# Effects of oral meloxicam administration to beef cattle receiving lipopolysaccharide administration or vaccination against respiratory pathogens[1]

**M. C. Rodrigues,*† R. F. Cooke,*[2,3] R. S. Marques,* S. A. Arispe,‡ D. H. Keisler,§ and D. W. Bohnert***

* Eastern Oregon Agricultural Research Center, Oregon State University, Burns 97720; † Programa de Pós-Graduação em Zootecnia, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, SP, Brazil, 18618-970; ‡Malheur County Extension Office, Oregon State University, Ontario, 97914; § Division of Animal Sciences, University of Missouri, Columbia 65211

**ABSTRACT:** This study evaluated the effects of oral meloxicam administration on metabolic, inflammatory, and acute-phase responses of beef cattle receiving a lipopolysaccharide (LPS) challenge (Exp. 1; d −1 to 6) or vaccinated against respiratory pathogens (Exp. 2; d 7 to 21). Twenty-one Angus steers ($n$ = 11) and heifers ($n$ = 10) were housed in individual pens on d −15 and were offered free-choice water, mineral-vitamin mix, and hay until d 21. In Exp. 1, cattle were ranked on d −1 by sex and BW and assigned to 1) oral meloxicam administration (1 mg/kg BW daily) from day −1 to 6 (MEL8), 2) oral meloxicam administration (1 mg/kg BW) on d 0 and oral lactose monohydrate administration (1 mg/kg BW) on d −1 and from d 1 to 6 (MEL1), or 3) oral lactose monohydrate administration (1 mg/kg BW daily) from d −1 to 6 (CON). On d 0, cattle received an intravenous LPS bolus (0.5 μg/kg BW) concurrently with treatment administration. Rectal temperature (RTEMP) was assessed, and blood samples were collected at −2, 0, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96, 120, and 144 h relative to LPS administration. No treatment effects were detected ($P \geq 0.36$) for RTEMP, concentrations of serum tumor necrosis factor α (TNFα), plasma haptoglobin, cortisol, insulin, and leptin, as well as blood mRNA expression of *TNF*α and cyclooxygenase-2, although all variables increased ($P < 0.01$) across treatments after LPS administration. In Exp. 2, cattle received the same treatments that they were assigned to in Exp. 1 from d 7 to d 13 and were vaccinated against respiratory pathogens concurrently with treatment administration on d 8. Blood samples were collected, and RTEMP was assessed as in Exp. 1 in addition to 168, 240, and 336 h relative to vaccination. No treatment effects were detected ($P \geq 0.26$) for RTEMP, the same plasma and serum variables evaluated in Exp. 1, and serum concentrations of antibodies against *Mannheimia haemolytica* or serum titers against bovine respiratory syncytial virus, bovine herpesvirus-1, bovine viral diarrhea virus-1, and parainfluenza-3 virus. All variables increased ($P < 0.01$) across treatments after vaccination, except for serum TNFα and titers against bovine viral diarrhea virus-1 ($P \geq 0.40$). Collectively, this study found no evidence that oral meloxicam administration, at the doses and intervals utilized herein, mitigated the metabolic, inflammatory, and acute-phase reactions elicited by LPS administration or vaccination against respiratory pathogens.

**Key words:** beef cattle, inflammation, lipopolysaccharide, meloxicam, vaccination



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## INTRODUCTION

Stressful procedures such as weaning, transport, and vaccination stimulate inflammatory and acute-phase responses in cattle (Carroll and Forsberg, 2007). These reactions are key components of the innate immune system but can be detrimental to cattle performance (Johnson, 1997). Meloxicam is a nonsteroidal anti-inflammatory drug commonly used in animal and

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[2]Corresponding author: reinaldo.cooke@oregonstate.edu

[3]Reinaldo Cooke is also affiliated as graduate professor to the Programa de Pós-Graduação em Zootecnia, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, SP, Brazil, 18618-970.

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human medicine (Coetzee et al., 2009). Guarnieri Filho et al. (2014) reported that oral meloxicam administration to cattle alleviated the acute-phase protein response and prevented the decrease in ADG, DMI, and G:F caused by transport and feedlot entry. However, research is still required to further understand the role of meloxicam on innate immune reactions (Van Engen et al., 2014) to biologically support its benefits to highly stressed beef cattle. One experimental model to characterize the effects of meloxicam on the bovine innate immune system is a bacterial lipopolysaccharide (**LPS**) challenge and subsequent evaluation of metabolic, inflammatory, and acute-phase variables (Carroll et al., 2009).

Vaccination against pathogens that cause bovine respiratory disease also elicited inflammatory and acute-phase protein reactions and reduced ADG, G:F (Arthington et al., 2013), and DMI (Rodrigues et al., 2015) in feeder cattle. Therefore, research to develop management interventions that benefit vaccine-induced immune protection and cattle performance is warranted (Arthington et al., 2013). On the basis of the benefits of meloxicam to highly stressed cattle (Guarnieri Filho et al., 2014), we hypothesized that oral meloxicam administration to cattle receiving either LPS or vaccinated against respiratory pathogens alleviates the resultant inflammatory and acute-phase responses. Hence, this study evaluated the effects of oral meloxicam administration on metabolic, inflammatory, and acute-phase parameters of beef steers and heifers receiving a bacterial LPS challenge (Exp. 1) or vaccinated against respiratory pathogens (Exp. 2).

## MATERIALS AND METHODS

Both experiments were conducted at Oregon State University's Eastern Oregon Agricultural Research Center (Burns Station). The animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee (number 4592)

Twenty-one Angus steers ($n$ = 11) and heifers ($n$ = 10) were used for Exp. 1 (d −1 to 6) and subsequently for Exp. 2 (d 7 to 21). Cattle were weaned on d −21 and exposed daily to halter training until d −2 to alleviate the impacts of human handling and weaning on the variables evaluated herein (Arthington et al., 2005; Cooke and Bohnert, 2011; Cooke et al., 2012). At weaning, cattle were vaccinated against *Clostridium* (2 mL subcutaneous [**s.c.**] injection of One Shot Ultra 7; Zoetis, Florham Park, NJ) and infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea types 1 and 2 viruses, and *M. haemolytica* (2 mL s.c. injection of Bovi-Shield Gold One Shot; Zoetis) and were administered an anthelmintic (s.c. injection at 1 mL/50 kg BW of Dectomax; Zoetis).

From d −15 to d 21, cattle were housed in individual pens (7 × 15 m) and received water, commercial mineral-vitamin mix, and mixed alfalfa-grass hay for ad libitum consumption. Hay samples were collected weekly, pooled across weeks, and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). These samples were analyzed by wet chemistry procedures for concentrations of CP (method 984.13; AOAC, 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY; AOAC, 2006), and NDF (Van Soest et al., 1991; modified for Ankom 200 fiber analyzer, Ankom Technology Corp.). Calculations for TDN used the equation proposed by Weiss et al. (1992), whereas $NE_m$ and $NE_g$ were calculated with the equations proposed by the NRC (2000). Hay nutritional profile was (DM basis) 63% TDN, 34% NDF, 24% ADF, 1.41 Mcal/kg of NEm, 0.83 Mcal/kg of NEg, and 20.0% CP. The mineral mix (Cattleman's Choice, Performix Nutrition Systems, Nampa, ID), contained 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 IU/kg of vitamin A, 13,000 IU/kg of vitamin D3, and 50 IU/kg of vitamin E.

### *Experiment 1*

***Animals and Treatments.*** On d −1, cattle were ranked by sex and BW (initial BW = 232 ± 4 kg, initial age = 223 + 2 d) and were assigned to 1 of 3 treatments: 1) oral meloxicam administration (1 mg/kg BW daily; Carlsbad Technologies Inc., Carlsbad, CA) from day −1 to 6 (**MEL8**), 2) oral meloxicam administration (1 mg/kg BW; Carlsbad Technologies Inc.) on d 0 and oral lactose monohydrate administration (1 mg/kg BW, excipient used in the manufacture of meloxicam tablets; Avantor Performance Materials, Center Valley, PA) on d −1 and from d 1 to 6 (**MEL1**), and 3) oral lactose monohydrate administration (1 mg/kg BW daily; Avantor Performance Materials) from d −1 to 6 (**CON**).

Meloxicam was originally presented in 15-mg tablets, which were ground daily using a commercial food processor (Soho Food Processor; West Bend Housewares, West Bend, WI) to ensure that MEL8 and MEL1 cattle received their exact dose according to their initial BW (average full BW obtained on d −2 and −1). Lactose monohydrate was administered to account for potential placebo effects. Meloxicam and lactose monohydrate were manually mixed with 50 mL of 0.9% saline until completely dissolved and were administered individually to cattle via oral drench at 0800 h to ensure complete consumption. Treatments were dissolved in saline with-

in 30 s before administration in 60-mL sterile syringes (Monoject Covidien Animal Health; Mansfield, MA). On d 0, all cattle received an intravenous bolus dose of bacterial LPS (0.5 μg/kg BW, *Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO) concurrently with treatment administration (Carroll et al., 2009). Bacterial LPS was dissolved into 10 mL of 0.9% saline immediately before challenge and was administered via 10-mL sterile syringes (Monoject Covidien Animal Health) according to cattle initial BW (average full BW obtained on d −2 and −1). The experimental length (d −1 to 6) was appropriate to evaluate the innate immune responses elicited by the LPS challenge without potential carryover effects to Exp. 2, given that the variables evaluated herein return to baseline levels within 5 d following LPS administration (Carroll et al., 2009).

The MEL8 and CON treatments are based on Guarnieri Filho et al. (2014) and previous research indicating that circulating concentrations of acute-phase proteins can be elevated for up to 5 d following a stress or pathogenic stimuli (Cooke et al., 2012; Rodrigues et al., 2015). Guarnieri Filho et al. (2014) also suggested that different lengths of meloxicam administration should be investigated. Accordingly, the MEL1 treatment was included to determine if a single meloxicam administration can mitigate the inflammatory and acute-phase responses elicited by the LPS challenge, which begins within 24 h after challenge (Carroll et al., 2009), whereas meloxicam has an elimination half-life of 28 h when orally administered to cattle at 1 mg/kg (Coetzee et al., 2009).

***Sampling.*** Cattle rectal temperature was assessed with a GLA M750 digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA) every 2 h from -2 to 8 h, every 6 h from 12 to 72 h, and every 24 h from 96 to 144 h relative to LPS administration. Blood samples were collected concurrently with rectal temperature assessment via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10-mL; Becton Dickinson, Franklin Lakes, NJ) with and without freeze-dried sodium heparin for plasma and serum collection, respectively. Blood samples were placed immediately on ice, centrifuged (2,500 × *g* for 30 min; 4°C) for plasma or serum harvest, and stored at −80°C on the same day of collection. All plasma samples were analyzed for plasma haptoglobin concentration according to colorimetric procedures described by Cooke and Arthington (2013). Plasma samples collected from 0 to 48 h were also analyzed for concentrations of cortisol, insulin, and leptin. Tumor necrosis factor α (**TNF**α) concentrations were analyzed in serum samples collected from 0 to 6 h based on the results reported by Carroll et al. (2009). Plasma concentrations of cortisol and insulin were determined using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Plasma concentrations of leptin were determined by radioimmunoassay according to procedures described by Delavaud et al. (2000). Serum concentrations of TNFα were determined using a bovine-specific commercial ELISA kit (RayBiotech, Inc., Norcross, GA). The intra- and inter-assay CV were, respectively, 2.7% and 3.7% for haptoglobin and 4.8% and 7.2% for TNFα. Plasma cortisol, insulin, and leptin concentrations were analyzed within a single assay. The intra-assay CV was 3.3% for cortisol, 2.1% for insulin, 4.2% for leptin.

**Table 1.** Primer sequences and accession number for all gene transcripts analyzed by quantitative real-time PCR in Exp. 1

| Target gene | Primer sequence[1] | Accession no. |
|---|---|---|
| β2-microglobulin | | NM_173893 |
| Forward | 5′-GGGCTGCTGTCGCTGTCT-3′ | |
| Reverse | 5′-TCTTCTGGTGGGTGTCTTGAGT-3′ | |
| Cyclooxygenase-2 | | AF031699 |
| Forward | 5′-AATCATTCACCAGGCAAAGG-3′ | |
| Reverse | 5′-TAGGGCTTCAGCAGAAAACG-3′ | |
| Tumor necrosis factor α | | NM_173966 |
| Forward | 5′-AACAGCCCTCTGGTTCAAAC-3′ | |
| Reverse | 5′-TCTTGATGGCAGACAGGATG-3′ | |

[1]Primer sequences obtained from Silva et al. (2008) for **β**2-microglobulin and cyclooxygenase-2 and Riollet et al. (2000) for tumor necrosis factor **α**.

Additional blood samples were collected at 0, 2, and 4 h relative to LPS administration into PAXgene tubes (Qiagen, Valencia, CA) for subsequent RNA isolation and analysis of *TNF*α, cyclooxygenase-2 (***COX2***), and β2-microglobulin mRNA expression in blood cells via real-time (**RT**) quantitative reverse transcription PCR. Upon collection, PAXgene tubes were stored at room temperature overnight and then at −80°C until RNA isolation. Total RNA was extracted from blood samples using the PAXgene Blood RNA Kit (Qiagen). Quantity and quality of isolated RNA were assessed via UV absorbance (UV Mini 1240; Shimadzu Scientific Instruments Inc., Columbia, MD) at 260 nm and 260/280 nm ratio, respectively (Fleige and Pfaffl, 2006). Extracted blood RNA (120 ng) was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems, Foster City, CA). Real-time PCR was completed using the Fast SYBR Green Master Mix (Applied Biosystems) and gene-specific primers (20 p*M* each; Table 1) with the StepOne Real-time PCR system (Applied Biosystems), according to procedures described by Cooke et al. (2008). At the end of each RT-PCR, amplified products were subjected to a dissociation gradient (95°C for 15 s, 60°C for 30 s, and 95°C for 15 s) to verify the amplification of a single product by denaturation at the anticipated temperature.

Responses were quantified on the basis of the threshold cycle ($C_T$), the number of PCR cycles required for target amplification to reach a predetermined threshold. The CT responses from *TNF*α and *COX2* were normalized to β2-microglobulin (Silva et al., 2008), and the CV for β2-microglobulin CT values across all samples was 4.2%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

### *Experiment 2*

***Animals and Treatments.*** Immediately after the last sampling of Exp. 1 on d 6, cattle (BW = 228 ± 4 kg, age = 230 ± 2 d) were assigned to Exp. 2. All cattle received the same treatment that they were assigned to in Exp. 1, and treatments were administered at 0800 h from d 7 d 13.

On d 8, cattle were revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea types 1 and 2 viruses, and *M. haemolytica* (2 mL s.c. of Bovi-Shield Gold One Shot; Zoetis) concurrently with treatment administration. The interval between the initial (d −21) and revaccination (d 7) followed the manufacturer's (Zoetis) recommendation for viral respiratory pathogens. Although revaccination against *M. haemolytica* is not required, this is a common practice in commercial feedlots because of the frequent lack of health history in high-risk receiving cattle (Richeson et al., 2008; Edwards, 2010). As in Exp. 1, the MEL8 and CON treatments are based on previous research from our group (Cooke et al., 2012; Guarnieri Filho et al., 2014). Given that leukocytes responsible for inflammatory and acute-phase responses are directly involved with antigen presentation to T cells (Durum and Muegge. 1996), excessive meloxicam administration may impair the innate immune responses required for proper vaccine efficacy. Therefore, MEL1 was included to determine if a single meloxicam administration concurrently with handling for vaccination can modulate the resultant inflammatory and acute-phase responses (Arthington et al., 2013; Rodrigues et al., 2015) without impairing vaccine efficacy.

***Sampling.*** Rectal temperature was assessed, and blood samples were collected for analyses of plasma and serum variables as in Exp. 1. Additional blood samples were collected at 168, 240, and 336 h relative to vaccination and were analyzed for plasma haptoglobin concentrations (Cooke and Arthington, 2013). Moreover, serum samples collected immediately prior (0 h) and at 168, 240, and 336 h following vaccination were also analyzed for concentrations of antibodies against *M. haemolytica* (Confer et al., 1996; Burciaga-Robles et al., 2010), as well as titers against bovine respiratory syncytial virus (**BRSV**), bovine herpesvirus-1 (**BHV-1**), bovine viral diarrhea virus-1 (**BVD-1**), and parainfluenza-3 virus (**PI3**) using a virus neutralization test (Rosenbaum et al., 1970; Oklahoma Animal Disease Diagnostic Laboratory, Stillwater). The intra- and interassay CV were, respectively, 4.2% and 4.3% for haptoglobin and 2.1% and 4.7% for TNFα. Plasma cortisol, insulin, and leptin concentrations were analyzed within a single assay. The intra-assay CV was, respectively, 5.1% for cortisol, 1.7% for insulin, and 2.6% for leptin.

**Table 2.** Metabolic, inflammatory, and blood mRNA responses from beef steers ($n$ = 11) and heifers ($n$ = 10) administered bacterial lipopolysaccharide (LPS; 0.5 μg/kg BW) and assigned to receive oral meloxicam (1 mg/kg BW daily) for 7 d (MEL8; $n$ = 7), lactose monohydrate for 7 d (CON; $n$ = 7), or meloxicam for 1 d and lactose monohydrate for 6 d (MEL1; $n$ = 7)[1]

| Item | CON | MEL8 | MEL1 | SEM | *P*-value |
|---|---|---|---|---|---|
| Metabolic and inflammatory responses | | | | | |
| Rectal temperature,[2] °C | 38.99 | 38.95 | 38.96 | 0.07 | 0.90 |
| Plasma cortisol, ng/mL | 30.4 | 30.6 | 30.1 | 2.7 | 0.98 |
| Plasma insulin, μIU/mL | 2.72 | 3.04 | 2.75 | 0.66 | 0.93 |
| Plasma leptin, ng/mL | 4.89 | 4.93 | 5.23 | 0.34 | 0.74 |
| Plasma haptoglobin, μg/mL | 0.26 | 0.29 | 0.28 | 0.05 | 0.95 |
| Serum TNFα,[3] ng/mL | 0.96 | 1.31 | 1.39 | 0.43 | 0.75 |
| Blood mRNA expression | | | | | |
| TNFα expression, relative fold change | 3.70 | 3.15 | 2.76 | 0.44 | 0.36 |
| COX2 expression,[4] relative fold change | 6.90 | 9.63 | 9.92 | 3.78 | 0.82 |

[1]Meloxicam and lactose monohydrate were manually mixed with 50 mL of 0.9% saline and administered individually to cattle via oral drench at 0800 h from d −1 to 6 of the experiment. On d 0, all cattle received an intravenous bolus dose of bacterial LPS (*Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO) concurrently with treatment administration (Carroll et al., 2009).

[2]Cattle rectal temperature was assessed (GLA M750 digital thermometer; GLA Agricultural Electronics, San Luis Obispo, CA), and blood samples were collected every 2 h from −2 to 8 h, every 6 h from 12 to 72 h, and every 24 h from 96 to 144 h relative to LPS administration.

[3]TNF**α** = tumor necrosis factor alpha.

[4]COX2 = cyclooxygenase-2.

### *Statistical Analysis*

Data from both experiments were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Animal was considered the experimental unit. All model statements contained the effects of treatment, time, sex, and all resultant interactions. Animal (treatment × sex) was used as the random variable. The specified term for the repeated statement was time, animal (treatment × sex) was included as the subject, and the covariance structure utilized was autoregressive, which provided the lowest Akaike information criterion and therefore the best fit.

**Table 3.** Rectal temperature (RTEMP), concentrations of plasma cortisol, plasma insulin, plasma leptin, plasma haptoglobin, and serum tumor necrosis factor alpha (TNF**α**), and blood mRNA expression (relative fold change) of *TNFα* and cyclooxygenase-2 (*COX2*) in beef cattle (steers, $n = 11$; heifers, $n = 10$) administered bacterial lipopolysaccharide (0.5 μg/kg BW)[1]

| Hour | RTEMP, °C | Cortisol, ng/mL | Insulin, μIU/mL | Leptin, ng/mL | Haptoglobin, μg/mL | TNFα, ng/mL | *TNFα mRNA* | *COX2 mRNA* |
|---|---|---|---|---|---|---|---|---|
| −2 | 39.09$^{c}$ | 33.8$^{c}$ | 1.57$^{d,e}$ | 4.94$^{b}$ | 104$^{e}$ | — | — | — |
| 0 | 39.11$^{c}$ | 22.7$^{e,d}$ | 1.47$^{d,e}$ | 4.84$^{b}$ | 79$^{e}$ | 0.26$^{b}$ | 2.10$^{b}$ | 3.60$^{b}$ |
| 2 | 39.72$^{b}$ | 46.9$^{b}$ | 7.01$^{a}$ | 4.78$^{b}$ | 163$^{d,e}$ | 2.86$^{a}$ | 4.98$^{a}$ | 19.6$^{a}$ |
| 4 | 40.77$^{a}$ | 55.6$^{a}$ | 5.67$^{a,b}$ | 4.87$^{b}$ | 156$^{d,e}$ | 0.54$^{b}$ | 2.56$^{b}$ | 2.55$^{b}$ |
| 6 | 39.54$^{b}$ | 37.5$^{c}$ | 4.96$^{b}$ | 4.93$^{b}$ | 109$^{e}$ | 0.31$^{b}$ | — | — |
| 8 | 39.12$^{c}$ | 21.2$^{e,d}$ | 3.37$^{c}$ | 5.04$^{b}$ | 129$^{e}$ | — | — | — |
| 12 | 38.92$^{d,c,e}$ | 24.3$^{e,d}$ | 2.85$^{d,c}$ | 5.56$^{a}$ | 158$^{d,e}$ | — | — | — |
| 16 | 38.82$^{d,f,e}$ | 20.0$^{e}$ | 1.53$^{d,e}$ | 5.38$^{a}$ | 272$^{d,c}$ | — | — | — |
| 24 | 38.33$^{h}$ | 22.9$^{e,d}$ | 0.99$^{e}$ | 4.96$^{b}$ | 497$^{b}$ | — | — | — |
| 36 | 38.83$^{d,e}$ | 23.6$^{e,d}$ | 0.98$^{e}$ | 5.02$^{b}$ | 580$^{a}$ | — | — | — |
| 48 | 38.76$^{d,g,f,e}$ | 25.7$^{d}$ | 0.82$^{e}$ | 4.92$^{b}$ | 615$^{a}$ | — | — | — |
| 60 | 38.97$^{d,c}$ | — | — | — | 431$^{b}$ | — | — | — |
| 72 | 38.51$^{h,g}$ | — | — | — | 431$^{b}$ | — | — | — |
| 96 | 37.99$^{i}$ | — | — | — | 332$^{c}$ | — | — | — |
| 120 | 38.66$^{g,f,e}$ | — | — | — | 223$^{d,e}$ | — | — | — |
| 144 | 38.56$^{h,g,f}$ | — | — | — | 162$^{d,e}$ | — | — | — |
| SEM | 0.10 | 2.4 | 0.65 | 0.22 | 52 | 0.32 | 0.34 | 2.90 |
| *P*-value | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |

$^{a–h}$Within columns, values with different superscripts differ ($P \leq 0.05$).

[1]Cattle received an intravenous bolus dose of bacterial lipopolysaccharide (*Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO) at 0 h, which was dissolved into 10 mL of 0.9% saline immediately before administration. Cattle rectal temperature was assessed (GLA M750 digital thermometer; GLA Agricultural Electronics, San Luis Obispo, CA), and blood samples were collected every 2 h from −2 to 8 h, every 6 h from 12 to 72 h, and every 24 h from 96 to 144 h relative to lipopolysaccharide administration

Results are reported as least squares means and are separated using PDIFF. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and $\leq 0.10$

## RESULTS AND DISCUSSION

### *Experiment 1*

No treatment effects were detected ($P \geq 0.36$) for rectal temperature, serum TNFα concentrations, plasma haptoglobin concentrations, and blood mRNA expression of *TNFα* and *COX2* (Table 2). Time effects were detected ($P < 0.01$) for all these variables (Table 3), indicating that the LPS administration elicited the expected innate immune response (Carroll and Forsberg, 2007) as reported by others (Carroll et al., 2009; Reuter et al., 2008; Waggoner et al., 2009). Upon a pathogenic stimulus such as LPS administration, the innate immune system elicits several reactions with the intent of controlling or eliminating the infection (Abbas and Lichtman, 2007). These include synthesis of proinflammatory cytokines such as TNFα from leukocytes and subsequent increase in body temperature via COX2 modulation and hepatic synthesis of acute phase proteins including haptoglobin (Carroll and Forsberg, 2007; Silva et al., 2008). Accordingly, rectal temperature increased ($P \leq 0.02$) from 2 to 6 h, plasma haptoglobin concentrations increased ($P \leq 0.05$) from 16 to 96 h, and serum TNFα concentrations and blood mRNA expression *TNFα* and *COX2* were increased ($P < 0.01$) at 2 h relative to LPS administration across all treatments (Table 3). However, oral meloxicam administration failed to mitigate these outcomes (Table 2), which does not support our hypothesis given that meloxicam is known to modulate COX2 and subsequent inflammatory and acute-phase outcomes (Beretta et al., 2005). These results also differ from previous research indicating that anti-inflammatory compounds alleviate innate immune responses elicited by LPS administration to cattle (Lohuis et al., 1991; Wagner and Apley, 2004; Myers et al., 2010), as well as research reporting reduced inflammatory and acute-phase variables when meloxicam was orally administered to beef cattle exposed to stressful situations (Guarnieri Filho et al., 2014; Van Engen et al., 2014).

No treatment effects were detected ($P \geq 0.74$) for plasma concentrations of cortisol, insulin, and leptin (Table 2). Time effects were detected ($P < 0.01$) for all these variables (Table 3), indicating that the LPS administration changed cattle metabolic responses as reported by others (Reuter et al., 2008; Carroll et al., 2009; Waggoner et al., 2009), whereas meloxicam administration did not impact these outcomes. Cortisol modulates early physiological responses following a pathogenic

**Table 4.** Metabolic, inflammatory, and serum antibody variables beef steers ($n = 11$) and heifers ($n = 10$) vaccinated against respiratory pathogens and assigned to receive oral meloxicam (1 mg/kg BW daily) for 7 d (MEL8; $n = 7$), lactose monohydrate for 7 d (CON; $n = 7$), or meloxicam for 1 d and lactose monohydrate for 6 d (MEL1; $n = 7$)[1]

| Item | CON | MEL8 | MEL1 | SEM | *P*-value |
|---|---|---|---|---|---|
| Metabolic and inflammatory responses | | | | | |
| Rectal temperature, °C | 39.19 | 39.06 | 39.13 | 0.06 | 0.29 |
| Plasma cortisol, ng/mL | 26.6 | 26.1 | 24.4 | 1.6 | 0.61 |
| Plasma insulin, μIU/mL | 1.84 | 1.92 | 1.91 | 0.38 | 0.98 |
| Plasma leptin, ng/mL | 5.00 | 5.29 | 5.44 | 0.25 | 0.46 |
| Plasma haptoglobin, μg/mL | 0.57 | 0.52 | 0.56 | 0.09 | 0.92 |
| Serum TNFα, ng/mL | 0.15 | 0.20 | 0.26 | 0.07 | 0.57 |
| Serum antibody variables | | | | | |
| Mannheimia haemolytica, ng/antibody bound | 0.87 | 0.70 | 0.96 | 0.17 | 0.53 |
| Bovine respiratory syncytial virus, titer $\log_2$ | 1.70 | 1.57 | 1.32 | 0.22 | 0.26 |
| Bovine herpesvirus-1, titer $\log_2$ | 0.79 | 0.66 | 0.75 | 0.28 | 0.90 |
| Bovine viral diarrhea virus-1, titer $\log_2$ | 1.35 | 1.45 | 1.17 | 0.21 | 0.62 |
| Parainfluenza-3 virus, titer $\log_2$ | 1.52 | 1.01 | 1.25 | 0.27 | 0.29 |

[1]Meloxicam and lactose monohydrate were manually mixed with 50 mL of 0.9% saline and administered individually to cattle via oral drench at 0800 h from d 7 to d 13 of the experiment. On d −21, all cattle were vaccinated against *clostridium* (2 mL subcutaneous [s.c.] injection of One Shot Ultra 7; Zoetis, Florham Park, NJ), parainfluenza-3 virus, infectious bovine rhinotracheitis virus, bovine viral diarrhea types 1 and 2 viruses, and *Mannheimia haemolytica* (2 mL s.c. injection of Bovi-Shield Gold One Shot; Zoetis) and were administered an anthelmintic (s.c. injection at 1 mL/50 kg BW of Dectomax; Zoetis). On d 8, all cattle were revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea types 1 and 2 viruses, and *M. haemolytica* (2 mL s.c. of Bovi-Shield Gold One Shot; Zoetis) concurrently with treatment administration. Cattle rectal temperature was assessed (GLA M750 digital thermometer; GLA Agricultural Electronics, San Luis Obispo, CA), and blood samples were collected every 2 h from −2 to 8 h, every 6 h from 12 to 72 h, every 24 h from 96 to 168 h, and at 240 and 336 h relative to vaccine administration on d 8.

**Table 5.** Rectal temperature (RTEMP), concentrations of plasma cortisol, plasma insulin, plasma leptin, plasma haptoglobin, and serum tumor necrosis factor alpha (TNF**α**) in beef cattle (steers, $n = 11$; heifers, $n = 10$) vaccinated against respiratory pathogens[1]

| Hour | RTEMP, °C | Cortisol, ng/mL | Insulin, μIU/mL | Leptin, ng/mL | Haptoglobin, μg/mL | TNFα, ng/mL |
|---|---|---|---|---|---|---|
| −2 | $38.56^{i,j,k}$ | $22.5^{e,f,g}$ | $1.26^{f,g}$ | $5.06^{d,e,f}$ | $176^{h}$ | — |
| 0 | $38.69^{g,h,i}$ | $18.4^{g}$ | $1.50^{d,e,f,g}$ | $4.78^{f}$ | $285^{g,h}$ | 0.23 |
| 2 | $39.54^{d}$ | $19.8^{e,f,g}$ | $1.20^{g}$ | $5.07^{d,e,f}$ | $181^{h}$ | 0.20 |
| 4 | $39.84^{c,b}$ | $33.5^{a}$ | $1.67^{d,e,f}$ | $4.98^{d,e,f}$ | $241^{g,h}$ | 0.19 |
| 6 | $40.17^{a}$ | $30.6^{a,b}$ | $1.93^{c,d,e}$ | $4.89^{e,f}$ | $191^{h}$ | 0.19 |
| 8 | $40.30^{a}$ | $29.2^{a,b,c}$ | $2.32^{bc}$ | $5.64^{b,c}$ | $258^{gh}$ | — |
| 12 | $39.94^{b}$ | $28.1^{b,c,d}$ | $2.70^{a,b}$ | $6.14^{a}$ | $405^{f,g}$ | — |
| 16 | $39.47^{d}$ | $27.3^{b,c,d}$ | $3.10^{a}$ | $5.73^{b}$ | $502^{e,f}$ | — |
| 24 | $39.02^{ef}$ | $25.5^{c,d,e}$ | $1.81^{d,e}$ | $5.29^{c,d}$ | $870^{c,d}$ | — |
| 36 | $39.65^{cd}$ | $23.9^{def}$ | $1.94^{c,d,e}$ | $5.24^{c,d,e}$ | $1049^{a,b}$ | — |
| 48 | $39.08^{e}$ | $24.2^{d,e,f}$ | $1.40^{e,f,g}$ | $4.94^{e,f}$ | $1098^{a,b}$ | — |
| 60 | $38.62^{h,i,j}$ | — | — | — | $1108^{a,b}$ | — |
| 72 | $38.41^{k}$ | — | — | — | $1205^{a}$ | — |
| 96 | $38.87^{e,f,g}$ | — | — | — | $976^{b,c}$ | — |
| 120 | $38.45^{j,k}$ | — | — | — | $695^{d,e}$ | — |
| 144 | $38.79^{f,g,h}$ | — | — | — | $518^{e,f}$ | — |
| 168 | $38.52^{i,j,k}$ | — | — | — | $262^{g,h}$ | — |
| 240 | $38.52^{i,j,k}$ | — | — | — | $241^{g,h}$ | — |
| 336 | $39.08^{e}$ | — | — | — | $238^{g,h}$ | — |
| SEM | 0.09 | 1.8 | 0.28 | 0.19 | 90 | 0.04 |
| *P*-value | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.97 |

[a–k]Within columns, values with different superscripts differ ($P \leq 0.05$).

[1]Cattle were revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea types 1 and 2 viruses, and *Mannheimia haemolytica* (2 mL subcutaneous of Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ) at 0 h. Cattle rectal temperature was assessed (GLA M750 digital thermometer; GLA Agricultural Electronics, San Luis Obispo, CA), and blood samples were collected every 2 h from −2 to 8 h, every 6 h from 12 to 72 h, every 24 h from 96 to 168 h, and at 240 and 336 h relative to vaccine administration.

stimulus, such as LPS administration, as an effector molecule on subsequent proinflammatory and acute-phase reactions (Steiger et al., 1999; Carroll et al., 2009; Cooke et al., 2012). Insulin and leptin synthesis are increased during an inflammatory response (Eizirik et al., 1995; Andersson et al., 2001; Roelfsema et al., 2001) with the intent of increasing energy utilization by the body to restore homeostasis (Waggoner et al., 2009). Leptin is also involved with activation and maturation of leukocytes and subsequent synthesis of proinflammatory cytokines (Matarese et al., 2005; Fernández-Riejos et al., 2010). Accordingly, plasma cortisol concentrations increased ($P \leq 0.03$) from 2 to 4 h, plasma insulin concentrations increased ($P \leq 0.05$) from 2 to 8 h, and plasma leptin concentrations increased ($P \leq 0.04$) from 12 to 16 h relative to LPS administration across all treatments (Table 3). Contrary to our findings, anti-inflammatory compounds have been shown to impact circulating concentrations of cortisol and insulin in cattle experiencing an inflammatory process (Cooke et al., 2012; Farney et al., 2013). Nevertheless, Guarnieri Filho et al. (2014) and Van Engen et al. (2014) also reported that oral meloxicam administration to beef cattle exposed to stressful situations did not impact circulating cortisol concentrations.

Collectively, Exp. 1 found no evidence that oral meloxicam administration at the doses and intervals utilized herein prevented or at least alleviated the metabolic, inflammatory, and acute-phase reactions elicited by LPS administration at 0.5 μg/kg BW to beef cattle. Perhaps the LPS administration resulted in biological responses that overwhelmed the anti-inflammatory capability of MEL1 and MEL8, or these treatments impacted innate immune responses not evaluated in the present experiment, such as synthesis and circulating concentrations of eicosanoids and bradykinin (Myers et al., 2010). Therefore, research is still warranted to fully elucidate the impacts of meloxicam on stress- and pathogen-induced innate immune reactions in cattle.

### *Experiment 2*

No treatment effects were detected ($P \geq 0.29$) for rectal temperature, serum TNFα concentrations, and plasma haptoglobin concentrations (Table 4). Time effects were detected ($P < 0.01$) for rectal temperature and plasma haptoglobin concentrations (Table 5) but not for serum TNFα concentrations ($P = 0.97$). The vaccine administered in this experiment contained a freeze-dried preparation of modified-live virus strains, a product from whole cultures of inactivated *M. haemolytica*, and a proprietary adjuvant formulation (Zoetis) to elicit a greater immune protection to target antigens (McKee et al., 2007; Coffman et al., 2010). Adjuvants and the viral fraction of vaccines stimulate recruitment of antigen-presenting leukocytes to the site of vaccine delivery, which in turn synthesize proinflammatory cytokines and stimulate inflammatory and acute-phase protein responses (Heegaard et al., 2000; Tizard, 2004; Carroll and Forsberg, 2007). Accordingly, rectal temperature increased ($P \leq 0.05$) from 2 to 48 h, whereas plasma haptoglobin concentrations increased ($P \leq 0.05$) from 16 to 144 h across all treatments relative to vaccine administration (Table 5), indicating that vaccination against respiratory pathogens elicited an innate immune response (Carroll and Forsberg, 2007), as previously reported (Arthington et al., 2013; Rodrigues et al., 2015). Rodrigues et al. (2015) also suggested that vaccination against respiratory pathogens does not increase serum TNFα concentrations in a peak manner within 6 h after vaccination, which was the sampling interval adopted herein for serum TNFα analysis based on previous research with LPS administration (Carroll et al., 2009). Nevertheless, as in Exp. 1, meloxicam administration failed to mitigate the vaccine-induced increase in rectal temperature and plasma haptoglobin, differing from our hypothesis based on the anti-inflammatory properties of meloxicam (Beretta et al., 2005; Guarnieri Filho et al., 2014; Van Engen et al., 2014).

No treatment effects were detected ($P \geq 0.46$) for plasma cortisol, insulin, and leptin concentrations (Table 4). Time effects were detected ($P < 0.01$) for all these variables (Table 5), given that plasma cortisol concentrations increased ($P \leq 0.05$) from 4 to 16 h, whereas plasma insulin and leptin concentrations increased ($P \leq 0.04$) from 8 to 16 h across all treatments relative to vaccine administration (Table 5). Rodrigues et al. (2015) also reported transient increases in plasma cortisol, insulin, and leptin concentrations in beef cattle within 16 h following vaccination against respiratory pathogens and attributed these outcomes to the immunomodulatory and homeostatic roles of these hormones during inflammatory reactions (Steiger et al., 1999; Matarese et al., 2005; Waggoner et al., 2009). Hence, vaccination against respiratory pathogens altered cattle metabolic parameters in the present experiment as expected (Rodrigues et al., 2015), and meloxicam administration did not impact these outcomes as in Exp. 1.

Neutralizing antibody titers provide an indication of immune protection, disease prevention, and vaccine efficacy in cattle (Howard et al., 1989; Bolin and Ridpath, 1990; Richeson et al., 2008). On the basis of the hypothesized effects of meloxicam on leukocytes responsible for inflammatory and acute-phase responses, which are directly involved with antigen presentation to T cells (Durum and Muegge. 1996), neutralizing antibodies against respiratory pathogens were evaluated to determine if treatments would impact vaccine efficiency. No treatment effects were detected ($P \geq 0.26$) for serum concentrations of antibodies against *M. hae-*

**Table 6.** Serum concentrations of antibodies against *Mannheimia haemolytica*, as well as serum titers against bovine respiratory syncytial virus (BRSV), bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus-1 (BVD-1), and parainfluenza-3 virus (PI3) in beef cattle (steers, $n = 11$; heifers, $n = 10$) vaccinated against respiratory pathogens[1]

| Hour | *M. haemolytica* | BRSV | BHV-1 | BVD-1 | PI3 |
|---|---|---|---|---|---|
| 0 | 0.61[c] | 1.03[b] | 0.22[c] | 1.27 | 0.64[b] |
| 168 | 0.82[b] | 1.65[a] | 0.65[b] | 1.31 | 1.42[a] |
| 240 | 1.00[a] | 1.80[a] | 1.05[a] | 1.38 | 1.45[a] |
| 336 | 0.87[b] | 1.64[a] | 1.03[a] | 1.33 | 1.54[a] |
| SEM | 0.10 | 0.11 | 0.15 | 0.12 | 0.15 |
| *P*-value | <0.01 | <0.01 | <0.01 | 0.40 | <0.01 |

[a–c]Within columns, values with different superscripts differ ($P \leq 0.05$).

[1]Cattle were revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea types 1 and 2 viruses, and *M. haemolytica* (2 mL subcutaneous of Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ) at 0 h. Cattle rectal temperature was assessed (GLA M750 digital thermometer; GLA Agricultural Electronics, San Luis Obispo, CA), and serum samples were collected at 0, 168, 240, and 336 h relative to vaccine administration (h 0).

*molytica* or serum titers against BRSV, BHV-1, BVD-1, and PI3 (Table 4), corroborating the lack of treatment effects on metabolic, inflammatory, and acute-phase protein responses. Time effects were detected ($P < 0.01$) for all these variables (Table 6) but not for serum BVD-1 titers ($P = 0.40$). As expected, serum concentrations of *M. haemolytica* antibodies and serum titers against BRSV, BHV-1, and PI3 titers increased ($P \leq 0.04$) across all treatments when comparing samples collected before (h 0) and after vaccination (h 168, 240, and 336; Table 6). The lack of a time effect ($P = 0.40$) on serum titers against BVD-1 was unexpected but can be attributed to the fact that the elevated serum BVD-1 titers at 0 h likely originated from vaccination on d −21 (Dean et al., 2003; Fairbanks et al., 2004).

Collectively, Exp. 2 found no evidence that oral meloxicam administration, at the doses and intervals utilized herein, mitigated the metabolic, inflammatory, and acute-phase reactions elicited by vaccination against respiratory pathogens or impacted serum concentrations or titers of antibodies against respiratory pathogens. As in Exp. 1, perhaps vaccination elicited biological responses that overwhelmed the anti-inflammatory capability of MEL1 and MEL8, or these treatments impacted innate immune responses not evaluated herein (Myers et al., 2010). Therefore, efforts to develop strategies that mitigate inflammatory and acute-phase responses known to impair performance while maintaining or enhancing immune protection in cattle vaccinated against respiratory pathogens are still warranted, including evaluation of other dosages of meloxicam or different nonsteroidal anti-inflammatory drugs.

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