Exposure to lipopolysaccharide in utero alters the postnatal metabolic response in heifers^{1,2,3}

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ABSTRACT: This study was designed to determine the effect of prenatal lipopolysaccharide (LPS) exposure on the postnatal metabolic response to an LPS challenge in beef heifers. Pregnant, crossbred cows ($n = 50$) were assigned to a prenatal immune stimulation (PIS; $n = 25$; administered $0.1 \mu\text{g/kg BW}$ LPS subcutaneously 233 ± 15 d of gestation) or saline treatment group (Control; $n = 25$). Birth and weaning BW of calves were collected. There was not ($P > 0.05$) a treatment $\times$ gender interaction for birth weight or 205-d adjusted weaning BW. Treatment did not affect ($P > 0.05$) birth BW, but steers and heifers of PIS cows had greater ($P < 0.02$) 205-d adjusted weaning BW than offspring from Control cows. From the 2 prenatal treatment groups, heifer calves ($n = 12$ PIS, 11 Control) were identified at weaning (238 ± 15 d of age) to subsequently receive an LPS challenge. On d 0, heifers were fitted with indwelling jugular catheters and were moved into individual pens. On d 1, heifers (fed at 0600 h) were challenged i.v. with LPS ($0.5 \mu\text{g/kg BW}$) at 0 h (1000 h). Blood samples were collected at 30-min intervals from -2 to 8 h and again at 24 h relative to the LPS challenge. There was a treatment $\times$

time interaction ($P < 0.01$) for cortisol; PIS heifers had greater cortisol from 4 to 6.5 h post-LPS challenge ($P < 0.001$). There was a treatment $\times$ time interaction ($P = 0.04$) for serum glucose such that glucose was greater ($P = 0.01$) in PIS than Control heifers at 0.5 h, but was greater in Control than PIS heifers at 2, 4.5, and 7 h post-LPS challenge. This resulted in overall time ($P < 0.01$) and treatment ($P < 0.01$) effects such that Control heifers had greater glucose concentrations than PIS heifers. There was a tendency ($P = 0.10$) for a treatment $\times$ time interaction for serum NEFA, such that NEFA was greater in Control than PIS heifers at -2, -1.5, and 7 h relative to the LPS challenge ($P \leq 0.02$). Also, there were time ($P < 0.01$) and treatment effects ($P < 0.01$) for NEFA with Control heifers having greater NEFA than PIS heifers. Serum BUN was affected by a treatment $\times$ time interaction ($P < 0.01$). Concentrations of BUN were greater in PIS heifers from -1.5 to -1 h, 1 to 2 h, at 4 h, and from 5 to 24 h relative to the LPS challenge. These results demonstrate postnatal growth and the metabolic responses of weaned beef calves can be significantly altered with a single exposure to LPS in utero.

Key words: cattle, cortisol, lipopolysaccharide, metabolism, prenatal stress

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INTRODUCTION

Prenatal stress can influence various aspects of the postnatal stress and immune responses. These effects are dependent on the timing of the stressor during gestation, as well as the sex of the offspring (Howerton and Bale, 2012). Increased hypothalamic-pituitary-adrenal (HPA) axis sensitivity as well as behavioral changes have been observed in response to prenatal stress (Lay et al., 1997b; Jarvis et al., 2006; Kohman et al., 2008). Further, immune function such as changes in leukocyte and antibody responses have been altered in response to prenatal stress (Couret et al., 2009a). However, there is limited literature utilizing cattle as a model, and none that directly evaluates the potential metabolic consequences of prenatal immune stimulation. Lay et al. (1996) and Burdick Sanchez et al. (2013) reported changes in the stress and immune responses of calves born to dams transported multiple times during gestation or when exposed to adrenocorticotrophic hormone (ACTH). As cattle are exposed to stressors and immune challenges throughout the production cycle, not excluding gestation, it is necessary to better understand the effects of prenatal stress and prenatal immune stimulation on the offspring.

Metabolism plays a major role in the ability of the immune system to successfully clear pathogens. While often overlooked, a significant amount of energy is necessary for immune activation and function. Kluger and Rothenburg (1979) reported the necessity for a 10 to 13% increase in ME to increase body temperature 1°C. However, this does not represent the additional energy required for other actions of the immune system, such as production of acute phase proteins and immunoglobulins. Thus, to understand the response of the body to an infection, including metabolic and energy demands that drive available energy, we must take a multidisciplinary approach. Therefore, this study was designed to determine if prenatal exposure to lipopolysaccharide (LPS) alters the postnatal metabolic response to an exogenous LPS challenge in beef heifers.

MATERIALS AND METHODS

Experimental Design

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 at the University of Florida (IACUC study 201307950).

Pregnant crossbred cows ($n = 50$) were selected from a single herd and managed similarly prior to allocation to treatments in the current study. Cows were

no more than 25% *Bos indicus* and ranged from 4 to 11 y of age (average parity of 5.8 ± 1.8 calvings). All cows were bred by natural service, with 8 different sires distributed randomly across the herd. Cows were randomly allotted to 1 of 2 treatments: 1) prenatal immune stimulation [PIS, $n = 25$; administered $0.1 \mu\text{g/kg BW}$ of LPS (*E. coli* O111:B4, Sigma Aldrich, St. Louis, MO) subcutaneously at 233 ± 19 d of gestation], and 2) saline group (Control, $n = 25$). Cows were administered LPS or saline on September 25, 2013. Vaginal temperature recording devices (Burdick et al., 2012) were inserted 2 d prior and removed 5 d post-LPS administration; 4 additional temperature recording devices were used to record ambient temperature. Cows grazed a common bahiagrass pasture from the time of LPS administration to weaning. Calves were born from October to December 2013. At birth all calves were tagged, weighed, and birth date was recorded, and male calves were surgically castrated. Day of gestation for LPS administration to dams was computed from calf birth date assuming a constant gestation length of 283 d. On April 8, 2014, calves were dewormed (fenbendazole 10% oral drench; 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), and *Clostridium chauvoei*, *clostridium speticum*, *clostridium haemolyticum*, *clostridium novyi*, *clostridium sordelli*, and *clostridium perfringens* types B, C and D (Ultrabac 8; Zoetis). On June 4, 2014, calves were administered a booster vaccination of Bovi-Shield Gold 5 and Ultrabac 8. Heifer calves were weaned and weaning BW recorded on July 7, 2014, to allow sufficient time to halter break heifers prior to LPS challenge on August 13, 2014. Steer calves were weaned and weaning BW recorded on July 16, 2014.

From these treatments, heifer calves ($n = 12$ PIS and 11 Control) were identified at weaning (238 ± 15 d of age) to subsequently receive an LPS challenge. Heifer calves from the Control group averaged 217 ± 5 kg BW and PIS heifers averaged 211 ± 5 kg BW. On d 0, heifers were fitted with indwelling jugular vein catheters and were moved into individual pens ($2.5 \text{ m} \times 6 \text{ m}$) in a covered barn. Heifers were allowed ad libitum access to feed and water throughout the study. On d 1, heifers were challenged intravenously with LPS ($0.5 \mu\text{g/kg BW}$) at 0 h (1000 h). Whole blood samples were collected at 30-min intervals from -2 to 8 h and again at 24 h relative to the LPS challenge. Whole blood was collected into tubes containing no additive (Sarstedt, Inc., Newton, NC), and allowed to clot at room temperature for 30 min prior to centrifugation at $1,500 \times g$

for 20 min at 4°C. Isolated serum was stored at -80°C until analyzed for cortisol, glucose, NEFA, and blood urea nitrogen (BUN) concentrations.

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) by comparison of unknowns to standard curves generated with known concentrations of cortisol. The minimum detectable cortisol concentration was 45.4 pg/mL, and the intra- and interassay coefficients of variation were 7.7% and 18.2%, respectively.

Glucose concentrations were determined by modification of the enzymatic Autokit Glucose (Wako Diagnostics, Richmond, VA) to fit a 96-well format as previously described (Burdick Sanchez et al., 2014a). Briefly, 300 µL of prepared working solution was added to 2 µL of serum or prepared standards in a 96-well plate. Plates were incubated at 37°C for 5 min and absorption was recorded at 505 nm. The plate reader used for this assay (BioTek Powerwave 340; BioTek Instruments, Winooski, VT) has an incubating and timing feature and therefore ensured the sample absorbances were read immediately following the 5-min incubation. Concentrations of glucose were determined by comparing unknown samples to a standard curve of known glucose concentrations. The minimum detectable concentration was 3.8 mg/dL and the intra- and interassay coefficients of variation were 12.4% and 16.6%, respectively.

Concentrations of NEFA 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., 2014a). Briefly, 200 µL of the prepared Color Reagent A were added to 5 µL of serum or prepared standards in a 96-well plate. Plates were incubated at 37°C for 5 min and the absorbance was read using a spectrophotometer at 550 nm. Next, 100 µL of prepared Color Reagent B was added to all wells on the 96-well plate. Plates were incubated for an additional 5 min and read for a second time using a plate reader at 550 nm. The plate reader used for this assay has an incubating timing feature, ensuring the sample absorbances were read immediately following the 5-min incubation. A final absorbance was obtained by subtracting the first reading, which was multiplied by a factor of 0.67 to account for changes in volume, from the second reading. The final absorbance values were used for all calculations (i.e., standard curve, sample concentrations). Concentrations of NEFA were determined by comparing unknown samples to a stan-

Figure 1. Effect of lipopolysaccharide (LPS) administration (0.1 mg/kg BW at 233 ± 19 d of gestation) on vaginal temperature of cows. Control = 25, Prenatal immune stimulation (PIS) = 25. Data are presented as the LSM. SEM is ± 0.06°C. *Treatments differ by $P \leq 0.01$.

dard curve of known NEFA concentrations. The minimum detectable concentration was 0.0014 mmol/L and the intra- and interassay coefficients of variation were 6.4% and 8.4%, respectively.

Serum concentrations of BUN were determined by a colorimetric assay according to the manufacturer directions (K024-H1, Arbor Assays, Ann Arbor, MI) by comparison of unknown samples to standard curves generated with known concentrations of urea nitrogen. The minimum detectable BUN concentration was 0.065 mg/dL and the intra- and intercoefficients of variation were 3.6% and 7.9%, respectively.

Statistical Analysis

Body weight data of calves were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with fixed effects of treatment, gender, and their interaction. Vaginal temperature of cows and metabolic parameter data of heifers were analyzed by the MIXED procedure of SAS specific for repeated measures (SAS Inst. Inc.). Treatment, time, and their interaction were included as fixed effects with cows or heifers within treatment as the subject. Specific treatment comparisons were made using the PDIF option in SAS, with $P \leq 0.05$ considered significant and $0.05 < P < 0.10$ considered a tendency. All data are presented as the LSM ± SEM.

RESULTS

Performance

Subcutaneous administration of LPS to cows in late gestation resulted in an average increase ($P < 0.01$) in vaginal temperature of 0.5°C in PIS versus Control cows for 7 h post-injection indicating the LPS injection elicited a mild febrile response (Fig. 1). Day

Table 1. Pre-weaning growth performance of calves from cows administered saline or lipopolysaccharide in late gestation

Item	Steers		Heifers		SEM	P-value ¹		
	Control ²	PIS	Control	PIS		Trt	Gender	T×G
LPS ³ administration, d of gestation	232.1	233.5	239.8	228.8	6.1	0.40	0.78	0.27
Birth weight, kg	33.3	37.3	31.7	31.9	1.7	0.18	0.03	0.24
Pre-weaning ADG, kg/d	0.84	0.92	0.81	0.84	0.02	0.02	0.04	0.28
205-d adjusted weaning weight, kg	205.0	226.0	198.4	204.6	6.4	0.02	0.02	0.20

¹Trt = effect of treatment; T×G = effect of treatment × gender interaction.

²Control = cows administered saline in late gestation ($n = 11$); Prenatal immune stimulation (PIS) = cows administered LPS (0.1 mg/kg BW *E. coli* 0111:B4; $n = 12$) at 233 ± 19 d of gestation.

³LPS = lipopolysaccharide.

of gestation in which LPS was administered to cows did not influence performance (Table 1). There was no treatment × gender interaction ($P \geq 0.20$) for any of the pre-weaning growth measures. Male calves had greater ($P < 0.03$) BW at birth than female calves (35.3 vs. 31.8 ± 1.1 kg, respectively), but birth BW between Control and PIS calves was similar ($P > 0.15$). Both treatment and gender affected pre-weaning ADG and 205-d adjusted weaning BW of calves. Steer calves had greater ($P < 0.04$) pre-weaning ADG (0.88 vs. 0.83 ± 0.02 kg/d, respectively) and 205-d adjusted weaning BW (215.5 vs. 201.5 ± 4.2 kg, respectively) than heifer calves. The PIS calves had greater ($P < 0.03$) pre-weaning ADG (0.88 vs. 0.83 ± 0.02 kg/d, respectively) and 205-d adjusted weaning BW (215.3 vs. 201.7 ± 4.0 kg, respectively) than Control calves.

LPS Challenge

Serum concentrations of cortisol were affected by a treatment × time interaction ($P < 0.01$; Fig. 2). Specifically, cortisol concentrations were greater in PIS heifers compared with Control heifers from 4 to 6.5 h following administration of LPS ($P \leq 0.01$). There were overall treatment ($P < 0.01$) and time ($P < 0.01$) effects, such that cortisol was greater in PIS heifers (20.5 ± 0.4 ng/mL) than in Control heifers (18.2 ± 0.4 ng/mL). For the time effect, basal cortisol concentrations, measured from -2 to 0 h, were less in PIS (5.9 ± 0.4 ng/mL) compared with Control heifers (7.9 ± 0.4 ng/mL).

There was also a treatment × time interaction ($P = 0.04$) for serum glucose concentrations (Fig. 3). Glucose concentrations were greater in PIS heifers at 0.5 h ($P < 0.01$), yet lesser at 2, 4.5 and 7 h ($P \leq 0.02$) compared with Control heifers. There were overall treatment ($P < 0.01$) and time ($P < 0.01$) effects where PIS heifers had less glucose (63.4 ± 1.0 mg/dL) than Control heifers (68.6 ± 1.1 mg/dL), while concentrations initially increased post-LPS before decreasing below baseline concentrations.

Serum NEFA concentrations were affected by treatment ($P < 0.01$) and time ($P < 0.01$; Fig. 4). Specifically, PIS heifers had decreased NEFA concentrations (0.278 ± 0.008 mmol/L) compared with Control heifers (0.316 ± 0.009 mmol/L). Concentrations of NEFA increased at 0.5 h ($P = 0.02$; 0 h vs. 0.5 h) and were elevated from 3.5 h through the end of the study ($P \leq 0.01$). Additionally, there was a tendency ($P = 0.10$) for a treatment × time interaction, such that NEFA concentrations were decreased in PIS heifers compared with Control heifers at -2, -1.5, and 7 h relative to the LPS challenge ($P \leq 0.03$). As a result, baseline NEFA concentrations, measured from -2 to 0 h, were decreased ($P < 0.001$) in PIS heifers (0.199 ± 0.013 mmol/L) compared with Control heifers (0.297 ± 0.014 mmol/L).

There was a treatment × time interaction ($P < 0.01$) for serum BUN concentrations, such that BUN was increased in PIS heifers from -1.5 to -1 h, from 1 to 2 h, at 4h, and from 5 to 24 h ($P \leq 0.03$; Fig. 5) relative to the LPS challenge at 0 h. There was also overall treatment ($P < 0.01$) and time ($P < 0.01$) effects where PIS heifers (12.12 ± 0.13 mg/dL) had greater BUN concentrations than Control heifers (9.45 ± 0.16 mg/dL). Serum BUN concentrations increased at 5.5 h ($P = 0.02$; 0 h vs. 5.5 h), and remained elevated through the remainder of the challenge ($P < 0.01$; 0 h vs. 24 h).

DISCUSSION

Evidence from human and rodent models demonstrates alterations in the fetal environment can have long-lasting effects in the offspring, including behavior, cardiovascular, and metabolic changes (Viltart and Vanbesien-Mailliot, 2007). Cattle are exposed to stressors and immune challenges several times during their lifetime, including weaning, transportation events, vaccination, and gestation is no exception. While previous studies in cattle have focused on stress induced by transportation or exogenous activation of the HPA axis (Lay et al., 1996; Price et al., 2015), the effects an acute infection during gestation has on the offspring has not

Figure 2. Effect of prenatal lipopolysaccharide (LPS) exposure (0.1 µg/kg BW at 233 ± 19 d of gestation) on the cortisol response to a postnatal LPS challenge (0.5 µg/kg BW). Control $n = 11$, Prenatal immune stimulation (PIS), $n = 12$. Data are presented as the LSM. *Treatments differ by $P \leq 0.01$.

Figure 3. Effect of prenatal lipopolysaccharide (LPS) exposure (0.1 µg/kg BW at 233 ± 19 d of gestation) on the glucose response to a postnatal LPS challenge (0.5 µg/kg BW). Control $n = 11$, Prenatal immune stimulation (PIS), $n = 12$. Data are presented as the LSM ± SEM. *Treatments differ by $P \leq 0.02$.

been studied. The data from the current study demonstrate clear metabolic and growth differences in heifer calves exposed to LPS once during late gestation.

Body weights of male rats born from LPS-treated dams were greater than control mice from 4 to 12 wk of age in a study reported by Nilsson et al. (2001). Additionally, Lay et al. (1997b) reported greater birth BW in calves exposed to ACTH during gestation than Control calves. While prenatal stress typically is reported to decrease offspring BW, as a result of intrauterine growth restriction, this was not observed in the current study. However, Reynolds et al. (2010) stated it is possible that long-term effects remain regardless of whether differences in birth BW are observed in response to prenatal stress. In support of this and the data from our study, Pacheco-Lopez et al. (2013) reported adult male mice exposed to maternal immune activation in utero had increased fat deposition. However, there was no difference in BW of lambs exposed to LPS 1 or 3 times during late gestation (Fisher et al., 2010). Lay et al. (1997a) report of larger pituitary glands found in fetal calves born via cesarean section at 266 d of gestation may explain greater birth weights in calves born to maternally stressed cows, potentially due to an increase in growth hormone secretion. While birth BW was not different in the current study, this may partially explain the greater ADG and 205-d adjusting weaning BW in prenatally stressed calves. However, this requires further investigation.

In the current study, baseline cortisol concentrations were decreased in PIS heifers compared with Control heifers. This is in contrast to a study in pigs, which found no difference in cortisol values in offspring from sows exposed to social stress during early or late gestation (Couret et al., 2009b). Cortisol is a product of the activation of the HPA axis. Upon stimulation of stress centers within the brain, corticotropin-releasing hor-

mone (CRH) is released from the hypothalamus, stimulating the release of ACTH from the anterior pituitary. Once in circulation, ACTH stimulates the production of glucocorticoids (i.e., primarily cortisol in mammals). Production of cortisol negatively regulates the HPA axis. In response to LPS, cortisol release is typically one of the first responses. It can therefore be hypothesized that increases in cortisol, as a result of LPS administered to pregnant dams, impacted aspects of the HPA axis. Indeed, altered HPA axis responsiveness has been reported in several studies in both rodent and cattle models (Lay et al., 1996; Lay et al., 1997b; Hashimoto et al., 2001) and cortisol has been reported to increase in response to LPS in pregnant ewes (Kabaroff et al., 2006). This is confirmed by the current study as cortisol concentrations were elevated for an extended period of time in PIS heifers compared with Control heifers. As stated above, Lay et al. (1997a) found greater pituitary gland weights in fetal calves born by cesarean section at 266 d of gestation and exposed to transportation multiple times during gestation, which is supportive of the altered HPA axis responsiveness observed in the current study. Studies in rodents have also demonstrated increased corticosterone in response to a postnatal LPS challenge in rats exposed to restraint stress prenatally (Hashimoto et al., 2001). However, a study in pigs exposed to restraint stress in utero found a decrease in cortisol concentrations following a postnatal LPS challenge in barrows compared with non-stressed controls, in contrast to the current study (Collier et al., 2011). Additionally, there was no difference in cortisol responses in female lambs exposed to LPS in utero, but the cortisol response was prolonged in male lambs (Fisher et al., 2010). Thus, there appears to be some species, sex and/or stressor-specific effects on the response to stress in utero on cortisol responsiveness to a postnatal LPS challenge.

Figure 4. Effect of prenatal lipopolysaccharide (LPS) exposure (0.1 $\mu\text{g/kg}$ BW at 233 ± 19 d of gestation) on the NEFA response to a postnatal LPS challenge (0.5 $\mu\text{g/kg}$ BW). Control $n = 11$, Prenatal immune stimulation (PIS), $n = 12$. Data are presented as the LSM $\pm$ SEM. *Treatments differ by $P \leq 0.03$.

Figure 5. Effect of prenatal lipopolysaccharide (LPS) exposure (0.1 $\mu\text{g/kg}$ BW at 233 ± 19 d of gestation) on the blood urea nitrogen (BUN) response to a postnatal LPS challenge (0.5 $\mu\text{g/kg}$ BW). Control $n = 11$, Prenatal immune stimulation (PIS), $n = 12$. Data are presented as the LSM $\pm$ SEM. *Treatments differ by $P \leq 0.03$.

A study in rats found a decrease in corticosterone receptors 21 and 90 d after birth in the hippocampus of offspring exposed to prenatal stress (Henry et al., 1994). A decrease in receptors may allow for a more robust cortisol response due to a decrease in the negative feedback regulation of glucocorticoids. Thus, prenatal stress appears to have long-term effects on aspects of the HPA axis, and likely influences the responsiveness to various stressors. Additionally, a study in which prenatally stressed pigs were exposed to a postnatal restraint stress found an increase in CRH mRNA expression in the amygdala and the paraventricular nucleus in the brain, suggesting overexpression of CRH (Jarvis et al., 2006). Another study in rats found that adrenalectomy of dams prior to exposure to a stress prevented the elevation in corticosteroids observed in non-adrenalectomized mice, suggesting the rise in maternal cortisol following exposure to stress during gestation affects the postnatal HPA axis sensitivity of the offspring (Barbazanges et al., 1996). While cortisol was not measured in the cows in the current study, an increase in cortisol concentrations has been previously documented in response to LPS administration in cattle (Carroll et al., 2009; Burdick et al., 2011). Furthermore, studies in rodents have determined both restraint stress and LPS exposure during gestation can decrease the expression of 11 β -hydroxysteroid dehydrogenase 2 (11 β -HSD2) in the placenta, an enzyme that deactivates active cortisol (corticosterone in rodents) to cortisone (or 11-dehydrocorticosterone in rodents; Mairesse et al., 2007; Straley et al., 2014). Thus, it can be assumed an increase in circulating cortisol concentrations in the cows, induced by administration of LPS during gestation, likely had profound effects on the HPA axis, potentially including down-regulation of glucocorticoid receptors and 11 β -HSD2,

resulting in a greater cortisol response in postnatally-challenged heifer calves.

In lambs, it is estimated 90 Kcal $\cdot$ d $^{-1}$ $\cdot$ kg $^{-1}$ is the energy consumption of fetal tissues in late pregnancy (130 d of gestation), supplied mostly by glucose (Battaglia and Meschia, 1978). While administration of LPS typically results in hyperglycemia, followed by a period of hypoglycemia (Burdick Sanchez et al., 2014a,b), it poses the question of whether these changes in glucose concentrations within the dam during late pregnancy affects fetal metabolism. The decrease in glucose is compounded by decreases in feed intake typically observed following LPS challenge (Zebeli et al., 2011). A decrease in maternal circulating glucose concentrations likely results in decreased fetal uptake of glucose, possibly having long-lasting effects on postnatal responses (Battaglia and Meschia, 1978). Indeed, a study in rats reported a decrease in glucose in the amniotic fluid 2 h following LPS administration on gestational d 19 (Serriere et al., 2011). As stated by Battaglia and Meschia (1978), hypoglycemia in the dam results in hypoglycemia in the fetus, altering the glucose requirements of the fetus. Thus, the decreased glucose concentrations observed in PIS heifer calves exposed to LPS may be a result of an alteration in their glucose requirements caused by prenatal LPS exposure.

Glucocorticoids, such as cortisol, are extremely important in the modulation of glucose homeostasis. Long et al. (1940) described studies in which the adrenal gland was removed, decreasing circulating glucose concentrations. Increasing cortisol typically leads to an increase in glycogenolysis and increasing serum glucose concentrations, yet in the current study glucose concentrations in PIS heifers were less than Control heifers, which was opposite of what was observed for cortisol

concentrations. These data suggest prenatal exposure to LPS may have altered the relationship between glucose and cortisol, which requires further study.

Baseline glucose values did not differ between Control and PIS heifers in the current study. This contrasts studies in rodents which found greater basal glucose concentrations in rats exposed to prenatal stress during the last week of gestation (Vallee et al., 1996; Nyirenda et al., 2001). This difference between studies is likely due to species differences and/or the type and duration of prenatal stressor used. A study in male lambs exposed to dexamethasone during gestation found a decreased glucose response to a postnatal i.v. glucose tolerance test (De Blasio et al., 2007). To more clearly determine the influence of maternal LPS exposure on metabolic responses, more specific testing (i.e., glucose tolerance and insulin sensitivity tests) should be utilized in the future.

There is limited data regarding the effect of prenatal stress on NEFA and BUN responses. A study that utilized guinea pigs administered a psychosomatic stressor during late gestation found decreased NEFA concentrations at birth in the plasma of fetuses from stressed dams as compared to control dams (Dauprat et al., 1985). The authors suggested tissues may be less sensitive to factors that activate lipolysis, such as cortisol. This supports data in the current study where PIS heifers had decreased NEFA concentrations yet increased cortisol concentrations compared with the Control heifers. Additionally, prenatal exposure to dexamethasone decreased lipolysis in lambs through modulating insulin actions on glucose and fatty acid metabolism (De Blasio et al., 2007). This supports observations of decreased glucose and NEFA responses in the current study. Further, an increased responsiveness to insulin action may lead to increased adiposity, and thus an increase in BW. This is one of the main concerns of prenatal stress in humans with regard to obesity, diabetes, and cardiovascular disease. An increase in circulating concentrations of BUN can be reflective of increased protein catabolism. It is possible the decreased glucose and NEFA concentrations observed in PIS heifer calves caused increased protein catabolism to provide adequate energy during the postnatal immune response. However, as stated earlier, different approaches are necessary to more fully understand the implications of prenatal stress on the postnatal metabolic response.

Conclusion

Prenatal LPS exposure altered several aspects of the stress and metabolic responses to postnatal LPS challenge. These alterations included decreased baseline cortisol values and increased and extended corti-

sol response, decreased baseline NEFA concentrations, reduced glucose and NEFA responses, and increased baseline and post-LPS challenge BUN concentrations. These data suggest prenatal LPS exposure may have altered the stress responsiveness of heifer calves. Further, prenatal stress may have resulted in changes in energy storage depots or may have altered the manner energy is redistributed or partitioned in response to an immune challenge. It is important to note differences in cortisol and the metabolic response were due to a single, low-dose administration of LPS during late gestation. Additionally, PIS calves had greater ADG and 205-d adjusted weaning weight. Future studies must determine if these metabolic changes affect post-weaning cattle growth, health, and productivity.

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