Short communication: Supply of methionine during late pregnancy enhances whole-blood innate immune response of Holstein calves partly through changes in mRNA abundance in polymorphonuclear leukocytes

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ABSTRACT

The supply of methionine (Met) in late pregnancy can alter mRNA abundance of genes associated with metabolism and immune response in liver and polymorphonuclear leukocytes (PMN) of the neonatal calf. Whether prenatal supply of Met elicits postnatal effects on systemic inflammation and innate immune response of the calf is not well known. We investigated whether enhancing the maternal supply of Met via feeding ethyl-cellulose rumen-protected Met (RPM) was associated with differences in calf innate immune response, mRNA abundance in PMN, and systemic indicators of inflammation during the first 50 d of life. Calves ($n = 14$ per maternal diet) born to cows fed RPM at 0.09% of diet dry matter per day (MET) for the last 28 ± 2 d before calving or fed a control diet with no added Met (CON) were used. Blood for biomarker analysis and isolation of PMN for innate immune function assays and mRNA abundance was harvested at birth (before colostrum feeding) and at 7, 21, and 50 d of age. Whole blood was challenged with enteropathogenic bacteria (*Escherichia coli* 0118:H8), and phagocytosis and oxidative burst of neutrophils and monocytes were quantified via flow cytometry. Although concentration of haptoglobin and activity of myeloperoxidase among calves from both maternal groups increased markedly between 0 and 7 d of age, followed by a decrease to baseline at d 21, the responses were lower in MET compared with CON calves. Nitric

oxide concentration decreased markedly between 0 and 7 d, regardless of maternal group, but MET calves tended to have lower overall concentrations during the study. In vitro phagocytosis in stimulated neutrophils increased markedly over time in both CON and MET calves, but responses were overall greater in MET calves. Oxidative burst in both neutrophils and monocytes increased over time regardless of maternal treatment. The mRNA abundance of lactate dehydrogenase (*LDHA*), signal transducer and activator of transcription 3 (*STAT3*), and S100 calcium binding protein A8 (*S100A8*) in PMN was overall greater in MET calves. Overall, data suggest that increasing the maternal supply of Met during late pregnancy could affect the neonatal calf inflammatory status and innate immune response. Although changes in mRNA abundance could play a role in coordinating the immune response, the exact mechanisms merit further study

Key words: methyl donor, immune function, nutrition, pregnancy

Short Communication

The development of the immune system in calves, including PMN functions such as phagocytosis and respiratory burst, is a slow process that requires several months of life to reach a mature state (Kampen et al., 2006). As the primary mediators of the innate immune response, PMN represent the first line of defense against pathogens, and a carefully orchestrated and complex set of signal transduction events leads to their recruitment (Kobayashi and DeLeo, 2009; Futosi et al., 2013). Considering also that passive immunity acquired from colostrum remains active only up to approximately 21 d of age, the neonatal stage encompasses a wide win-

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dow of susceptibility to stress and immune challenges (Chase et al., 2008; Ranade et al., 2014).

Recent research has focused on how manipulation of maternal nutrition during late pregnancy alters or can improve calf health and reduce stress during neonatal and weaning periods (Osorio et al., 2013b; Jacometo et al., 2016). A nutritional strategy that our group has been researching is to enhance the maternal supply of methionine (Met) by feeding rumen-protected methionine (**RPM**) during the last 3 to 4 weeks prepartum. Methyl donors serve as precursors of S-adenosylmethionine and are important sources of the antioxidants glutathione (GSH) and taurine, both of which have been associated with decreased oxidative stress and inflammatory status (as judged by plasma biomarkers and liver tissue glutathione; Osorio et al., 2014; Zhou et al., 2017; Batistel et al., 2018).

Tremendous advances have been made over the years in our knowledge of the nutritional physiology of the calf (Blum, 2006). However, the extent to which prenatal maternal nutrition affects the molecular profiles of inflammatory pathways and PMN function in the neonatal animal are not well defined. In an *in vitro* evaluation of the effect of Met supplementation in neonatal Holstein calf PMN, Abdelmegeid et al. (2017) concluded that an "ideal" supply of Lys relative to Met (2.9:1) decreased pro-inflammatory signaling and affected the antioxidant systems. These data underscored an important role of Met supply in establishing an adequate immune response during the neonatal period, presumably leading to better immunological status *in vivo*. The potential role of maternal nutrition on immune adaptations of the neonatal calf likely encompasses key metabolic organs (Li et al., 2015; Du et al., 2018; Zhu et al., 2019). The primary aim of the present study was to generate additional *in vivo* data related to oxidative burst, phagocytosis capacity, plasma biomarkers, and mRNA abundance related to certain aspects of immune function. To address these objectives, a subset of calves from the entire cohort of animals in the study of Batistel et al. (2017) was used.

The Institutional Animal Care and Use Committee at the University of Illinois Urbana-Champaign (protocol 14270) approved all experimental procedures. Details of the maternal treatments were described previously (Batistel et al., 2017). Briefly, 60 multiparous Holstein cows were fed with a basal control diet (**CON**; 1.47 Mcal/kg of DM, 15.6% CP), with no addition of methionine, or the basal diet plus ethyl-cellulose rumen-protected methionine (**MET**; Mepron, Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany) during the last 28 d of pregnancy. The supplemental methionine was top-dressed on the total mixed diet at a rate of 0.09% of DM. This supply of Met was based on experiments

demonstrating a benefit of achieving a Lys:Met ratio close to 2.8:1 in terms of production performance and health (Osorio et al., 2013a; Zhou et al., 2016).

After parturition, calves were removed from their dams immediately, and cows were milked in the parlor, where the volume of colostrum was recorded and IgG content estimated at room temperature (22°C) based on specific gravity, with a bovine colostrometer (Nasco, Fort Atkinson, WI; catalog number C10978N). As in previous studies (McGuirk and Collins, 2004), only calves with maternal colostrum containing at least 50 mg/dL of IgG were used in the study. Calves were fed 3.8 L of fresh colostrum from their respective dams using nipple bottles within 6 h after birth. If the dam produced less than 3.8 L, the calves were removed from the study.

Details of animal housing and milk replacer were described previously (Xu et al., 2018). Briefly, calves were housed in individual outdoor hutches and fed twice daily with a milk replacer (Advance Excelerate, Milk Specialties, Carpentersville, IL; 28.5% CP, 15% fat) until 35 d of age. At this point they were switched to once-daily feeding until weaning (42 d of age). From d 1 until 56 d of life, calves had *ad libitum* access to a starter grain mix (Ampli-Calf STR 20 R50, Purina, St. Louis, MO; minimum 19.9% CP, maximum 13.5% NDF, maximum 9.0% ADF).

Blood samples were collected from the jugular vein ($n = 14$ calves per group) at birth and at 7, 21, 42, and 50 d of age and used for blood biomarkers (Table 1), PMN isolation, and measurement of phagocytosis capacity and oxidative burst activity. The vacutainer tubes used contained lithium heparin, and plasma was obtained by centrifugation at $1,900 \times g$ for 15 min at 4°C and stored at -80°C until further analysis. Aspartate aminotransferase (**AST**, catalog number 0018257540), bilirubin (catalog number 0018254640), gamma-glutamyl transferase (GGT, catalog number 0018254240), and haptoglobin in plasma were measured with kits purchased from Instrumentation Laboratory SpA (Werfen Co., Milan, Italy) using a clinical auto-analyzer (ILAB 650, Instrumentation Laboratory). The d-ROMs test (catalog code MC002) purchased from Diacron International (Grosseto, Italy) was used to measure reactive oxygen metabolites (ROM). Ferric-reducing antioxidant power (FRAP) was used to measure total antioxidant capacity using the colorimetric method of Benzie and Strain (1996). Activity of myeloperoxidase was measured via colorimetry based on the reaction of myeloperoxidase (**MPO**) contained in the plasma sample with hydrogen peroxide. Nitric oxide (NOx), nitrite, and nitrate concentrations were determined using the methods described by Trevisi et al. (2013). Retinol and tocopherol were extracted with hexane and analyzed by reverse-

Table 1. Plasma biomarkers and PMN function from birth through 50 d of age in calves born to dams fed a basal control diet (CON) or the basal diet plus ethylcellulose rumen-protected methionine (MET) during the last 28 d of pregnancy¹

Item ²	Maternal diet	Day of age					SEM	P-value		
		0	7	21	42	50		Maternal (M)	Day (D)	M × D
Bilirubin, $\mu\text{mol/L}$	CON	11.6	6.25	3.17	2.29	1.06	1.45	0.72	<0.01	0.68
	MET	11.2	7.29	3.27	2.62	0.94	1.40			
GGT, U/L	CON	11.7	201	52.3	21.9	23.8	19.2	0.53	<0.01	0.33
	MET	10.2	207	60.9	26.9	24.9	21			
AST, U/L	CON	36.6	50.7	50.8	69.7	107 ^a	103	0.37	<0.01	0.02
	MET	38.0	48.8	49.7	73.1	91.4 ^b	99.2			
Inflammation										
Haptoglobin, g/L	CON	0.26	0.76	0.22	0.31	0.30	0.06	0.05	<0.01	0.23
	MET	0.27	0.52	0.22	0.25	0.19	0.07			
MPO, U/L	CON	37.6	472	325	352 ^a	384 ^a	24.5	0.37	<0.01	0.04
	MET	32.4	487	348	279 ^b	322 ^b	24.5			
Oxidative status										
Paraoxonase, U/mL	CON	4.78	19.6	40.9	64.5	74.9	3.79	0.61	<0.01	0.87
	MET	3.44	22.0	41.7	67.9	79.2	3.79			
ROM, mg of H ₂ O ₂ /100 mL	CON	5.72	22.4	20.6	18.8	18.6	1.01	0.48	<0.01	0.74
	MET	6.28	23.1	20.8	18.6	19.3	1.07			
FRAP, $\mu\text{mol/L}$	CON	234	144	121	137	149	10.3	0.27	<0.01	0.88
	MET	220	143	120	129	138	9.68			
NOx, $\mu\text{mol/L}$	CON	297	135 ^a	88.9	75.1	78.3	33.0	0.08	<0.01	<0.01
	MET	278	98.2 ^b	77.3	80.7	74.0	30.8			
Nitrite, $\mu\text{mol/L}$	CON	4.87	12.4	27.8	16.0	13.4	4.63	0.76	<0.01	0.64
	MET	5.40	10.4	31.1	16.7	10.4	4.98			
Nitrate, $\mu\text{mol/L}$	CON	292	121	52.2	53.7	62.7	39.3	0.21	<0.01	0.56
	MET	272	84.8	45.5	56.6	55.6	36.6			
Retinol, $\mu\text{g}/100\text{ mL}$	CON	10.4	16.4	18.0	24.1	32.9	1.69	0.80	<0.01	0.81
	MET	11.0	17.3	18.0	22.7	34.7	1.69			
Tocopherol, $\mu\text{g/mL}$	CON	0.60	1.97	3.45	3.42	2.27	0.25	0.34	<0.01	0.06
	MET	0.65	2.60	4.16	2.81	2.25	0.25			
PMN function, %										
Monocytes										
Oxidative burst	CON	15.0	23.4	25.4	29.1	28.2 ^a	2.09	0.43	<0.01	0.03
	MET	15.1	27.4	23.9	26.5	34.4 ^b	1.93			
Phagocytosis	CON	24.6	23.4	30.8	34.9	38.4	2.15	0.76	<0.01	0.99
	MET	24.1	23.0	30.2	33.6	37.3	2.08			
Neutrophils										
Oxidative burst	CON	40.2	56.6	50.3	62.1	69.4	4.11	0.27	<0.01	0.99
	MET	43.4	60.9	55.3	66.6	73.7	3.87			
Phagocytosis	CON	52.5	65.5 ^b	71.8	76.2 ^b	79.8 ^b	2.45	0.04	<0.01	0.05
	MET	55.5	71.8 ^a	77.4	84.7 ^a	89.8 ^a	2.39			

^{a,b}Means within a column differ ($M \times D$ $P < 0.05$). Longitudinal profiles for each group are depicted in Supplemental Figures S1 and S2 (<https://doi.org/10.3168/jds.2018-15676>).

¹MET diet consisted of Mepron at 0.09% of diet DM (Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany). Calves were weaned after 42 d of age.

²GGT = gamma-glutamyl transferase; AST = aspartate aminotransferase; MPO = myeloperoxidase; ROM = reactive oxygen metabolites; FRAP = ferric-reducing ability of plasma; NOx = nitric oxide.

phase HPLC using Spherisorb ODS-2, 3 μm , in a 150 $\times$ 4.6 mm column (Alltech, Deerfield, IL); a UV detector set at 325 (for retinol) or 290 nm (for tocopherol); and 80:20 methanol:tetrahydrofuran as the mobile phase.

Neutrophils were isolated from blood collected in acid citrate dextrose (ACD)-containing evacuated tubes. Samples were centrifuged at 600 $\times g$ for 30 min at 4°C. Twenty-five milliliters of cold deionized water were added to lyse the red blood cells, followed by addition of 5 mL of 5 $\times$ PBS. Samples were centrifuged at 200 $\times$

g for 10 min at 4°C and the supernatants decanted. Two subsequent washings using 10 mL of 1 $\times$ PBS at 4°C were performed, with samples centrifuged at 500 $\times g$ for 5 min at 4°C and supernatants decanted. An aliquot (20 μL) was used for PMN quantification and viability using a granulocyte primary antibody (CH138A, Veterinary Microbiology and Pathology, Washington State University, Pullman, WA), followed by a second antibody (Goat Anti-Mouse IgM, Human ads-PE, Southern Biotech, Birmingham, AL). Cells were fixed with 150

μL of 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO) and preserved at 4°C until flow cytometry reading (LSR II, Becton Dickinson, San Jose, CA).

Total RNA was extracted using the miRNeasy kit (Qiagen, Hilden, Germany) according to manufacturer's protocols. Samples were treated on-column with DNaseI (Qiagen). The RNA concentration was measured using a NanoDrop ND-1000 (NanoDrop Technologies, Rockland, DE), and RNA quality was measured using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA). All samples used in the analysis were diluted with nuclease-free water to a concentration of 100 ng RNA/ μL concentration. Quantitative PCR (qPCR) was performed using 4 μL diluted cDNA combined with 6 μL of a mixture composed of 5 μL SYBR Green master mix (Applied Biosystems, Waltham, MA), 0.4 μL each of 10 μM forward and reverse primers, and 0.2 μL nuclease-free water, in a MicroAmp Optical 384-Well Reaction Plate (Applied Biosystems). Additional details are reported in Supplemental Data (<https://doi.org/10.3168/jds.2018-15676>).

Phagocytosis capacity and oxidative burst activity of peripheral monocytes and neutrophils were determined simultaneously upon challenge with enteropathogenic bacteria (*Escherichia coli* 0118:H8). We incubated 200 μL of blood with 40 μL of 100 μM dihydrorhodamine 123 (Sigma-Aldrich) and 40 μL of propidium iodine-labeled bacteria (109 cfu/mL) at 38.5°C for 10 min. After red blood cells were lysed with ice-cold MilliQ water (Millipore, Billerica, MA), cells were resuspended in PBS solution. Subsequently, monocytes were marked with APC anti-CD14 antibody (catalog number 301808; Biolegend, San Diego, CA), and neutrophils were stained with CH138A primary anti-bovine granulocyte monoclonal antibody (catalog number BOV2067, Washington State University) and PE-labeled secondary antibody (catalog number 1020-09S, Southern Biotech). The cells were re-suspended in PBS solution for flow cytometry analyses (LSR II; Becton Dickinson). Data are reported as percentages of CD14/CH138A-positive cells with phagocytosis and oxidative burst capability.

Data were analyzed using PROC MIXED of SAS (version 9.4; SAS Institute Inc., Cary, NC). Fixed effects in the model were maternal diet (M), day (D), and their interactions ($M \times D$). Random effect was calf within M. The exponential correlation covariance structure SP (POW) for repeated measures was used for the analysis because time intervals were not evenly spaced. Data that were not normally distributed according to the Shapiro-Wilk test were log₂-scale transformed. The transformed data were subjected to statistical analysis to determine treatment effects and were back-transformed for presentation purposes. Statistical

differences were declared significant at $P < 0.05$ and tendencies at $P < 0.10$.

Compared with CON calves, the interaction $M \times D$ for MPO ($P = 0.04$; Table 1) and AST ($P = 0.02$) was due to MET calves having lower concentrations of MPO at 42 and 50 d, and lower AST at 50 d (Supplemental Figure S1; <https://doi.org/10.3168/jds.2018-15676>). In addition, MET calves tended ($M P = 0.08$) to have an overall lower concentration of NOx and haptoglobin ($M P = 0.05$) compared with CON calves. Nitric oxide concentration also had an $M \times D$ interaction, where at 7 d after birth MET calves had lower ($P < 0.01$, Table 1) NOx concentrations compared with CON calves. A tendency ($P = 0.06$) for an $M \times D$ interaction in tocopherol concentration was detected, due to MET calves having greater concentrations at 7 and 21 d of age (Table 1), whereas at 42 d this trend changed in an opposite way (CON > MET, $P < 0.01$) due to a drastic decrease for MET calves between 21 and 42 d of age.

The lower concentrations of haptoglobin, NOx, and AST, coupled with lower MPO activity and greater concentrations of tocopherol in MET calves could be responses to adaptation to extrauterine life. In fact, the early period is particularly stressful, encompassing a rapid need to adapt to drastic physiologic changes (first 7 d of life) along with the gradual microbial colonization of the gut (21 to 50 d of age; Loores et al., 2002; Rey et al., 2014). The fact that MPO increased markedly at 7 d of age is suggestive of an activation of the oxidant status response (Jacometo et al., 2016). Given the lack of reference values for MPO in calves, it is difficult to establish with absolute confidence whether the values reported here denote oxidative stress. At least in non-ruminants, changes in the level and activity of MPO in the circulation are associated with inflammation and oxidative stress (Ndrepepa, 2019).

In vitro neutrophil phagocytosis and oxidative burst were greater overall ($M P < 0.05$; Table 1) in stimulated blood from MET calves compared with CON. In addition, neutrophil phagocytosis had an $M \times D$ interaction ($P < 0.05$; Table 1) for MET, compared with CON calves having greater neutrophil phagocytosis starting from d 7 through 50 d of age (Supplemental Figure S2; <https://doi.org/10.3168/jds.2018-15676>). The phagocytosis response, along with temporal increase in MPO concentration, could be interpreted as an indicator of a better innate immune responsiveness to an infectious challenge. By phagocytizing, killing, and digesting invading pathogens, neutrophils and macrophages represent the first line of defense of the innate immune system (Goff and Horst, 1997).

The mRNA abundance of *TLR2* tended to be greater ($M P = 0.07$; Table 2) in MET calves, and this group

Table 2. Messenger RNA abundance in PMN from birth to 50 d of age in calves born to dams fed a basal control diet (CON) or the basal diet plus ethylcellulose rumen-protected methionine (MET) during the last 28 d of pregnancy¹

Gene ²	Maternal diet	Day of age						P-value		
		0	7	21	42	50	SEM	Maternal (M)	Day (D)	M × D
<i>TLR2</i>	CON	1.84	0.89	0.96	0.96	0.89	0.47	0.07	0.02	0.36
	MET	2.43	1.91	0.73	1.25	1.69	0.66			
<i>TLR4</i>	CON	2.33	1.38	1.06	1.29	0.87	0.68	0.37	<0.01	0.55
	MET	2.63	2.37	0.81	1.38	1.55	0.77			
<i>NFKB1</i>	CON	1.29	1.17	0.98	1.47	1.30	0.19	0.28	<0.01	0.36
	MET	1.51	1.38	0.97	1.27	1.50	0.20			
<i>IL10</i>	CON	2.36	0.55	0.42	0.56	0.54	0.50	0.59	<0.01	0.56
	MET	1.81	0.71	0.32	0.50	0.45	0.38			
<i>IL1B</i>	CON	0.78	1.06	0.76	1.37	1.05	0.30	0.22	<0.01	0.39
	MET	0.68	1.77	0.78	1.53	1.78	0.39			
<i>S100A8</i>	CON	1.93	0.66	0.37	0.62	0.52	0.48	0.03	<0.01	0.70
	MET	2.83	1.47	0.46	0.83	0.93	0.65			
<i>STAT3</i>	CON	2.04	0.88 ^b	0.69	1.00	0.93	0.34	0.01	<0.01	<0.01
	MET	2.49	2.50 ^a	0.76	1.06	1.39	0.49			
<i>TNF</i>	CON	1.08	0.98	1.22	1.47	1.67	0.22	0.90	<0.01	0.25
	MET	0.86	0.88	1.44	1.64	1.64	0.22			
<i>RXRA</i>	CON	1.80	0.81	0.71	1.61	1.30	0.32	0.68	<0.01	0.27
	MET	2.56	0.96	0.78	1.17	1.31	0.42			
<i>DDX58</i>	CON	0.51	1.70	0.61	1.27	1.17	0.49	0.39	<0.01	0.84
	MET	0.69	1.52	1.03	1.39	1.27	0.46			
<i>SOD1</i>	CON	0.86	0.93	1.25	1.46	1.31	0.21	0.07	<0.01	0.95
	MET	0.64	0.79	1.11	1.13	1.18	0.17			
<i>SOD2</i>	CON	1.15	1.18	0.56	0.90	0.67	0.37	0.18	<0.01	0.77
	MET	1.49	2.20	0.68	1.29	1.43	0.72			
<i>MPO</i>	CON	0.52	0.35	0.62	1.61	0.79	0.47	0.15	<0.01	0.10
	MET	0.75	0.58	0.41	1.91	2.36	0.69			
<i>NOX1</i>	CON	0.50	0.41	0.62	2.45	2.60	0.84	0.27	<0.01	0.54
	MET	0.27	0.41	0.70	1.14	1.88	0.65			
<i>NOS2</i>	CON	3.09	0.28	0.53	1.69	0.84	1.12	0.96	<0.01	0.19
	MET	1.31	0.32	0.36	2.39	1.88	0.86			
<i>SLC2A1</i>	CON	2.95	1.81 ^a	2.03 ^b	2.61	2.48	0.39	0.57	<0.01	0.04
	MET	2.78	1.25 ^b	2.89 ^a	2.62	3.32	0.46			
<i>LDHA</i>	CON	1.53	1.12	0.82	1.03	1.01	0.18	0.06	<0.01	0.62
	MET	1.81	1.61	0.92	1.13	1.33	0.21			
<i>GSR</i>	CON	1.41	1.05	0.93	1.24	0.92	0.24	0.33	0.03	0.31
	MET	1.54	1.34	0.86	1.24	1.55	0.27			
<i>GSS</i>	CON	1.27	1.51	1.26	1.42	1.23	0.20	0.35	<0.01	0.31
	MET	1.36	2.04	1.17	1.28	1.50	0.29			
<i>GPX1</i>	CON	1.09	0.99	1.04	1.20	1.29	0.17	0.07	0.22	0.15
	MET	1.70	1.69	1.21	1.17	1.58	0.25			

^{a,b}Means within a column differ ($M \times D P < 0.05$). Longitudinal profiles for each group are depicted in Supplemental Figure S3 (<https://doi.org/10.3168/jds.2018-15676>).

¹MET diet consisted of Mepron at 0.09% of diet DM (Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany). Calves were weaned after 42 d of age.

²*TLR2* = toll-like receptor 2; *TLR4* = toll-like receptor 4; *NFKB1* = nuclear factor kappa B subunit 1; *IL10* = interleukin 10; *IL1B* = interleukin 1-β; *S100A8* = S100 calcium binding protein A8; *STAT3* = signal transducer and activator of transcription 3; *TNF* = tumor necrosis factor; *RXRA* = retinoic-X-receptor α; *DDX58* = DExD/H-box helicase 58; *SOD1* = superoxide dismutase 1; *SOD2* = superoxide dismutase 2; *MPO* = myeloperoxidase; *NOX1* = NADPH oxidase 1; *NOS2* = nitric oxide synthase 2; *SLC2A1* = solute carrier family 2 member 1; *LDHA* = lactate dehydrogenase A; *GSR* = glutathione-disulfide reductase; *GSS* = glutathione synthetase; *GPX1* = glutathione peroxidase 1.

showed a greater abundance of *STAT3* ($M P = 0.01$; Table 2) and *S100A8* ($M P = 0.03$; Table 2; most evident in the first week of life) compared with CON calves. We found no significant interactions (Supplemental Figure S3; <https://doi.org/10.3168/jds.2018-15676>). Activation of TLR can induce a pro-inflammatory response, including synthesis of proteins that compose extracel-

lular traps, such as *S100A8* (Lee and Hwang, 2006; Moyes et al., 2010).

It is noteworthy that MET calves tended ($M P = 0.07$) to have greater expression of *GPX1*. At least in non-ruminants, the maturation of phagocytic cells into monocytes and granulocytes upon a challenge is associated with an increase in *GPX1* mRNA abundance

(Shen et al., 1994), suggesting that transcription and production of GPX represent a way to protect PMN from toxic oxygen radicals and to enhance the elimination of as many pathogens as possible before dying from these same destructive products. Because in some human diseases, overexpression of *GPX1* and other antioxidant defense enzymes is considered a good health prognosis (Tome et al., 2005), upregulation of *GPX1* in MET calves also could be considered beneficial against oxidative status.

Calves in the MET group had an overall upregulation ($M P = 0.05$) of *LDHA* primarily during the first 7 d of life. In non-ruminant leukocytes, the protein encoded by this gene is primarily involved in the interconversion of pyruvate to lactate during glycolysis (Ganapathy et al., 2009). Activation of the inflammatory cascade in rats enhanced glucose uptake, pentose phosphate pathway activity, and lactate output from peritoneal leukocytes (Haji-Michael et al., 1999). Thus, lactate is considered an indicator of glucose metabolism by leukocytes, primarily via the pentose phosphate pathway, which generates reducing equivalents to support phagocytosis (Moyes, 2015). The upregulation of *LDHA* in MET calves during the first 7 d of life suggested a greater capacity for the conversion of pyruvate to lactate, but it cannot be discerned whether the rate of glucose utilization in PMN was affected.

Although absence of data on passive transfer of immunoglobulins is a limitation in the present study, overall, the data underscore the potential for maternal supply of Met during the last 30 d prepartum to enhance the ability of circulating innate immune cells of the calf to respond to a pathogen challenge in early life. The underlying mechanisms may include alterations in mRNA abundance (Loor et al., 2013). However, the existence of other molecular (e.g., epigenetic) or physiologic (e.g., glucose or nutrient uptake) mechanisms induced by the greater maternal supply of Met could not be discerned and should be studied in the future.

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