Breed-specific differences in the immune response to lipopolysaccharide in ewes

Jessalyn M. Hadfield,* Elizabeth C. Bowdridge,* Ida Holásková,[†] Ted H. Elsasser,[‡] and Robert A. Dailey*¹

*Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, WV 26506; [†]West Virginia Agriculture and Forestry Experiment Station, Morgantown, WV 26506-6108; and [‡]Agricultural Research Service, Animal Biosciences and Biotechnology Laboratory, USDA, Beltsville, MD 20705

ABSTRACT: Innate immune response to a lipopolysaccharide (LPS) challenge varies among sheep breeds. How different breeds respond to bacterial infections impacts management practices of sheep producers. Hence, clinical response, acute-phase response, and gene expression of pro- and anti-inflammatory markers in peripheral white blood cells (WBCs) were examined after an LPS challenge in Dorset and Suffolk ewes. Ewes received either PBS or 2.5 µg/kg LPS (i.v.) 4 to 5 d after onset of synchronized estrus. Blood was collected via jugular venipuncture intermittently for 24 h to determine WBC counts. Rectal temperatures and observations of behavioral/physical appearances were recorded hourly. After LPS, WBCs decreased the first hour ($P = 0.0001$) and rectal temperatures ($P < 0.0001$) increased through 4 h; both returned toward normal 6 h after challenge. Suffolk ewes exhibited greater changes in temperature ($P = 0.03$) and behavioral/physical responses ($P < 0.0001$) than Dorset ewes and had an enhanced acute-phase response demonstrated by increased concentrations of plasma haptoglobin ($P = 0.04$), as well as cortisol concentrations ($P = 0.03$). Real-time PCR was completed on buffy coat

homogenates for expression of pro-inflammatory [*CXCL8*, *IL-6*, interferon gamma (*IFNG*), complement component 3 (*C3*), toll-like receptor 4 (*TLR4*), prostaglandin synthase 2 (*PTGS2*)] and anti-inflammatory [*IL-10*, superoxide dismutase 2 (*SOD2*), forkhead box P3 (*FOXP3*), peroxisome proliferator-activated receptor gamma (*PPARG*), mannose receptor C type 1 (*MRC1*), transforming growth factor β (*TGF β*)] genes. After LPS treatment, gene expressions increased for *CXCL8* ($P = 0.0003$), *TLR4* ($P = 0.004$), *SOD2* ($P < 0.0001$), and *C3* ($P = 0.003$), while *PPARG* ($P = 0.006$) and *MRC1* ($P = 0.003$) decreased. Overall, Dorset ewes had greater expression of *TLR4* ($P = 0.003$), *IL-10* ($P = 0.045$), *PPARG* ($P = 0.002$), *FOXP3* ($P = 0.001$), and *SOD2* ($P = 0.0002$), whereas Suffolk ewes had greater expression of *IL-6* ($P = 0.0007$), *IFNG* ($P = 0.02$), *PTGS2* ($P = 0.0002$), and *C3* ($P = 0.008$). Suffolk ewes also displayed greater expression of *IL-6* ($P = 0.002$) and *C3* ($P = 0.0004$) in response to LPS. In conclusion, differences in gene expression may explain the enhanced inflammatory response in Suffolk ewes and may predispose Suffolk ewes to be more responsive to bacterial infection than Dorset ewes.

Key words: acute-phase reaction, breed, gene expression, lipopolysaccharide, sheep, temperature response

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INTRODUCTION

The innate immune system of a host evolved to identify pathogens by recognizing pathogen-associated molecular patterns (PAMPs).

¹Corresponding author: rdailey@wvu.edu

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Lipopolysaccharide (LPS) is a PAMP on Gram-negative bacteria such as *Escherichia coli* (*E. coli*), which binds to toll-like receptor 4 (TLR4) initiating activation of intracellular signaling cascades and the expression of inflammatory mediators including IL-1 β , tumor necrosis factor alpha (TNF α), IL-6, interferon gamma (IFNG), CXCL8, and prostaglandins (PGs) (Turner et al., 2012). Immune mediators stimulate cells to upregulate inflammation locally, while systemically they induce the febrile response, activate the hypothalamic-pituitary-adrenal (HPA) axis, and initiate production of acute-phase proteins (APPs), such as haptoglobin (Hp) (Chrousos, 1995; Gabay and Kushner, 1999; Kabarroff et al., 2006). Differential immune responses occurred among sheep breeds after bacterial and parasitic infection (Stuen et al., 2011; Bowdridge et al., 2015; Jacobs et al., 2015, 2016). Moreover, Dorset and Suffolk ewes appeared to show differential responses to LPS challenge (Nikpour, 2017). The objectives of this study were: 1) to examine the inflammatory response to an LPS challenge in Dorset and Suffolk ewes to determine if 1 breed would have an enhanced immune response to bacterial infection and 2) to characterize gene expression in peripheral white blood cells (WBCs) during a LPS-induced inflammatory response to examine if differences in gene expression between breeds could be associated with the severity of the inflammatory response.

MATERIALS AND METHODS

All procedures were in compliance with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* and approved by the West Virginia University Animal Care and Use Committee (WVU IACUC # 13-0404.3). All animals were housed at the West Virginia University Animal Science Farm (Stewartstown Road, Morgantown, WV) under the same management. In fall 2015, 21 Dorset ewes (BW = 71.8 $\pm$ 1.4 kg) and 32 Suffolk ewes (BW = 76.7 $\pm$ 2.1 kg) that were 3 to 5 years old were synchronized for estrus with a 5-d controlled intravaginal drug releasing insert (CIDR; Pfizer, New York, NY) and 20 mg of prostaglandin F_{2 α} (i.m.; Lutalyse, Pharmacia and UpJohn, New York, NY) at CIDR removal (Dixon et al., 2007). Estrous synchronization ensured similar hormonal stage of all ewes, as reproductive hormones are known to affect the inflammatory response (Lewis, 2003). On day 4 after estrus, ewes were weighed (without food or water restriction), sorted into pens in the barn, and given hay and

water free choice. Within breed, ewes were assigned at random to be challenged with either 2.5 μ g/kg of LPS (i.v., *E. coli* O55:B5, Sigma-Aldrich, St Louis, MO) (Elsasser et al., 2004) or 3 mL PBS on day 5 after estrus. This dose of LPS has been used previously (Elsasser et al., 2004; Graham, 2014; Nikpour, 2017). The 4 treatment groups were: Dorset + PBS n = 10, Dorset + LPS n = 11, Suffolk + PBS n = 16, and Suffolk + LPS n = 16. Rectal temperatures and behavioral and mucosal responses (lethargy, coughing, vaginal discharge, nasal discharge, and inappetence) were recorded hourly for 12 h.

Blood Collection

Jugular samples (~8 mL) were collected via venipuncture into EDTA-treated blood collection tubes (WorldWide Life Sciences Division, Inc., Bristol, PA) at CIDR withdrawal (~2 d before expected estrus) and at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 9, 12, and 24 h after LPS challenge and stored on ice until transported to the laboratory. White blood cell counts in 20 μ L of whole blood from each ewe were obtained at 0, 1, 2, 3, 6, 9, and 12 h using a Beckman Coulter Counter (Beckman Coulter Z2 series model # 9914591-D, Pasadena, CA). Samples were then centrifuged at 700 $\times$ g for 20 min at 4 $^{\circ}$ C, and plasma was collected and stored at -80 $^{\circ}$ C.

Quantitative PCR

Buffy coats were collected and pooled at random from 2 to 4 ewes to form 4 pooled samples for each treatment group (Dorset + PBS, Dorset + LPS, Suffolk + PBS, Suffolk + LPS) at each time point (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 9, 12, and 24 h). Cells in the buffy coat were resuspended in PBS, and any remaining red blood cells were removed using ACK lysis buffer (Lonza catalog #10-584E). After washing with PBS, WBCs were transferred to polypropylene tubes and homogenized in 1.5 mL RNA-bee (Tel-Test, Friendswood, TX) and stored at -80 $^{\circ}$ C until total RNA extraction and cDNA preparation as described by Sommers (2014). The subset of time points selected for analyses of specific genes from 15 time samples were selected based on a preliminary (unpublished) experiment that determined the approximate time frame of when expression changed and returned to normal (Table 1).

Genes measured are involved in the initiation or recovery from an inflammatory response or associated with certain immune cell phenotypes.

Table 1. Times when each protein and gene were measured in blood samples (plasma or white blood cells) after treatment

Protein or gene ¹	Time of blood sample (h)														
	0	0.25	0.5	0.75	1	1.5	2	2.5	3	4	5	6	9	12	24
C3	x	x	x	x	x	x	x		x			x			
Cortisol	x				x		x		x			x	x	x	
CXCL8	x								x	x		x	x		
Foxp3	x				x		x		x			x		x	x
GAPDH	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Hp	x						x					x		x	x
IFNG	x		x			x			x			x		x	x
IL-6	x	x		x		x	x		x			x			
IL-10	x					x			x			x		x	
MRC1	x				x							x		x	x
PPARG	x				x				x			x	x	x	x
PTGS2	x	x	x	x	x	x	x		x						
SOD2	x					x			x			x	x	x	x
TGFβ	x					x			x	x		x	x	x	
TLR4	x					x			x			x	x		
TNFα	x			x		x	x		x						

¹Complement component 3: C3; forkhead box P3: Foxp3; glyceraldehyde 3-phosphate dehydrogenase: GAPDH; haptoglobin: Hp; interferon gamma: IFNG; interleukin-6: IL-6; interleukin-8: CXCL8; interleukin-10: IL-10; mannose receptor type C 1: MRC1; peroxisome proliferator-activated receptor gamma: PPARG; prostaglandin synthase 2: PTGS2; superoxide dismutase 2: SOD2; transforming growth factor beta: TGFβ; toll-like receptor 4: TLR4; tumor necrosis factor alpha: TNFα.

Real-time PCR was completed on buffy coat homogenates for expression of pro-inflammatory [*CXCL8*, *IL-6*, interferon gamma (*IFNG*), complement component 3 (*C3*), toll-like receptor 4 (*TLR4*), prostaglandin synthase 2 (*PTGS2*)] and anti-inflammatory [*IL-10*, superoxide dismutase 2 (*SOD2*), forkhead box P3 (*FOXP3*), peroxisome proliferator-activated receptor gamma (*PPARG*), mannose receptor C type 1 (*MRC1*), transforming growth factor β (*TGFβ*)] genes.

Primers (Chauhan et al., 2014; Sommers, 2014; Jacobs et al., 2016) were purchased from Integrated DNA Technologies (Coralville, IA); associated GenBank accession numbers and primer sequences are in Table 2. Samples were analyzed in duplicate using a Bio-Rad CFX96 (Bio-Rad, Hercules, CA) as described by Sommers (2014). Relative fold changes in expression of candidate genes were obtained using the 2-ΔΔCt method (Livak and Schmittgen, 2001). The Ct values were used to calculate ΔCt values for genes of interest [Ct (test) – Ct (reference)]. The reference gene for normalization was GAPDH. Levels of GAPDH were comparable across breed and treatment. The ΔΔCt values were obtained in reference to the sample collected at CIDR removal. Graphical and statistical representation for each gene is based on fold changes with respect to GAPDH.

Steroid and Protein Assays

To measure cortisol and Hp, plasma samples were pooled to match the buffy coat (qPCR) for each treatment group (Dorset + PBS, Dorset + LPS, Suffolk + PBS, Suffolk + LPS) at each time point measured. Concentrations of cortisol were assessed at 0, 1, 2, 3, 6, 9, and 12 h by ELISA (Immuno-Biological Laboratories, Inc., Minneapolis, MN) according to the manufacturer's instructions with a sensitivity of 2.5 ng/mL and an intra- and inter-assay CV of 4.7% and 11.8%, respectively. Briefly, 20 μL of each control and standard were added to an antibody-coated microtiter plate in duplicate followed by 20 μL of each sample. Enzyme conjugate (200 μL) was then added to each well and the plate was incubated at room temperature for 60 min. The plate was then manually washed 3 times followed by the addition of substrate solution (100 μL) to each well and an incubation of 15 min at room temperature. Stop solution (100 μL per well) was then added and absorbance was read at 450 nm. Haptoglobin was assessed at 0, 2, 6, 12, and 24 h using a sheep ELISA (Mybiosource, San Diego, CA) with a sensitivity of 15.6 ng/mL and intra- and inter-assay CV of 3.5% and 16.4%, respectively, in samples diluted 1:2,000 in provided diluent buffer, according to manufacturer's instructions. In brief, 100 μL of standards were added to

Table 2. Primer sequences of the housekeeping and genes of interest

GenBank ¹	Forward primer (5'-3')	Reverse primer (5'-3')	Length (bp)
C3 XM_004022821.1	GCACTGTCCACCAACCTCA	ATCAGGCTTCTGCTTCTCCA	87
CXCL8 NM_001009401.1	GGATTACAGAGTTCCTGTTCA	CTGTGAGGTAGAAAGATGACTGAGA	86
Foxp3 FJ491732.1	GAAACAGCACATTCCCAGAGT	GGATGAGGGTGGCATAGGT	90
GAPDH HM043737	CAGGAGCACGAGAGGAAGAG	AATGTATGGAGGTCGGGAGA	91
IFNG NM_001009803.1	ATGACCTGTCGCCAAAATC	GCAGGCAGGAGAACCATTAC	97
IL-6 NM_001009392.1	TAACCACTCCAGCCACACAC	GATAACCTTTGCGTTCTTTACCC	80
IL-10 U11421.1	TTTCCTGACTGCCCTCTAA	GCTCCCTGGTTTCTCTTCCT	105
MRC1 HM099914.1	GGAGGGAACACAAGGGATG	GCAAGGAAGGGTCAGGTTG	94
PPARG NM_001100921.1	CTTGACGGGAAAGACGACA	GCTGATGTGCTTGAACCTTGATT	97
PTGS2 U68486	CTACCCGCCTCATATTCCTG	CCAAATGGTGGCATACTCA	94
SOD2 NM_001280703	TCACAGCATCTTCTGGACAA	TGCTCCTTATTGAAGCCAAG	177
TGFβ NM_001009400.1	GAAGTGTGTGTTTCGTCAGC	AGAAATTGGCGTGGTAGCC	92
TLR4 NM_001135930.1	GGCATCATCTTCATCGTCCT	CCACTCCAGGTAGGTGTTCC	99

¹Complement component 3: C3; forkhead box P3: Foxp3; glyceraldehyde 3-phosphate dehydrogenase: GAPDH; interferon gamma: IFNG; interleukin-6: IL-6; interleukin-8: CXCL8; interleukin-10: IL-10; mannose receptor type C 1: MRC1; peroxisome proliferator-activated receptor gamma: PPARG; prostaglandin synthase 2: PTGS2; superoxide dismutase 2: SOD2; transforming growth factor beta: TGFβ; toll-like receptor 4: TLR4.

an antibody-coated microtiter plate in duplicate followed by 100 µL of diluted sample. The plate was then incubated for 30 min at room temperature and then manually washed 4 times. Enzyme-antibody conjugate (100 µL per well) was then added followed by a 15-min incubation in the dark at room temperature. The plate was then washed manually 4 times before the addition of TMB substrate solution (100 µL per well) and 10 min of incubation in the dark at room temperature. Stop solution (100 µL per well) was added and absorbance was read at 450 nm. Concentrations of TNFα in individual plasma samples were assessed at 0, 0.75, 1.5, 2, and 3 h using 100 µL duplicates assayed by RIA (Kenison et al., 1990) with a sensitivity of 44 pg/mL and CV <10%.

Statistical Analysis

Data were analyzed using JMP Version Pro 12 and SAS Version 9.3 (SAS Institute, Cary, NC). Continuous data not normally distributed were log-transformed. Rectal temperatures and WBCs were analyzed by repeated measures ANOVA for effects of breed, treatment, time, and their

interactions. Concentrations of Hp, cortisol, and TNFα, and fold changes in gene expression were analyzed by repeated measures with irregular spacing ANOVA for effects of breed, treatment, time, and their interactions. Least square means comparisons of significant interactions were made specifically comparing only the breeds within the treatment and time combinations, using slicing option in Proc Mixed of SAS. The Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995) was used to control the error rate due to multiple ANOVAs for the list of 12 genes, applying false discovery rate of 0.10. Due to the low frequency of individual mucosal and behavioral responses (nasal discharge, vaginal discharge, diarrhea, lethargy, loss of appetite, coughing, and heavy breathing) during the 12-h posttreatment, the number of symptoms observed being experienced by each ewe were summed and classified as frequency of sickness responses and analyzed by the generalized linear model using Poisson distribution after log transformation for the effect of breed, treatment, and the interaction. Significance criterion alpha level was 0.05. All results are reported as mean ± SEM.

RESULTS AND DISCUSSION

Being a PAMP, LPS is highly immunogenic and stimulates innate immune cells to produce pro-inflammatory cytokines and eicosanoids that regulate the acute phase response (APR) (Calder, 2003). Treatment with LPS increased ($P < 0.0001$) rectal temperature, which peaked (1.2 ± 0.2 °C) at hour 4 (Fig. 1A), decreased WBCs (Fig. 1B) attaining a nadir at hour 1 ($P < 0.0001$), increased ($P = 0.003$) concentrations of cortisol (Fig. 1C) that remained elevated until 6 to 12 h ($P < 0.0001$), increased tumor necrosis α (TNF α) to its maximum ($P < 0.0001$) at hour 2 (Fig. 1D), and increased sickness responses

(4.4 ± 0.7 vs. 0.1 ± 0.1 , $P < 0.0001$, Fig. 1F). Expressions of *CXCL8* (Fig. 2B, $P = 0.0003$), *SOD2* (Fig. 2F, $P < 0.0001$), *TLR4* (Fig. 2H, $P = 0.004$), and *C3* (Fig. 2A, $P = 0.003$) were increased after LPS, while expression of *PPARG* (Fig. 2E, $P = 0.006$) and *MRC1* (Fig. 2D, $P = 0.003$) were decreased. Activation of complement and TLR4 are known to initiate production of pro-inflammatory cytokines (IL-1 β , TNF α , and IL-6) (Turner et al., 2012), indeed TNF α levels increased after LPS challenge (Fig. 1D). Increased expression of the antioxidant *SOD2* (Fig. 2F) may be associated with the production of reactive oxygen species (ROS), and

Figure 1. Acute-phase response (mean $\pm$ SEM) to LPS or PBS in Dorset and Suffolk ewes over time (0 to 24 h). — Dorset + PBS Dorset + LPS — Suffolk + PBS - - - Suffolk + LPS (A) Rectal temperature: LPS $P < 0.0001$; LPS $\times$ Breed $P = 0.03$; Time $P = 0.0005$; LPS $\times$ Time $P = 0.0001$. (B) Number of WBCs: LPS $P < 0.0001$; Time $P < 0.0001$; LPS $\times$ Time $P < 0.0001$. (C) Plasma cortisol concentrations: Breed $P = 0.03$; LPS $P = 0.003$; Breed $\times$ LPS $P = 0.03$; Time $P < 0.0001$; LPS $\times$ Time $P < 0.0001$; Breed $\times$ LPS $\times$ Time $P = 0.009$. (D) Plasma tumor necrosis α (TNF α) concentrations: LPS $P < 0.0001$; Time $P < 0.0001$; LPS $\times$ Time $P < 0.0001$; Breed $\times$ LPS $\times$ Time $P = 0.04$. (E) Plasma Hp concentrations: Breed $P < 0.0001$; Breed $\times$ LPS $P = 0.04$; Time $P = 0.04$. (F) Average number of sickness symptoms 0 to 12 h: Breed $P < 0.001$; LPS $P < 0.001$; Breed $\times$ LPS $P < 0.001$. For change in temperature, total WBCs, TNF α , and sickness responses: $n = 10$ Dorset + PBS ewes, $n = 11$ Dorset + LPS ewes, $n = 16$ Suffolk + PBS ewes, $n = 16$ Suffolk + LPS ewes. For cortisol and Hp: $n = 4$ pooled samples for each treatment group (Dorset + PBS, Dorset + LPS, Suffolk + PBS, Suffolk + LPS).

Figure 2. Expression of genes (mean $\pm$ SEM) in Dorset and Suffolk ewes treated with LPS or PBS over time. — Dorset + PBS Dorset + LPS — Suffolk + PBS - - Suffolk + LPS Fold change in mRNA expression of genes in peripheral WBCs were normalized to GAPDH and referenced to expression levels at CIDR withdrawal. For each gene, $n = 4$ pooled samples for each treatment group (Dorset + PBS, Dorset + LPS, Suffolk + PBS, Suffolk + LPS) at each time point. (A) Complement component 3 (C3): LPS $P = 0.003$; Breed $P = 0.008$; Time $P < 0.001$; Breed $\times$ LPS $P < 0.001$; LPS $\times$ Time $P < 0.001$. (B) Interleukin-8 (CXCL8): LPS $P < 0.001$; Time $P < 0.001$; LPS $\times$ Time $P = 0.014$. (C) Forkhead box P3 (Foxp3): Breed $P < 0.001$; LPS $\times$ Time $P < 0.001$. (D) Mannose receptor C type 1 (MRC1): LPS $P = 0.003$; Time $P < 0.001$. (E) Peroxisome proliferator-activated receptor gamma (PPARG): LPS $P = 0.006$; Breed $P = 0.002$; Time $P = 0.006$; LPS $\times$ Time $P = 0.005$. (F) Superoxide dismutase 2 (SOD2): LPS $P < 0.001$; Breed $P < 0.001$; Time $P < 0.001$; LPS $\times$ Time $P < 0.001$. (G) Transforming growth factor beta (TGFβ): Breed $P = 0.08$; Time $P = 0.02$; Breed $\times$ Time $P = 0.01$. (H) Toll-like receptor 4 (TLR4): LPS $P = 0.003$; Breed $P = 0.004$; Time $P = 0.01$; LPS $\times$ Time $P = 0.01$.

increased expression of *CXCL8* (Fig. 2B) demonstrates involvement of neutrophils (Graham, 2014) which use ROS to control bacteria. Finally, decreased *PPARG* (Fig. 2E) and *MRC1* (Fig. 2D) expression indicates a shift towards a pro-inflammatory environment and a switch from tolerogenic to immunogenic immune cell phenotypes (Ricote et al., 1998; Faveeuw et al., 2000; Genoet et al., 2004; Appel et al., 2005; Gordon and Martinez, 2010).

Suffolk showed clinical display of sickness more frequently over 12-h posttreatment than Dorset (6.6 ± 0.7 vs. 1.2 ± 0.6 , $P = 0.003$, Fig. 1F). Concentrations of cortisol increased soon after LPS administration in the Suffolk and remained elevated through hour 3 before decreasing to normal by hour 12, whereas concentrations did not increase until hour 2, peaked at hour 3, and decreased to normal by hour 6 in Dorset ($P = 0.008$). Suffolk had a delayed $\text{TNF}\alpha$ response ($P = 0.04$) and greater overall concentrations of Hp (0.12 ± 0.01 vs. 0.04 ± 0.004 mg/mL, $P < 0.0001$; Fig. 1E), a general marker of inflammation (Cray et al., 2009).

Dorset had greater expression (Fig. 3) of *IL-10* ($P = 0.045$), *TLR4* ($P = 0.003$), *SOD2* ($P = 0.0002$), *PPARG* ($P = 0.002$), and *Foxp3* ($P = 0.0001$) than Suffolk ewes. Interleukin-10 downregulates expression of pro-inflammatory cytokines and also stimulates and reinforces changes in both the innate and adaptive immune system. This includes changes such as inducing regulatory phenotypes in dendritic cells, macrophages, and T cells (Fiorentino et al., 1991a, 1991b; Maynard and Weaver, 2008).

Additionally, IL-10 is produced by regulatory T cells (Tregs) to mediate some of their suppressive functions (Maynard and Weaver, 2008; Shevach, 2009; Huber et al., 2011). Lower mRNA expression of *Foxp3*, the transcription factor for Tregs, in Suffolk could be due to smaller Treg numbers or loss of their suppressive functions and conversion into effector T cells in response to exposure to pro-inflammatory cytokines (Gao et al., 2012). In conjunction with transforming growth factor β ($\text{TGF}\beta$), IL-6 induces naïve CD4^+ cells to polarize into pro-inflammatory Th17 cells. However, $\text{TGF}\beta$ in conjunction with IL-2 induces Treg polarization; IL-6 can inhibit this $\text{TGF}\beta$ function (Robertson et al., 2013). Regulatory T cells are not terminally differentiated, however, and can turn into Th1 or Th17 cells if exposed to high concentrations of pro-inflammatory cytokines, such as IL-6, IFNG, or IL-1 (Gao et al., 2012). Suffolk ewes tended to have greater *TGF* β expression and had greater expression of *IL-6* and *IFNG*, meaning naïve CD4^+ cells and Tregs will become inflammatory T cell subsets. Reduced *SOD2* could impact the ability to control ROS, while lower *PPARG* activity sets Suffolk ewes to have a prolonged inflammatory response and possibly an increased chance of these immune cells switching from tolerogenic to immunogenic phenotypes (Szanto and Nagy, 2008).

Suffolk ewes had greater mRNA expression of pro-inflammatory *IFNG* (Fig. 3, $P = 0.02$), *C3* (Fig. 3, $P = 0.008$), *IL-6* (Fig. 3, $P = 0.0007$), and *PTGS2* (Fig. 3, $P = 0.0002$), along with greater expression of *IL-6* (Fig. 4, $P = 0.002$) and *C3*

Figure 3. Expression of genes (mean $\pm$ SEM) affected by the main effect of breed. Gene expression in peripheral WBCs averaged over LPS and PBS for each breed, normalized to GAPDH and referenced to expression levels at CIDR withdrawal. For each gene, $n = 8$ samples for each breed (Dorset, Suffolk) at all of the time points marked in Table 1 for that gene. The number (n) of observations for each gene was attained by summation of the product of the number of pooled samples for each breed by number of time points: *IL-10* ($n = 40$, $P = 0.045$), toll-like receptor 4 (*TLR4*) ($n = 40$, $P = 0.003$), superoxide dismutase 2 (*SOD2*) ($n = 56$, $P = 0.0002$), peroxisome proliferator-activated receptor gamma (*PPARG*) ($n = 56$, $P = 0.002$), forkhead box P3 (*Foxp3*) ($n = 56$, $P = 0.0001$), interferon gamma (*IFNG*) ($n = 56$, $P = 0.02$), complement component 3 (*C3*) ($n = 72$, $P = 0.008$), *IL-6* ($n = 56$, $P = 0.0007$), prostaglandin synthase 2 (*PTGS2*) ($n = 64$, $P = 0.0002$), transforming growth factor beta (*TGF* β) ($n = 56$, $P = 0.08$).

Figure 4. Expression of interleukin-6 (IL-6) and change in temperature from hour 0 (mean $\pm$ SEM) for the time points both of these factors were measured. Fold change in IL-6 expression in peripheral WBCs was normalized to GAPDH and referenced to expression levels at CIDR withdrawal, $n = 4$ pooled samples for each treatment group (Dorset + PBS, Dorset + LPS, Suffolk + PBS, Suffolk + LPS) at each time point. For change in temperature, $n = 10$ Dorset + PBS ewes, $n = 11$ Dorset + LPS ewes, $n = 16$ Suffolk + PBS ewes, $n = 16$ Suffolk + LPS ewes. IL-6: Breed $P < 0.001$; LPS $\times$ Breed $P = 0.002$. Rectal temperature: LPS $P < 0.0001$; LPS $\times$ Breed $P = 0.03$; Time $P = 0.0005$; LPS $\times$ Time $P = 0.0001$.

(Fig. 2A, $P = 0.0004$) in response to LPS. Along with the decreased anti-inflammatory mRNA for *IL-10* (Fig. 3, $P = 0.045$), decreased expression of *PPARG* (Figs. 2E and 3, $P = 0.002$) and *Foxp3* (Figs. 2C and 3, $P = 0.0001$) indicates that Suffolk are set to have a more rapid and prolonged inflammatory response. While specific PGs were not measured, LPS is known to stimulate production of prostaglandin E_2 (Herath et al., 2009). The cytokines IL-1 β , TNF α , and IL-6 and prostaglandin E_2 acting in the hypothalamus increase temperature (Netea et al., 2000; Evans et al., 2015), discomfort (Konsman et al., 2002), cortisol, via activation of the HPA axis, and Hp, which is produced in response to cortisol and IL-6. Indeed, Suffolk ewes challenged with LPS had greater expression of IL-6 (Fig. 4), a greater temperature response (Fig. 4), cortisol (Fig. 1C), and sickness response (Fig. 1F) compared to Dorset ewes.

CONCLUSION

Differences in gene expression of molecules involved in the inflammatory response may be responsible for the more rapid, intense, and prolonged inflammatory response to LPS challenge in Suffolk compared to Dorset ewes and predispose Suffolk ewes to have a more severe immune response to bacterial infection.

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