ABSTRACT

Previous stressors and systemic inflammation may increase the intestine’s susceptibility to hindgut acidosis (HGA). Therefore, our experimental objectives were to evaluate the effects of isolated HGA on metabolism, production, and inflammation in simultaneously immune-activated lactating cows. Twelve rumen-cannulated Holstein cows (118 ± 41 d in milk; 1.7 ± 0.8 parity) were enrolled in a study with 3 experimental periods (P). Baseline data were collected during P1 (5 d). On d 1 of P2 (2 d), all cows received an i.v. lipopolysaccharide (LPS) bolus (0.2 µg/kg of body weight; BW). During P3 (4 d), cows were randomly assigned to 1 of 2 abomasal infusion treatments: (1) control (LPS-CON; 6 L of H2O/d; n = 6) or (2) starch infused (LPS-ST; 4 kg of corn starch + 6 L of H2O/d; n = 6). Treatments were allocated into 4 equal doses (1.5 L of H2O or 1 kg of starch and 1.5 L of H2O, respectively) and administered at 0000, 0600, 1200, and 1800 h daily. Additionally, both treatments received i.v. LPS on d 1 and 3 of P3 (0.8 and 1.6 µg/kg of BW, respectively) to maintain an inflamed state. Effects of treatment, time, and their interaction were assessed.

Repeated LPS administration initiated and maintained an immune-activated state, as indicated by increased circulating white blood cells (WBC), serum amyloid A (SAA), and LPS-binding protein (LBP) during P2 and P3 (29%, 3-fold, and 50% relative to P1, respectively) for both abomasal infusion treatments. Regardless of abomasal treatment, milk yield and dry matter intake were decreased throughout P2 and P3 but with lesser severity following each LPS challenge (54, 44, and 37%, and 49, 42, and 40% relative to baseline on d 1 of P2, d 1 and d 3 of P3, respectively). As expected, starch infusions markedly decreased fecal pH (5.56 at nadir vs. 6.57 during P1) and increased P3 fecal starch relative to LPS-CON (23.7 vs. 2.4% of dry matter). Neither LPS nor starch infusions altered circulating glucose, insulin, nonesterified fatty acids, or β-hydroxybutyrate, although LPS-ST cows had decreased blood urea nitrogen throughout P3 (16% relative to LPS-CON). Despite the striking reduction in fecal pH, HGA had no additional effect on circulating WBC, SAA, or LBP. Thus, in previously immune-activated dairy cows, HGA did not augment the inflammatory state, as indicated by a lack of perturbations in production, metabolism, and inflammatory biomarkers.

Key words: abomasal infusion, lipopolysaccharide, intestinal barrier dysfunction

INTRODUCTION

Systemic inflammation is routinely associated with many stressful events experienced by dairy cows, including heat stress and feed restriction (Kvidera et al., 2017b; Proudfoot et al., 2018; Al-Qaisi et al., 2020). In the absence of overt mammary gland, uterus, or lung infections, the mechanism behind stress-induced inflammation appears to be a compromised gastrointestinal tract (GIT) barrier. Transition cows are at particular risk for intestinal barrier dysfunction considering their exposure to multiple concurrent and successive (stacked) stressors associated with calving (i.e., dietary shift, tissue trauma, and so on). Potentially starting as early as dry-off (Mezzetti et al., 2020; Goetz et al., 2022), these repeated insults may exacerbate the necessary inflammation associated with parturition, resulting in a hyper-inflammatory state (Trevisi and Minuti, 2018). In fact, an augmented inflammatory response during early lactation may play a central mechanistic role in the development of some metabolic disorders, such as ketosis or subclinical hypocalcemia (Horst et al., 2021). Thus, there is economic and pragmatic merit in better characterizing sources of GIT hyperpermeability, especially considering the profound energy and nutrient demands of activated leukocytes (Johnson, 2012; Kvidera et al., 2017a).

Systemic inflammation emanating from the recognition of GIT-derived antigens is a well-characterized
consequence of SARA (Gozho et al., 2005; Li et al., 2016; Zhao et al., 2018); yet, the site(s) at which barrier dysfunction occurs remains unclear. In general, it is presumed that the postruminal GIT may be more susceptible to damage by the chemical and physical alterations associated with excessive fermentation in the large intestine [i.e., hindgut acidosis (HGA)]; Gressley et al., 2011; Plaizier et al., 2018]. This hypothesis is grounded in the stark anatomical and buffering capacity differences between the rumen and hindgut, as the rumen is reinforced with 4 multilayered strata of squamous epithelium, whereas the hindgut has only a single columnar epithelial layer covered in mucous (Gressley et al., 2011; Steele et al., 2016). In support, Khafipour et al. (2009a,b) observed a systemic inflammatory response to grain-induced but not alfalfa pellet–induced SARA, despite similar rumen conditions (i.e., reduced pH, increased endotoxin, and so on), suggesting that inflammation was emanating postruminally and potentially from HGA. Further, Bissell and Hall (2010) reported an increase in systemic inflammation and severe morbidity (Mary Beth Hall, USDA-ARS, Madison, WI; personal communication) following the induction of isolated HGA in dry cows, and we recently uncovered a potential relationship between postpartum fecal pH and inflammation in early lactation (Rodriguez-Jimenez et al., 2019). Thus, HGA may play a mechanistic role in the exacerbated inflammatory response of poorly transitioning dairy cows.

Despite the aforementioned evidence, we and others have been unsuccessful in eliciting systemic inflammation or an immune-activated phenotype with isolated HGA in dairy cows and pigs (Mainardi et al., 2011; Mayorga et al., 2021; van Gastelen et al., 2021a,b; Abeyta et al., 2023a,b,c; Piantoni et al., 2023). However, as stated previously, the parturipotent period is accompanied with myriad stressors that may either directly compromise the intestinal barrier or create a stacked stressor scenario that increases the susceptibility to HGA (Trevisi and Minuti, 2018; Horst et al., 2021). Systemic LPS and inflammation (regardless of the source) can directly cause or exacerbate GIT barrier dysfunction (Ding et al., 2004; Hietbrink et al., 2009; Cao et al., 2018), and this has implications for the transition cow that could have subclinical metritis (Mateus et al., 2003) or mastitis (Vels et al., 2009). Thus, we can postulate that the inflamed transition cow is more susceptible to HGA. Therefore, our objectives were to evaluate the effects of HGA in previously immune-activated lactating dairy cows with the hypothesis that HGA would augment the production, metabolic, and inflammatory responses to LPS.

**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, mid-lactation dairy cows (118 ± 41 DIM; 1.7 ± 0.8 parity; 683 ± 42 kg of BW) were utilized in an experiment conducted over 2 replications in November, 2019. Cows were moved into individual box stalls (4.57 × 4.57 m) at the Iowa State University Dairy Farm (Ames) and allowed 3 d to acclimate to the experimental settings. On d 2 of acclimation, all cows were implanted with jugular catheters as previously described by Horst et al. (2020b). The trial consisted of 3 experimental periods (P), during which all cows were fed ad libitum once (0800 h) and milked twice (0600 and 1800 h) daily in their stalls. Feed refusals were measured at 0730 h daily to determine DMI. During P1 (5 d), baseline data were generated. Immediately following the 0600 h milking on d 1 of P2 (2 d), all cows received an i.v. LPS bolus (0.2 µg/kg of BW, *Escherichia coli* O55:B5; Sigma Aldrich) to initiate the “stacked stressor” event. During P3 (4 d), cows were balanced by milk yield, DIM, parity, and presumed pregnancy status and assigned to 1 of 2 abomasal infusion treatments: (1) control (LPS-CON; 6 L of H2O/d; n = 6) or (2) starch infused [LPS-ST; 4 kg of corn starch (98.5% DM) + 6 L of H2O/d; n = 6]. Respective abomasal infusion treatments were divided into 4 equal doses and administered daily at 0000, 0600, 1200, and 1800 h; thus, LPS-ST cows received 1 kg of corn starch, and both LPS-CON and LPS-ST received 1.5 L of H2O per infusion. In addition to their respective abomasal infusion treatments, all cows received i.v. LPS boluses on d 1 (0.8 µg/kg of BW) and d 3 (1.6 µg/kg of BW) of P3 to maintain a chronic inflammatory state, as we previously reported (Horst et al., 2020a).

Milk yield was recorded following each milking, and milk samples were collected on d 4 and 5 of P1 and daily during P2 and P3 and stored at 4°C with a preservative (bronopol tablet; D&F Control Systems) until compositional analysis by Dairy Lab Services (Dubuque, IA) by infrared analysis (Official Method 972.16; AOAC International, 1995). Cows were fed a diet formulated to meet or exceed predicted energy, protein, vitamin, and mineral requirements (NRC, 2001; Table 1), and had ad libitum access to clean drinking water throughout the experiment. Samples of the TMR 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). The spectroscopic feed analyses were based on chemical
Respiration rate (RR) and rectal temperature (Tr) were collected following each milking. Respiration rate was collected by measuring flank movements for 15 s and multiplying by 4 to calculate breaths per minute. Rectal temperature was measured using a digital thermometer (GLA M700 Digital Thermometer).

Abomasal Infusions

Infusions were facilitated using approximately 3 m of polyvinyl chloride-reinforced braided vinyl tubing (0.5-inch outer diameter, 0.25-inch inner diameter; Eastman Chemical Co.) fitted with a plastisol flange at the end (~11 cm diameter) to allow for continuous placement. The flange had several drilled holes (~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. 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 performed 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 ~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 and daily during P2 and P3 for complete blood count (CBC) analysis. Blood samples were also collected 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 (Becton Dickinson Co.) and were subsequently centrifuged at 1,500 × g for 15 min at 4°C before being aliquoted into microcentrifuge tubes for storage at −20°C until analysis. For CBC data, a 3-mL blood sample was collected in K$_2$EDTA tubes (Becton Dickinson Co.) and stored at 4°C for ~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 Inc.; 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 6.1%, 9.1 and 4.0%, 11.8 and 5.1%, 11.1 and 5.4%, 12.1 and 5.0%, 15.3 and 3.8%, and 16.2 and 4.2%, respectively.

Fecal Analysis

Fecal samples (~250 g) were collected rectally twice daily (0600 and 1800 h) on d 4 and 5 of P1 and throughout P2 and P3; however, 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., Arcadia, WI) for starch analysis using a YSI biochemistry analyzer (AOAC Official Method 2014.10; AOAC International, 2014; Yellow Springs Instrument Inc.). Following collection, samples were scored using a 1 to 5 manure scoring system (Zaaijer et al., 2005; 1 = runny and thin; 5 = dry and stiff) and subsequently processed for fecal pH analysis by the laboratory.

Table 1. Ingredients and composition of diet

<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient (% of DM)</td>
<td></td>
</tr>
<tr>
<td>Corn silage</td>
<td>34.4</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>13.5</td>
</tr>
<tr>
<td>Straw</td>
<td>1.8</td>
</tr>
<tr>
<td>Ground corn</td>
<td>28.8</td>
</tr>
<tr>
<td>SoyPlus</td>
<td>2.7</td>
</tr>
<tr>
<td>Corn gluten feed</td>
<td>4.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>6.6</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>8.2</td>
</tr>
<tr>
<td>Chemical analysis (% of DM unless noted)</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>31.7</td>
</tr>
<tr>
<td>CP</td>
<td>15.9</td>
</tr>
<tr>
<td>Fat</td>
<td>4.7</td>
</tr>
<tr>
<td>NDF</td>
<td>25.2</td>
</tr>
<tr>
<td>ADF</td>
<td>16.7</td>
</tr>
<tr>
<td>NEl (Mcal/kg of DM)</td>
<td>1.69</td>
</tr>
</tbody>
</table>

1Values represent an average of ration nutrient summary reports collected throughout the trial. Diet moisture averaged 44.4%.

2Dairy Nutrition Plus.

3Average nutrient levels: 0.9% Ca, 0.4% P, 0.3% Mg, 0.2% S, 1.2% K, 0.4% Na, 0.5% Cl, 85 mg/kg Zn, 48 mg/kg Mn, 4 mg/kg Fe, 15 mg/kg Cu, 0.8 mg/kg Co, 0.4 mg/kg Se, 0.8 mg/kg I, 6,635 IU/kg vitamin A, 1,327 IU/kg vitamin D, and 27 IU/kg vitamin E.
Liver Biopsies

Liver biopsies were collected 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 lack of phenotypic and inflammatory 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 (calculated as described; VanBaale et al., 2005), voluntary and calculated DMI, milk variables, metabolites, insulin, CBC metrics, SAA, LBP, thermal indices, fecal pH, fecal score, and P3 fecal starch. 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 Institute Inc.). Cow nested within treatment was used as the random variable. For P2 fecal starch, 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. All data are reported as least squares means and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$. During P1 and P2, data were analyzed using the treatments cows were destined to receive during P3 (LPS-CON vs. LPS-ST), despite all animals being treated similarly. One cow in the LPS-ST treatment developed severe hypocalcemia following the first LPS bolus during P2 (before abomasal infusions during P3) and thus her data were removed from the entire data set.

RESULTS

As expected, abomasal starch infusions markedly decreased fecal pH during P3 (0.82 units relative to LPS-CON; $P < 0.01$; Figure 1A), with the nadir occurring on d 1 (1.01-unit-decrease relative to baseline). Lipo polysaccharide administration decreased fecal score for LPS-ST on d 1 of P2 (36% relative to LPS-CON; Figure 1B) before returning to baseline on d 2, whereas fecal score slightly decreased from d 1 to d 2 but remained similar to baseline on both days for LPS-CON ($P < 0.01$). During P3, no effect of starch infusions on fecal score was detected ($P > 0.15$). Relative to LPS-CON, fecal starch markedly increased in LPS-ST throughout P3 (23.7 vs. 2.4% of DM; $P < 0.01$; Figure 1C).

Regardless of treatment, repeated LPS administration decreased milk yield throughout P2 and P3 (36 and 29%, respectively; $P < 0.01$; Figure 2A) relative to baseline, with progressively less severity following each LPS infusion (53% on d 1 of P2, and 44 and 37% on d 1 and 3 of P3, respectively, relative to P1; $P < 0.01$). Dry matter intake followed a similar pattern such that it decreased following LPS administration throughout P2 and P3 (35 and 31% relative to baseline, respectively; $P < 0.01$; Figure 2B), with progressively less severe reductions with each LPS bolus (49% on d 1 of P2, and 42 and 40% on d 1 and 3 of P3, respectively, relative to P1; $P < 0.01$). However, there were no additional negative effects of starch infusions on milk yield or DMI during P3 ($P > 0.35$). By design, there was a treatment × period interaction for calculated DMI (voluntary DMI + 3.94 kg DM from starch infusions in LPS-ST) such that both treatments decreased similarly throughout P2 (35% relative to P1; Table 2) but only the LPS-ST cows increased relative to P2 during P3 ($P < 0.01$).

There were no effects of starch infusions on ECM, or milk fat concentrations, their yields, lactose, MUN, or SCS (Table 2). Milk fat increased for both treatments during P2 (23% relative to baseline; $P < 0.01$) but returned to baseline concentrations during P3. In contrast, milk fat yield decreased during P2 and P3 (27% relative to P1; $P < 0.01$). There was a tendency for milk protein content to be increased in LPS-ST throughout the entire experiment (6% relative to LPS-CON; $P = 0.06$). Additionally, milk protein content increased during P2 for both treatments (5% relative to P1; $P < 0.01$). Milk protein yield decreased from P1 to P2 for both treatments, and although treatments were similar to each other, protein yield decreased with more severity during P2 in LPS-ST relative to LPS-CON (41 vs. 28% relative to P1, respectively; $P < 0.03$). Milk lactose concentrations decreased for both treatments during P2 (7% relative to P1; $P < 0.01$) but returned to baseline by P3. Further, regardless of abomasal treatment, ECM decreased throughout P2 and P3 (30 and 29% relative to P1, respectively; $P < 0.01$). Milk urea nitrogen initially increased for both treatments during P2 (18% relative to baseline; $P < 0.01$) before decreasing below baseline concentrations during P3 (13%). Similarly, during P2, SCS increased (30% relative to baseline; $P = 0.05$) before decreasing back to baseline concentrations during P3.
There were no effects of treatment or time on circulating glucose, insulin, or BHB \((P > 0.31; \text{Table 3})\). There was a tendency for a treatment × period interaction for circulating NEFA such that it increased and decreased from P1 to P2 for LPS-ST and LPS-CON, respectively \((P = 0.08; \text{Table 3})\), but it remained similar between groups throughout P2 and P3. Overall, there were no effects of treatment or period on circulating BUN \((P > 0.24; \text{Table 4})\). In response to LPS, concentrations of SAA and LBP increased during P2 and P3 relative to P1 \((3\text{-fold and } 50\%, \text{respectively}; P < 0.01; \text{Table 4})\) for both abomasal treatments. Rectal temperature remained unaltered over time and due to starch \((P > 0.14; \text{Table 4})\). However, RR decreased for both treatments throughout P2 and P3 \((5 \text{ breaths/min relative to P1}; P < 0.01; \text{Table 4})\). Overall, there were no effects of

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**Figure 1.** Fecal pH (A), score (B), and starch concentration (C) in immune-activated lactating dairy cows abomasally infused with either water (LPS-CON) or 4 kg/d corn starch (LPS-ST). P1 represents an average of measurements obtained before i.v. LPS administration and the administration of respective abomasal infusion treatments (d 1–4 of P1). P2 represents data obtained when both treatments were given an i.v. bolus of LPS (0.2 µg/kg of BW; indicated with arrow) on d 1 of P2. During P3, both treatments received 2 additional i.v. LPS boluses on d 1 and 3 (0.8 and 1.6 µg/kg of BW, respectively; indicated with arrows), and respective abomasal treatments were administered split into 4 equal doses at 0000, 0600, 1200, and 1800 h daily. During P1 and P2, data were analyzed using the treatments cows were destined to receive during P3 (LPS-CON vs. LPS-ST), despite all animals being treated similarly. In (A), P2 treatment: \(P > 0.77\), day: \(P > 0.34\), and treatment (Trt) × day: \(P > 0.91\), and P3 treatment × day: \(P > 0.36\). In (B), P2 treatment: \(P > 0.10\), and day: \(P > 0.25\), and P3 treatment: \(P > 0.15\), day: \(P > 0.22\), and treatment × day: \(P > 0.38\). In (C), P2 treatment: \(P > 0.96\) and P3 d: \(P > 0.75\) and treatment × day: \(P > 0.39\). Results are expressed as LSM ± SEM and considered significant if \(P \leq 0.05\) and a tendency in \(0.05 < P \leq 0.10\).
DISCUSSION

Inflammation is a well-characterized sequela of rumen acidosis (Gozho et al., 2005; Khafipour et al., 2009b; Li et al., 2016), but the region(s) in which antigen translocation occurs remains uncertain. Relative to the rumen, the hindgut may be more susceptible to the chemical and physical insults accompanying excessive fermentation, which is supported by the relative lack of anatomical and buffering defenses in the postruminal GIT (Gressley et al., 2011; Steele et al., 2016). Previous work indicates that HGA may contribute to an exacerbated inflammatory response in early lactation via its purported effects on postruminal barrier function (Bisell and Hall, 2010; Rodriguez-Jimenez et al., 2019). However, we and others have failed to replicate these findings in isolated HGA models (Mainardi et al., 2011; van Gastelen et al., 2021a,b; Abeyta et al., 2023a,b; Piantoni et al., 2023), which may be explained by a lack of “stacked stressors” in the experimental designs. For instance, circulating LPS (regardless of origin) can disrupt intestinal barrier integrity from the basolateral side (Ding et al., 2004; Hietbrink et al., 2009; Cao et al., 2018), and thus, pre-existing inflammation may intensify the negative pathology of HGA. Therefore, experimental objectives were to evaluate whether HGA would exacerbate the inflammatory status of immune-activated dairy cows with the hypothesis that abomasal starch infusions would decrease production, alter metabolism, and augment the inflammatory response associated with repeated i.v. LPS infusions.

Based on our previous experiment (Horst et al., 2020a; Rodriguez-Jimenez et al., 2020), 3 increasing doses of i.v. LPS were administered 48 h apart to initiate (P2) and sustain (P3) a systemic inflammatory response before and in concurrence with abomasal infusions. The elevated circulating acute phase proteins (APP; SAA and LBP) and various WBC during P2 and P3 relative to baseline agree with our previous trial (Horst et al., 2020a) and demonstrate that a chronic inflammatory state was achieved. Of note, however, is the elevation of these metrics (particularly SAA) above what is considered “healthy” (Humblet et al., 2006; Ceciliani et al., 2012) during baseline in both treatments. Although unclear, it anecdotally appears that the liver biopsies may have caused an immune response during P1 before i.v. LPS infusion or abomasal infusions. Liver biopsies were not molecularly analyzed because ST infusions did not appear to cause or intensify an immune-activated phenotype (i.e., inflammation, production changes, and so on).
Table 2. Effects of hindgut acidosis on production parameters and milk variables in immune-activated dairy cows¹

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>SEM</th>
<th>Trt</th>
<th>P</th>
<th>Trt × P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECM (kg/d)</td>
<td>LPS-CON</td>
<td>LPS-ST</td>
<td>LPS-CON</td>
<td>LPS-ST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40.7</td>
<td>39.6</td>
<td>30.2</td>
<td>25.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated DMI² (kg/d)</td>
<td>20.6ab</td>
<td>22.0a</td>
<td>15.2d</td>
<td>12.6d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk variable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fat (%)</td>
<td>4.19</td>
<td>3.99</td>
<td>5.15</td>
<td>4.90</td>
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<tr>
<td>Fat yield (kg/d)</td>
<td>1.62</td>
<td>1.46</td>
<td>1.23</td>
<td>1.06</td>
<td></td>
<td></td>
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<tr>
<td>Protein (%)</td>
<td>3.07</td>
<td>3.25</td>
<td>3.19</td>
<td>3.44</td>
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<td></td>
</tr>
<tr>
<td>Protein yield (kg/d)</td>
<td>1.08b</td>
<td>1.22b</td>
<td>0.78b</td>
<td>0.72b</td>
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<tr>
<td>Lactose (%)</td>
<td>4.69</td>
<td>4.71</td>
<td>4.34</td>
<td>4.45</td>
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<tr>
<td>MUN (mg/dL)</td>
<td>11.4</td>
<td>11.8</td>
<td>13.3</td>
<td>14.1</td>
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<tr>
<td>SCS</td>
<td>3.4</td>
<td>1.9</td>
<td>4.0</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹All cows were administered with 3 separate i.v. LPS boluses on d 1 of period (P) 2, and d 1 and 3 of P3 (0.2, 0.8, and 1.6 µg/kg of BW, respectively). 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 (LPS-CON; abomasal infusion of 6 L of H₂O/d; n = 6) or (2) starch infused (LPS-ST; abomasal infusion of 4 kg of corn starch mixed in 6 L of H₂O/d; n = 6). Abomasal infusion treatments were split into 4 equal doses and administered at 0000, 0600, 1200, and 1800 h daily.

Table 3. Effects of hindgut acidosis on metabolism metrics in immune-activated lactating dairy cows¹

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>SEM</th>
<th>Trt</th>
<th>P</th>
<th>Trt × P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>LPS-CON</td>
<td>LPS-ST</td>
<td>LPS-CON</td>
<td>LPS-ST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>76.9</td>
<td>79.2</td>
<td>76.4</td>
<td>82.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (µg/L)</td>
<td>0.69</td>
<td>0.61</td>
<td>0.75</td>
<td>0.78</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NEFA² (µEq/L)</td>
<td>240b</td>
<td>128b</td>
<td>224w</td>
<td>218w</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BHB (mg/dL)</td>
<td>6.4</td>
<td>5.3</td>
<td>7.1</td>
<td>6.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>8.1</td>
<td>7.7</td>
<td>9.5</td>
<td>9.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹All cows were administered with 3 i.v. LPS boluses on d 1 of period (P) 2, and d 1 and 3 of P3 (0.2, 0.8, and 1.6 µg/kg of BW, respectively). 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 (LPS-CON; abomasal infusion of 6 L of H₂O/d; n = 6) or (2) starch infused (LPS-ST; abomasal infusion of 4 kg of corn starch mixed in 6 L of H₂O/d; n = 6). Abomasal infusion treatments were split into 4 equal doses and administered at 0000, 0600, 1200, and 1800 h daily.

Table 4. Effects of hindgut acidosis on immune metrics and thermal indices in immune-activated dairy cows¹

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>SEM</th>
<th>Trt</th>
<th>P</th>
<th>Trt × P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC³ (×10³/µL)</td>
<td>LPS-CON</td>
<td>LPS-ST</td>
<td>LPS-CON</td>
<td>LPS-ST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.97</td>
<td>10.04</td>
<td>11.83</td>
<td>12.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils (×10³/µL)</td>
<td>3.02</td>
<td>3.76</td>
<td>6.15</td>
<td>7.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (×10³/µL)</td>
<td>4.65</td>
<td>5.15</td>
<td>4.33</td>
<td>4.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes (×10³/µL)</td>
<td>0.53</td>
<td>0.59</td>
<td>0.57</td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAA³ (µg/mL)</td>
<td>394</td>
<td>275</td>
<td>1,084</td>
<td>1,118</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBP⁴ (µg/mL)</td>
<td>8.1</td>
<td>7.5</td>
<td>11.1</td>
<td>12.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹All cows were administered with 3 separate i.v. LPS boluses on d 1 of period (P) 2, and d 1 and 3 of P3 (0.2, 0.8, and 1.6 µg/kg BW, respectively). Data from periods 1 and 2 represent an average of cows destined to be assigned to 1 of 2 abomasal infusion treatments (Trt) during P3: (1) control (LPS-CON; abomasal infusion of 6 L of H₂O/d; n = 6) or (2) starch infused (LPS-ST; abomasal infusion of 4 kg of corn starch mixed in 6 L of H₂O/d; n = 6). Abomasal infusion treatments were split into 4 equal doses and administered at 0000, 0600, 1200, and 1800 h daily.

²Nonesterified fatty acids.

³SAA = serum amyloid A.

⁴LBP = LPS-binding protein.
The rescue of milk yield and DMI with each progressive LPS bolus indicates endotoxin tolerance developed with repeated LPS infusions. Endotoxin tolerance is a well-documented phenomenon by which repeated LPS exposure results in a diminished proinflammatory response to each successive administration (Beeeson, 1946; Biswas and Lopez-Collazo, 2009). Interestingly, our results corroborate with Dickson et al. (2019), who documented that milk yield was one of the first metrics to display tolerance to a continuous and increasing i.v. LPS exposure despite the sustained inflammation. Regardless, we hypothesized that adding HGA to an already immune-activated cow (as a “stacked stressor” model) would exacerbate the pre-existing inflammation, which could still be evaluated given the inflammatory state during P3.

In agreement with our previous studies (Abeyta et al., 2023a,b,c), abomasal starch infusions markedly decreased fecal pH (5.56 at nadir), which indicates that our goal of inducing and segregating HGA was achieved. Nevertheless, HGA did not augment inflammation as indicated by unaltered APP, blood count metrics, and production between treatments. This is consistent with findings from our group and others (Mainardi et al., 2011; van Gastelen et al., 2021a,b; Abeyta et al., 2023a,b,c; Piantoni et al., 2023) and builds upon the existing evidence demonstrating that pH changes in the hindgut in isolation from altered rumen dynamics do not appear to alter postruminal barrier integrity. However, this does not eliminate the potential for rumen acidosis to cause postruminal epithelial damage via alternative mechanisms (discussed below).

In line with our previous experiments (Abeyta et al., 2023a,b,c), the substantial increase in fecal starch concentrations suggests that the capacity for enzymatic hydrolysis and fermentation in the small and large intestines, respectively, was exceeded. Using an equation formulated by Nennich and colleagues (2005), we estimated that approximately 1.28 kg/d (32%) of the infused starch was excreted during P3, suggesting that ~68% of the abomasally infused starch was degraded postruminally. Interestingly, this value is slightly lower than estimations from our previous abomasal infusion experiments (84–85% of infused starch disappeared; Abeyta et al., 2023a,b) and the average postruminal starch disappearance formulated via meta-analysis by Sanz-Fernandez et al. (2020; 85 ± 10%). Although unclear, this may be attributed to diminished pancreatic digestive enzyme secretion following i.v. LPS infusions. For instance, Vaccaro and colleagues (1996) described substantially decreased pancreatic enzyme output in rats administered i.p. LPS, and pancreatic amylase, trypsin, and chymotrypsin secretion was strikingly lower in patients with sepsis relative to healthy controls (Tribl et al., 2003). This is supported by in vivo and in vitro work that described decreased mRNA expression of amylase and considerable damage and apoptosis in the pancreas of LPS-treated rats (Vaccaro et al., 2000). The potential link between systemic inflammation and a reduced capacity for postruminal enzymatic digestion has practical implications for many aspects of diet formulation.

Despite clear indications of extensive hindgut fermentation, metabolic parameters were largely unchanged by abomasal starch infusions. Circulating glucose is under tight homeostatic regulation (Baumgard et al., 2017); thus, it is not too surprising that we did not observe changes associated with starch infusions, particularly because this has been a relatively consistent response across studies (Abeyta et al., 2023a,b,c). Absorbed glucose first undergoes visceral metabolism before appearing in the systemic circulation and, due to the large metabolic demands of the splanchnic bed, the intestinal glucose flux rate is commonly zero or even negative in ruminants (Reynolds, 2002). Additionally, the aforementioned evidence that small intestinal starch digestibility may have been decreased by LPS infusions suggests that less glucose was absorbed in the current model. This is supported by a lack of insulin and NEFA responses, which typically increase and decrease, respectively, in response to starch infusions (Knowlton et al., 1998; Abeyta et al., 2023a,b), although this is not always consistent (van Gastelen et al., 2021a; Abeyta et al., 2023c).

Blood urea nitrogen decreased following starch infusions, and this is a consistent response across studies (Gressley and Armentano, 2007; van Gastelen et al., 2021a,b; Abeyta et al., 2023a,b). An increase in nonstructural carbohydrate availability allows for rapid microbial proliferation in the GIT, thus sequestering available nitrogen within the intestinal lumen to support microbial growth (Van Soest, 1982). Consequently, less absorbed ammonia results in less substrate for hepatic ureagenesis (Røjen et al., 2012) and this ultimately reduces BUN. Others have demonstrated the capacity for blood urea to cross through the intestinal epithelium in ruminants (postruminal; Reynolds and Huntington, 1988; Huntington, 1989), pigs (Van Leeuwen et al., 1995), rabbits (Xiao et al., 2012), rats (Younes et al., 2001), and humans (Wolpert et al., 1971). Blood urea may therefore provide a valuable N source to the hindgut microbiome. Thus, the reduction in BUN described herein is likely explained by a combination of reduced ammonia entry to the hepatic urea cycle and the recruitment of BUN to the hindgut lumen to increase N availability for microbial growth.

Despite the lack of an inflammatory response with isolated HGA, excessive alimentary fermentation ap-
pears to have the capacity to alter postruminal barrier function, as indicated by injury to the small intestinal villi in dairy cows (Lai et al., 2022) and substantial damage to the colonic epithelium in goats (Tao et al., 2014). These findings mirror other studies which demonstrate that increased supplementation of non-structural carbohydrates may cause inflammation or morbidity in rodents (Ten Bruggencate et al., 2003; Geier et al., 2007), horses (Fagliari et al., 1998; Kwon et al., 2013), rabbits (Gidenne et al., 2004; Zhou et al., 2021a), and fish (Zhang et al., 2020; Zhou et al., 2021b). Apparently, excessive postruminal fermentation in isolation from altered rumen dynamics is not responsible for hindgut barrier dysfunction in dairy cows, as was previously hypothesized by our group and others (Khafipour et al., 2009b; Plaizier et al., 2018; Abeyta et al., 2023a,b,c). Although unclear, this may be explained by altered luminal butyrate concentrations, which rise with increased hindgut fermentation (Mao et al., 2012; Westreicher-Kristen et al., 2018; van Gastelen et al., 2021b). Butyrate serves as the main energy source for intestinal cells (Bergman, 1990) but also possesses anti-inflammatory properties (Hamer et al., 2008). Additionally, in vivo experiments indicate butyrate supplementation preserves intestinal barrier function by modulating tight junction protein expression and increasing mucin secretion (Han et al., 2015; Chen et al., 2018; Gonzalez et al., 2019). Further, oral butyrate supplementation alleviated oxidative stress and intestinal injury (Hou et al., 2014) and reduced intestinal permeability (Leonel et al., 2013) in porcine and rodent colitis models, respectively. Thus, increased butyrate availability may be a mechanism by which the hindgut barrier was preserved in our and others’ HGA experiments (van Gastelen et al., 2021a,b; Abeyta et al., 2023a,b,c). Regardless, the previously mentioned detrimental effects of SARA on small and large intestinal epithelia suggest that some aspect of altered rumen function during acidosis affects epithelial integrity of the whole GIT. Although the mechanistic reasonings remain uncertain, interesting findings from Ametaj et al. (2010) and Saleem et al. (2012) suggest that acidosis increases ruminal concentrations of potentially noxious compounds such as ethanol, methyl-amines, N-nitrosodimethylamine, and bioactive amines—molecules that cause intestinal hyperpermeability and other pathologies (Souliotis et al., 2002; Bala et al., 2014). Thus, the concoction of potentially toxic compounds produced during rumen acidosis may compromise postruminal GIT barrier health. The importance of identifying sources of immune activation in dairy cows lies in the potential economic and animal welfare detriments associated with the energetic demands of an activated immune system. Thus, future work needs to better characterize the mechanisms underlying postruminal intestinal damage associated with rumen acidosis.

CONCLUSIONS

Excessive hindgut fermentation ostensibly damages postruminal barrier integrity; however, previous studies have not reported inflammation or an immune-activated phenotype (i.e., reduced milk yield, DMI, febrile response) with isolated HGA. Systemic inflammation has direct negative impacts on intestinal barrier function. Thus, we hypothesized that an already inflamed cow may become more susceptible to the stress of HGA, especially considering that transition cows are likely immune-activated before an abrupt change to a higher starch diet postpartum. However, in contrast to our hypothesis, HGA did not appear to augment pre-existing inflammation in dairy cows repeatedly administered i.v. LPS. The recent accumulation of ruminant HGA literature suggests that postruminal damage does not occur with excessive hindgut fermentation in isolation from altered rumen function. However, a variety of potentially toxic compounds are produced in the rumen during SARA, and these may be responsible for altering postruminal GIT health. Thus, future work better characterizing the impacts of acidic rumen fluid on the postruminal GIT is warranted.

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REFERENCES


AOAC International. 1996. AOAC official method 973.18: Fiber (acid detergent) and lignin in animal feed. AOAC International.


Fagliari, J. J., D. McClenahan, O. A. Evanson, and D. J. W.

Chen, G., X. Ran, B. Li, Y. Li, D. He, B. Huang, S. Fu, J. Liu, and


Tao, S., Y. Duanmu, H. Dong, J. Tian, Y. Ni, and Z. Zhao. 2014. A high-concentrate diet induced colonic epithelial barrier disruption is associated with the activating of cell apoptosis in lactating


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