Rapid Communication: Use of pressure mat gait analysis in measuring pain following normal parturition in dairy cows

Michael D. Kleinhenz,^{*,†,⊕} Patrick J. Gorden,^{*} Michael Burchard,^{*} Joshua A. Ydstie,^{*} and Johann F. Coetzee^{*,‡,1}

^{*}Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011; [†]Department of Clinical Science, Kansas State University, Manhattan, KS 66506; and [‡]Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506

ABSTRACT: Research investigating the pain of normal parturition is lacking as there are few objective methods for measuring pain. The objective of this research was to describe the gait of cows following eutocia using pressure mat gait analysis; and if meloxicam alters the gait of cows. Twenty Holstein cows within 26 h of unassisted calving were enrolled into the study. Treatment groups included: 1) postpartum cows administered meloxicam (MEL; $n = 10$); and 2) postpartum cows administered placebo (PLBO; $n = 10$). Meloxicam was administered by oral bolus at 1 mg/kg within 26 h of calving. Placebo cows were given an oral bolus of dry whey powder within 26 h of calving. A commercially available floor mat-based pressure/force measurement system was used to compare ambulation between treatment groups. Cows were walked across the mat before treatment administration, and 2, 4, 6, 8, 12, 24, 48, and 72 h posttreatment. The percent of total force, percent

total contact pressure, and percent total impulse of the rear limbs were calculated. Outcome measures were statistically analyzed using repeated measures, with the cow serving as the experimental unit. Cows in the MEL group placed 48.9% (95% CI: 47.4% to 50.5%) of total force on the rear limbs compared to 46.3% (95% CI: 44.7% to 47.9%) in PLBO cows ($P = 0.02$). Total impulse on their rear limbs for the MEL cows was 50.5% (95% CI: 48.6% to 52.4%) compared to 46.7% (95% CI: 44.8% to 48.7%) for the PLBO cows ($P = 0.01$). No differences in contact pressure of the rear limbs were observed ($P = 0.27$). The PLBO cows had a longer gait 101.3 cm (95% CI: 95.9% to 106.6 cm) vs. 90.8 cm (95% CI: 85.4% to 96.1 cm) ($P = 0.03$). These findings show meloxicam-treated cows have altered weight distribution to the rear limbs as measured by pressure mat gait analysis, suggesting meloxicam may effectively treat postpartum pain.

Key words: animal welfare, dairy cattle, gait, nonsteroidal anti-inflammatory drug; post-parturient

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INTRODUCTION

There has been an increase in research focused on ways to improve dairy cow health and well-being in the immediate postpartum period.

Nonsteroidal anti-inflammatory drugs (NSAID) have been investigated for their pharmacological effects in the reduction of inflammation associated with calving (Laven et al., 2012). Practitioners regard calving to be a painful event, but studies investigating the pain associated with normal parturition are deficient (Remnant et al., 2017).

Meloxicam is an NSAID that acts as a cyclooxygenase (COX) inhibitor. Injectable meloxicam is

¹Corresponding author: jcoetzee@vet.k-state.edu

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approved in the European Union and Canada as an adjunctive therapy to treat acute respiratory infection, diarrhea, pain relief associated with dehorning, and acute mastitis (EMEA, 2009). Recently, pressure mat gait analysis was used as a validated method of pain assessment for the Food and Drug Administration (FDA) approval of transdermal flunixin meglumine for the control of pain associated with foot rot (US FDA, 2017). The objectives of this research were 1) to investigate the use of pressure mat gait analysis to describe the gait of cows in the immediate postpartum period and 2) determine the effects of meloxicam on gait in cows administered oral meloxicam in the immediate postpartum period. Our hypothesis was that meloxicam administration in the immediate postpartum period would be effective at treating postpartum pain as evidenced by altered gait of post-parturient dairy cows measured by contact force, contact pressure, step impulse, and gait length.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee at Iowa State University (Protocol 4-16-8243-B).

Animals and Study Design

The methods used for treatment allocation and enrollment have been previously published by [Gorden et al. \(2018\)](#).

The study population consisted of 20 Holstein cows enrolled within 26 h of calving. To be eligible for enrollment, cows had to be free of lameness (score ≤ 2 out of 5) when assessed using a visual lameness scoring system described by [Sprecher et al. \(1997\)](#); have no history of receiving meloxicam in the past 30 d; and had a calving ease score < 2 . The latter enrollment criterion was to eliminate excessive pain associated with dystocia. Postpartum cows were randomly assigned into their treatment group on the day of calving using the RAND function in a spreadsheet program (Microsoft Excel, Richmond, WA). Postpartum cows were assigned to receive either meloxicam (MEL; $n = 10$; Meloxicam tablets 15 mg, Cadila Healthcare Ltd, India) at 1 mg/kg by oral bolus or a placebo bolus containing whey protein powder (PLBO; $n = 10$; Body Fortress Super Advance whey protein, Healthwatchers, Inc., Bohemia, NY). All treatments were administered as an oral bolus with the treatment article within a gelatin bolus (size #07, Torpac Inc., Fairfield, NJ). Treatment occurred immediately after the midday (noon) milking.

On the day of calving, cows were evaluated for inclusion criteria. If inclusion criteria were met, the cow was weighed for treatment dosing and placed onto the research study. All evaluations, body weight determinations, and time 0 (T0) collections were completed prior to the 12:00 p.m. milking on the day of enrollment.

Cows were group housed in a free-stall barn bedded with recycled manure solids. Cows were feed a total mixed ration with ad libitum access to water. Cows were milked by study personnel using farm protocols at 4:00 a.m., 12:00 p.m., and 8:00 p.m.

Gait Analysis

Gait analysis was completed using a commercially available floor mat-based system (MatScan, Tekscan, Inc., South Boston, MA). Data were collected prior to treatment (T0) and 2, 4, 6, 8, 12, 24, 48, and 72 h after treatment. Video synchronization was used to ensure consistent gait between and within cows for each time point. Research software (HUGEMAT Research 5.83, Tekscan, Inc., South Boston, MA) was used to determine force, contact pressure, and impulse (area under the force vs. time curve) using methods previously described ([Kotschwar et al., 2009](#); [Schulz et al., 2011](#); [Coetzee et al., 2014](#)). The percent of total force, percent total contact pressure, and percent total impulse of the rear limbs were determined using the equation:

$$\begin{aligned} \% \text{ Force Rear} = & [(\text{Left Rear Force} + \text{Right Rear Force}) \\ & + (\text{Left Rear Force} + \text{Right Rear Force} \\ & + \text{Left Front Force} + \text{Right Front Force})] * 100 \end{aligned}$$

Statistical Analysis

Contact force was used to determine the number of cows needed per treatment group. The study was designed to have a statistical power of 0.80 assuming an effect size in differences of 122 kg force with a SE of 36.8 and as reported by [Coetzee et al. \(2014\)](#). A sample size of 10 animals per treatment group was determined.

Statistical analysis was performed using commercially available computer software (JMP Pro 13, SAS Institute, Cary, NC). Statistical analysis was completed using repeated measures, with the cow serving as the experimental unit. Fixed effects were treatment (MEL/PLBO), time, and treatment by time interaction. Interaction between groups was completed using Tukey's HSD. Statistical significance was established when $P \leq 0.05$.

RESULTS

The mean body weight $\pm$ SD of cows enrolled were as follows: MEL 699 ± 117 kg; and PLBO 686 ± 46 kg.

Results from pressure mat analysis are summarized in Table 1. The mean step force of the rear limbs was 393.4 kg (95% CI: 327.2% to 459.5 kg) for the MEL cows and 331.3 kg (95% CI: 265.2% to 397.5 kg) for the PLBO cows ($P = 0.15$). There was evidence of a treatment effect on the % of total force by the rear limbs applied to the mat ($P = 0.02$) with the MEL cows applying 48.9% (95% CI: 47.4% to 50.5%) of the force on their rear limbs. The PLBO group placed 46.3% (95% CI: 44.7% to 47.9%) of force on their rear limbs. The MEL cows had a mean rear pressure of 7.1 kg/cm² (95% CI: 5.8% to 8.5 kg/cm²) compared to 6.2 kg/cm² (95% CI: 4.8% to 7.6 kg/cm²) for the PLBO group ($P = 0.27$).

Cows in the MEL group had 50.5% (95% CI: 48.6% to 52.4%) of the total impulse on their rear limbs compared to 46.7% (95% CI: 44.8% to 48.7%) for the PLBO group ($P = 0.01$). No treatment by time interaction was appreciated ($P = 0.53$). The PLBO had a longer gait [101.3 cm (95% CI: 95.9 to 106.6 cm)] compared to the MEL [90.8 cm (95% CI: 85.4 to 96.1 cm)] ($P = 0.03$; Fig. 1).

DISCUSSION

There is a paucity of literature on the pain associated with normal calving events. The percent of total force by the rear limbs was determined to account for differences in cow body weight and distribution of weight by each limb. These findings show that cows treated with meloxicam following calving distribute more weight to the rear limbs compared to placebo cows. When gait analysis was used following surgical castration, a change in the force applied by the front limbs compared to rear

limbs was found with castrated calves placing more force on their front limbs (Kleinhenz et al., 2018). This change was attributed to the calf shifting its weight to the front limbs, away from the castration site following the procedure, as the change in weight distribution was not seen in uncastrated controls.

The pressure mat system utilized to determine outcome variables in this study has been used in castration and lameness studies (Kotschwar et al., 2009; Schulz et al., 2011; Coetzee et al., 2014; Kleinhenz et al., 2018). Additionally, the technology used in the current study is similar to that used for the label claim for pain control by transdermal flunixin meglumine (US FDA, 2017). In the pivotal approval study, flunixin-treated calves applied more force on the affected foot while walking across the mat after 8 h compared to placebo control. Thus, pressure mat gait analysis is considered a validated pain measure in cattle.

Cows in the MEL group had shorter gait lengths compared to PLBO group. An explanation of this observation may be that meloxicam lowered the pain associated with udder edema associated with calving. Meloxicam has been shown to improve pain sensitivity of the mammary gland of cows

Figure 1. Mean gait (cm) for Holstein cows given meloxicam (1.1 mg/kg) within 26 h of parturition (MEL) or placebo within 26 h of parturition (PLBO). * $P \leq 0.05$.

Table 1. Summary of pressure mat outcomes (LS mean $\pm$ SEM) for Holstein cows given meloxicam (1.1 mg/kg) within 26 h of parturition (MEL) or placebo within 26 h of parturition (PLBO)

Parameter	LS mean ¹ $\pm$ SEM		P-value		
	MEL	PLBO	Treatment	Time	Treatment $\times$ time
Force rear limbs (kg)	393.4 $\pm$ 31.4	331.3 $\pm$ 31.4	0.15	0.95	0.25
% Force on rear limbs	48.9 ^a $\pm$ 0.7	46.3 ^b $\pm$ 0.7	0.02	0.08	0.81
Contact pressure rear limbs (kg/cm ²)	7.1 $\pm$ 0.6	6.2 $\pm$ 0.6	0.27	0.97	0.23
% Contact pressure on rear limbs	49.5 $\pm$ 0.1	49.8 $\pm$ 0.1	0.17	0.16	0.65
Impulse rear limbs (kg $\times$ s)	252.7 $\pm$ 19.6	213.0 $\pm$ 19.6	0.11	0.39	0.12
% Impulse on rear limbs	50.5 ^a $\pm$ 0.9	46.7 ^b $\pm$ 0.9	0.01	0.15	0.52
Gait (cm)	90.9 ^a $\pm$ 2.5	101.4 ^b $\pm$ 2.5	0.03	0.78	0.31

^{a,b}Superscripts with different letters are significant ($P \leq 0.05$).

¹Least square means.

with induced endotoxic mastitis (Fitzpatrick et al., 2013). Furthermore, cows with induced mastitis have been shown to avoid weight shifting (Chapinal et al., 2013). The authors speculate it is in an effort to decrease friction rubbing between the leg and inflamed udder. The longer observed gait observed in the PLBO cows may be due to exaggerated steps to minimize leg-udder friction and contact.

Meloxicam and sodium salicylate have been shown to significantly increase milk production over the entire lactation when given following calving (Carpenter et al., 2016). These data make the administration of meloxicam an enticing way to increase production with a single intervention. Neither meloxicam nor salicylate are approved by the US FDA for use in lactating dairy cows and thus constitute an extra-label drug use (ELDU). However, ELDU would be restricted to relieving pain and suffering under the Animal Medicinal Drug Use Clarification Act (AMDUCA) and not to enhancement of production (US FDA, 1996).

Studies investigating meloxicam administration on cow behavior in the postpartum period have yielded equivocal results (Newby et al., 2013; Mainau et al., 2014). Timing of meloxicam administration was similar to the current study, but route of administration and dose was different. The study presented here used oral administration at 1 mg/kg compared to 0.5 mg/kg by subcutaneous injection in the previously referenced studies. In cattle, oral meloxicam given at 1.0 mg/kg has 100% bioavailability, and reaches a maximum concentration of 3.1 µg/mL (Coetzee et al., 2009).

The data presented here are the first to characterize the gait of cows following eutocia using floor-based pressure mat analysis technology. The changes in weight distribution when measured by pressure mat analysis show meloxicam may be beneficial to cows immediately following calving. Further research is needed to elicit details on pain associated with normal parturition and the role NSAID therapy may have in cows during the immediate postpartum period.

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