Short communication: A comparison of 2 nonsteroidal antiinflammatory drugs following the first stage of a 2-stage fistulation surgery in dry dairy cows

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ABSTRACT

Postoperative pain and its management following fistulation surgery in cattle are poorly understood. The purpose of this study was to compare 2 nonsteroidal antiinflammatory drugs (NSAID) as potential postoperative pain management treatments following the first stage of a 2-stage fistulation surgery. A randomized complete block design trial was conducted in dry Holstein cows ($n = 10$) following fistulation surgery. Ketoprofen (3 mg/kg of body weight i.m.) was administered on the day of surgery and 24 h later, whereas meloxicam (0.5 mg/kg of body weight s.c.) was administered once only on the day of surgery. Outcomes evaluated at 0, 2, 9, 24, 26, and 33 h postsurgery were heart rate, respiration rate, rectal temperature, and infrared temperature around the surgical site. Outcomes evaluated on the day of surgery and d 1 following surgery and compared with the average for the 4 d before surgery were lying activity (total lying time, total time spent lying on the left side, and percentage of time lying on the left side) and feed intake. A difference was observed in dry matter intake on d 1 but this effect was not different on d 0 compared with presurgical averages. A difference was observed in time spent lying on the left side and a difference was observed in heart rate following the first stage of fistulation surgery compared with presurgical averages. The infrared temperature readings around the surgical site were significantly greater in the hours following surgery compared with presurgical averages. The respiration rate increased over time after 24 h postsurgery compared with presurgical values. Although it was clear that the surgery is painful, the drug effects were more difficult to explain. On d 0 and 1, the meloxicam-treated cows ate 3 kg more but spent 101 min/d less time lying on their left

side compared with ketoprofen-treated cows. The first stage of a 2-stage fistulation surgery was considered painful based on changes in heart rate, respiration rate, infrared temperature readings, dry matter intake, and time spent lying on the left side. It is clear that left flank surgery is painful and that NSAID can improve outcomes associated with that pain, but we cannot make recommendations as to which NSAID to choose based on these results.

Key words: ketoprofen, meloxicam, pain, welfare

Short Communication

One approach to placement of a permanent rumen fistula involves a 2-stage surgery. First, a left-flank laparotomy is performed under local anesthesia while clamping a portion of the rumen outside of the body through an incision. One week later, after the body wall has adhered to the rumen and the clamped part of the rumen has been necrotized and removed, a rubber cannula is fitted into the fistula. It has been reported that laparotomy in humans is a procedure that requires pain management (Luks et al., 1999; Nicholson and Tiruchelvam, 2001; Rosen et al., 2001). In a previous study by Newby (2012), the effects of ketoprofen versus saline were evaluated following the first stage of a 2-stage fistulation surgery in lactating dairy cows. It was found that the surgery itself proved to be painful based on a significant decrease in DMI, milk production, and time spent lying on the surgical side (the left side), and a significant increase in heart rate, respiration rate, infrared temperature readings around the surgical site, as well as serum haptoglobin concentration. The administration of a label dose of ketoprofen once following surgery on d 0, and once 24 h later provided some beneficial analgesic effects to the animals. Ketoprofen-treated animals spent significantly more time lying on the left side on d 0 and 1, had significantly less tail-flicking behavior on d 1, and had a tendency toward higher milk production following surgery compared with control

Received January 15, 2013.

Accepted May 16, 2013.

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animals. However, even though the effects of postsurgical pain were not completely alleviated by ketoprofen, it is clear that analgesia is required following the first stage of a 2-stage fistulation surgery. Because nutritionist researchers at the University of Guelph required 10 fistulated cows in their last trimester of gestation, an opportunity existed to compare 2 nonsteroidal antiinflammatory drugs (NSAID) following surgery and provide a follow-up study to the previous fistulation study by Newby (2012).

The use of NSAID for pain relief, and to reduce inflammation as well as prostaglandin synthesis, has been successfully demonstrated after surgery in rodents (Pairet and Ruckebusch, 1989; de Winter et al., 1998). Nonsteroidal antiinflammatory drug therapy has also been used to alleviate signs of visceral pain in cattle (Constable et al., 1997). Roughan and Flecknell (2003) evaluated the analgesic effects of meloxicam or carprofen compared with saline administered 1 h preoperatively following a midline laparotomy in rats. They concluded that a dose of 1 to 2 mg/kg of meloxicam or any dose of carprofen effectively reduced the pain behavior (e.g., back arching, fall/stagger, twitch, writhing, and grooming) compared with the 0.5 mg/kg meloxicam and saline groups.

The plasma half-life of ketoprofen given intramuscularly is 2 h and 80% of the dose is eliminated in the urine within 24 h of administration (Merial-Canada, 2002), whereas the plasma half-life of meloxicam given subcutaneously is 23 to 27 h for low-milk-yield cows and 17.5 h for high-milk-yield cows (EMEA, 2007). It is hypothesized that a longer-lasting NSAID, approved for use in cattle in Canada (Compendium of Veterinary Products, 2012), such as meloxicam, would further alleviate postsurgical pain compared with a shorter-lasting one. Thus, the purpose of this study was to compare a label dose of 3 mg of ketoprofen/kg administered on d 0 and 1 following the first stage of a 2-stage fistulation surgery to a label dose of 0.5 mg/kg dose of meloxicam administered once only on d 0 in dry Holstein cows.

This study was conducted at the Elora Dairy Research Centre at the University of Guelph (Guelph, Ontario, Canada) and was approved by the University's Animal Care Committee (Animal Utilization Protocol no. 11R093). Cows were housed in individual tie-stalls (2.0 × 1.2 m; bedding of wood shavings over a pasture mat) with feed dividers to allow for feed intake recording 4 d before surgery. Cow pairs were then moved to individual pens (3.5 × 3.1 m; straw pack bedding) on the day of the first stage of the fistulation surgery and remained in the pen for the week following the first stage. Because of space restrictions within the barn, the cows were then moved to a second set of tie-stalls following the second stage (cannula placement) for 1

wk before returning to their original designated tie-stall. Cows were fed twice daily at 0730 and 1300 h according to the routine feeding procedure at the Elora Dairy Research Centre, except for the day of surgery when the morning feeding was delayed in the morning until after surgery completion. Diets were fed as a TMR and contained haylage, corn silage, and hay for the forage base, and high-moisture corn, protein, and mineral supplement for the concentrate. Samples of the diet were collected twice weekly and frozen at -20°C for later analysis. Dry matter intake calculations were based on the amount offered,orts, and DM analysis of sampled diets. All clinical health events and treatments that may have occurred outside the surgery were recorded for each cow enrolled in the study. To administer NSAID treatments, all cows' weights were estimated by the veterinarian upon enrollment in the study and the mean estimated weight ($\pm$ SD) for the ketoprofen group was 713 ($\pm$ 56) kg, and for the meloxicam group was 632 ($\pm$ 33) kg. The estimate for the weight averages on day of surgery was 673 ($\pm$ 61) kg and the actual weight determined approximately 37 ($\pm$ 21) d after surgery was 673 ($\pm$ 27) kg.

A randomized clinical trial was conducted with 10 healthy dry Holstein cows, at the end of their first lactation, in their third trimester of gestation [on average, 53 ($\pm$ 12 SD) d before calving], and which underwent a 2-stage fistulation surgery (described elsewhere; Newby, 2012). The first stage consisted of a rumen clamp procedure. A sedative (xylazine; 0.015–0.02 mg/kg i.v.) was administered 30 min before surgery, followed by administration of a proximal paravertebral block with 2% lidocaine, followed by final skin preparation. A 20-cm vertical incision was made immediately behind the rib cage and approximately 40 to 50 cm down from the vertebrae. The rumen was pulled through this opening and clamped in a wooden clamp and secured with 6 vertical mattress sutures in the skin. Before releasing the cow from the head-gate, all cows received a dose of penicillin given intramuscularly (20,000 IU/kg) and were given their randomly assigned treatment. Enrolled cows were assigned in pairs based on similar lactation and their expected calving due date (mean due date difference $\pm$ SD = 29 ± 27 d) each week. Cows within each pair were randomly assigned to 1 of 2 treatments: ketoprofen or meloxicam. The ketoprofen group received 3 mg of ketoprofen/kg of BW as per label instructions (Anafen; Merial Canada Inc., Baie d'Urfé, Québec, Canada) intramuscularly at the time of surgery completion (d 0), and 24 h following surgery. The meloxicam group received 0.5 mg of meloxicam/kg of BW (Metacam; Boehringer Ingelheim Canada Ltd., Burlington, Ontario, Canada) subcutaneously once as per product label instructions (EMEA, 2007) at the

time of surgery completion. The cows were monitored daily 4 d before surgery and postsurgical measurements included daily DMI (d 0 and 1), when the NSAID were either administered or are known to be effective. Measurements taken on d 0 before surgery, and at 2, 9, 24, 26, and 33 h postsurgery included physiological outcomes (heart rate, respiration rate, and rectal temperature), infrared temperature readings at 4 points around the surgical site (top, bottom, and right and left of the surgical site) and at a control point (at the edge of the shaved area by the L2 and L3 transverse processes) using a digital temperature reader (Mastercraft; Canadian Tire Corp. Ltd., Guelph, Ontario, Canada) held 20 cm away from the skin. All cows were fitted with a 3-axis accelerometer (Hobo Pendant G logger; Hoskin Scientific Ltd., Burnaby, British Columbia, Canada; Ledgerwood et al., 2010) attached to the right hind leg and recording at 1-min intervals for 4 d before surgery and on d 0 and 1 postsurgery. Days started at 0500 h. Total daily lying time (the sum of time spent lying both right and left from the raw data) and time spent lying on the left side were calculated for each cow from the data collected from the accelerometer. The data output from the accelerometer was manipulated as described in Ledgerwood et al. (2010) for the 60-s sampling interval using a 1-event filter for potentially erroneous readings of lying or standing events. The time for d 0 started following surgery completion and lasted until 24 h postsurgery and for d 1 started at 24 h postsurgery and lasted until 48 h after surgery completion. None of the cows sustained illnesses or required additional treatment as a result of the surgery over the next 14 d.

All descriptive statistics, model building, and analyses were performed with Stata Intercooled 10.1 software (StataCorp, College Station, TX). Mixed multivariable models were built for each of the following outcomes: DMI, heart rate, respiration rate, rectal temperature, infrared temperature, and lying behavior (total time spent lying on the left side, percentage of time lying on the left side, and total daily lying time). All models included a random intercept for animals to account for multiple measurements being taken from each cow. All tests were 2-sided and significance was based on $\alpha < 0.05$. For the DMI models, cow weight was used as a covariate and kept in the model if it was significant. For all models, interactions between treatment and any significant covariates in the final model were tested. Two types of mixed linear regression models were built for each outcome. The first set of models (surgical effect) was designed to test for the effects of the first stage of surgery on the outcome. Time was modeled as a categorical variable and the presurgical values category was the referent, except for the heart rate model, where time was modeled as a continuous variable because it

met the linearity assumptions. The second set of models (treatment effect) was designed to test for the effect of treatment administered on d 0 and d 1. As such, the time was included as a categorical variable for d 0 and 1 only for the DMI and lying behavior models, and as a categorical variable for d 0 presurgery and 2, 9, 24, 26, and 33 h postsurgery for the respiration rate and rectal temperature models, and as continuous for the heart rate model. Treatment was controlled in each model and the presurgical average values (4 d before surgery for the DMI and lying behavior, and d-0 presurgical values for the other physiological measures) were included as a covariate in the models. For the infrared readings, the 4 reading positions were tested individually against the control position to determine which readings were significantly different from the control. Only the positions that were significantly different from the control position were averaged and used as the infrared reading outcome and these included the bottom, right, and left readings around the surgical site. We examined the standardized residuals to identify outliers at the observation level and BLUP for any outliers at the cow level. Normality and homogeneity of variance were assessed for the observation-level standardized residuals as well as the BLUP.

The acute effects of the first stage of a 2-stage fistulation surgery resulted in a significant decrease in DMI on d 1 (Table 1 and Figure 1) and in a decrease in time spent lying on the surgical side (left side) following surgery (Table 1 and Figure 2), suggesting post-operative pain in these animals. The negative effects of the surgery on DMI and time spent lying on the left side were similar to those reported previously for this surgery (Newby, 2012). Interestingly, the DMI on d 0 was not significantly different compared with the presurgical average (Table 1 and Figure 1), suggesting that a beneficial effect of NSAID administration on d 0 may have existed. Cows treated with the longer-lasting meloxicam had a higher DMI compared with ketoprofen-treated animals on d 0 and d 1 (Table 1 and Figure 1). These results are in agreement with a suite of evidence that NSAID attenuate reduction in feed intake associated with both painful procedures [dehorning (Heinrich et al., 2010); LDA (Newby et al., 2013)] and illness (neonatal calf diarrhea complex; Todd et al., 2010).

Although DMI indicates that meloxicam was more effective, animals treated with ketoprofen had a tendency to spend more time lying on their left side, indicating they were more willing to lie on the surgical side (Table 1 and Figure 2). These results highlight how drugs may differentially affect indicators of pain (e.g., meloxicam had a positive effect on DMI and ketoprofen on willingness to lie on the wound). A possible explanation

Figure 1. Daily mean DMI (kg; $\pm$ SE) by treatment group around the first stage of fistulation surgery (laparotomy and clamp placement of the rumen; $n = 10$). Presurgical average was the average of the DMI for 4 d presurgery, d 0 was the day of surgery and the first injection day, and d 1 was the day of the second injection for the ketoprofen group only. * indicates significant difference for that day from presurgical average values ($P < 0.05$) and the letters (a and b) indicate a significant difference between treatment groups on d 0 and 1 ($P < 0.05$).

for lower lying time on the left side in the meloxicam group could be that these cows spent more time standing and eating compared with the ketoprofen-treated cows. However, no differences were observed in the percentage of time spent lying on the left side between treatment groups [model coefficient (β) = -1.93% ; $P = 0.78$; 95% CI: -15.70 to 11.84 ; Figure 3], nor were significant differences observed in the total daily lying time between treatment groups (meloxicam vs. ketoprofen: $\beta = -182.8$ min/d; 95% CI: -389.9 to 24.3 ; $P = 0.08$; mean $\pm$ SD: presurgery = 845 ± 89 min/d; d 0 = 588 ± 250 min/d; d 1 = 827 ± 129 min/d). One cow

Figure 2. Mean time spent lying on the left side (min/d; $\pm$ SE) by treatment group around the first stage of fistulation surgery (laparotomy and clamp placement of the rumen; $n = 10$). Presurgical average was the average of the time spent lying on the left side for 4 d presurgery, d 0 was the day of surgery and the first injection day, and d 1 was the day of the second injection for the ketoprofen group only. * indicates significant difference from the presurgical average ($P < 0.05$) and the letters (a and b) indicate a tendency between treatment groups on d 0 and 1 ($P = 0.05$).

was found to be an outlier and had a standing time of 22.2 h. Although we do not believe this was a recording error, it was a highly unusual value for this population of cows. We noted that if this cow was removed from the analysis, the effect of total daily lying time between treatment groups became smaller and the P -value increased (meloxicam group: $\beta = -138.3$ min/d; $P = 0.21$; 95% CI: -352.9 to 76.2). Further investigation with an increased sample size would be required to compare the time budget between cows treated with meloxicam or ketoprofen following surgery.

In the present study, significant increases following surgery were observed in heart rate (HR) and in the infrared temperature (IRT) reading compared with

Table 1. Model coefficient β , 95% CI, and P -value for the variables of interest of either the surgical effect model or the treatment effect model for the different outcomes (DMI and time spent lying on the left side) for 10 Holstein cows assigned to receive ketoprofen (3 mg/kg of BW) by intramuscular injection at the time of surgery (d 0) and 24 h later (d 1), or to receive meloxicam (0.5 mg/kg of BW) by subcutaneous injection at the time of surgery

Model	Outcome	Variable	β	95% CI	P -value
Surgical effect ¹	DMI (kg)	d 0	-1.5	-3.6 to -0.60	0.16
		d 1	-2.6	-4.7 to -0.54	0.01
	Time spent lying on the left side (min/d)	d 0	-249.9	-340.9 to -159.0	<0.001
		d 1	-195.8	-286.8 to -104.9	<0.001
Treatment effect ^{1,2}	DMI (kg)	Meloxicam	3.0	0.04 to 6.0	0.05
	Time spent lying on the left side (min/d)	Meloxicam	-100.9	-202.8 to 1.1	0.05

¹While controlling for treatment and time as a categorical variable in all models, and for BW in the DMI model; the presurgical values category was the referent for the time variable.

²While controlling for presurgical values as a covariate; ketoprofen was the referent for the treatment variable.

Table 2. Model coefficient β , 95% CI, and *P*-value for the variables of interest of the surgical effect model for the physiological outcomes (heart rate, respiration rate, and infrared temperature) for 10 Holstein cows assigned to receive ketoprofen (3 mg/kg of BW) by intramuscular injection at the time of surgery (d 0) and 24 h later (d 1), or to receive meloxicam (0.5 mg/kg of BW) by subcutaneous injection at the time of surgery¹

Model	Outcome	Variable	β	95% CI	<i>P</i> -value
Surgical effect ²	Heart rate (beats/min)	Time	0.38	0.21 to 0.55	<0.001
		2 h	-3.6	-8.5 to 1.3	0.15
	Respiration rate (breaths/min)	9 h	2.4	-2.5 to 7.3	0.34
		24 h	5.2	0.32 to 10.1	0.04
		26 h	8.4	3.5 to 13.3	0.001
		33 h	8.6	3.7 to 13.5	0.001
	Infrared temperature (°C)	2 h	3.6	2.9 to 4.2	<0.001
		9 h	1.9	1.2 to 2.5	<0.001
		24 h	2.3	1.6 to 3.0	<0.001
		26 h	2.7	2.0 to 3.4	<0.001
		33 h	2.7	2.1 to 3.4	<0.001

¹The treatment effect model is not shown because no significant treatment effects were observed.

²While controlling for treatment and time as a categorical variable, except for the heart rate model where time was modeled as a continuous variable; presurgical values were the referent for the categorical time variable for the respiration rate and infrared temperature models.

presurgical values (Table 2). The respiration rate (**RR**) significantly increased, but only after 24 h following surgery (Table 2), possibly because, by this time, the anesthetic effects of the nerve blocks had completely disappeared. However, no differences were found between treatment groups for any of these outcomes. These physiological changes were also observed in the fistulation study comparing saline and ketoprofen (Newby, 2012) and indicate that the NSAID and dos-

ages used in this study may not have been adequate to alleviate pain based on HR, RR, and IRT as indicators.

In conclusion, the first stage of a 2-stage fistulation surgery was considered painful due to the decreases in DMI and in time spent lying on the left side, as well as the increases in HR and RR and IRT around the surgical sites following deliberate tissue damage associated with a laparotomy. This study compared ketoprofen with meloxicam following surgery, 2 NSAID approved for use in cattle in Canada (Compendium of Veterinary Products, 2012). Meloxicam-treated animals ate more than ketoprofen-treated animals following the first stage of the procedure but ketoprofen-treated animals spent more time lying on the left side compared with meloxicam-treated animals. There seem to be beneficial effects of meloxicam on DMI. The reduction of the decrease in feed intake following surgery seen in the meloxicam group may have some implications when it comes to reducing metabolic stress and decreased risk of developing ketosis, although no animals became ill in the current work. Beneficial effects of ketoprofen also seem to exist on time spent lying on the surgical side in the days following surgery. The differences between these 2 effects are difficult to explain. Further research is needed to determine the appropriate dosage of NSAID required for sufficient pain relief. However, at this point in time, no recommendation can be made to choose between ketoprofen and meloxicam following the first stage of a 2-stage fistulation surgery.

Figure 3. Mean percentage ($\pm$ SE) of time spent lying on the left side when lying down by treatment group around the first stage of fistulation surgery (laparotomy and clamp placement of the rumen; $n = 10$). Presurgical average was the average of the percentage of time lying on the left side for 4 d presurgery, d 0 was the day of surgery and the first injection day, and d 1 was the day of the second injection for the ketoprofen group only. * indicates significant difference from the presurgical average ($P < 0.05$).

ACKNOWLEDGMENTS

The authors thank the Elora Dairy Research Centre (Guelph, Ontario, Canada) staff for their help during this project. Funding for this study was generously provided by the Ontario Ministry of Agriculture and Rural

Affairs (Guelph, Ontario, Canada) and by a Natural Sciences and Engineering Research Council (Ottawa, Ontario, Canada) Industrial Post-Graduate Scholarship II.

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