Effect of timing of subcutaneous meloxicam administration on indicators of pain after knife castration of weaned calves¹

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ABSTRACT: The newly revised Canadian Codes of Practice for the management of beef cattle requires that as of 2018, calves older than 6 mo of age be castrated using pain control. Castration is a husbandry procedure commonly done without pain control, and there is a lack of agreement on an effective pain mitigation strategy specific to castration. The aim of this study was to identify the optimal time of administration of meloxicam prior to castration. Thirty-four Angus and Angus crossbred bull calves (282 ± 28.0 kg BW) were randomly assigned to 1 of 3 treatments receiving a single s.c. injection of meloxicam (0.5 mg/kg BW): 6 h (6H; $n = 11$), 3 h (3H; $n = 12$), or immediately (0H; $n = 11$) before knife castration. Measurements included visual analog scale (VAS), head movement (HM), accelerometer movement (AM) and strain gauge exertion force (EF) on the squeeze chute, stride length (SL), lying and standing behavior, salivary cortisol (SC), haptoglobin, serum amyloid A (SAA), substance P (SP), and scrotal temperature (ST). Samples were collected on d -7, -5, -2, -1, and immediately before castration (T0) and 30, 60, 120, and 240 min and 1, 2, 5, 7, 14, 21, and 28 d after castration, except for VAS, AM, EF, and HM, which were obtained at the

time of castration. A time $\times$ treatment effect ($P = 0.01$) was observed for SP, where 0H had lower concentrations than 3H and 6H calves 1 d after castration, whereas 3H calves tended to have greater levels than 6H calves 5 d after castration. Mean ST was greater ($P < 0.01$) in 6H calves compared to 0H and 3H calves 120 min after castration, whereas 6H and 3H calves had greater ST compared to 0H calves 240 min after castration. On d 1 after castration, 6H calves had greater ST than 0H and 3H calves, whereas 0H calves had greater ST compared to 3H and 6H calves on d 28 after castration. The SL tended ($P = 0.09$) to be shorter in 3H and 6H calves than 0H calves 30, 60, 120, and 240 min after castration. Number of peaks from the AM between 2 and 3 SD above or below the mean were greater ($P = 0.03$) in 3H and 6H calves than in 0H calves. No treatment differences ($P > 0.10$) were observed for the number of peaks and area for AM and EF, VAS, HM, SC, or haptoglobin. On the basis of these results, the optimal time to administer s.c. meloxicam in 7- to 8-mo-old knife-castrated calves is immediately before castration (0H), as evidenced by fewer indicators of pain and inflammation compared to 3H and 6H calves.

Key words: beef, behavior, castration, meloxicam, pain, physiology

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INTRODUCTION

In western Canada, approximately 1% of ranch calves are castrated at 6 mo of age or older (Moggy et al., 2017). However, in the United States bulls arriving at feedlots account for approximately 0.9%, and the majority (91.2%) of feedlots castrate these animals (USDA, 2013). Castration at weaning without pain control is an animal welfare issue as older animals show greater behavioral and physiological changes associated with pain and distress (Bretschneider, 2005). As of January 2018 the Canadian Beef Codes of Practice (National Farm Animal Care Council, 2013) requires pain control for castrating calves older than 6 mo of age (Schwartzkopf-Genswein et al., 2012); however, no standard pain mitigation protocols have been established.

An effective way to manage the pain of castration could be to provide preventive analgesia. Meloxicam is a nonsteroidal anti-inflammatory drug and an attractive analgesic option for postoperative pain in livestock because of the ease of administration (s.c.) and long-lasting effect (half-life of 22 h; Stock and Coetzee, 2015). Oral meloxicam has been shown to reduce the acute inflammatory response in yearling bulls after surgical castration (Roberts et al., 2015). The s.c. route of administration may be more practical than oral or i.v. administration, but to our knowledge there are no studies examining when s.c. meloxicam should be administered relative to castration to produce optimal analgesia. A pharmacokinetic study showed that peak plasma levels of meloxicam are achieved 12 h after oral administration (Coetzee et al., 2009).

Therefore, the objective of this study was to compare the effect of preemptive meloxicam given s.c. at 3 time points prior to surgical castration on indicators of pain associated with inflammation in 7- to 8-mo-old beef calves. We hypothesize that the administration of s.c. meloxicam 6 h prior to castration would be more effective at reducing pain indicators after castration compared to 3 and 0 h prior to castration.

MATERIALS AND METHODS

This protocol was approved by the Lethbridge Research Centre Animal Care Committee (ACC number 1428); all animals were cared for in accordance with the Canadian Council of Animal Care guidelines (Canada Council of Animal Care, 2009).

Animal Housing and Management

Thirty-four 7- to 8-mo-old Angus and Angus crossbred bull calves (282 ± 28.0 kg BW) were used in a 28-d experiment. Cows and calves were transported separately to the Agriculture and Agri-Food Canada Lethbridge

Research Centre (LRC; Lethbridge, AB, Canada) during the first week after birth. Calves used in the present experiment were control calves from a previous castration experiment. Prior to weaning, calves were housed in a 76.9-ha pasture adjacent to the LRC feedlot facility with a perennial grass pasture consisting of brome grass, orchard and creeping red fescue, and annual cereal crops, including oats and spring and fall triticale. Calves were weaned at 7 to 8 mo of age, 3 wk prior to the start of the trial, to adapt the calves to the experimental pens. Calves were vaccinated with a 7-way clostridial vaccine (Ultrabac/Somubac, Zoetis Canada Inc., Kirkland, QC, Canada) and housed in 2 experimental pens (40.2×27.4 m) with a 24.4×2.45 m concrete apron in front of the feed bunk. Calves were fed once a day at 0900 h a total mixed ration consisting of 80% barley silage, 17% dry-rolled barley, and 3% supplement with vitamins and minerals to meet beef cattle requirements (NRC, 2016). Water was available ad libitum in a centrally located trough ($80 \times 40 \times 50$ cm). Animal weights on d -1 were used to equally distribute animals across pens and treatments, and calves were randomly assigned to 1 of 3 treatments receiving a single s.c. injection of meloxicam (0.5 mg/kg BW): 6 h (6H; $n = 11$), 3 h (3H; $n = 12$), or immediately (0H; $n = 11$) before surgical castration.

Calves were restrained in a hydraulic squeeze chute (Cattlelac Cattle, Reg Cox Feedmixers Ltd, Lethbridge, AB, Canada), where they were sampled and castrated. To avoid castrating calves at different time points during the day, 6H calves and 3H calves were run through the chute 6 h and 3 h prior to castration to administer the corresponding s.c. meloxicam dose with a 16-gauge, 1-inch needle in the neck of the calves, whereas the 0H group received s.c. meloxicam immediately before castration. The same veterinarian performed surgical castration on all the calves by making a latero-lateral incision on the scrotum with a Newberry castration knife (Syrvet Inc., Waukegan, IA). Testicles were then externalized one at a time from the scrotum, and an emasculator was used to crush and cut the spermatic cords.

Physiological Parameters

Blood Samples. Blood samples were collected via jugular venipuncture into vacuum tubes (BD Vacutainer; Becton Dickinson, Franklin Lakes, NJ) on d -7, -5, -2, and -1; immediately before castration (T0); and 30, 60, 120, and 240 min and 1, 2, 5, 7, 14, 21, and 28 d after castration. Additional samples were collected 60 and 240 min and on d 1, 2, and 5 after castration to determine plasma concentrations of meloxicam. These samples were collected into 10-mL lithium heparin tubes (BD Vacutainer; Becton Dickinson) and centrifuged at 0 °C for 15 min at $1.5 \times g$, and the serum

was decanted and stored at -80°C for further analysis using high-pressure liquid chromatography (Agilent 1100 Pump, Column Compartment, and Autosampler, Santa Clara, CA) with mass spectrometry detection (LTQ, Thermo Scientific, San Jose, CA) at Iowa State University's College of Veterinary Medicine (Ames, IA). The limit of quantitation of the analysis was 1.0 ng/mL, with a limit of detection of 0.3 ng/mL.

Samples to determine concentrations of substance P (SP) were collected on d -1, at T0, and at 30, 60, 120, and 240 min and on d 1, 2, 5, 7, 14, 21, and 28 into a 6-mL vacuum tube with EDTA (BD Vacutainer; Becton Dickinson). The sample was then centrifuged at 0°C for 15 min at $1.5 \times g$, and the serum was decanted and frozen at -80°C for further SP analysis, which was previously described by Van Engen et al. (2014) with some modifications at Iowa State University's College of Veterinary Medicine (Ames, IA). Nonextracted plasma samples were analyzed in duplicate with a double-antibody RIA using a purchased primary antibody (Substance-P (3-11) Antibody for Immunohistochemistry Human, Rat, Mouse, #H-061-05, Phoenix Pharmaceutical, Burlingame, CA). The range of detection for SP was between 5 and 320 pg/mL, with an average $R^2 = 0.98$. The coefficient of variation for intra-assay variability was 8.2%, and the interassay variability was calculated at 20.6%. The limit of detection was 10 pg/mL, and the limit of quantitation was 20 pg/mL.

Samples to determine complete blood cell count were collected on d -7, -5, -2, and -1; at T0; and at 30, 60, 120, and 240 min and on d 1, 2, 5, 7, 14, 21, and 28 after castration. The sample was collected in a 6-mL EDTA tube (BD vacutainer; Becton Dickinson) for complete blood count using a HemaTrueHematology Analyzer (Heska, Lobeland, CO), which measured white blood cells (WBC), red blood cells (RBC), platelet count, and the neutrophil:lymphocyte (N:L) ratio.

Blood samples to measure haptoglobin were collected on d -1, 1, 2, 5, 7, 14, 21, and 28, whereas serum amyloid A (SAA) was collected on d -1, 1, 2, 5, and 7. The sample was collected into 10-mL nonadditive tubes (BD Vacutainer; Becton Dickinson), which were centrifuged at 4°C for 15 min at $1.5 \times g$, and the serum was decanted and frozen at -80°C for further analysis. Haptoglobin concentrations were analyzed using a Roche Cobas c501 biochemistry analyzer (Roche Diagnostics, Laval, QC, Canada) using a Tridelta bovine haptoglobin calibrator (TP801CAL, Tridelta, Maynooth, Ireland) and 2 levels of in-house controls (bovine serum pools) daily and 2 levels of Tridelta controls weekly. Samples to determine SAA concentrations were collected on d -1, 1, 2, 5, and 7 and analyzed using an enzyme-linked immune sorbent assay (Tridelta Phase range SAA kit, TP 807, Tridelta Development, Maynooth, Ireland).

Salivary Cortisol. Saliva samples to determine cortisol concentration were collected on d -7, -5, -2, and -1 before castration; at T0; and at 30, 60, 120, and 240 min and 1, 2, 5, 7, 14, 21, and 28 d after castration. Samples were collected with a cotton swab and immediately stored in a plastic tube and frozen at -20°C for further cortisol analysis using an enzyme immunoassay kit (Salimetrics, State College, PA). Interassay and intra-assay variability values were 14.1% and 5.9%, respectively.

Hair Cortisol. Hair from the forehead was clipped and stored in plastic bags at room temperature on d -1 and on d 28 after castration for further cortisol analysis. Hair samples were processed as described in detail by Moya et al. (2014), and cortisol was quantified using an enzyme-linked immunosorbent assay (Salimetrics). Values for interassay and intra-assay variability were 8.3% and 5.8%, respectively.

Scrotal Temperature. Thermographic images of the scrotal area were collected on d -7, -5, and -2; at T0; and at 30, 60, 120, 240 min and on d 1, 2, 5, 7, 14, 21, and 28 after castration. Images were taken from behind the calves 1 m from the scrotal area using a FLIR I60 infrared camera (FLIR Systems, Burlington, ON, Canada) and analyzed with FLIR Tools version 5.1 (FLIR Systems) using an emissivity coefficient of 0.98 to measure scrotal temperature (ST).

Rectal Temperature. Rectal temperature was measured on d -7, -5, -2, -1, 0, 1, 2, 5, 7, 14, 21, and 28 using a digital thermometer (GLA Agricultural Electronics, M750 Livestock Thermometer, San Luis Obispo, CA).

Performance. Animals were weighed on d -1, 1, 2, 5, 7, 14, 21, and 28 in the squeeze chute. Initial BW was the weight collected on d -1, and the final BW was the weight collected on d 28.

Lesion Score. Scrotal swelling was evaluated using an 11-point scale as described by Molony et al. (1995) and then collapsed into a 5-score scale. The scores were as follows: 4, presence of suppurative discharge with inflammatory response that required intervention; 3, presence of pus with increasing inflammatory response; 2, increasing degree of swelling with obvious erythema but without pus; 1, increasing degree of swelling without obvious erythema; and 0, no swelling, inflammation, or infection visible or palpable.

Behavioral Parameters

Behavior Scored and Observed during Castration. The visual analog scale (VAS) consisted of a 10-cm line (the far right indicating extreme pain and the far left indicating no pain) in which 2 experienced observers marked their perception of the amount of pain the calf was exhibiting during castration. The distance

Table 1. Ethogram of behaviors recorded¹

Behavior	Definition
Eating	When the head of the calf was in the feeder
Lying	Either lateral (lying with hip and shoulder on the ground with at least 3 limbs extended) or ventral (lying in sternal recumbency with legs folded under the body or 1 hind or front leg extended) lying
Walking	Walking forward more than 2 steps
Standing	Standing on all 4 legs
Foot stamping	Hind legs are lifted and forcefully placed on the ground or kicked outward while standing; 1 leg lifted and 1 lowering was counted as 1
Head turning	When the head is turned and touches the side of the calf's body, including head turning to groom
Tail flicking	Forceful tail movement beyond the widest part of the rump; movement to 1 side is counted as 1 action
Kneeling	Front legs are bent on the ground while back legs are extended

¹Modified from Molony et al. (1995).

from the start of the line to the mark was measured to the nearest 0.5 cm and was used as an indicator of pain response to castration. In addition, the frequency of urination, defecation, vocalization, and leg movement was recorded. Observers were blind to the treatments.

Head movement (HM) was recorded using a video camera placed in front and to the right side of the head gate to record HM during castration. Videos were imported into Kinovea (General Public License) version 2 motion tracking software, and the middle of the hairline of the muzzle was used as a reference point to track total HM distance.

In addition, the squeeze chute was equipped with three 1-dimensional accelerometers, each measuring a different movement (vertical, horizontal, and lateral), and the right and the left head gates were equipped as previously described by Schwartzkopf-Genswein et al. (1997) with 2 strain gauges to measure the force exerted when the animals pushed against or pulled away from the head gate. Baseline measurements were collected for 20 s for all calves on d -1 when animals were restrained in the squeeze chute and head gate. The forces from the 3 accelerometers were added to obtain the overall accelerometer force, and the forces measured from the right and left strain gauges were also added to obtain an overall force. The variables evaluated included number of peaks (defined as the change in direction of the analog signal) and total area (total area under peaks). Accelerometer and strain gauge forces were summarized to obtain the total number of peaks between 1 and 2 SD, 2 and 3 SD, and 3 SD above and below the mean, as well as total area above and below 1, 2, and 3 SD from the mean.

Stride Length. Calves were recorded walking along a 1 × 3 m alley on d -7, -5, -2, and -1; immediately after castration; and 30, 60, 120, 180, and 240 min after castration, as well as on d 1, 2, 5, and 7 and weekly thereafter until d 28. The software GOM Player (GOM Lab, Gretech Corporation, Seoul, South Korea) was then used to take 2 pictures of the hind legs when both feet were flat on the ground, 1 with the right foot in

front of the left and another picture with the left foot in front of the right. The distance between both hind feet was measured using a modified method previously described by Currah et al. (2009) using ImageJ (Bethesda, MD), and the average was used for further analysis.

Behavioral Observations in Experimental Pens.

Behavior was recorded using 2 Avigilon cameras (2.0MP HD IR Bullet Camera, Avigilon, Vancouver, BC, Canada) located on the north and south sides of the experimental pens mounted on 6-m poles. Numbered penning tags were placed on the back of the calves using tag cement (Livestock Identification tag cement, W.J. Ruscoe Company, Akron, OH) to identify calves. Two experienced observers did focal sampling from continuous recordings (Martin and Bateson, 2007) of standing, lying, walking, eating, tail flicking, foot stamping, and head turning (Table 1) using The Observer XT (Noldus Information Technology, Wageningen, The Netherlands). These behaviors were scored on the day of castration for a period of 3 h, between 4 and 7 h after castration, and during the same time of day on d 2 and 3 after castration for 6 calves per treatment. Because of technical problems the behavior for d 1 was not recorded. Interrater and intrarater reliabilities were 0.99 and 0.98 for the 2 experienced observers, who were blinded to treatments.

Standing and Lying Behavior. On d -7 calves were fitted with Hobo data loggers (Onset Computer Corporation, Bourne, MA) that were covered in plastic film to protect the device from rain and attached to the left hind leg immediately below the hocks with 10.2-cm-wide latex flexible cohesive bandage (Latex Flexible Cohesive Bandage, Professional Preference, Calgary, AB, Canada) to automatically measure the duration of lying and standing. Hobos were changed every 7 d and placed on the alternate hind limb. Information obtained from the Hobos was summarized into standing and lying percentage, mean standing and lying bout duration (min/d), and standing and lying bouts (number/d; University of British Columbia Animal Welfare Program, 2013).

Feeding Behavior. All calves were fitted with a radio-frequency ear tag, and each pen was equipped with a GrowSafe feed bunk monitoring system (GrowSafe Systems, Airdrie, AB, Canada) with 5 feeding tubs that recorded feeding behavior for each individual calf 24 h a day during a 28-d period. The GrowSafe system consists of 2 antennas located in the feed bunk that detect individual ear tags when calves are within 50 cm of the feed bunk and record feeding behavior information (Schwartzkopf-Genswein et al., 1999). The electronic monitoring system records feed intake (kg/d), bunk attendance frequency (visits/d), and bunk attendance duration (min/d), which can be used to calculate feeding time (min/d), DMI (kg/d), feeding rate (g/min), meal frequency (number/d), meal duration (min/meal), and meal size (kg/meal). For this study a meal criterion of 300 s was selected as it has been previously used for a wide range of cattle sizes (Sowell et al., 1998; Schwartzkopf-Genswein et al., 2003).

Statistical Analysis

A power analysis was conducted for the primary outcomes (salivary cortisol and stride length) with the SD of each parameter observed in a previous study under the same experimental conditions (Moya et al., 2014) and an α of 0.05 and a power of 0.08. The power analysis indicated that at least 7 to 11 calves per treatment were necessary to detect expected differences among treatments. MIXED MODELS (SAS, version 9.4, SAS Inst. Inc., Cary, NC) was used to assess the effects of time of meloxicam administration on all variables. The mean baseline for variables measured before castration was used as a covariate for variables measured after castration. Calves were the experimental unit as treatments were mixed within the pen. All parameters, except for VAS, HM, ADG, and initial and final BW, were analyzed using a mixed repeated measures model PROC MIXED (SAS, version 9.4, SAS Inst. Inc.). Covariance structures included compound symmetry, unstructured, and autoregressive order 1. Time and treatment were considered fixed effects and were tested for interactions, whereas pen and calves nested within pen were considered random effects. Physiological parameters were log transformed, percentage data were arcsine transformed, and behavioral parameters were square root transformed when data did not follow a normal distribution. Because of significant differences in baseline levels for SAA, the values for the different time points were subtracted from the baseline values. Significance was established at $P \leq 0.05$, and tendencies were $0.05 < P \leq 0.10$. When interactions were significant ($P \leq 0.05$), treatment means were separated with a post hoc test us-

Figure 1. Least squares means and SEM of scrotal temperature of immediately before and 30, 60, 120, and 240 min after surgical castration of Angus and Angus crossbred bulls receiving s.c. meloxicam 6, 3, and 0 h before castration. Least squares means with differing superscripts differ ($P \leq 0.05$).

ing the PDIF option in SAS. The least squares means were generated after adjusting for covariates, and normality was tested using PROC UNIVARIATE (SAS, version 9.4, SAS Inst. Inc.). The feeding information was collapsed from days to weeks for analysis. The days on which the different treatment groups presented a particular swelling score were analyzed using a Wilcoxon-Mann-Whitney test (SAS, version 9.4, SAS Inst. Inc.), and medians and 95% distribution-free confidence limits were calculated using the univariate procedure (SAS, version 9.4, SAS Inst. Inc.). IBM SPSS statistics for Windows, version 22.0 (IBM Corp., Armonk, NY) was used to calculate the intraclass correlation coefficient with a 95% CI to determine behavior interrater and intrarater reliabilities. Reliability was calculated by comparing scores of students and an experienced observer (interreliability) of eight 2-h-long videos and comparing scores of students of the same video but evaluated at different time points (intrareliability).

RESULTS

Day of Castration

Physiology. Meloxicam concentration presented a treatment $\times$ time interaction ($P < 0.01$), where 6H and 3H calves had greater meloxicam levels ($1,796 \pm 112.7$ and $1,572 \pm 112.7$ ng/mL, respectively) than 0H calves (858 ± 112.7 ng/mL) 60 min after castration, but no differences ($P \geq 0.10$) were seen between treatments at 240 min after castration (6H: $1,519 \pm 72.6$ ng/mL; 3H: $1,630 \pm 72.6$ ng/mL; 0H: $1,623 \pm 72.6$ ng/mL). No treatment or interaction effects ($P > 0.10$) were observed for SP or salivary cortisol concentrations 30, 60, 120, and 240 min after castration; however, there was an overall increase (time effect; $P < 0.01$) in SP and salivary cortisol concen-

Table 2. Least squares means ($\pm$ SEM) of samples collected immediately before castration and at 30, 60, 120, and 240 min after castration for meloxicam, substance P (SP), salivary cortisol, scrotal temperature (ST), visual analog scale (VAS), head movement (HM), accelerometer movement and strain gauge exertion force peak number and total area (TA), and stride length (SL) of surgically castrated Angus and Angus crossbred bulls receiving s.c. meloxicam 6, 3, and 0 h before castration¹

Item	Treatment (T)			SEM ²	<i>P</i> -value		
	0H	3H	6H		T	Time	T × time
Physiology							
Meloxicam, ³ ng/mL	1,241.2 ^b	1,601.5 ^a	1,657.7 ^a	0.07	<0.01	<0.01	<0.01
SP, pg/mL	125.7	129.7	129.6	0.04	0.74	<0.01	0.10
Salivary cortisol, nmol/L	5.3	5.4	5.4	0.12	0.98	<0.01	0.25
ST, °C	29.7 ^b	29.2 ^b	31.3 ^a	0.60	0.04	0.34	<0.01
Behavior							
VAS, ⁴ cm	2.7	2.5	3.3	0.08	0.09	—	—
Urination, <i>n</i>	1.0	1.0	1.0	0.02	0.99	—	—
Defecation, <i>n</i>	1.0	1.0	1.0	0.01	0.35	—	—
Leg movements, <i>n</i>	2.4	2.6	2.7	0.14	0.54	—	—
Vocalizations, <i>n</i>	1.0	1.0	1.1	0.07	0.18	—	—
HM, ⁵ cm	23.0	27.1	24.1	3.42	0.70	—	—
Accelerometers ⁶							
Peaks above and below the mean between 1 and 2 SD, <i>n</i>	1,698.5	2,722.0	2,001.8	9.50	0.19	—	—
Peaks above and below the mean between 2 and 3 SD, <i>n</i>	484.4 ^b	1,209.7 ^a	1,485.7 ^a	9.12	0.03	—	—
Peaks above and below the mean for 3 SD, <i>n</i>	413.4	1,759.1	1,420.8	7.38	0.07	—	—
TA above and below 1 SD, V × s	148.3	180.9	172.9	1.44	0.15	—	—
TA above and below 2 SD, V × s	27.1	43.0	44.0	0.94	0.41	—	—
TA above and below 3SD, V × s	21.0	26.5	24.9	0.74	0.58	—	—
Strain gauges ⁷							
Peaks above and below the mean between 1 and 2 SD, <i>n</i>	4,000.3	2,451.1	3,591.8	9.20	0.58	—	—
Peaks above and below the mean between 2 and 3 SD, <i>n</i>	2,015.7	1,401.9	1,572.7	7.79	0.75	—	—
Peaks above and below the mean for 3 SD, <i>n</i>	2,930.5	3,409.5	1,237.6	13.3	0.23	—	—
TA above and below 1 SD, V × s	1,672.7	1,486.8	1,094.8	4.28	0.50	—	—
TA above and below 2 SD, V × s	589.4	861.7	442.6	5.18	0.47	—	—
TA above and below 3 SD, V × s	301.1	596.7	240.7	4.33	0.37	—	—
SL, ⁸ cm	49.1	47.6	47.7	0.84	0.09	<0.01	0.44

^{a,b}Least squares means within a row with differing superscripts differ ($P \leq 0.05$).

¹Values represent the mean of the corresponding sampling times.

²The values presented correspond to nontransformed means; however, SEM and *P*-values correspond to ANOVA analysis using the Napierian logarithm or square-root + 1 transformed data.

³Meloxicam = mean of 60- and 240-min sample plasma meloxicam concentrations.

⁴VAS = VAS collected during castration.

⁵HM = head movement collected during castration.

⁶Accelerometer = accelerometer peak number and total area collected during castration.

⁷Strain gauges = strain gauge peak number and total area collected during castration.

⁸SL = stride length collected immediately after and 30, 60, 120, and 240 min after castration.

trations. A treatment $\times$ time interaction ($P < 0.01$; Fig. 1) was observed for ST, where 6H and 0H calves had greater ST than 3H calves at T0 and 30 min after castration. Sixty minutes after castration 6H calves had greater ST than 3H calves; however, no differences were observed between both groups and 0H calves. The 6H calves had greater ST than 0H and 3H calves 120 min after castration, whereas 6H and 3H calves had greater temperatures than 0H calves 240 min after castration (Table 2).

Behavior. No treatment effects ($P > 0.10$) were observed for VAS, urination, defecation, leg movement,

vocalizations, or HM during castration. Stride length increased (time effect; $P < 0.01$) during the minutes following castration regardless of treatment and tended ($P = 0.09$) to be shorter in 3H and 6H calves than 0H calves 30, 60, 120, and 240 min after castration. Number of peaks between 2 and 3 SD recorded by the squeeze chute accelerometers was greater ($P = 0.03$) in 3H and 6H calves than 0H calves and tended to be greater ($P = 0.07$) above 3 SD in 3H and 6H calves compared to 0H calves. No treatment differences ($P > 0.10$) were observed for total number of peaks and total area for accelerometers and strain gauges.

Figure 2. Least squares means and SEM for substance P concentrations on d 1, 2, 5, 7, 14, 21, and 28 after surgical castration of Angus and Angus crossbred bulls receiving s.c. meloxicam 6, 3, and 0 h before castration. Least squares means with differing superscripts differ ($P \leq 0.05$).

Days 1 to 28 after Castration

Physiology. Meloxicam concentrations decreased ($P < 0.01$) on d 1 (566 ± 26.5 ng/mL), 2 (197 ± 26.5 ng/mL), and 5 (9.6 ± 26.57 ng/mL) after castration; however, no effects or interactions ($P > 0.10$) were observed. Substance P had a treatment $\times$ time interaction ($P < 0.01$; Fig. 2), where the 6H and 3H calves presented greater SP concentrations than 0H calves on d 1 after castration, whereas 3H calves tended to have greater SP concentrations than 6H calves on d 5 after castration. The N:L ratio was greater ($P = 0.05$) in 0H calves compared to 3H and 6H calves on d 1, 2, 5, 7, 14, and 28 after castration. The N:L ratio and WBC increased (time effect; $P < 0.01$) up to d 2 regardless of treatment and returned to baseline levels after d 2. Platelets decreased (time effect; $P < 0.01$) for all treatments after castration until d 2 and then increased, reaching the highest concentrations on d 21, whereas RBC did not present an obvious pattern. Haptoglobin, SAA, and salivary cortisol concentrations increased (time effect; $P < 0.01$) after castration regardless of treatment, reaching the highest concentrations on d 5 for haptoglobin and on d 2 for SAA and salivary cortisol.

Scrotal temperature had a treatment $\times$ time interaction ($P < 0.01$; Fig. 3) where 6H calves had greater temperatures than 0H and 3H calves on d 1 after castration, whereas 0H calves had greater temperatures than 3H and 6H calves on d 28 after castration. Body temperature increased (time effect; $P < 0.01$) up to d 2 for all treatments and then went back to baseline levels, spiking again on d 28. No treatment or interaction effects ($P > 0.10$) were observed for performance (Table 3) or lesion scores (Table 4).

Behavior. Stride length, standing, and walking increased (time effect; $P < 0.01$), whereas lying decreased after castration for all treatments. Foot stamping presented a treatment $\times$ time interaction ($P < 0.01$; Fig. 4), where 3H calves had a greater number of foot stamps

Figure 3. Least squares means and SEM of scrotal temperature on d 1, 2, 5, 7, 14, 21, and 28 after surgical castration of Angus and Angus crossbred bulls receiving s.c. meloxicam 6, 3, and 0 h before castration. Least squares means with differing superscripts differ ($P \leq 0.05$).

on d 0 compared to 0H and 6H calves and a lower number of foot stamps on d 3 compared to 6H calves, but no differences were seen between both groups and 0H calves. Tail flicks tended ($P = 0.09$) to be greater for 0H calves compared to 3H calves on d 0, 2, and 3; however, no differences were seen between both groups and 6H calves. Standing and lying behavior as well as feeding behavior had a time effect ($P < 0.01$). Standing and lying duration increased during the first week after castration and followed an inconsistent pattern during subsequent weeks for all treatments. Standing percentages increased, whereas lying percentages decreased during the first days following castration regardless of treatment, and standing and lying bouts followed an inconsistent pattern. Dry matter intake increased for all treatments over time, but no obvious pattern was observed for feeding time, feeding rate, meal frequency, or meal size (Table 5). No adverse event happened during the trial.

DISCUSSION

Tissue damage can cause central sensitization, which is a heightened response of the spinal cord neurons and can lead to increased pain from either painful (hyperalgesia) or nonpainful (allodynia) stimuli (Ochroch et al., 2003). Abram and Yaksh (1993) demonstrated that central sensitization, or “windup,” could not be prevented by inhalation anesthesia alone, highlighting the importance of using preemptive analgesia, which has the potential to eliminate central sensitization. If preemptive analgesia is effective, it would be a valuable tool to put into practice before painful husbandry procedures, as it would reduce postoperative pain, consequently improving animal welfare.

Previous studies have reported a lack of considerable behavioral changes after burdizzo castration

Table 3. Least squares means ($\pm$ SEM) of samples taken days after castration for meloxicam, substance P (SP), complete blood count (CBC), haptoglobin, serum amyloid A (SAA), salivary cortisol, hair cortisol, scrotal temperature (ST), rectal temperature (Temp), and performance (initial BW, final BW, and ADG) of surgically castrated Angus and Angus crossbred bulls receiving s.c. meloxicam 6, 3, and 0 h before castration¹

Item	Treatment (T)			SEM ²	P-value		
	0H	3H	6H		T	Time	T $\times$ time
Meloxicam ³ , ng/mL	296.2	225.4	255.9	0.14	0.88	<0.01	0.38
SP ⁴ , pg/mL	116.9	119.9	120.2	0.03	0.52	<0.01	0.01
CBC ⁵							
N:L ratio	1.2 ^a	0.9 ^b	0.9 ^b	0.03	0.05	<0.01	0.78
WBC, 10 ⁹ /L	11.6	11.1	11.0	0.05	0.36	<0.01	0.97
RBC, 10 ¹² /L	8.2	8.4	8.1	0.17	0.16	<0.01	0.45
Platelets, 10 ⁹ /L	542.2	483.4	520.4	0.43	0.14	<0.01	0.33
Haptoglobin ⁶ , g/L	1.8	1.9	2.1	0.14	0.47	<0.01	0.12
SAA ⁷ , μ g/mL	19.2	23.7	27.0	0.80	0.68	<0.01	0.69
Salivary cortisol ⁸ , nmol/L	3.5	3.4	3.3	0.04	0.97	<0.01	0.96
Hair cortisol ⁹ , nmol/L	1.9	2.0	1.7	0.08	0.14	0.74	0.21
ST ¹⁰ , °C	34.9	34.8	35.2	0.30	0.65	<0.01	<0.01
Temp ¹¹ , °C	39.7	39.6	39.6	0.05	0.61	<0.01	0.66
Performance							
Initial BW (d -1), kg	283.3	280.3	283.1	8.72	0.96	—	—
Final BW (d 28), kg	303.6	301.3	309.8	8.46	0.76	—	—
ADG, kg/d	0.7	0.7	0.9	0.18	0.12	—	—

^{a,b}Least squares means within a row with differing superscripts differ ($P \leq 0.05$).

¹Values represent the mean of the corresponding sampling days.

²The values presented correspond to nontransformed means; however, SEM and P -values correspond to ANOVA analysis using the Napierian logarithm or square-root + 1 transformed data.

³Meloxicam = plasma meloxicam concentration collected on d 1, 2, and 5 after castration.

⁴Substance P = substance P collected on d 1, 2, 5, 7, 14, 21, and 28 after castration.

⁵CBC = CBC collected on d 1, 2, 5, 7, 14, 21, and 28 after castration. WBC = white blood cells; RBC = red blood cells.

⁶Haptoglobin = haptoglobin collected on d 1, 2, 5, and 7 after castration.

⁷SAA = serum amyloid A collected on d 1, 2, 5, and 7 after castration.

⁸Salivary cortisol = salivary cortisol collected on d 1, 2, 5, 7, 14, 21, and 28 after castration.

⁹Hair cortisol = hair cortisol collected on d -8 and 28.

¹⁰ST = scrotal temperature collected on d 0, 1, 2, 5, 7, 14, 21, and 28 after castration.

¹¹Temp = rectal body temperature collected on d 0, 1, 2, 5, 7, 14, 21, and 28 after castration.

at the time of weaning (Lambertz et al., 2014, 2015), suggesting that weaning and castration should be done at the same time to reduce labor costs (Lambertz et al., 2014). Although castrating and weaning calves at the same time would optimize management, caution should be taken when interpreting the results, as the previously mentioned study assessed behavioral changes in burdizzo-castrated calves, whereas clamp castration has been reported to be the method that causes the least amount of pain and distress compared to ring, band, and surgical castration (Stafford et al., 2002). In the present study, castration was performed 3 wk after weaning to eliminate potential stressors that could mask the effect of castration on physiological and behavioral indicators. One limitation of this study may be that breed effect on the behavioral and physiological parameters measured could not be accounted for because the breed of the calf sires was unknown.

In the present study, a lack of differences in VAS scores, urination, defecation, leg movement, vocalization, strain gauge exertion force, and HM during castration was expected, as calves did not receive an anesthetic to block the pain during the procedure; therefore (despite individual pain tolerance differences), all calves experienced a similar amount of pain. However, we expected to see treatment differences in physiological and behavioral parameters after castration, as we predicted that meloxicam concentrations in plasma would be greater in the 6H calves at the time of castration and therefore would be more effective at reducing pain and inflammation compared to the 3H and 0H calves. Greater escape behavior (number of peaks measured from the accelerometers on the squeeze chute) observed in 3H and 6H calves was likely due to an increase in agitation as these animals had to go through the chute twice, once to receive the medication and a second time

Table 4. Lesion scores of surgically castrated Angus and Angus crossbred bulls receiving s.c. meloxicam 6, 3, and 0 h before castration from d 1 to 28 after castration

Score	Treatment ¹									P-value
	0H			3H			6H			
	<i>n</i>	Median	95% FCL	<i>n</i>	Median	95% FCL	<i>n</i>	Median	95% FCL	
4	8	1	1–14	7	1	1–14	7	2	1–14	0.54
3	10	2	1–28	10	5	1–21	13	5	1–28	0.80
2	6	5	5–14	10	14	5–28	8	14	2–28	0.13
1	4	18	14–28	4	18	14–28	5	14	14–28	0.96

¹FCL = distribution-free 95% confidence limits.

to be castrated. It is not likely that the time of meloxicam administration had an effect on the amount of escape behavior occurring in the squeeze chute because meloxicam does not block the pain experienced at the time of castration but works by decreasing peripheral and central sensitization by reducing prostaglandin production (Burian and Geisslinger, 2005). Behavioral differences were limited to foot stamping, although a tendency was observed for tail flicks. A lack of differences in feeding behavior has been previously reported in 213-d-old calves receiving ketoprofen alone or after multiple i.m. injections following sham, band, and surgical castration (Moya et al., 2014).

Coetzee et al. (2008) suggested SP may be a good measurement to differentiate between stress caused by nociception and stress caused by handling on the basis of a study in which greater SP concentrations were reported in 4- to 6-mo-old surgically castrated calves compared to a noncastrated group, whereas no differences were observed in plasma cortisol concentrations. Similar results were obtained in the present study, in which SP concentrations differed across treatments on d 1 after castration and no differences were observed for salivary cortisol across treatments, although sampling times vary between studies. A possible explanation for the difference observed between treatments on d 1 could be that 6H and 3H calves had to go through the chute twice before castration. The 0H calves went through the chute only once at the time of castration; therefore, the extra handling could have increased SP levels in 6H and 3H calves as it is also associated with stress. Another possible explanation is that 0H calves benefited from receiving the drug immediately before castration, which is in accordance with findings from a previous study assessing the effectiveness of administering oral meloxicam 12 h or immediately before dehorning (Allen et al., 2013). Calves that received meloxicam 12 h prior to dehorning presented low prostaglandin E₂ (PGE₂) concentrations up to 12 h after dehorning, whereas calves that received meloxicam immediately before dehorning had reduced PGE₂ up to 3 d following the procedure. The authors suggested

Figure 4. Least squares means and SEM of foot stamping on d 0, 2, and 3 after surgical castration of Angus and Angus crossbred bulls receiving s.c. meloxicam 6, 3, and 0 h before castration. Least squares means with differing superscripts differ ($P \leq 0.05$).

this could have been due to a longer duration of action of meloxicam when administered immediately before dehorning and could be the reason why 0H calves had lower SP concentrations.

Scrotal temperature was greatest in the 6H calves between T0 to 240 min postcastration and d 1, suggesting that this group presented greater scrotal inflammation up to 1 d after castration. Although ST results were not expected, the findings were similar to the SP results, suggesting that 0H and 3H calves could have benefited from a longer exposure to meloxicam after castration. A similar finding was reported in a human study evaluating the effects of morphine administered 1 h prior (i.m.) to surgery, at the time of induction (i.v.), or at the time of incision closure (i.v.) in females undergoing hysterectomy (Richmond et al., 1993). Twenty-four hours postsurgery the induction group requested less morphine than the incision closure group; however, the induction group had greater VAS pain scores on movement than the incision group 48 h postsurgery, suggesting that the “extra morphine” was effective at controlling pain 2 d postsurgery (Katz, 1995). These findings are in agreement with stride length and foot stamping results, which indicate that 0H calves experienced less pain and distress compared to 3H and 6H calves.

Table 5. Least squares means ($\pm$ SEM) of stride length (SL), behavioral observations, standing and lying behavior, and feeding behavior of surgically castrated Angus and Angus crossbred bulls receiving s.c. meloxicam 6, 3, and 0 h before castration¹

Item	Treatment (T)			SEM ²	P-value		
	0H	3H	6H		T	Time	T $\times$ time
SL, ³ cm	48.1	48.0	47.6	0.69	0.48	<0.01	0.22
Behavioral observations ⁴							
Lying	32.1	43.7	25.6	1.32	0.81	<0.01	0.20
Standing	121.8	115.3	114.8	0.70	0.85	<0.01	0.16
Walking	12.3	8.0	10.1	0.21	0.12	<0.01	0.29
Eating	42.4	40.3	35.5	0.65	0.47	0.20	0.38
Tail flick	311.4	61.3	186.4	2.69	0.09	0.45	0.16
Foot stamp	4.4	8.1	7.1	0.33	0.64	0.99	<0.01
Head turning	10.2	7.0	5.9	0.29	0.18	0.57	0.13
Standing/lying behavior ⁵							
Standing duration, min	182.0	196.1	176.2	0.41	0.82	<0.01	0.47
Lying duration, min	75.0	76.9	78.7	0.57	0.53	<0.01	0.93
Standing time, %	52.5	51.5	53.1	0.01	0.60	<0.01	0.77
Lying time, %	47.5	48.5	46.9	0.01	0.60	<0.01	0.77
Standing bouts	9.7	9.1	9.1	0.06	0.64	<0.01	0.49
Lying bouts	12.4	11.9	10.7	0.08	0.19	<0.01	0.63
Feeding behavior ⁶							
DM feed intake, kg/d	8.7	8.5	8.6	0.20	0.76	<0.01	1.00
Feeding time, min/d	203.7	199.6	203.4	5.48	0.80	<0.01	0.99
Feeding rate, g/min	43.1	44.5	43.4	1.26	0.43	<0.01	0.55
Meal frequency, meal/d	9.6	8.8	9.2	0.49	0.15	<0.01	0.53
Meal duration, min/meal	32.5	26.0	26.2	5.74	0.41	0.47	0.53
Meal size, kg/meal	1.0	1.1	1.1	0.04	0.29	<0.01	0.36

¹Values represent the mean of the corresponding sampling days.

²The values presented correspond to nontransformed means; however, SEM and P-values correspond to ANOVA analysis using square-root plus 1 transformed data for behavioral observations and standing and lying behavior.

³SL = stride length collected at d 1, 2, 5, 7, 14, 21, and 28 after castration.

⁴Behavioral observations = behavioral observation collected on d 0, 2, and 3 after castration.

⁵Standing and lying behavior were collected every day; however, data from sampling days (d -1, 7, 14, 21, and 28) were excluded because of incomplete data.

⁶Feeding behavior = feeding behavior information collected daily throughout the experiment.

The N:L ratio was the only parameter that was elevated in the 0H calves; however, all calves presented values above the normal range (0.3 to 0.6; Smith, 2008). A possible explanation for the increased N:L ratio for all calves during the first 28 d after castration could be stress associated with surgical castration. A typical stress leucogram is characterized by neutrophilia and lymphopenia caused by endogenous or exogenous steroid release (Jones and Allison, 2007). However, lymphocyte values were within the normal range (2.5 to $7.5 \times 10^3/\mu\text{L}$; Smith, 2008), whereas the neutrophil values were above the normal range for all treatments, therefore affecting the N:L ratio. Even though 0H calves presented greater levels, it is unlikely for this finding to be associated with the time of meloxicam administration as meloxicam's effect should last for only 44 ± 3 h (Stock and Coetzee, 2015), whereas differences in N:L ratio were observed up to 28 d after castration. We speculate that the neutrophilia seen across treatments could

be due to noninfective inflammation or subclinical secondary infections to castration (Roland et al., 2014).

Inflammation, infection, and trauma can increase acute-phase proteins as bacterial toxins and tissue injury secrete cytokines, which have the potential of activating hepatocytes and initiating acute-phase protein production (Petersen et al., 2004). Although acute-phase protein response varies between individual animals (Jacobsen et al., 2004), haptoglobin and SAA have been suggested to be accurate indicators of inflammatory conditions in cattle (Horadagoda et al., 1999). In the present study no differences were detected in haptoglobin or SAA levels; however, all treatment groups had greater haptoglobin and SAA concentrations than normal values (HP: <0.1 g/L; SAA: 1.3 ± 0.4 mg/L; Ceciliani et al., 2012), indicating that meloxicam did not eliminate the inflammatory response mediated by acute-phase proteins. On the basis of previous findings from Brown et al. (2015), who

reported a reduction in haptoglobin levels in weaned surgically castrated calves receiving oral meloxicam compared to nonmedicated calves, we speculate that s.c. meloxicam reduced the acute-phase inflammatory response; however, we cannot be certain of this as a control group not receiving meloxicam would be necessary to be able to make this comparison.

Our hypothesis for this study was based on the prediction that absorption of s.c. meloxicam would be faster than oral administration, which has been reported to take 12 h to reach peak plasma concentrations (Coetzee et al., 2009). On the basis of this information, we estimated that peak plasma levels would be reached 6 h after administration. However, meloxicam concentrations did not differ between treatments at 240-min sampling; therefore, assuming that peak plasma levels were reached before 240 min and that peak plasma levels are equivalent to peak tissue levels, we would not expect to see differences between treatments after 240 min. Conversely, it is possible that peak meloxicam levels could have occurred after 240-min sampling or that differences seen between treatments could be related to the total time the calves were exposed to the drug after the onset of tissue damage, which would explain the differences in physiological and behavioral parameters observed hours and 1 d after castration. The lack of differences in the majority of physiological and behavioral parameters could be due to underpowered sample sizes, as sample size was not calculated for all outcomes in addition to a large individual animal variation. Other possibilities include times of meloxicam administration not being substantially different from one another, response variables not being sensitive enough, or time of s.c. administration having no effect.

On the basis of our results, there is little evidence that providing meloxicam subcutaneously before castration had any additional benefit, and contrary to our hypothesis, 0H calves presented fewer indicators of pain and distress compared to 6H and 3H calves, probably because of the increased exposure to meloxicam. Administering s.c. meloxicam immediately before castration has the added benefits of increasing the exposure time to meloxicam, handling the calves once, consequently reducing extra labor costs for the producer, and, more importantly, reducing unnecessary stress to the calves. However, there is a need for a pharmacokinetic study using s.c. meloxicam to determine when peak plasma levels are reached.

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