy cows.

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