Prenatal immune stimulation alters the postnatal acute phase and metabolic responses to an endotoxin challenge in weaned beef heifers[1,2]

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ABSTRACT: This study evaluated whether administration of lipopolysaccharide (LPS) at each trimester of gestation would alter the acute phase (APR) and metabolic responses to a postnatal LPS challenge in weaned heifers. Pregnant crossbred multiparous cows (n = 50) were randomized into prenatal immune stimulation (PIS; n = 24; administered 0.1 µg/kg BW LPS subcutaneously at 71 ± 2, 170 ± 2 and 234 ± 2 d of gestation) and saline (CON; n = 26) groups. From these treatment groups, heifer calves (n = 12 PIS and 11 CON) were identified at weaning (244 ± 3 d of age) to receive an LPS challenge. On d 0, heifers were fitted with vaginal temperature (VT) devices, jugular catheters, and moved into individual stalls. On d 1, heifers were challenged i.v. with LPS (0.5 µg/kg BW) at 0 h. Blood samples were collected and sickness behavior scores (SBS) recorded at 0.5 h intervals from −2 to 8 h and at 24 h relative to LPS challenge. Serum was analyzed for cortisol, cytokines, glucose, non-esterified fatty acids (NEFA), and serum urea nitrogen (SUN) concentrations. Baseline VT was lesser in PIS heifers from −11 to −5 h pre-LPS (treatment × time: $P < 0.01$) compared to the CON; however, the post-LPS VT response did not differ between treatments ($P = 0.89$). There was a treatment × time interaction ($P < 0.01$) for SBS with PIS heifers having lesser SBS from 0.5 to 2 h post-LPS compared to CON. There was a treatment × time interaction ($P = 0.03$) for cortisol with PIS heifers having greater cortisol at 0.5, 3, 3.5, 5.5 and 6.5 h post-LPS compared to CON. There were treatment × time interactions for the post-LPS cytokine responses ($P \leq 0.05$). Specifically, PIS heifers had greater TNF-α from 1.5 to 2 h, yet less TNF-α at 3 h than CON ($P < 0.01$), and PIS heifers had greater IFN-γ from 3.5 to 5.5 h post-LPS than CON ($P < 0.01$). In contrast, IL-6 was less in PIS than CON heifers from 1.5 to 8 h post-LPS ($P < 0.001$). Glucose concentrations were greater in PIS heifers at −1 h, but less at 2, 3 and 5.5 h compared to CON (treatment × time: $P < 0.01$). Serum NEFA concentrations were greater ($P = 0.04$) in PIS than CON heifers. There was a treatment × time interaction ($P < 0.01$) for SUN with PIS heifers having greater SUN concentrations at −2, −1.5, 2, 3, 6.5 and 24 h than CON. These data demonstrate that in utero exposure to multiple

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low doses of endotoxin has lasting physiological and immunological effects when the offspring encounter a similar postnatal immunological insult.

Key words: acute phase response, cattle, immune, lipopolysaccharide, metabolism, prenatal stress

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INTRODUCTION

The negative consequences of prenatal exposure to various stressful stimuli has been evaluated extensively in multiple species including humans (Lobel, 1994), primates (Clarke and Schneider, 1993; Schneider et al., 2002), rodents (McCormick et al., 1995; Maccari et al., 2003), swine (Haussmann et al., 2000; Lay et al., 2008), and cattle. In cattle, prenatal exposure to various stressful stimuli such as heat stress (Strong et al., 2015; Monteiro et al., 2016), metabolic stress (Ling et al., 2018), nutritional restriction (Bellows and Short, 1978; Blecha et al., 1981) and transportation (Lay et al., 1997; Littlejohn et al., 2016; Littlejohn et al., 2020) have been reported to have lasting effects on postnatal biological pathways including reproduction, growth, metabolism, stress responses, innate immunity, and adaptive immunity. Additionally, prior work has demonstrated that prenatal exposure to transportation stress can alter genome-wide DNA methylation (Littlejohn et al., 2018), thus demonstrating that the ability to re-program physiological, immunological and behavioral responses of cattle is within our reach. However, there has been a void in beef cattle research that has focused on how in utero stimuli may be utilized to re-program the immune system of beef cattle to better prepare them for the current production system environment.

In an effort to capitalize on the ability to re-program physiological/immunological systems during gestation, our laboratory has utilized prenatal immune stimulation (PIS) as a means to alter the immune system of calves in utero (Carroll et al., 2017) in a manner that would translate to a more robust innate immune system in the postnatal calf. The hypothesis was that by exposing fetal calves in utero to a relatively mild immunological challenge, it would better prepare their immune system for subsequent immunological challenges postnatally. Data from this prior study demonstrated that a single low-dose, subcutaneous administration of lipopolysaccharide (LPS) to pregnant cows in the last third of gestation was able to alter the innate immune response, metabolic response, and performance of heifer calves challenged with the same endotoxin at weaning (Burdick Sanchez et al., 2017; Carroll et al., 2017). Thus, these prior data provided the foundation for continuing to explore how in utero immune stimulation could be utilized to enhance immune function and health of beef cattle in their postnatal environment. Therefore, our laboratory sought to determine if administration of LPS during all three trimesters of gestation would elicit even greater alterations in the immune and metabolic responses of calves to a postnatal LPS challenge.

MATERIALS AND METHODS

All experimental procedures were in compliance with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* and approved by the Institutional Animal Care and Use Committee of the University of Florida (IACUC Protocol #201508806).

Pregnant Angus × Brangus crossbred cows (n = 50; 527 ± 46 kg body weight) were selected from a single herd managed similarly prior to allocation to treatments in the current study. Cows had an average parity of 5.8 ± 2.6 calvings. Cows were randomly allotted to one of two treatments: 1) prenatal immune stimulation [PIS; n = 24; administered 0.1 µg/kg BW of LPS (*Escherichia coli* O111:B4; Sigma Aldrich, St. Louis, MO) subcutaneously at 71 ± 2, 170 ± 2, and 234 ± 2 d of gestation] and 2) saline group (CON; n = 26; administered an equal volume of sterile saline subcutaneously at 71 ± 2, 170 ± 2, and 234 ± 2 d of gestation). The dose of LPS administered and route of administration was selected in order to produce a physiological response in the dam while avoiding abortion of the fetus (Carroll et al., 2017). Cows grazed a common bahiagrass pasture from the time of LPS administration to weaning. Calves were born October through December and birth date was recorded. Day of gestation for LPS administration to dams was computed from calf birth date assuming a constant gestation length of 283 d. At

150 ± 14 d of age (PIS calves 149 ± 13 d of age; CON calves 150 ± 12 d of age) all calves were dewormed (5 mg of fenbendazole/kg BW; Safe Guard, Merck Animal Health, Madison, NJ), and vaccinated against *Mannheimia haemolytica* type A1 (One Shot; Zoetis, Florham Park, NJ), infectious bovine rhinotracheitis virus, bovine virus diarrhea virus types 1 and 2, parainfluenza3 virus, and bovine respiratory syncytial virus (Bovi-Shield Gold 5; Zoetis, Florham Park, NJ), and *Clostridium chauvoei*, *Clostridium speticum*, *Clostridium haemolyticum*, *Clostridium novyi*, *Clostridium sordelli*, and *Clostridium perfringens* types B, C and D (Ultrabac 8; Zoetis, Florham Park, NJ). At 214 ± 14 d of age all calves were administered a booster vaccination of Bovi-Shield Gold 5 and Ultrabac 8. Calves were weaned and weaning weight recorded at 245 ± 14 d of age to allow sufficient time to halter break the heifers prior to the LPS challenge at 279 ± 14 d of age. Calculated adjusted 205-d weaning weights did not differ between PIS or CON calves (172 vs. 173 ± 4 kg, respectively; $P = 0.84$) or between heifers and bulls (171 vs. 172 ± 7 kg, respectively; $P = 0.70$).

From these treatments, 23 heifer calves ($n = 12$ PIS and 11 CON) were identified at weaning (245 ± 14 d of age) to subsequently receive an LPS challenge. Heifer calves from the CON group averaged 240 ± 5 kg BW and PIS heifers averaged 233 ± 4 kg BW. On d 0 (35 d after weaning), heifers were fitted with indwelling vaginal temperature (VT) recording devices that measured VT continuously at 5-min intervals (Burdick et al., 2012) and jugular vein catheters. Heifers were then moved into individual pens (2.5 m × 6 m) in a covered barn. Heifers were allowed access to feed until 1 h prior to collection of the first blood sample and had ad libitum water access throughout the study. On d 1, heifers were challenged intravenously with LPS (0.5 µg/kg BW) at 0 h (1000 h). Sickness behavior scores (SBS) were recorded and whole blood samples were collected at 0.5 h intervals from −2 to 8 h and again at 24 h relative to the LPS challenge at 0 h. Whole blood for serum was collected into Sarstedt tubes containing no additive (Sarstedt, Inc., Newton, NC), and were allowed to clot at room temperature for 0.5 h prior to centrifugation at 1500 × *g* for 20 min at 4 °C. Isolated serum was stored at −80 °C until analyzed for cortisol, cytokine, glucose, NEFA, and urea nitrogen (SUN) concentrations.

Sickness Behavior Scores

A trained observer assessed and recorded each heifer's SBS by visual observation following the collection of each blood sample (Carroll et al., 2017). Heifers were scored on a scale of 1 to 4 using 0.25-unit increments. Specifically, heifers scored as 1 maintained normal maintenance behavior; heifers scored as 2 were calm but with head distended and increased respiration; heifers scored as 3 displayed clinical signs of sickness, increased respiration and drool, while heifers scored as 4 were observed lying on side with labored breathing and frothing at the mouth. Intervention would occur on any heifer with a SBS of 4. Heifers were scored by the same observer (blinded to treatment) throughout the study who has over 23 yr of animal behavior experience and 10 yr experience observing sickness behavior in cattle.

Serum Analysis

All serum analyses were performed in duplicate. Serum cortisol concentrations were determined using a commercially available enzyme immunoassay kit according to the manufacturer's directions (Arbor Assays, Ann Arbor, MI USA) by comparison of unknowns to standard curves generated with known concentrations of cortisol. Intra- and inter-assay coefficients of variation were less than 12.9% and 9.4%, respectively.

Serum cytokine concentrations (TNF-α, IFN-γ, and IL-6) were determined by a custom bovine 3-plex sandwich-based chemiluminescence ELISA kit (Searchlight-Aushon BioSystems, Inc., Billerica, MA). All intra-assay coefficients of variation were less than 11.6% and all inter-assay coefficients of variation were less than 14.3% for all assays.

Glucose concentrations were determined by modification of the enzymatic Autokit Glucose (Wako Diagnostics, Richmond, VA, USA) to fit a 96-well format as previously described (Burdick Sanchez et al., 2014). Concentrations of glucose were determined by comparing unknown samples to a standard curve of known glucose concentrations. The intra- and inter-assay coefficients of variation were less than 7.2% and 12.7%, respectively.

Concentrations of NEFAs were determined by modification of the enzymatic HR Series NEFA-HR (2) assay (Wako Diagnostics, Richmond, VA, USA) to fit a 96-well format as previously described (Burdick Sanchez et al., 2014). Concentrations of NEFAs were determined by comparing unknown samples to a standard curve of known NEFA concentrations. The intra- and inter-assay coefficients of variation were less than 5.5% and 5.6%, respectively.

Concentrations of SUN were determined by a colorimetric assay according to the manufacturer's directions (K024-H1; Arbor Assays, Ann Arbor, MI) by comparison of unknowns to standard curves generated with known concentrations of urea nitrogen. The intra- and inter-coefficients of variation were less than 2.4% and 4.5%, respectively.

Statistical Analysis

Prior to analysis, vaginal temperature data were averaged into 1-h intervals. All data were analyzed by the MIXED procedure of SAS specific for repeated measures (Version 9.3, SAS Inst. Inc., Cary, NC, USA). Treatment, time, and the treatment × time interaction were included as fixed effects with heifer within treatment included as the experimental unit. Autoregressive 1 was the covariance structure used based on having the lowest AICC fit statistic value. Vaginal temperature was analyzed in two time periods: baseline (−12 to 0 h) and post-challenge (0–23 h) due to the presence of a significant baseline treatment difference. When main effects were significant, means were separated using the PDIFF option in SAS, with $P \leq 0.05$ considered significant and $0.05 < P \leq 0.10$ considered a tendency. All data are presented as the LSM ± SEM.

RESULTS

Vaginal temperature was measured at 5-min intervals from 12 h prior to 23 h following administration of LPS and was collapsed into 1-h intervals prior to analysis (Figure 1). There was a treatment × time interaction ($P < 0.01$) for baseline VT such that baseline VT was lesser in heifers in the PIS treatment group from −11 to −5 h pre-challenge compared to the CON group; however, the post-LPS VT response did not differ between treatments ($P = 0.89$). An overall effect of time ($P < 0.01$) was observed, such that VT increased in response to administration of LPS and returned to baseline within 6 h post-challenge. Sickness behavior scores were elevated (treatment × time: $P < 0.01$) in CON compared to PIS heifers immediately after administration of LPS (0.5 h) until 2 h post-LPS (treatment × time: $P < 0.01$; Figure 2).

There was a treatment × time interaction for serum cortisol concentrations ($P = 0.03$). Specifically, cortisol was greater ($P < 0.05$) in PIS compared to CON heifers at 0.5, 3, 3.5, 5.5, and 6.5 h following LPS administration (Figure 3). Serum TNF-α concentrations were greater in PIS than CON heifers from 1.5 to 2 h but were greater in CON than PIS heifers at 3 h (treatment × time: $P < 0.01$; Figure 4A). There was a bi-phasic TNF-α response in CON heifers but not in PIS

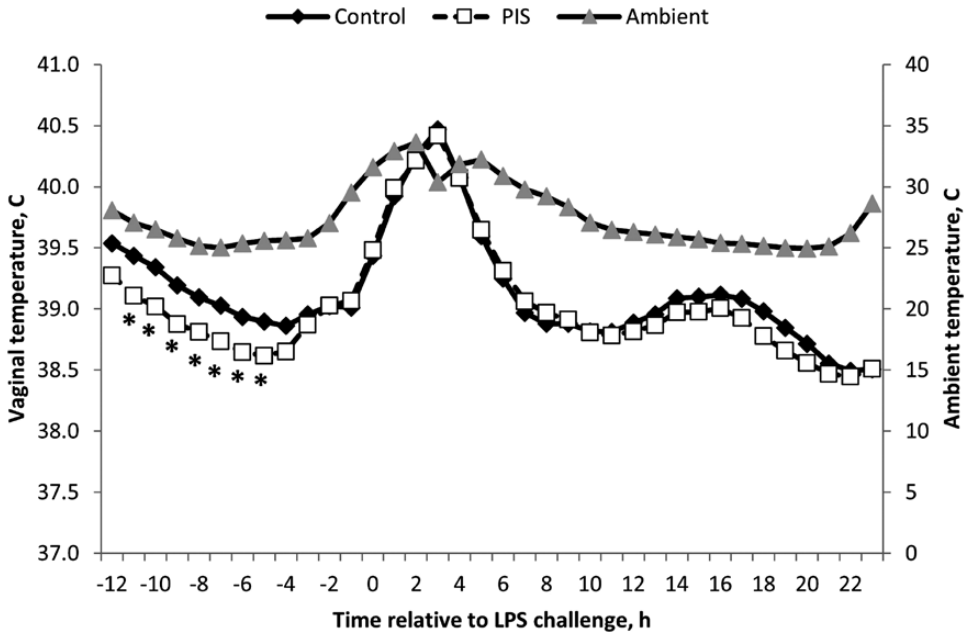

Figure 1. Effect of administered lipopolysaccharide (LPS) three times during gestation (0.1 µg/kg cow BW at 71, 170, and 234 ± 2 d of gestation; PIS) or saline (CON) on the postnatal vaginal temperature response to LPS challenge (0.5 µg/kg calf BW) in weaned heifers. There was a treatment × time interaction ($P < 0.01$) for baseline values such that PIS heifers had lesser vaginal temperature from −11 to −5 h. However, there was no treatment × time interaction following LPS administration ($P = 0.89$) but there was an effect of time ($P < 0.001$). *Treatments within timepoints differ $P \leq 0.05$. SEM ± 0.11 °C.

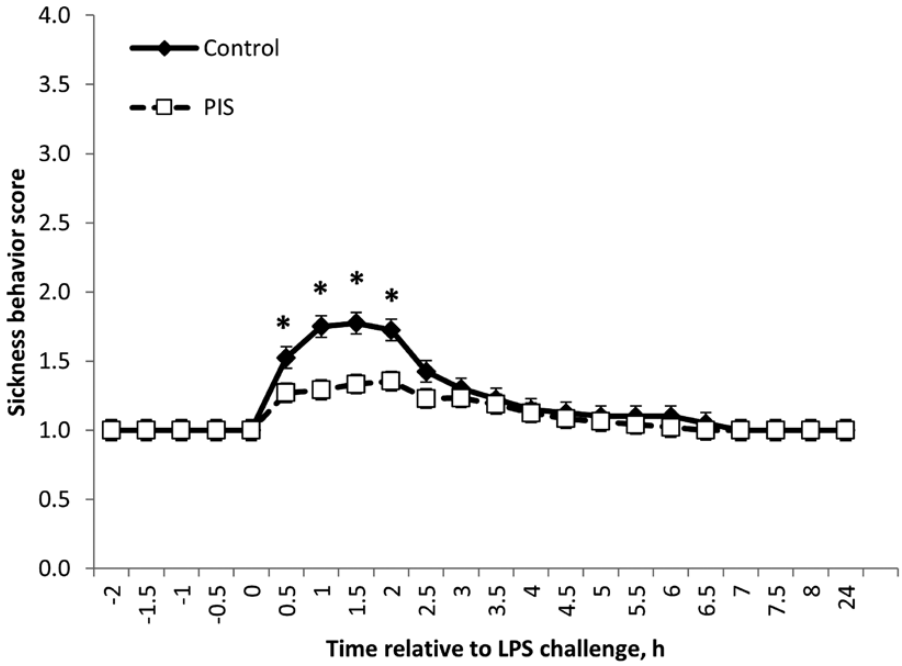

Figure 2. Effect of administered lipopolysaccharide (LPS) three times during gestation (0.1 µg/kg cow BW at 71, 170, and 234 ± 2 d of gestation) on the postnatal sickness behavior response to LPS challenge (0.5 µg/kg calf BW) in weaned heifers. There was a treatment × time interaction (P = 0.007) such that sickness behavior was greater in CON calves compared to PIS calves from 0.5 to 2 h post-challenge. *Treatments within timepoints differ $P \leq 0.05$. SEM ± 0.08.

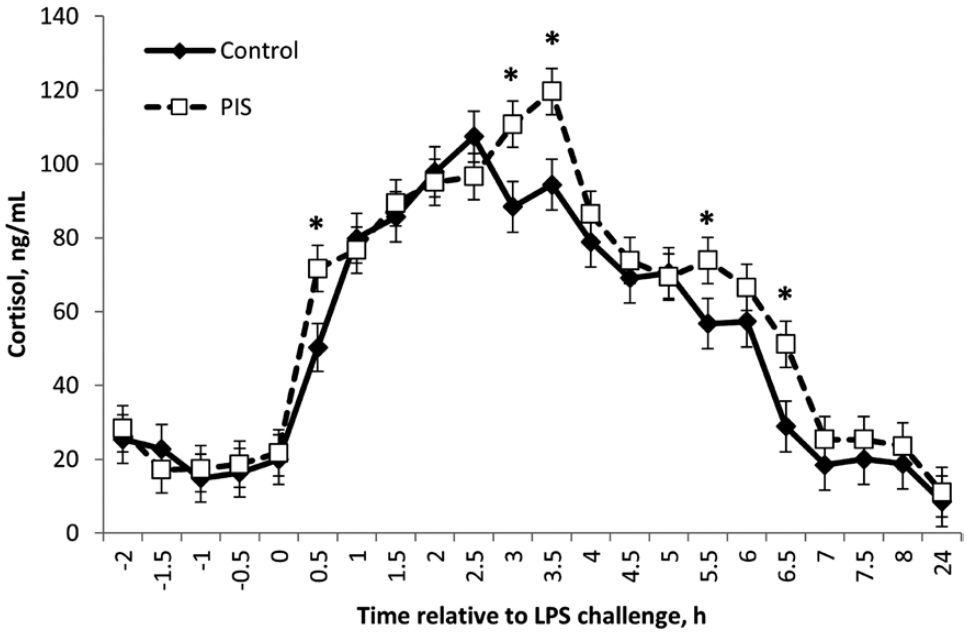

Figure 3. Effect of administered lipopolysaccharide (LPS) three times during gestation (0.1 µg/kg cow BW at 71, 170, and 234 ± 2 d of gestation) on the postnatal serum cortisol response to LPS challenge (0.5 µg/kg calf BW) in weaned heifers. There was a treatment × time interaction (P = 0.03). *Treatments within timepoints differ $P \leq 0.05$. SEM ± 6.86 ng/mL.

heifers. Similarly, there was a treatment × time interaction ($P < 0.01$) for serum IL-6 such that PIS heifers had reduced IL-6 concentrations from 1.5 through 8 h post-challenge compared to CON heifers (Figure 4B). A treatment × time interaction was also observed for serum IFN-γ concentrations (P = 0.04). Yet, in contrast to IL-6, serum IFN-γ concentrations were greater ($P < 0.01$) in PIS heifers compared to CON heifers from 3.5 to 5.5 h post-challenge (Figure 4C).

There was a treatment × time interaction for serum glucose concentrations ($P < 0.01$) where glucose was greater in PIS than CON heifers at −1 h but was greater in CON than PIS heifers at 2, 3, and 5.5 h post-challenge (Figure 5). Serum NEFA concentrations were affected by treatment (P = 0.04)

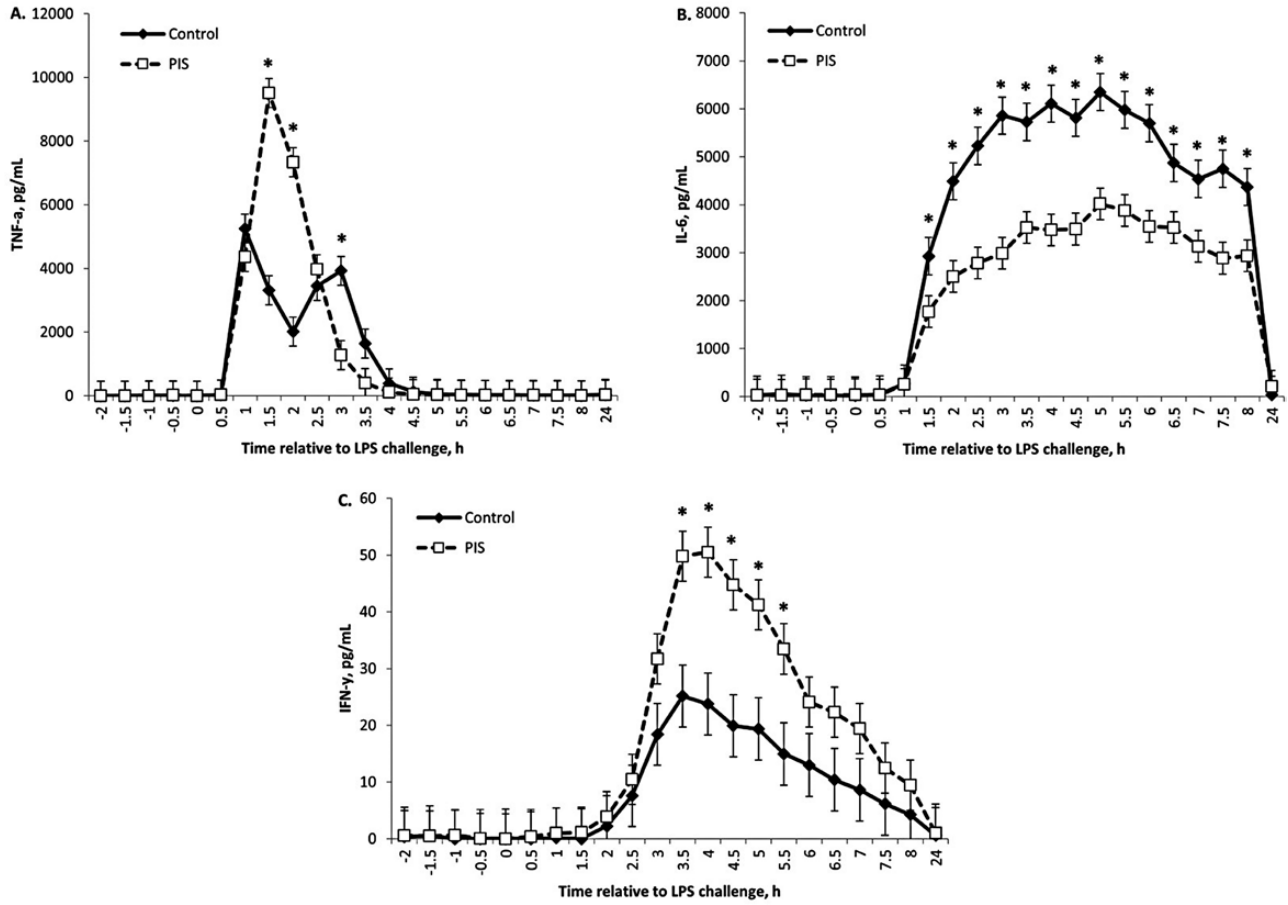

Figure 4. Effect of administered lipopolysaccharide (LPS) three times during gestation (0.1 µg/kg cow BW at 71, 170, and 234 ± 2 d of gestation) on the postnatal serum (A) TNF-α, (B) IL-6, and (C) IFN-γ response to LPS challenge (0.5 µg/kg calf BW) in weaned heifers. There was a treatment × time interaction for TNF-α, IL-6 and IFN-γ ($P \leq 0.04$). *Treatments within timepoints differ $P \leq 0.05$. SEM ± 453.98, 386.91, and 5.52 pg/mL for TNF-α, IL-6 and IFN-γ, respectively.

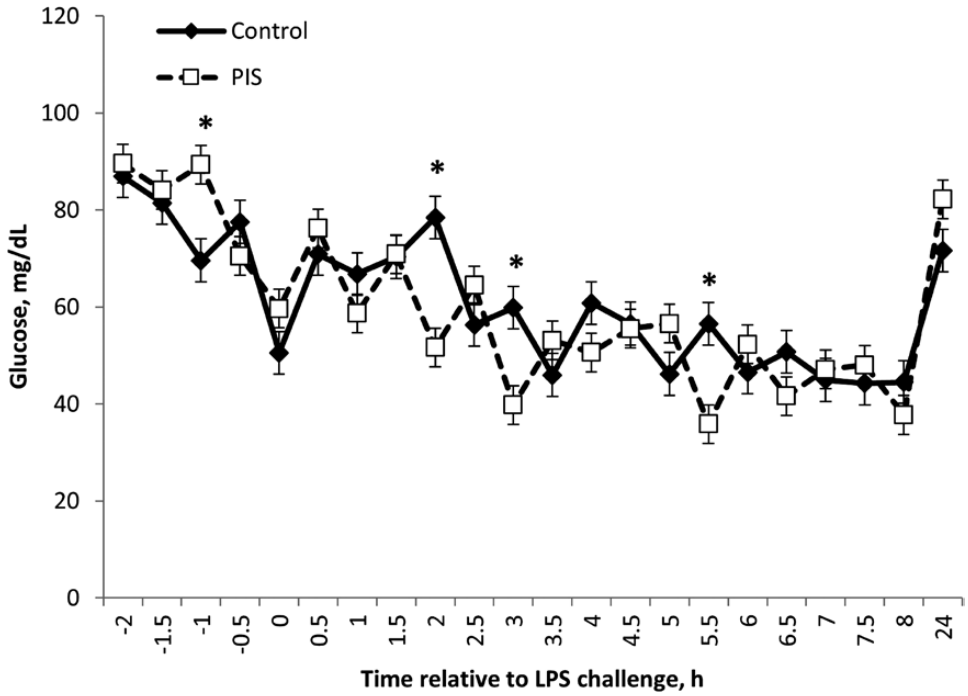

Figure 5. Effect of administered lipopolysaccharide (LPS) three times during gestation (0.1 µg/kg cow BW at 71, 170, and 234 ± 2 d of gestation) on the postnatal serum glucose response to LPS challenge (0.5 µg/kg calf BW) in weaned heifers. There was a treatment × time interaction ($P < 0.001$). *Treatments within timepoints differ $P \leq 0.05$. SEM ± 4.41 mg/dL.

and time ($P < 0.01$), but there was no treatment x time interaction ($P = 0.42$; Figure 6). Serum concentrations of NEFA were greater in PIS heifers than CON heifers. Serum urea nitrogen concentration was affected by a treatment × time interaction ($P < 0.07$) such that SUN was greater in PIS heifers compared to CON heifers at −2 and −1.5 h prior to the challenge, and 2, 3, 6.5, and 24 h post-challenge (Figure 7).

DISCUSSION

As expected, heifers from both groups displayed a rapid and transient febrile response which is characteristic of an endotoxin challenge (Carroll et al., 2009). The PIS heifers displayed a lesser baseline VT during the pre-challenge time period compared to the CON heifers. However, the febrile response profile was similar between both groups and were not different after endotoxin administration. The ability to modulate the febrile response is a significant biological event given that fever is a highly conserved fundamental response in vertebrates (Evans et al., 2015). In a prior study (Carroll et al., 2017), PIS and Control heifers displayed dissimilar febrile responses with the PIS heifers having a decreased, yet prolonged febrile response likely explained by lesser concentrations of TNF- α and a prolonged elevation of IL-6 observed in the prior study. In the current study where the febrile response was not altered by in utero exposure to endotoxin, greater peak concentrations of TNF- α and lesser peak concentrations of IL-6 were observed. While both TNF- α and IL-6 are often considered to be endogenous pyrogenic messengers, the potential fever response generated by greater concentrations of TNF- α in the PIS heifers may have been subdued by the lesser concentrations of IL-6. Prior studies utilizing IL-6 deficient mice have demonstrated that TNF- α induced febrile responses are in fact IL-6 dependent (Sundgren-Andersson et al., 1998). However, it should not be overlooked that potential differences in the febrile response may have been masked by an elevated ambient temperature that coincided with the peak febrile response in the current study. A peak ambient temperature of 33.6 °C was recorded 0.5 h prior to peak VT responses observed in both groups of heifers.

Sickness behavior is another highly conserved biological response that is critical to survival in animals and can be defined as a combination of various physiological and behavioral responses controlled by pro-inflammatory cytokines during an infection (Dantzer et al., 1996). From

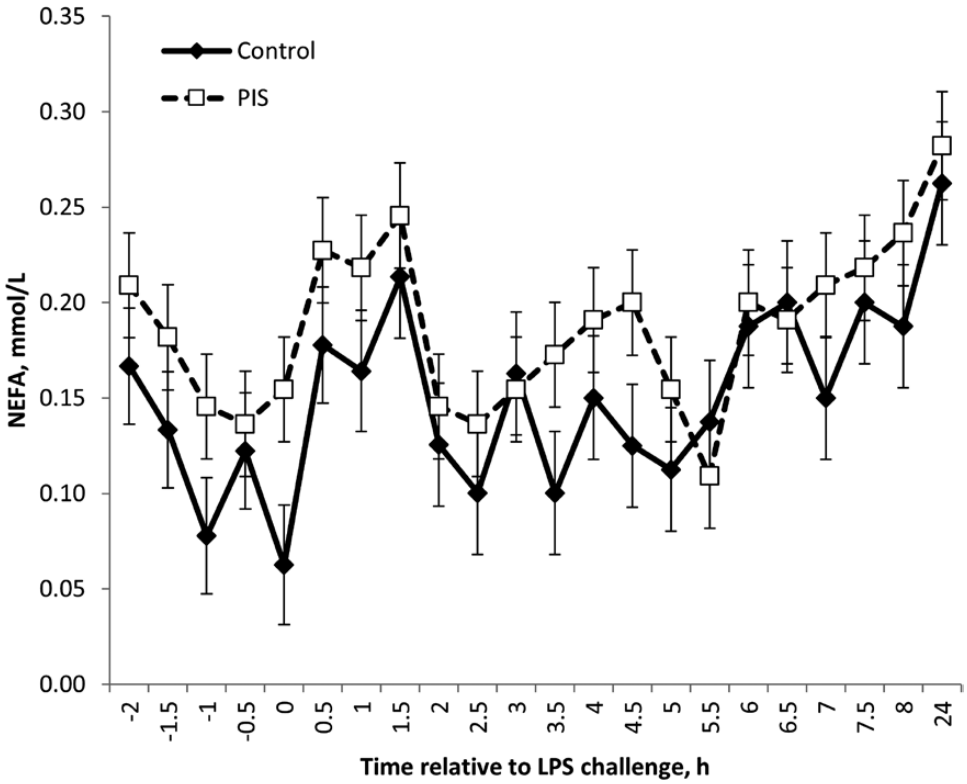

Figure 6. Effect of administered lipopolysaccharide (LPS) three times during gestation (0.1 µg/kg cow BW at 71, 170, and 234 ± 2 d of gestation) on the postnatal serum NEFA response to LPS challenge (0.5 µg/kg calf BW) in weaned heifers. There was a treatment ($P = 0.04$) and time ($P < 0.001$) effect for serum NEFA concentrations. SEM ± 0.03 mmol/L.

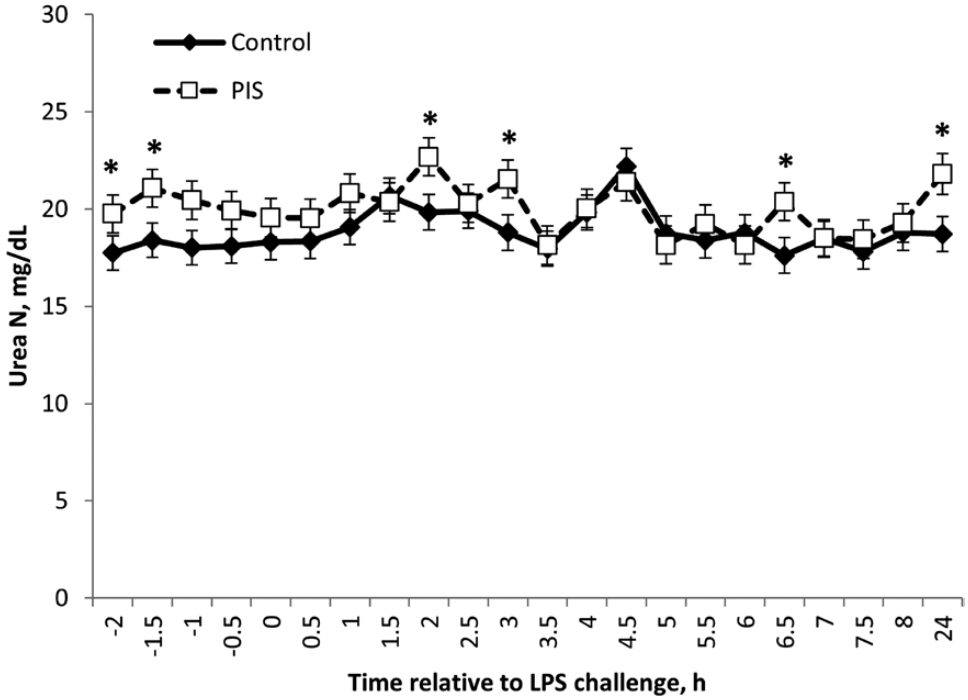

Figure 7. Effect of administered lipopolysaccharide (LPS) three times during gestation (0.1 µg/kg cow BW at 71, 170, and 234 ± 2 d of gestation) on the postnatal serum urea N response to LPS challenge (0.5 µg/kg calf BW) in weaned heifers. There was a treatment × time interaction ($P = 0.007$). *Treatments within timepoints differ $P \leq 0.05$. SEM ± 0.98 mg/dL.

an evolutionary perspective, sickness behaviors evolved as a way for animals to preserve energy resources during times of infection. The febrile response is very energetically demanding, and thus conserving energy resources by altering behavior during a fever allows energy from non-essential processes to be redirected to support the febrile response and other immunological activities (Evans et al., 2015; Harden et al., 2015). In the current study, SBS were greater in the CON heifers as compared to the PIS heifers from 0.5 to 2 h post-LPS challenge. Greater SBS in the CON heifers would be consistent with the greater concentrations of IL-6 observed in these heifers as IL-6 has been reported to be an important mediator of LPS-induced sickness behavior (Bluthé et al., 2000). Acute SBS is often an indicator of the severity to which cattle respond to an endotoxin challenge. These data are somewhat consistent with our prior study in which control heifers had a greater SBS at 30 min post-LPS compared to their PIS (Carroll et al., 2017). However, in both the current study and our prior study, SBS were relatively low compared to SBS observed in other LPS challenge studies utilizing steer calves (Burdick et al., 2011; Littlejohn et al., 2019). Lesser SBS recorded in these two studies compared to prior studies could be due to the sexually dimorphic responses we have previously reported in cattle following an endotoxin challenge (Carroll et al., 2015) and/or the fact that the calves were halter broke prior to the endotoxin challenge which may have altered their overall sickness behavior.

Cortisol concentrations in the current study were greater at multiple time points in the PIS heifers compared to the CON heifers. While cortisol is often thought of as an indicator of the severity of stress experienced by cattle, there are two other important roles for cortisol during an immune response. First, cortisol is the primary glucocorticoid in most mammals responsible for increases in blood glucose concentrations via stimulating the liver to convert fat and protein to intermediate metabolites that are ultimately converted to glucose for energy (Kuo et al., 2015). The energetic demand of the immune system is substantial and increasing energy availability aids in the host's ability to mount an adequate immune response (Bird, 2019). Secondly, cortisol suppresses the inflammatory response and immune system to prevent excessive and chronic stimulation which could be fatal to the host (Wolkow et al., 2015). The elevated cortisol concentrations at multiple time points observed in the current study are consistent with elevated cortisol reported for PIS heifers in our prior study (Burdick Sanchez et al., 2017).

While there were no main treatment effects for the proinflammatory cytokine (i.e., TNF- α, IL-6, and IFN-γ) responses in the current study, there were treatment × time interactions that resulted

in remarkably different peak concentrations between the PIS and CON heifers. Greater peak concentrations of TNF- α and IFN- γ were observed in the PIS heifers compared to the CON heifers. However, peak concentrations of IL-6 were lesser in the PIS heifers compared to the CON heifers. Interestingly, while TNF- α concentrations reached a greater peak in the PIS heifers compared to the CON heifers, concentrations returned to baseline values more rapidly in the PIS heifers. In the CON heifers, TNF- α concentrations remained elevated for a longer duration, and displayed a biphasic response, thus suggesting continued stimulation and an inability to promptly resolve the immunological insult. The lesser IL-6 peak response in the PIS heifers may be an indicator that these heifers were better able to tolerate or clear the endotoxin. While IL-6 plays a key role in inflammation and the stimulation of acute phase proteins, it is also an important linkage between the innate and adaptive immune system via promoting differentiation of naïve CD4+ T cells (Tanaka et al., 2014). Additionally, IL-6 has been reported to have a primary anti-inflammatory role and is associated with the inhibition of LPS-induced TNF- α production (Aderka et al., 1989). Thus, a reduced IL-6 response would seemingly correspond to a lesser need to recruit the adaptive immune system to help eliminate the endotoxin.

As with TNF- α, IFN- γ peak concentrations were greater in the PIS heifers compared to the CON heifers. Given that IFN- γ is a primary activator of macrophages and stimulates natural killer cells and neutrophils, it is an important early defense mechanism for the host against infections (Schroder et al., 2004). Thus, elevated peak IFN- γ concentrations in the PIS heifers may be indicative of a primed immune response or "memory" innate immune response to subsequent endotoxin (Morris et al., 2015). The proinflammatory cytokine data observed in the current study is inconsistent with observations from our prior work in which PIS heifers had greater IL-6 concentrations, and TNF- α and IFN- γ concentrations did not differ between PIS and Control heifers (Carroll et al., 2017). However, the proinflammatory cytokine response in the current study in conjunction with the lesser SBS observed in the PIS heifers from 0.5 to 2 h after the LPS challenge may indicate that the endotoxin challenge was more severe in the CON heifers.

As in our prior study (Burdick Sanchez et al., 2017) where differences in serum concentrations of glucose were observed between the PIS and CON heifers, the magnitude of the difference was relatively minor and may not translate to significant biological effects during the endotoxin challenge. Similarly, NEFA concentrations also differed between the PIS and CON heifers, but again these relatively minor differences are probably not biologically significant during the course of an immune challenge. An interesting observation, however, is that in our prior study, PIS heifers had less baseline serum NEFA concentrations compared to the control heifers whereas in the current study the PIS heifers had greater baseline serum NEFA concentrations compared to the CON heifers. Interestingly, the relationship between IL-6 and NEFA concentrations were opposite in our first study where IL-6 concentrations were greater and NEFA concentrations were lesser in PIS compared to Control versus in the current study where IL-6 concentrations were lesser and NEFA concentrations were greater. However, the relationships between IL-6 and NEFA concentrations observed in both of our studies are consistent with prior research that has reported that IL-6 is associated with increased free fatty acids oxidation (Petersen et al., 2005).

The only metabolic marker response that was consistent between our prior study and the current study was SUN concentrations which were elevated in the PIS heifers in both studies. Increased circulating concentrations of SUN may be reflective of increased protein intake and/or increased protein catabolism from tissue breakdown (Hammond, 1983). While feed intake was not recorded in either study, it is difficult to determine the source of the increased SUN in the PIS heifers in either study.

It is interesting to note that both consistencies and inconsistencies were observed between our prior study and the current study. While some consistent responses due to prenatal immuno-stimulation might be expected due to the highly conserved nature of the overall innate immune response, it's not surprising that inconsistent physiological and immunological responses were also observed due to differences in endotoxin exposure. In our prior study, the cows were only exposed to endotoxin at a single time point during gestation (i.e., 3rd trimester), whereas in the current study the cows were exposed to endotoxin in each trimester. Whether the differences observed between the offspring from our two studies reflects a relationship between endotoxin exposure and gestational development and/or the development of endotoxin tolerance in the fetus remains unknown.

Prior work in preterm fetal sheep has demonstrated that acute, high-dose endotoxin exposure induces a differential physiological response

compared to either chronic or repeated endotoxin exposure (Mathai et al., 2013). These authors also suggested that low-dose systemic endotoxin exposure may induce a level of self-tolerance which can alter the systemic inflammatory response. Likewise, several rodent studies have demonstrated that immune stimulation at different gestational stages can differentially alter postnatal central nervous system, inflammatory and behavioral responses (Zuckerman and Weiner, 2005; Meyer et al., 2006; Fortier et al., 2007). Thus, while both of our studies used the same dose of LPS (i.e., 0.1 µg/kg BW) the developmental stage of the fetus varied as well as the number of times the cows were exposed to the LPS. Therefore, the similarities in some biological markers, as well as the dissimilarities in other biological markers following the postnatal endotoxin challenge in the offspring from these two studies is not unwarranted.

CONCLUSIONS

The results from the current study, as well as those from our prior research, highlight physiological and immunological responses in beef cattle that can be altered by in utero exposure to various endotoxin regimes. In both studies, significant differences in various aspects of the pro-inflammatory and innate immune responses were observed, thus demonstrating an ability to re-program the innate immune system. Given that the innate immune response is the most evolutionarily conserved arm of the immune system, the ability to re-program it during gestational development is a significant step forward in efforts aimed at generating beef cattle with a more robust immune system.

Additionally, these results in conjunction with our prior research demonstrate the need to further explicate the subtleties associated with in utero development of innate immunity in cattle. The data generated from these studies irrefutably demonstrate the potential for re-programming the innate immune system while simultaneously highlighting the need for additional exploration into the timing associated with pre-natal immune stimulation as it relates to immunological development in the fetus.

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