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Effect of a single subcutaneous injection of meloxicam on chronic indicators of pain and inflammatory responses in 2-month-old knife and band-castrated beef calves housed on pasture

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HIGHLIGHTS

- Effects of meloxicam on chronic indicators of pain after castration.
- Cow-calf proximity and home range as novel indicators of pain post-castration.
- Knife and banded calves experienced more acute and chronic pain, respectively.
- Meloxicam did not reduce indicators of pain at the time-points assessed.
- Proximity and home range have the potential to be used as indicators of pain.

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ABSTRACT

One hundred and thirty-one 2 mo. old pasture housed Angus cross bull calves were evaluated for 62 d over two years (Year 1: $n = 69$, 134.1 ± 20.37 kg BW; Year 2: $n = 62$, 118.1 ± 15.49 kg BW) to determine 1) the effects of a subcutaneous (s.c) injection of meloxicam on indicators of long term pain after castration and 2) the potential use of cow-calf proximity and home range as indicators of pain. Calves were randomly assigned to treatments using a 3 × 2 factorial design including castration - sham (CT; $n = 47$), band (BA; $n = 46$) or knife (KN; $n = 38$) castration and medication – s.c. meloxicam (M; $n = 66$) or s.c. lactated ringers solution (NM; $n = 65$). Measurements included performance, scrotal temperature, swelling (WS) and healing (WH) scores, and pain sensitivity, collected on d -1, 6, 13, 20, 34, 48, and 62 post-castration. Suckling, lying, standing and walking duration, and head-turning, lesion-licking, foot-stamping and tail-flick frequencies were collected immediately following and up to 2-d after castration. Cow-calf proximity and home range were obtained from d 0 to 2 and from d 14 to 16. With the exception of suckling, no medication ($P > 0.05$) effects were found. Greater ($P < 0.05$) pain sensitivity was observed in KN from d 6 to 34 and on d 62, and in BA from d 6 to 62 compared to CT calves. Knife calves showed an earlier (d 20) absence of inflammatory responses (WS; $P < 0.05$) than BA (d 34) and overall, KN calves had greater ($P < 0.05$) standing, walking, and head turning than BA and CT. Knife and BA had greater ($P < 0.05$) foot stamping than CT for the first 2 h post-castration, but KN exhibited greater ($P < 0.05$) frequencies between 9 and 11 h (d 0) compared to BA and CT, and had greater ($P < 0.05$) tail flicks from d 0 to 2 than CT. Banded calves were closer to their dams on d 15 while KN calves and their dams had a reduced home range on d 0 than CT cow-calf pairs. Although meloxicam did not reduce indicators of pain (with exception of suckling behavior), our results suggest that knife castration causes greater acute pain, while band castration resulted in greater chronic-pain. Cow-calf proximity and home range have some potential to be used as pain indicators post-castration.

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1. Introduction

Painful routine husbandry practices used by beef cattle operations in North America, such as castration, are a major animal welfare concern (Lemos Teixeira et al., 2018). Some of the reasons for the continued use of this practice include reduced aggression towards humans and other animals, unwanted breeding, and improved carcass quality (Price et al., 2003; Bolado-Sarabia et al., 2018). It is well known that knife and band castration can reduce feed intake and growth, increased acute and chronic pain-related behaviors and inflammatory responses (Coetzee, 2013; Marti et al., 2018a; Meléndez et al., 2017a). Therefore, Stafford (2007) recommended that both acute and chronic pain should be minimized during castration by providing analgesia while pain is probable to occur.

A survey of cow-calf producers in Western Canada reported that the majority (95 %) of respondents castrated calves 3-mo of age or younger but only 10% of the respondents administered a pain control prior to castration (Moggy et al., 2017). To date, oral meloxicam (a nonsteroidal anti-inflammatory drug; NSAID) is the only drug labelled in Canada to mitigate castration related pain (Meléndez et al., 2019) in cattle. Oral meloxicam is an attractive option for producers because it has a relatively long duration of action (elimination half-life of up to 27 h; Coetzee et al., 2009; Meléndez et al., 2019) compared to other NSAIDS providing up to 72 h of pain relief after band and knife castration (Olson et al., 2016). However, researchers had stated that oral meloxicam may have some limitations such as unpredictable absorption in the gut (Meléndez et al., 2019) as well as potential problems with dosing associated with incomplete intake of the product when given to cattle as a drench. In addition, a previous study conducted by our research group found that calves receiving a single (0.5 mg/kg) s.c. injection of meloxicam at the time of castration reached therapeutic concentrations sooner (Tmax = 3.7 h) and had longer elimination half-life (16.2 h) than calves receiving a single (1 mg/kg) administration of oral meloxicam (Tmax = 24 h; elimination half-life = 15.2 h; Meléndez et al., 2019). Consequently, subcutaneous (s.c.) meloxicam appears to provide a more practical and effective alternative to oral meloxicam for on farm use although relatively few studies have been conducted assessing its effectiveness in mitigating pain in cattle.

The effectiveness of s.c. meloxicam in mitigating castration related pain post-operatively in calves of various ages is well documented (Meléndez et al., 2018a, b; Marti et al., 2018a, b). The majority of studies in the literature have assessed acute physiological and behavioral indicators of pain post-castration, typically within several minutes of and up to several days after its administration. However, few studies have assessed the pain mitigation effects of meloxicam and other NSAIDS, following castration or other painful procedures, on longer term indicators of pain such as inflammation, behaviour and performance outcomes (Pang et al., 2006; Marti et al., 2018a, b; Daniel et al., 2020). This is likely because most studies were designed to conduct pain assessments corresponding to the duration of action of the drug. The duration of action is determined by the elimination half-life which is verified by measuring the rate of drug clearance from the blood. However, few if any studies have assessed NSAID concentrations and clearance from tissue levels, although previous researchers have stated that oral meloxicam reduced respiratory related morbidity in feedlot calves (Coetzee et al., 2012) suggesting the effectiveness of the drug may be prolonged and could impact long term indicators of pain such as inflammation. In addition, previous studies provide some evidence that NSAIDs can mitigate pain well beyond their duration of action (based on half-life). For example, week old, castrated calves that were not provided pain control had greater Substance P concentrations (biomarker of pain, inflammation and stress) and tail flicks frequencies 7 days post-castration compared to those administered s.c. meloxicam at the time of castration (Meléndez et al., 2018a). Another study found that calves of the same age and administered the same NSAID as in the previous study, tended to have greater feeding durations than non-medicated calves for up to 34 days post castration while non-medicated calves had greater hair cortisol concentrations on d 56 post-castration than medicated calves (Marti et al., 2018a). In contrast, Marti et al. (2018b) reported that meloxicam was not effective in reducing chronic indicators of pain such as inflammation or rate of wound healing over a 42-d period post-castration in 2-mo old calves. Therefore studies looking at the effects of pain mitigating drugs beyond their determined duration of action are needed to better understand any residual or unforeseen effects.

Most of the previously cited studies were conducted with calves that were housed in a feedlot setting following castration to facilitate ease of repeated handling for sample collection. However, calves are typically housed on pasture after castration. It is possible the feedlot environment may affect the outcomes of some pain indicators such as the length of the expression of certain behaviors (e.g., suckling, walking, lying, and standing) as well as the incidence and degree of post-operative inflammation and associated complications such as abscessing due to a greater risk of contamination with manure (Petherick et al., 2015). The current study was conducted with pasture housed calves to improve relevance to industry and remove potential confounding effects associated with being housed in a feedlot environment.

To date, very few studies have assessed the relationship between pain in calves post-castration and cow behavior (such as grazing) and cow-calf proximity. Previous studies indicate that pain experienced by offspring can modulate their mother's behavior suggesting it has potential to be used as a novel indicator of pain in animals. For example, ewes expressed greater maternal care (e.g., sniffling, licking, and/or nursing) towards their offspring that had experienced a painful event (tail docking and castration) than if they had not (Hild et al., 2011; Futro et al., 2015).

Thus, the aim of this study was to determine 1) the effects of a subcutaneous (s.c) injection of meloxicam on indicators of long term pain after castration in calves housed on pasture and 2) the potential use of cow-calf proximity and home range as indicators of pain post-castration. We hypothesized that 1) a s.c. injection of meloxicam administered immediately prior to castration would reduce physiological and behavioral indicators of long term stress/pain and inflammatory responses at the wound site, 2) castrated calves administered s.c. meloxicam would have similar proximities and home ranges to uncastrated calves, but different proximities and home ranges from non-medicated castrated calves and, 3) dams of medicated castrated calves would graze and explore the pasture in a similar manner to the dams of uncastrated calves due to the lack of change in calf behavior related to the pain mitigating effects of meloxicam.

2. Materials and methods

The Animal Care Committees of the Lethbridge Research and Development Centre (LeRDC) (ACC 1618) and the University of Calgary (AC16-0066) approved all the procedures described in this study. Animals were cared for in accordance with the Canadian Council of Animal Care guidelines (CCAC, 2009).

2.1. Animal housing and management

One hundred and forty-four 2-mo-old Angus and Angus crossbred bull calves were evaluated over two consecutive years, 2016 (Year 1: $n = 72$; 134.1 ± 20.37 kg BW) and 2017 (Year 2: $n = 72$; 116.8 ± 15.21 kg BW). Cow-calf pairs were transported approximately 40 km from a local ranch to Agriculture and Agri-Food Canada LeRDC (AB, Canada) during the first week after birth.

Each year, cow-calf pairs were managed in 2 groups (36 calves per group) to be castrated on two 2 separate days, two weeks apart. Prior to the experimental period (approximately 6 wk before castration), cow-calf pairs were housed on a fall rye pasture (0.08 ha per animal). From d -6 (prior to castration) until weaning (d 96 and 120 post-castration for Years 1 and 2, respectively), all cow-calf pairs were housed in one of four paddocks stocked at approximately 0.27 ha per animal for a period of time ranging between 10 and 18 d per paddock. In order to assess cow-calf behavior (see sections 2.4.1 and 2.4.2), a subset of 12 cow-calf pairs from each castration group were housed separately from the other animals on a smaller paddock (0.33 ha per animal from d -6 to 2 post-castration and returned back to their original paddock on d 3 post-castration.

On the days prior to (d -6 and -1) and on the day of castration (d 0) as well as 6, 13, 20, 34, 48, and 62 d post-castration, cow-calf pairs were moved from the pasture to a portable handling facility (approximately 400 m from the center of the pasture) which consisted of a holding pen and a squeeze chute (Pearsons Livestock Equipment, Thedford, NE, USA). Calves were individually restrained in the squeeze chute for 3 to 5 min to collect the same samples as those collected post-castration (see sections 2.3 and 2.4), and then restrained on a tip table (Calf Roper, Ram-Bull Ltd, Barons, AB, Canada) so that the castration treatments could be imposed. An experienced veterinarian conducted all castrations which took between 0.5 to 2 min per calf. Calves could hear but not see their dams during sampling and castration procedures. All calves in the first and second year of the study were vaccinated with a 7-way clostridial vaccine (Ultrabac®/Somubac®, Zoetis Canada Inc., Kirkland, Canada) 13 and 6 d post-castration, respectively.

2.2. Experimental Design and Treatments

Each year, calves were equally distributed by body weight (BW) (obtained prior to castration; d -6) to treatments according to a 3 × 2 factorial design which included castration method (CAS) - sham (control; CT), band (BA), or - knife (KN); and medication (MED) - single dose of 0.5 mg/kg of s.c. meloxicam (Metacam 20 mg/mL, Boheringer Ingelhein, Burlington, Ontario, Canada) (M; $n = 36$) or a single s.c. administration of lactated ringers solution (Lactated Ringer's Irrigation, Baxter Canada, Mississauga, Ontario, Canada) (NM; $n = 36$) to yield six treatments: CT-M, CT-NM, BA-M, BA-NM, KN-M, KN-NM (12 calves per treatment per year).

Band castration was performed by placing a band (Elastrator Pliers and Rings, Kane Veterinary Supplies Ltd., Edmonton, Alberta, Canada) over the neck of the scrotum above the testicles using an elastrator (Elastrator Pliers and Rings, Kane Veterinary Supplies Ltd., Edmonton, Alberta, Canada). Knife castration consisted of making a latero-lateral incision in the scrotum with a Newberry castration knife (Syrvet Inc., Waukee, IA, USA) in order to externalize the testicles, while crushing and cutting of the spermatic cords was done using an emasculator. Uncastrated calves (sham treatment) were handled the same as band and knife castrated calves with the exception that they were not castrated but their testicles were manipulated for similar amounts of time as required to conduct either knife or band castration.

2.3. Physiological parameters

2.3.1. Performance

All animals were weighed in a squeeze chute (Pearsons Livestock Equipment, Thedford, NE, USA) on d -6, -1, 0, 6, 13, 20, 34, 48, and 62 post-castration and at the time of weaning. Growth performance parameters included BW obtained on d 62 (BW d 62, kg) as well as at the time of weaning (BW weaning, kg). Additionally, average daily gain (ADG) was calculated over the entire study period (ADG d 62, kg/d) and at weaning (d -1 to weaning; ADG weaning, kg/d).

2.3.2. Pain and inflammatory response

Pain sensitivity (PAIN) was recorded as the maximum pressure (g force) exerted before an animal responded as described by Lomax and Windsor (2013). Measurements were taken on d -1, 6, 13, 20, 34, 48, and 62 post-castration on the skin surrounding the wound using a Von Frey anesthesiometer (IITC-Life Science Instruments, Woodland Hills, CA, USA) as previously described by Lomax and Windsor (2013) and Marti et al. (2017b). Greater PAIN values were indicative of less pain sensitivity.

Inflammatory response parameters included maximum scrotal temperature (MST), wound swelling (WS), and wound healing (WH) assessed on d -1, 6, 13, 20, 34, 48, and 62 post-castration. In order to measure MST, thermographic images of the scrotal area were taken from behind the calves prior to scrotal manipulation at a distance of approximately 1 m, using a FLIR i60 infrared camera (FLIR Systems Ltd Burlington, Ontario, Canada). Images were processed using ThermaCam Quick View 1.3 (FLIR Systems Ltd Burlington, Ontario, Canada) as previously described by Marti et al. (2017b). An emissivity coefficient of 0.98 was used to analyze the images. The WS was scored in all KN and BA calves using a 5-point scale adapted from Molony et al. (1995) and described by Marti et al. (2017a). The WH in KN calves (WH-K) was scored using a 5-point scale previoulsy described by Mintline et al. (2014) while WH for BA calves (WH-B) was assessed using a 5-point scale as previously described by Marti et al. (2017a). Briefly, the WS categorizes the animals based on the clinical state of the scrotum (assessed in the area above the band and at the scrotal area for knife castrated calves); a WS score of 0 represents a scrotum with no swelling, inflammation, or infection while a score of 4, represents animals that required an intervention due to the severe swelling (such as draining an scrotal abscess). Similarly, WH categorizes the animals based on the incision state where a score of 1 is related to visible incision, exposed tissue, and presence or not to exudate and/or scabbing while for score of 5, incision, tissue, scabbing and/or exudate are no longer visible (Mintline et al., 2014).

Table 1
Ethogram of behaviors recorded prior to- and post- castration (adapted from Meléndez et al., 2017a).

Item	Definition
Duration behaviors	
Suckling	Suckling from its mother's udder
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
Standing	Standing on all 4 legs
Walking	Walking forward more than 2 steps
Frequency behaviors	
Head turning	Head turned and touching the side of the calf's body when standing
Lesion licking	Head turning to lick the wound site caused by the castration while standing
Foot stamping	Hind legs are lifted and forcefully placed on the ground or kicked outward while standing
Tail flicking	Forceful tail movement beyond the widest part of the rump when standing; movement to 1 side is counted as 1 action

2.4. Behavioral parameters

2.4.1. Calf behavior

A subset of 12 cow-calf pairs (4 pairs per treatment) was randomly selected from the first group castrated in each year and individually marked with matching penning tag numbers (Livestock Identification tag cement,W.J. Ruscoe Co., Akron, OH) glued on their backs. This was done to facilitate individual animal identification during behavioral observations (from d -1 to d 2 post-castration) when the pairs were housed separately from the other animals in another paddock as previously described on section 2.1. Twelve research personnel (visible to the cattle) were positioned within the pasture (10 to 50 m away from the animals) and each person video recorded (CX405 Handycam, Sony Corporation, Tokyo, Japan) a calf that was randomly assigned to them. On d -1 (baseline measurement) and 0 (castration day), calves were video recorded for 2-h period immediately after sampling and castration (TIME: 0 to 2 and 9 to 11 h after calf handling). On d 1 and 2 post-castration, calves were video recorded from 26 to 28, 33 to 35, 50 to 52 and 57 to 59 h post-castration, for a total of 16 h of recording for each calf. Indirect observations using Observer XT (Noldus Information Technology, Wageningen, The Netherlands) with focal animal sampling and continuous recordings (Martin and Bateson, 2007) were used to record duration- and frequency-behaviors as described in Table 1 (adapted from Molony et al., 1995 and Meléndez et al., 2017a). Behavioral responses were selected in the present study (Table 1) on the basis of what had been used in previous studies where treatment (castration and/or medication) effects had been observed (Meléndez et al., 2017a, 2018a). Two experienced observers (blind to treatments) recorded duration-behavior data, while 2 additional observers recorded frequency-behavior data. Inter- and intra-observer reliability were 0.93 and 0.90, respectively. Due to significant rainfall on the day of castration (d 0) for the first group of calves castrated in Year 2, behavioral data could not be collected for the first 2 h post-castration.

2.4.2. Cow-calf proximity and home range

A subset of 12 calves (and their dams) were randomly selected from the second group castrated in each year (a total of 4 cow-calf pairs per treatment). Calves were fitted with a Lotek 3300SR GPS collar (Lotek Engineering, Newmarket, Ontario, Canada) weighing 400 g while cows were fitted with a Lotek 3300LR GPS collar (Lotek Engineering, Newmarket, Ontario, Canada) weighing 950 g. Each collar was configured using a software interface (Lotek Engineering, Newmarket, Ontario, Canada). The operational schedule of the GPS collars recorded the geographical coordinates (latitude and longitude) every 30-min from d 0 to d 2 post-castration while housed separately from the larger group of animals (paddock 1; 0.33 ha per animal), and again from d 14 to 16 post-castration while they were housed together with the rest of the group in a larger paddock (paddock 2; 0.20 ha per animal). The GPS data was processed using ArcGIS software (Version 10.4.1; ESRI, 2011).

Cow-calf proximity, defined as an average distance (m) between the calf and dam at a given time was calculated using the following equation (1):

$$D=cos^{-1}[\cos(((90-lat_1)\times\pi)/180)\times\cos(((90-lat_2)\times\pi)/180)+\sin(((90-lat_1)\times\pi)/180)\times\sin(((90-lat_2)\times\pi)/180)\times\cos(((long_1-long_2)\times\pi)/180)]\times6371\times1000 \quad (1)$$

Where D is the distance (m) between each cow and its calf at a given time, and *lat* and *long* refer to latitude and longitude in decimal degrees for cow (lat_1; $long_1$) and calf (lat_2; $long_2$).

Home range, defined as the area (ha) used by an individual animal during its routine activities (Burt, 1943; Mills et al., 2018), was determined daily for each cow-calf pair and was calculated by creating a 100% Minimum Convex Polygon (MCP) which included all GPS fixes per animal; adapted from Van der Saag et al. (2018). The MCP estimation was calculated in R software version 3.4.1 (R Core Team, 2011) using the “adehabitatHR” (Calenge et al., 2006) and “sp” (Pebesma et al., 2005) packages.

2.4.3. Flight speed

Flight speed (FS), as previously described by Burrow et al. (1988), was measured at the time of exit from the squeeze chute on each animal on d -6 and -1 and was used as an indicator of calf reactivity by means of an electronic device (FarmTek, Inc., Wylie, TX). This included 2 pairs of photoelectric cells, a chronometer, and a small processor programmed to record the time taken by each calf to cover a 2.16 m distance immediately after the squeeze chute. Cattle with faster FS are deemed more excitable (Burrow et al., 1988) and more susceptible to stress (Burdick et al., 2011). Consequently, the average FS (of d -6 and -1) value was calculated and used to control (covariate) any potential reactivity effects on treatments as suggested by Petherick et al. (2015). The use of FS in this study was not intended to be employed as an independent variable but as an indicator of animal reactivity which is best measured the first few times the animal is handled through a facility (Burrow and Corbet, 2000).

2.5. Data management and statistical analysis

2.5.1. Animals

In Year 1, one calf died 2-wk after castration (BA-M) due to a clostridial infection, a second calf died (KN-NM) prior to the commencement of the trial due to poor health, and one calf (CT-NM) was removed from the trial prior to castration due to moderate lameness. In Year 2, one calf (BA-NM) died 1-wk after castration due to reasons unrelated to castration, and nine knife castrated calves (n = 4 and 5 KN-M and KN-NM, respectively) had scrotal abcesses that required draining on days 20, 34, and 48 post-castration. The abcesses were drained by the same veternarian that performed the castrations during the study. The additional stress/pain associated with draining the abscesses would have confounded our results such that differences observed in the physiological or behavioural measures taken could not be attributed to the castration/medication treatment alone. Therefore, all data from the 13 calves identified above were not included in the final dataset. Consequently, 131 calves were used in Years 1 and 2: CT-M ($n = 24$), CT-NM ($n = 23$), BA-M ($n = 23$), BA-NM ($n = 23$), KN-M ($n = 18$), and KN-NM ($n = 20$). For behavior measurements (described in section 2.4.1), the number of calves used per treatment in Years 1 and 2 was as follows: CT-M ($n = 4$), CT-NM ($n = 4$), BA-M ($n = 4$), BA-NM ($n = 4$), KN-M ($n = 2$), and KN-NM ($n = 2$).

2.5.2. GPS data management

Outliers were removed from the dataset by creating a paddock boundary file using a buffer command on ArcMap 10.4.1 (ESRI, 2011) of 6 m.Visual checking was further conducted to ensure the data was properly removed. In addition, all data obtained while animals were at the handling facilities for sampling collection and/or castration was removed from the database. Due to a slight shift in the times recorded between the GPS collars of the cows and calves, a script was developed in SAS (SAS 9.1, SAS Institute Inc., Cary, NC) to match the times recorded on the collars of cow-calf pairs applying a one-minute buffer. A match was considered only when the time difference between collars was less than one minute. Missing data represented 4.7% of the total data points ($n = 645$ from 13,824 data points). One calf (CT-NM) from Year 1 and five cows (1 CT-NM, 1 CT-M, 2 BA-NM, and 1 BA-M) from Year 2 had more than 50 % mising data due to technical issues with the GPS collars and were removed from the database. In addition, as previously stated in section 2.5.1, all KN calves with GPS ($n = 4$) assessed in the Year 2 had scrotal abcesses which could have affected their behaviors so the cow-calf data were removed from the database. For cow-calf proximity, if the number of GPS data points observed per day per calf represented less than 90 % of the number of data points observed for its dam (or *vice-versa*), data for both the cow and calf were removed from the database for only that day. Consequently, for cow-calf proximity and home range the database represented in both years included 15 and 36 cow-calf pairs, respectively: CT-M ($n = 3$ and 7), CT-NM ($n = 2$ and 6) BA-M ($n = 3$ and 7), BA-NM ($n = 3$ and 7), KN-M($n = 2$ and 4) and for KN-NM ($n = 2$ and 5).

2.5.3. Statistical analysis

All data analyses were carried out using the SAS software package (SAS 9.4, SAS Institute Inc., Cary, NC, USA). Outliers were identified and removed from the data set using the UNIVARIATE procedure of SAS and a schematic box plot. To ensure the assumptions for a parametric ANOVA were met, the normality of error distribution was verified using the UNIVARIATE procedure in SAS and homoscedasticity of variance for all dependent variables was performed with the GLM procedure of SAS using Levene’s test. Non-normally distributed behavioral data (suckling, lying, standing, and walking durations, head turning, lesion licking, foot

Table 2
Performance of uncastrated, band, and knife castrated 2 mo old Angus cross calves with and without a single administration of s.c. meloxicam assessed between d -1 and 62 post castration and at weaning.

Item[1]	Treatments[2]							P-value[3]		
	CT		BA		KN					
	NM	M	NM	M	NM	M	*SEM*	CAS	MED	CAS × MED
Initial BW, kg	129.3	128.3	126.4	127.0	123.5	126.1	6.61	—	—	—
BW d 62, kg	208.8	213.7	201.6	204.9	198.9	207.1	7.49	**0.05**	0.09	0.80
BW weaning, kg	281.7	287.7	282.0	276.4	271.8	280.3	5.77	0.14	0.45	0.22
ADG d 62, kg/d	1.17	1.18	1.02	1.03	1.02	1.10	1.08	**0.01**	0.47	0.80
ADG weaning, kg/d	1.06	1.05	1.20	1.22	1.09	1.13	0.05	**<0.01**	0.54	0.90

[1] Initial BW (obtained on d -1 prior to castration, kg); BW d 62 (obtained on d 62 post-castration, kg); BW weaning (obtained on d 96 and d 120 post-castration for year 1 and 2, respectively, kg); ADG d 62 (obtained from d -1 to d 62 post-castration, kg/d) and; ADG weaning (obtained from d -1 to d 96 and to d 120 post-castration for year 1 and 2, respectively, kg/d)
[2] CT: Uncastrated calves submitted to the same handling procedure as castrated ones; BA: Calves castrated using rubber ring; KN: Calves castrated using a Newberry knife; NM: single s.c. injection of lactated ringers at castration time; M: single injection of s.c. meloxicam (0.5 mg/kg) at castration time.
[3] CAS: Castration treatment effect; MED: Medication treatment effect. Bold *P*-values indicate significant effect at $P \leq 0.05$.

stamping and tail flicking frequencies) were square root + 1 transformed. The results were presented with the back transformed LSmeans ± standard error of *base-e* log as well as of square root + 1 transformed data.

2.5.3.1. Treatment effects on physiological and behavioral parameters. Animal was used as the experimental unit. For the performance parameters, a mixed-effects model (MIXED procedure of SAS) was performed including castration method (CAS), medication (MED) and their interactions as fixed effects, and group nested within year as a random effect, while average FS (assessed on d -6 and -1) was included as a covariate. For PAIN, MST, and calf behavior (suckling, lying, standing, and walking durations as well as head turning, lesion licking, foot stamping, and tail flick frequencies), a mixed-effects model (MIXED procedure of SAS) was performed including TIME as repeated measure, CAS, MED, TIME (and their interactions) as fixed effects, year was included as a random effect, and average FS (assessed on d -6 and -1) was included as a covariate. The covariance structure that best fit according to Schwarz's Bayesian information criterion was used. Multicollinearity tests between the covariate FS and each one of the other covariates were carried out by using the variance inflation factors (VIF) option. A post-hoc (Tukey) test was used to compare the adjusted means and the Kenward-Roger method for degrees of freedom. Main effects were considered significant at $P \leq 0.05$. The Wilcoxon-Mann-Whitney test was carried out to determine the effects of CAS (without including CT calves) and MED on the time to achieve each wound swelling (WS) and wound healing (WH) score as previously described by Marti et al. (2017a). The UNIVARIATE procedure of SAS was also used to calculate medians and the 95% distribution-free confidence limits. Significance was established at $Z \leq 0.05$.

2.5.3.2. Treatment effects on cow-calf proximity and home range. Animal was used as the experimental unit. Data was analysed separately for paddock 1 (d 0, 1, and 2) and paddock 2 (d 14, 15, and 16) using the same animals across the days. For both paddocks, a generalized linear mixed model (GLIMMIX procedure of SAS) was performed including CAS, MED, DAY (and their interactions) as fixed effects, while year and animal (and interaction) were included as a random effect. For cow-calf proximity, the statistical model specification (for both paddocks) included a gamma distribution and log funtion. For calf home range, the model specifications included (for both paddocks) an inverse gaussian distribution and reciprocal square function while for the cow home range, the model specification included (for both paddocks) a gamma distribution with a log function. The covariance structure that best fit according to Schwarz's Bayesian information criterion was used. A post-hoc test (Bonferroni correction) was used to compare the adjusted means and the main effects were considered significant at $P \leq 0.05$.

3. Results and discussion

No significant (medication × castration; $P > 0.05$) interactions were observed for any of the indicators of pain assessed in the current study and therefore the main effects of medication and castration will be discussed separately in the following sections.

3.1. Performance

3.1.1. Medication effects

Meloxicam did not improve any of the performance variables assessed (BW d 62, BW weaning, ADG d 62 or ADG weaning; $P > 0.05$; Table 2), possibly due to the short-lasting elimination half-life of 16.2 h (Meléndez et al., 2019). These findings are in agreement with previous studies in which meloxicam was administered to beef calves of similar age as the current study after band or knife castration. Marti et al. (2018b) reported no differences in BW d 42, BW weaning, and ADG after knife castration in 2-mo-old feedlot housed calves compared to non-medicated cohorts after the s.c. administration of meloxicam at the time of castration. Recently, Daniel et al. (2020) also reported a lack of differences between 3-mo-old medicated and non-medicated calves (2 mg/kg of oral meloxicam at the time of- and 14-d after band castration) on ADG assessed over 28-d while housed in pasture. Likewise, the majority of studies assessing the effects of analgesia (NSAID) alone, or in combination with anesthetics on calf performance post-castration did not improve weight gain in castrated compared to uncastrated calves (Ting et al., 2003; Gonzalez et al., 2010; Coetzee et al, 2012; Moya et al., 2014).

3.1.2. Castration treatment effects

Significant castration effects ($P \leq 0.05$; Table 2) were observed for all performance parameters with the exception of BW weaning. Uncastrated calves had greater ($P = 0.05$) BW d 62 (211.2 ± 6.95 kg) than BA and KN calves (203.3 ± 6.94 and 203.1 ± 7.08 kg, respectively). The CT calves also had greater ($P = 0.01$) ADG d 62 (1.17 ± 0.07 kg) and ADG weaning (1.21 ± 0.04 kg) than BA (1.03 ± 0.07 and 1.06 ± 0.04 kg, respectively) and KN calves (1.06 ± 0.07 and 1.11 ± 0.04 kg, respectively). These findings are expected due to the pain/stress associated with castration (Weary et al., 2006) as well as the lack of testosterone post-puberty affecting the growth rate of castrated compared to uncastrated calves (Bretschneider 2005; Marti et al., 2017a). Similar findings were reported in studies assessing the effects of castration on pasture (Petherick et al., 2015; Daniel et al., 2020) as well as feedlot housed (Marti et al., 2018b) beef calves at similar ages and at similar days post-procedure to the calves used in the present study.

In contrast, a study conducted in 2-mo-old feedlot housed knife- or band-castrated calves, reported no differences in BW and ADG obtained

Table 3
Wound swelling scores assessed during the entire experimental period (d 6, 13, 20, 34, 48, and 62 post-castration) in Angus and Angus crossbred calves band-castrated (BA) and knife-castrated (KN) with (M) and without (NM) a single s.c administration of meloxicam.

Score[1]	N[3]	Median[3]	95% FCL[4]	N[3]	Median[3]	95% FCL[4]	P-value[5]
Castration effect[2]		BA			KN		
4	0	—	—	0	—	—	—
3	11	20	13 - 48	4	10	6 - 34	0.10
2	29	13	6 - 34	14	13	6 - 20	0.25
1	41	34	6 - 48	36	6	6 - 48	**0.01**
0	40	48	34 - 62	33	48	20 - 62	**0.03**
Medication 2effect[2]		**M**			**NM**		
4	0	—	—	0	—	—	—
3	8	20	6 - 34	7	20	6 - 48	0.41
2	18	13	6 - 34	25	13	6 - 34	0.10
1	38	16.5	6 - 48	40	34	6 - 62	0.12
0	37	48	34 - 62	36	48	20 - 62	0.92

[1]Swelling score: 0) No swelling, inflammation or infection visible or palpable; 1) Increasing degrees of swelling without obvious erythema; 2) Increasing degree of swelling with obvious erythema but without pus; 3) Presence of pus with increasing inflammatory response 4) Presence of pus with inflammatory response that needs an intervention (adapted from Molony et al., 1995 and described by Marti et al., 2017a).
[2]BA: Calves castrated using rubber ring; KN: Calves castrated using a Newberry knife. NM: Calves castrated with a single s.c. injection of lactated ringers at castration time; M: Calves castrated with a single injection of s.c. meloxicam (0.5 mg/kg) at castration time.
[3]N: number of animals observed at each score; Median: median days to reach each swelling score relative to day of castration;
[4]FCL: 95% confidence limits distribution free.
[5]Treatment effect. Bold *P*-values indicate significant effect at $P \leq 0.05$.

Table 4
Wound healing score assessed during the entire experimental period (d 6, 13, 20, 34, 48, and 62 post-castration) in Angus and Angus crossbred calves band-castrated (BA) and knife-castrated (KN) with (M) and without (NM) a single s.c. administration of meloxicam.

Score[1]	N[3]	Median[3]	95% FCL[4]	N[3]	Median[3]	95% FCL[4]	P-value[5]
Castration effect[2]		BA			KN		
1	38	6	6	12	6	6	0.99
2	28	13	6 - 13	38	6	6 - 13	<0.01
3	37	13	13 - 34	27	34	13 - 48	<0.01
4	46	34	20 - 48	32	48	20 - 62	<0.01
5	20	62	48 - 62	30	62	34 - 62	0.36
Medication effect[2]		**M**			**NM**		
1	25	6	6	25	6	6	0.99
2	36	10	6 - 13	30	6	6 - 13	0.79
3	32	20	13 - 34	32	20	13 - 48	0.51
4	41	34	20 - 48	37	34	20 - 62	0.44
5	29	62	34 - 62	21	62	48 - 62	0.51

[1]Healing score - Band (Marti et al., 2017a): 1) Restriction of blood flow but scrotum is not necrotic; 2) Necrotic scrotum without purulent exudate; 3) Necrotic scrotum with purulent exudate; 4) Loss of necrotic tissue with presence of granular tissue; wound may have exudate either wet or dry. 5) Completely healed; the wound is no longer visible. Knife (Mintline et al., 2014): 1) Incision runs the length of the scrotum and tissue is exposed; the incision may have exudate, either wet or dry; 2) Incision greater than ¾ of the length of the scrotum and scabbing is present; the incision may have exudate either wet or dry; 3) Incision less than ¾ of the length of the scrotum; the incision may have exudate either wet or dry; 4) Incision less than ¼ of the length of the scrotum; small scab or discoloration is present; the incision may have exudate either wet or dry; 5) Completely healed.
[2]BA: Calves castrated using rubber ring; KN: Calves castrated using a Newberry knife. NM: Calves castrated with a single s.c. injection of lactated ringers at castration time; M: Calves castrated with a single injection of s.c. meloxicam (0.5 mg/kg) at castration time.
[3]N: number of animals observed at each score; Median: median days to reach each healing score relative to day of castration;
[4]FCL: 95% confidence limits distribution free.
[5]Treatment effect. Bold *P*-values indicate significant effect at $P \leq 0.05$.

49-d post-castration between castrated and uncastrated calves (Marti et al., 2017a). When we compared the differences between initial BW and BW at weaning for each one of the castration treatments (uncastrated, band-, and knife castration), we noticed that our findings were approximately 11.4, 17.2, and 15.3 kg greater, respectively, than those reported by Marti et al. (2017a). Similarly, uncastrated, band- and knife-castrated calves had lower ADG at weaning (0.44, 0.31, and 0.37 kg/d, respectively) (Marti et al., 2017a) than the same castration treatments in the present study (Table 2). Several factors can affect weight gain in beef calves including genetics, diet, metabolism, and environment (Fordyce et al., 1996; Mittal et al., 2019). However, it is possible that the differences in growth rate between studies may also be attributed to the fact that cows housed on pasture spent more time standing while grazing, allowing calves to suckle longer compared to cows housed in a feedlot. Although milk is the main diet of 2-mo-old calves, they begin to consume forage at about 2-mo of age (Marti et al., 2017a). Forage intake in young calves may contribute to early ruminal development (Baldwin et al., 2004), which may improve their rate of growth. Thus, even though the maintenance requirements of pasture-housed calves is greater than feedlot-housed calves (Maurya et al., 2012; Valente et al., 2013), it is hypothesized that unweaned calves housed on pasture may start eating greater amounts of grass earlier than those housed in a feedlot (fed hay) which may also explain differences in growth rates reported in the present and previous studies (Marti et al., 2017a).

3.2. Pain and inflammatory response

3.2.1. Medication effects

No medication effects ($P > 0.05$) were observed for PAIN, MST, WS ($Z > 0.05$; Table 3), or WH ($Z > 0.05$; Table 4) at any time-point over the 62 d assessment period post- knife or band-castration. Meloxicam functions by blocking cyclooxygenase, an enzyme responsible for producing prostaglandins, and subsequently an inflammatory response, which causes post procedural pain (Coetzee, 2013). The findings of the present study are contrary to our hypothesis that medicated calves would have reduced scrotal temperatures and wound swelling indicative of reduced inflammation and pain sensitivity but faster wound healing than non-medicated calves post-castration. Even though the elimination half-life of s.c. meloxicam is short (half-life = 16.2 h; Meléndez et al., 2019), other studies have shown the pain mitigating effects of meloxicam well past the period of time that it would be expected to have an effect based on its half-life. For example, reduced hair cortisol concentrations were found on d 56 post-castration in 1-wk old band-castrated calves administered s.c. meloxicam, suggesting the medication may have reduced the pain/stress associated with band application and scrotal sloughing (Marti et al., 2018a). In addition, a tendency for reduced scrotal swelling between 7 and 42 days after castration was found in knife castrated calves administered s.c. meloxicam compared to those that were non-medicated (Marti et al., 2018b). However, the authors reported a lack of medication effects up to d 42 post-castration on scrotal temperature and WH measurements, which is in agreement with the findings of the current study. It is possible (based on an elimination half-life of 16.2 h; Meléndez et al., 2019) that the effects of meloxicam would not be measurable beyond 48 h after its administration. Possible reasons for the lack of agreement among studies could be explained either by the method of wound swelling assessments (scrotal

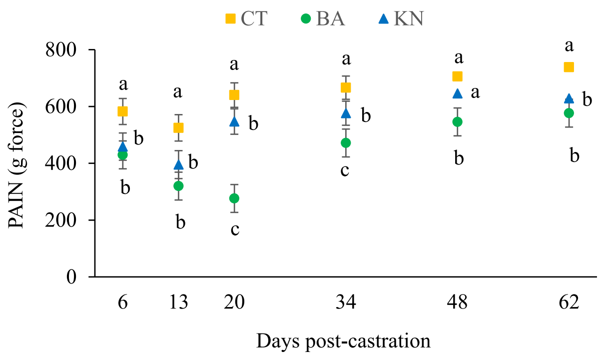

Fig. 1. Least square means and SEM for pain sensitivity obtained on d 6, 13, 20, 34, 48, and 62 post-castration of uncastrated (CT, n = 47), band (BA, n =46), and knife (KN, n = 38) castrated 2-mo old Angus and Angus crossbred calves housed in pasture. [a-c] Least square means with different superscript among treatments differ ($P \leq 0.05$).

circumference *versus* wound swelling score) or that the risk of infection and/or inflammation was greater in feedlot-housed calves compared to the pasture-housed calves due to increased exposure of scrotal lesions to manure contamination, which may have confounded or masked the medication effects.

3.2.2. *Castration treatment effects*

Significant castration × sampling day interactions ($P < 0.01$) were observed for PAIN (Fig. 1) and a castration effect ($P < 0.05$) was observed for MST. Significant differences ($Z < 0.05$) were found for WS (Table 3) and for WH-K and WH-B scores (Table 4). Knife castrated pasture-housed calves and feedlot-housed calves (Marti et al., 2017a) achieved a swelling score of 1 at a similar time point (7 d) post-castration. However, KN pastured calves were expected (at 95 % confidence limit) to achieve a wound swelling score of 0 (Table 3) and a wound healing score of 3 (Table 4), 2 weeks and 1 week earlier, respectively, than 2-mo old feedlot housed calves (Marti et al., 2017a, 2018b). Wound healing of KN calves was delayed, which may explain the greater ($P < 0.01$) pain sensitivity observed on d 6, 13, 20, 34 and 62 compared to CT calves (Fig. 1), suggesting that although KN calves wounds were completely healed on d 62 post-castration, animals were still experiencing some level of discomfort. This could be related to the trauma caused by the procedure resulting in hyperalgesia (exaggerated response to a painful stimulus) at the wound site in some KN calves or allodynia (a non-painful stimulus that becomes painful) in others (Stafford et al., 2002).

Band castrated pasture housed calves were expected to have a wound swelling score of 1 (at 95% confidence of the median) up to 5-wks after BA castration ($Z < 0.05$; Table 3). These findings may be related to the fact that the majority (approximately 72 %) of BA calves had sloughed their scrotums between d 13 and 34 (data not shown), increasing the risk of infection during this period of time. This may explain the greater ($P < 0.01$) pain sensitivity observed for BA calves between d 13 and 48 post-castration compared to KN and CT calves (Fig. 1). Researchers have previously reported that band castration causes a delayed pain/stress response which is normally observed when the testicles slough-off (Stafford et al., 2002; Gonzalez et al., 2010; Marti et al., 2017a) similar to the finding of the current study.

When comparing the findings between pasture-housed and feedlot-housed calves, we expected (at 95 % confidence limit) BA calves to achieve a wound healing score of 4 (Table 4), 1-wk earlier than band castrated calves housed in the feedlot (Marti et al., 2017a). However, swelling scores of 1 were achived on d 34 in the present study, while Marti et al. (2017a) reported swelling scores of 1 on d 28 post-castration. Differences between studies may be due to the fact that calves were only assessed bi-weekly from d 20 to 62 post-castration in the present study, while Marti et al. (2017a) did a weekly assesment. In addition, banded calves had lower ($P < 0.05$) MST than both KN and CT calves (37.1, 37.4, and 37.5 ± 0.17°C, respectively). Although the differences observed among treatments were small and may not have biological relevance, a reduction in scrotal temperature after band-castration is expected as a result of restriction of the blood flow to the testicles (Stafford et al., 2002; Meléndez et al., 2017a).

Our results suggest that KN and BA calves housed on pasture healed faster than calves housed in a feedlot, likely due to a reduced risk of infection in pasture housed calves. This assumption is supported by a lack of a wound swelling scores of 4 in the present study (Table 3), while Marti et al. (2017a) reported that 36 % of KN and 58 % of BA calves achieved wound swelling scores of 4 on d 7 and 21, respectively. Likewise, none of the BA calves in the present study had a WS score of 4 at any sampling point post-castration (Table 3), which is also contrary to Marti et al. (2017a) who found that 58 % of BA calves had a WS score of 4 by d 21 post-castration.

3.3. *Calf behavior*

3.3.1. *Medication effects*

A significant medication effect ($P < 0.05$; Table 5) was observed for suckling behavior, where M calves spent more time suckling from their

Table 5
Least square means (± SEM) of behavioral parameters (duration and frequency) obtained on d 0 (immediately post-castration) and d 1 and 2 post-castration in Angus and Angus crossbred calves uncastrated (CT), band-castrated (BA), and knife-castrated (KN) with (M) and without (NM) a single s.c. administration of meloxicam.

	Treatments[2]							P-value[3]						
	CT		BA		KN									
Item[1]	NM	M	NM	M	NM	M	*SEM*	CAS	MED	CAS × MED	TIME	CAS × TIME	MED × TIME	CAS × MED × TIME
Duration behavior, min														
Suckling	2.2	3.1	1.3	2.6	1.9	3.7	0.40	0.42	**0.04**	0.80	<0.01	0.27	0.80	0.48
Lying	45.0	50.6	47.7	46.8	46.5	40.9	1.63	0.76	0.93	0.60	<0.01	0.73	0.84	0.94
Standing	19.4	23.2	30.0	26.2	44.2	40.0	0.49	**0.02**	0.84	0.70	<0.01	0.17	0.61	0.97
Walking	4.6	4.8	8.8	7.4	10.1	8.7	0.28	0.03	0.55	0.80	<0.01	**<0.01**	0.41	0.99
Frequency behavior, n														
Head turning	2.6	0.8	1.7	2.3	4.7	5.5	0.28	**0.04**	0.71	0.37	0.07	0.12	0.86	0.83
Lesion licking	0.4	0.3	1.4	0.6	3.4	2.1	0.24	**0.05**	0.23	0.81	0.34	0.24	0.98	0.87
Foot stamping	0.7	1.0	6.5	2.6	3.7	5.1	0.62	0.23	0.77	0.64	<0.01	**0.03**	0.50	0.70
Tail flicking	47.7	69.2	439.6	190.7	763.6	1215.8	5.06	0.01	0.91	0.41	<0.01	**0.03**	0.26	0.64

[1] The values presented correspond to nontransformed least square means; SEM and P-values correspond to ANOVA analysis using square root + 1 transformed data.
[2] CT: Uncastrated calves submitted to the same handling procedure as castrated ones; BA: Calves castrated using rubber ring; KN: Calves castrated using a Newberry knife; NM: single s.c. injection of lactated ringers at castration time; M: single injection of s.c. meloxicam (0.5 mg/kg) at castration time.
[3] CAS: Castration treatment effect; MED: Medication treatment effect; TIME: Sampling time effect. Bold P-values indicate significant effect at $P \leq 0.05$.

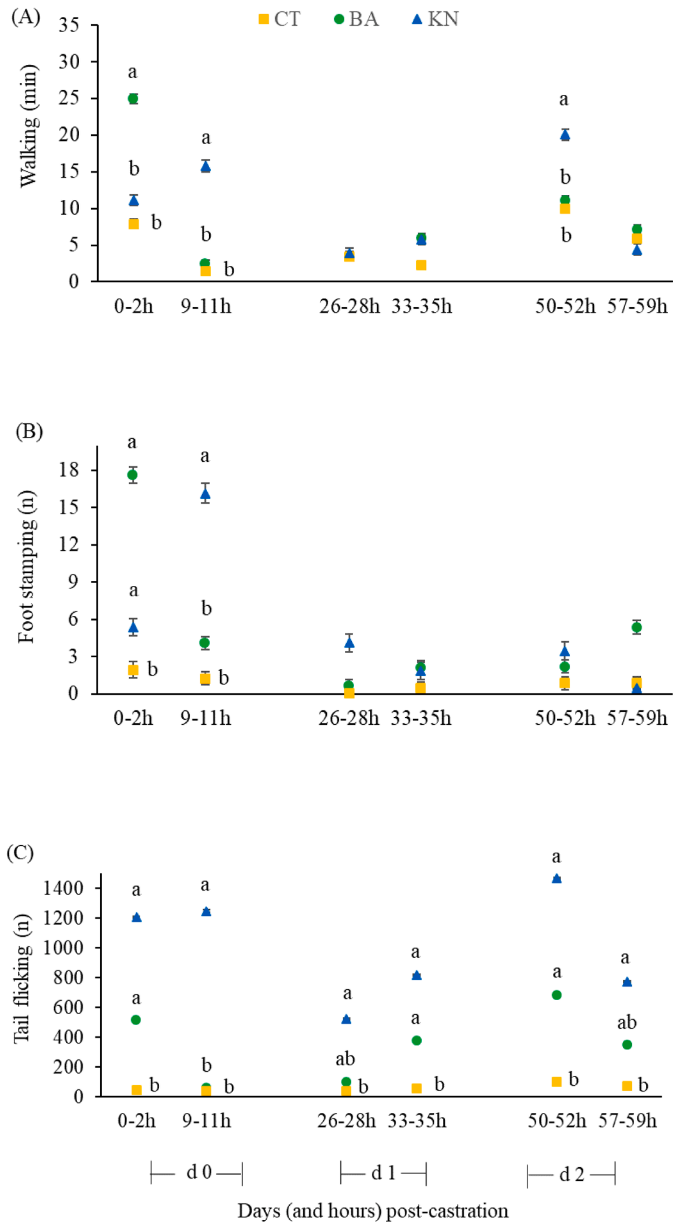

Fig. 2. Least square means and SEM for (A) walking, (B) foot stamping, and (C) tail flicking obtained on d 0, 1, and 2 (between 0 and 59 h) post-castration in uncastrated (CT, $n = 8$), band (BA, $n = 8$), and knife (KN, $n = 4$) castrated 2-mo old Angus and Angus crossbred calves housed on pasture. [a-b] Least square means with different superscripts among treatments differ ($P \leq 0.05$).

dams than NM calves (3.1 and 1.8 ± 0.37 min, respectively). The reason for differences between medicated and non-medicated calves is unknown and the magnitude of differences (1.3 min) may lack biological relevance. Therefore, more studies are necessary to better understand the effect of meloxicam on appetenace in calves. Contrary to our findings, no differences were found in eating behavior in 2-mo-old feedlot-housed calves assessed between 2 and 4 h and up to 7 d relative to knife castration, using the same treatment application as the present study (Meléndez et al., 2018b). However, it is important to note that Meléndez et al., (2018b), included suckling, ingesting hay or straw from the ground or the feeder as eating behaviour which could possibly explain the differences between studies. Curiously, in a previous study of our research group (Meléndez et al., 2018a) we found an increased suckling behaviour in 1-wk-old knife castrated calves compared to uncastrated calves, both receiving a single s.c. injection of meloxicam at the time of castration as well as greater suckling behaviour in uncastrated receiving meloxicam than uncastrated receiving lactated ringers (control). No medication effects ($P > 0.05$; Table 5) were observed for any of the other behaviors assessed in the present study. Contrary to our findings, Meléndez et al. (2018b) reported behavioral differences in lying and walking durations as well as frequecies of tail flicks and head turning between medicated and non-medicated calves suggesting that s.c. meloxicam was effective at mitigating indicators of acute pain from 2 to 4 h post- knife castration. Lack of agreement among studies could be attributed to the fact that assessments were conducted at different time points post-castration as well as the environmental setting (feedlot vs pasture).

3.3.2. *Castration treatment effects*

No differences ($P > 0.05$) were observed in suckling and lying durations up to 2 d post-castration among castrated and uncastrated calves (Table 5). Knife castrated calves had greater ($P < 0.05$) standing durations (42.3 ± 0.42 min) and head turning frequencies (5.1 ± 0.24 n) compared to BA (28.1 ± 0.32 min and 2.1 ± 0.18 n) and CT (21.3 ± 0.32 min and 1.6 ± 0.18 n) calves. Likewise, KN calves had greater ($P < 0.01$) walking durations on d 0 (9 – 11 h) and 2 (50-52h) post-castration (Fig. 2A) as well as greater ($P < 0.01$) foot stamp (Fig. 2B) and tail flicks frequencies (Fig. 2C) on d 0 (9 – 11 h) compared to BA and CT calves. Even though no significant differences ($P > 0.05$) were observed between castrated calves for tail flicks on d 1 and 2 (Fig. 2C) or for lesion licking over the three days of observations (Table 5), KN calves had more than twice as many frequencies for both behaviors as BA calves. Uncastrated calves had lower ($P = 0.05$) lesion licking frequencies than KN (0.34 and 2.72 ± 0.15 n, respectively) calves but uncastrated calves did not differ ($P > 0.05$) from BA (0.97 ± 0.15 n) calves. Overall, behavioral outcomes suggest that KN calves might have experienced greater discomfort and/or pain for up to 2 d post-procedure compared to band castration (Fig 2). However, no significant differences ($P > 0.05$) were observed among castration treatments for lying or suckling behaviors on d 1 or 2 post-castration (Table 5). This is in partial agreement with Meléndez et al. (2017a) who found that KN calves spent less time eating and lying down while more time standing and walking 2 to 4 h post-castration compared to BA and CT calves. As discussed, Meléndez et al., (2017a), included suckling behavior as well as ingesting hay or straw as eating behavior, and also assessed the calves' behavior at different time points (from 2 to 4 h post-castration and for 4 min every 10 min over the 3-h period on d 1, 2, 3, and 7 post-castration) than the current study, which could possibly explain differences in the outcomes between studies. Another possible reason for the lack of agreement between studies may be the fact that successive handling in the previous study immediately post-castration (at 0, 60 and 120 min) combined with being housed in a feedlot might have interfered in the length of expressions of those behaviors when compared to the current study where animals were handled only once on the day of castration and housed on pasture.

On the other hand, the lack of differences between BA and CT calves on d 0, 1, and 2 post-castration which is in agreement with Meléndez et al. (2017a), suggest that the amount of pain and/or discomfort experienced by the banded calves during the first 2-d post-castration was not enough to be detected by the pain-related behaviors used in the current study. However, this assumption was not supported by the findings reported by Petherick et al. (2015). The authors reported that 3-mo old pasture housed calves castrated using rubber rings exhibited more tail flicks than surgically and uncastrated calves during the first 4-h post-castration, and surgically castrated calves spent less time walking for the first 3-days post-castration than rubber ring and uncastrated calves. However, in the current study, surgically castrated calves had more tail flicks than uncastrated calves on d 0, 1, and 2, but not in most of the days of observation when compared to banded calves. Discrepancies among studies is likely related to differences in when behaviors were assessed, Petherick et al. (2015) recorded behavior at

Table 6
Least square means (± SEM) of behavioral parameters obtained by using GPS tracking collars (cow-calf proximity and home range) recorded on d 0 (immediately post-castration) and d 1, and 2 (paddock 1) and on d 14, 15, and 16 (paddock 2) post-castration in Angus and Angus crossbred cows and their calves uncastrated (CT), band-castrated (BA), and knife-castrated (KN) with (M) and without (NM) a single s.c. administration of meloxicam.

	Treatments[2]							P-value[3]						
	CT		BA		KN									
Item[1]	NM	M	NM	M	NM	M	*SEM*	CAS	MED	CAS × MED	DAY	CAS × DAY	MED × DAY	CAS × MED × DAY
Cow-calf proximity, m														
Paddock 1	42.9	38.3	37.5	33.2	36.9	32.1	7.55	0.60	0.38	0.99	0.03	0.48	0.27	0.47
Paddock 2	125.5	117.5	100.8	78.4	104.9	117.5	20.6	0.28	0.18	0.41	<0.01	**<0.01**	0.81	0.81
Cows' home range, ha														
Paddock 1	5.5	5.4	5.8	5.7	5.1	5.2	0.37	0.40	0.38	0.94	<0.01	**<0.01**	**<0.01**	0.47
Paddock 2	12.2	11.7	11.9	10.9	12.8	10.8	3.05	0.96	0.30	0.87	<0.01	0.99	0.93	0.33
Calves' home range, ha														
Paddock 1	5.3	5.1	4.8	5.2	4.7	4.5	0.52	0.29	0.98	0.62	<0.01	**0.05**	0.94	0.89
Paddock 2	13.3	10.3	8.7	10.3	10.3	8.7	2.54	0.96	0.31	0.07	<0.01	0.21	0.55	0.16

[1] Paddock 1: 0.33 ha per animal; Paddock 2: 0.20 ha per animal.
[2] CT: Uncastrated calves submitted to the same handling procedure as castrated ones; BA: Calves castrated using rubber ring; KN: Calves castrated using a Newberry knife; NM: single s.c. injection of lactated ringers at castration time; M: single injection of s.c. meloxicam (0.5 mg/kg) at castration time.
[3] CAS: Castration treatment effect; MED: Medication treatment effect; DAY: Sampling day effect. Bold P-values indicate significant effect at $P \leq 0.05$.

0–40 min, 68–230 min, and 238–440 min (d 1, 2, and 3, respectively) post-castration while behavior was assessed on d 0 (0-120 min and 540-660 min), 1 (1,560-1,680 min and 1,980-2,100 min) and, 2 (3, 000-3,120 min and 3,420-3,540 min) post-castration in the current study.

3.4. Cow-calf proximity and home range

3.4.1. Medication effects

No significant medication ($P > 0.05$; Table 6) effects were observed for cow-calf proximity assessed while they were housed on paddocks 1 and 2. A significant medication × day interaction ($P < 0.01$; Table 6) was observed for cow home range from d 0 to 2 post-castration (paddock 1) however, no differences ($P > 0.05$) were observed between medication treatments within each sampling day. These findings were unexpected as we hypothesized that castrated calves administered meloxicam would have similar proximities and home ranges to uncastrated calves, but different proximities and home ranges from non-medicated castrated calves for 48 h following castration based on meloxicam half-life (Coetzee et al., 2012). We also hypothesized that the dams of medicated castrated calves would graze and explore the pasture in a similar manner to the dams of uncastrated calves due to the lack of changes in their calves' behavior related to the pain mitigating effects of meloxicam (Meléndez et al., 2018b). These hypothesis were based on the fact that knife castrated calves of similar age (4-5 mo-old) as the present study and administered oral meloxicam 2 h before castration showed an increased number of steps (measured by accelerometer fitted in the calves' legs) up to d 3 post-castration, compared to non-medicated calves (Olson et al., 2016). To date, there are no studies assessing the effects of (s.c.) meloxicam on cow-calf proximity post-castration. However, similar to our findings, a previous study using oral meloxicam prior to knife-castration in 6- to 8-mo old pasture-housed calves fitted with GPS collars found no differences in the home ranges (using a 95% MCP calculation) of medicated and non-medicated calves over a 7-d period post-castration (Van der Saag et al., 2018). Possible explanations for the lack of drug effect on cow-calf proximity as well as cow-calf home range include: 1) the cow-calf bond is strong therefore the pair would exhibit synchronized activites which may have masked any treatment effect 2) the sampling interval used for the GPS fixes (30-min) may have been too infrequent to detect treatment differences or 3) the number of animals evaluated for cow-calf proximity (CT = 5, BA = 6, and KN = 4) and home range (CT = 13, BA = 14, and KN = 9) was not sufficient to detect treatment differences 5) cow-calf proximity is not a good indicator of pain and 6) the drug was effective beyond 48 h after administration.

3.4.2. Castration treatment effects

No differences ($P > 0.05$; Table 6) were observed for cow-calf proximity while animals were housed on paddock 1 (d 0, 1, and 2 post-castration) among castration treatments (Fig. 3A). These findings were unexpected. We hypothesized that the distance between KN calves and their dams would be different compared to either BA and CT calves in the first 2 d post-castration, due to pain-related changes in walking, standing and lying. This assumption was based on findings in sheep showing that the maternal care (such as licking, sniffing, and vigilance behavior) ewes give their lambs increased when the lambs displayed pain-related behaviors (i.e. lying, head turning, foot stamping, and tail flicks). The authors reported that the maternal care expressed by the ewes was also modulated by the severity of pain their lambs experienced (Futro et al., 2015). Similar results have been reported in female rats who were shown to increase maternal care (licking and grooming) towards their offspring when the offspring were exposed to a painful stimuli (Blass et al., 1995; Walker et al., 2003).

Based in our findings, BA calves had closer proximity (castration × day interaction; $P < 0.01$; Table 6) to their dams on d 15 post-castration (paddock 2) compared to KN calves (Fig. 3A). These findings may be explained by the fact that BA calves had increased pain sensitivity on d 20 post-castration compared to KN calves (Fig. 1). Studies have shown that suckling increases oxytocin concentations in the dam's milk (Lupoli et al., 2001) resulting in reduced pain/stress responses in their calves (Meléndez et al., 2018a). Therefore, it is possible that BA calves remained in close proximity to their dams to mitigate castration related pain. On the other hand, a previous study reported no changes in the behavior of Angus cows (e.g. sniffing and licking) towards their nursing calves for the 30-min period after rubber ring castration (Turner et al., 2020). The authors stated that the lack of differences was possibly overshadowed by the general inactivity of the 1-d old calf or by the physical stress of calving.

In contrast, significant castration treatment × day interactions ($P \leq 0.05$; Table 6) were observed for the home ranges of KN calves (Fig. 3B) and their dams (Fig. 3C) where KN cow-calf pairs had reduced (lower 100% MCP values; $P < 0.01$) ranges compared to CT cow-calf pairs on d 0. These findings suggest that the trauma caused by the procedure affected the amount of walking observed in the calves on the day of castration which is also supported by the behavioral results of this study. Our findings indicate that cow-calf home range may be a useful indicator of pain following castration in beef cattle and future studies should include using a larger sample size to determine the consistency of these findings.

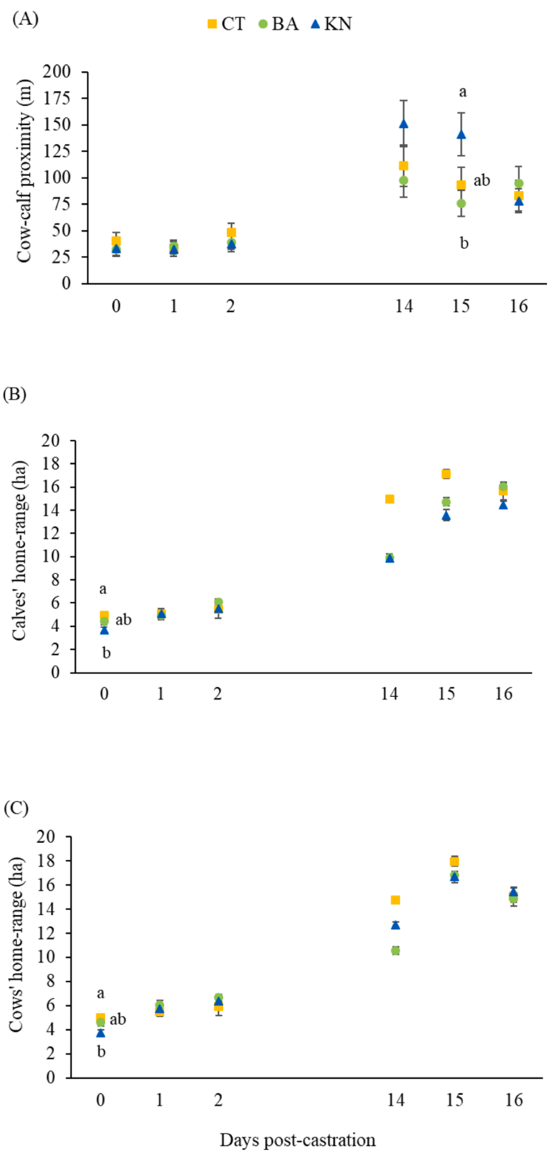

Fig. 3. Least square means and SEM for (A) cow-calf proximity, (B) calves' home-range, and (C) cows' home-range obtained on d 0, 1, 2, 14, 15, and 16 post-castration of uncastrated (CT, $n = 8$), band (BA, $n = 8$), and knife (KN, $n = 4$) castrated 2-mo old Angus and Angus crossbred calves housed in pasture. [a-b] Least square means with different superscript among treatments differ ($P \leq 0.05$).

4. Conclusions

With the exception of suckling behavior, meloxicam did not reduce any of the physiological or behavioral indicators of pain assessed in this study. Further studies are required to evaluate the effect of s.c. meloxicam used in 2-mo-old calves housed on pasture increasing the number of sampling points for indicators of pain within the first week post-castration. Knife and band castration caused pain and/or inflammatory responses over the 62 d assessment period post-procedure. Knife castrated calves exhibited more indicators of acute pain while band castrated calves experienced longer term pain. This is based on the absence of inflammatory responses (wound swelling) in knife castrated calves which occurred 2 weeks earlier than in band castrated calves and greater pain sensitivity for the first 2-weeks post-castration in knife *versus* the third week post-castration in banded calves. Overall, knife calves had greater standing and walking duration, as well as greater head turning, and tail flick frequencies than band castrated calves for up to 2 d post-castration. Increased proximity of BA calves to their dams on d 15, combined with a reduced home range observed in KN calves on the day of castration support that knife castration causes greater acute pain while band castration results in longer term pain. In addition, the reduced home-range observed in the dams of KN calves on the day of castration suggested that calf pain-related behavior may alter how cows explore the pasture. However, the lack of differences in cow-calf proximity between castrated and uncastrated calves suggest that further studies are necessary using a larger sample size and reduced GPS sampling intervals to better understand the relationship between proximity and home-range measurements and calf pain.

CRediT authorship contribution statement

Désirée Gellatly: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Sonia Marti:** Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Project administration. **Edmond A. Pajor:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Funding acquisition. **Daniela M. Meléndez:** Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing. **Diego Moya:** Conceptualization, Methodology, Validation, Writing - original draft, Writing - review & editing. **Eugene D. Janzen:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Xiaohui Yang:** Software, Formal analysis, Data curation, Writing - original draft. **Mohammad R.M. Milani:** Formal analysis, Writing - review & editing, Data curation. **Karen S. Schwartzkopf-Genswein:** Conceptualization, Methodology, Validation, Resources, Investigation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare they have no financial or personal relationship with people or organizations that would create a conflict of interest.

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References

Baldwin, R.L., McLeod, K.R., Klotz, J.L., 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J. Dairy Sci. 87, E55–E65. https://doi.org/10.3168/jds.S0022-0302(04)70061-2.

Blass, E.M., Shide, D.J., Zawmon, C., Sorrentino, J., 1995. Mother as shield–differential effects of contact and nursing on pain responsivity in infant rats–evidence for non opioid mediation. Behav Neurosci 109, 342–353. https://doi.org/10.1037//0735-7044.109.2.342.

Bolado-Sarabia, J.L., Pérez-Linares, C., Figueroa-Saavedra, F., Tamayo-Sosa, A.R., Barreras-Serrano, A., Sánchez-López, E., García-Reynoso, I.C., Ríos-Rincón, F.G., Rodríguez-Poché, M.Y., García-Vega, L.A., Gallegos, E., Castro-Osuna, P., 2018. Effect of immunocastration on behaviour and blood parameters (cortisol and testosterone) of Holstein bulls. Austral. J. Vet. Sci. 50, 77–81. https://doi.org/10.4067/S0719-81322018000200077.

Bretschneider, G., 2005. Effects of age and method of castration on performance and stress response of beef male cattle: A review. Livest. Prod. Sci. 97, 89–100. https://doi.org/10.1016/j.livprodsci.2005.04.006.

Burdick, N.C., Randel, R.D., Carroll, J.A., Welsh, T.H., 2011. Interactions between Temperament, Stress, and Immune Function in Cattle. Intern. J. Zool. https://doi.org/10.1155/2011/373197. Article ID 373197.

Burrow, H.M., Corbet, N.J., 2000. Genetic and environmental factors affecting temperament of zebu and zebu-derived beef cattle grazed at pasture in the tropics. Austral. J. Agric. Res 51. https://doi.org/10.1071/AR99053.

Burrow, H., Seifert, G., Corbet, N., 1988. A new technique for measuring temperament in cattle. Proc. Aust. Soc. Anim. Prod 17, 154–157. http://www.asap.asn.au/livestocklibrary/1988/Burrow88.PDF. accessed 14 May 2020.

Burt, W.H., 1943. Terrioriality and home range concepts as applied to mammals. J Mammal 24 (13), 346–352. https://doi.org/10.2307/1374834.

Calenge, C., 2006. The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. Ecol. Model. 197, 516–519. https://doi.org/10.1016/j.ecolmodel.2006.03.017.

Canada Council of Animal Care (CCAC), 2009. CCAC guidelines on: Animal use protocol review. CCAC, 2009. http://www.ccac.ca/Documents/Standards/Guidelines/Farm_Animals.pdf. accessed 14 May 2020.

Coetzee, J.F., Edwards, L.N., Mosher, R.A., Bello, N.M., O'Connor, A.M., Wang, B., KuKanich, B., Blasi, D.A., 2012. Effect of oral meloxicam on health and performance of beef steers relative to bulls castrated on arrival at the feedlot. J. Anim. Sci. 90, 1026–1039. https://doi.org/10.2527/jas.2011-4068.

Coetzee, J., 2013. Assessment and management of pain associated with castration in cattle veterinary clinics of North America. Food Animal Practice 29, 75–101. https://doi.org/10.1016/j.cvfa.2012.11.002.

Daniel, J.A., Crane, A., Krawczel, P.D., Coetzee, J.F., Whitlock, B.K., 2020. Effect of oral meloxicam administration on growth performance and behavior of pre-weaning age calves following band castration. Transl. Anim. Sci. 4, 1082–1090. https://doi.org/10.1093/tas/txaa021.

Environmental Systems Research Institute (ESRI), 2011. ArcGIS Desktop: Release 10in ESRI, editor. ESRI, Redlands, California, USA.

Fordyce, G., Howitt, C.J., Holroyd, R.G., O'Rourke, P.K., Entwistle, K.W., 1996. The performance of Brahman-Shorthorn and Sahiwal-Shorthorn beef cattle in the dry tropics of northern Queensland - Scrotal circumference, temperament, ectoparasite resistance, and the genetics of growth and other traits in bulls. Aust. J. Experim. Agric. 36, 9–17. https://doi.org/10.1071/EA9960009.

Futro, A., Masłowska, K., Dwyer, C.M., 2015. Ewes direct most maternal attention towards lambs that show the greatest pain-related behavioural responses. PLoS ONE 10 (7), e0134024. https://doi.org/10.1371/journal.pone.0134024.

González, L.A., Schwartzkopf-Genswein, K.S., Caulkett, N.A., Janzen, E., McAllister, T.A., Fierheller, E., Schaefer, A.L., Haley, D.B., Stookey, J.M., Hendrick, S., 2010. Pain mitigation after band castration of beef calves and its effects on performance, behavior, Escherichia coli, and salivary cortisol. J. Anim. Sci. 88, 802–810. https://doi.org/10.2527/jas.2008-1752.

Hild, S., Clark, C.C.A., Dwyer, C.M., Murrell, J.C., Mendl, M., Zanella, A.J., 2011. Ewes are more attentive to their offspring experiencing pain but not stress. Appl. Anim. Behav. Sci. 132, 114–120. https://doi.org/10.1016/j.applanim.2011.04.003.

Lemos Teixeira, D., Larraín, R., Melo, O., Hötzel, M.J., 2018. Public opinion towards castration without anaesthesia and lack of access to pasture in beef cattle production. PLoS ONE 13 (1), e0190671. https://doi.org/10.1371/journal.pone.0190671.

Lomax, S., Windsor, P.A., 2013. Topical anesthesia mitigates the pain of castration in beef calves. J. Anim. Sci. 91, 4945–4952. https://doi.org/10.2527/jas.2012-5984.

Lupoli, B., Johansson, B., Uvnäs-Moberg, K., Svennersten-Sjaunja, K., 2001. Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and their calves. J. Dairy Res. 68, 175–187. https://doi.org/10.1017/S0022029901004721.

Marti, S., Meléndez, D.M., Pajor, E.A., Moya, D., Heuston, C., Gellatly, D., Janzen, E.D., Schwartzkopf-Genswein, K.S., 2017a. Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: II. Chronic pain. J. Anim. Sci. 95, 4367–4380. https://doi.org/10.2527/jas2017.1763.

Marti, S., Schwartzkopf-Genswein, K.S., Janzen, E.D., Meléndez, D.M., Gellatly, D., Pajor, E.A., 2017b. Use of topical healing agents on scrotal wounds after surgical castration in weaned beef calves. Can Vet J 58 (10), 1081–1085. PubMed PMID: 28966358; PubMed Central PMCID: PMC5603928.

Marti, S., Meléndez, D.M., Pajor, E.A., Moya, D., Gellatly, D., Janzen, E.D., Schwartzkopf-Genswein, K.S., 2018a. Effect of a single dose of subcutaneous meloxicam prior to band or knife castration in 1-wk-old beef calves: II. Inflammatory response and healing. J. Anim. Sci. 96, 4136–4148. https://doi.org/10.1093/jas/sky291.

Marti, S., Meléndez, D.M., Pajor, E.A., Moya, D., Gellatly, D., Janzen, E.D., Schwartzkopf-Genswein, K.S., 2018b. Effect of a single dose of subcutaneous meloxicam before knife castration alone or combined with hot-iron branding on scrotal healing, inflammatory response and behaviour in 2 mo old beef calves. Can. J. Anim. S. https://doi.org/10.1139/CJAS-2018-.

Martin, P., Bateson, P., 2007. Recording methods. Measuring behaviour: an introductory guide, Third ed. Cambridge University Press.

Maurya, V.P., Sejian, V., Kumar, K., Singh, G., Naqvi, S.M.K., 2012. Walking stress influence on livestock production. Environmental stress and amelioration in livestock production. German: Springer-Verlag GMbH Publisher, pp. 75–95. https://doi.org/10.1007/978-3-642-29205-7_4.

Meléndez, D.M., Marti, S., Pajor, E.A., Moya, D., Heuston, C., Gellatly, D., Janzen, E.A., Schwartzkopf-Genswein, K.S., 2017a. Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: I. Acute pain. J. Animal Sci. 95, 4352–4366. https://doi.org/10.2527/jas2017.1762.

Meléndez, D., Marti, S., Pajor, E.A., Moya, D., Gellatly, D., Janzen, E.A., Schwartzkopf-Genswein, K.S., 2018a. Effect of a single dose of meloxicam prior to band or knife castration in 1 week old beef calves: I. Acute Pain. J. Anim. Sci. 96, 1268–1280. https://doi.org/10.1093/jas/sky034.

Meléndez, D., Marti, S., Pajor, E.A., Moya, D., Gellatly, D., Janzen, E.A., Schwartzkopf-Genswein, K.S., 2018b. Effect of subcutaneous meloxicam on indicators of acute pain and distress after castration and branding in 2-mo-old beef calves. J. Anim. Sci. 96, 1268–1280. https://doi.org/10.1093/jas/sky245.

Meléndez, D.M., Marti, S., Pajor, E.A., Sidhu, P.K., Gellatly, D., Janzen, E.D., Schwinghamer, T.D., Coetzee, J.F., Schwartzkopf-Genswein, K.S., 2019. Pharmacokinetics of oral and subcutaneous meloxicam: Effect on indicators of pain and inflammation after knife castration in weaned beef calves. PLoS ONE 14 (5), e0217518. https://doi.org/10.1371/journal.pone.0217518.

Mills, E.C., Poulsen, J.R., Fay, J.M., Morkel, P., Clark, C.J, Meier, A., Beirne, C., White, L. J.T., 2018. Forest elephant movement and habitat use in a tropical forest-grassland mosaic in Gabon. PLoS ONE 13 (7), e0199387. https://doi.org/10.1371/journal.pone.0199387.

Mintline, E.M., Varga, A., Banuelos, J., Walker, K.A., Hoar, B., Drake, D., Weary, D.M., Coetzee, J.F., Stock, M.L., Tucker, C.B., 2014. Healing of surgical castration wounds: a description and an evaluation of flunixin. J. Anim. Sci. 92, 5659–5665. https://doi.org/10.2527/jas.2014-7885.

Mittal, P.K., Gottam, G.S., Gupta, B., Bilochi, D.R., 2019. The effect of climate change on productivity and reproductive and health performance of livestock: A review. J. Entomol. Zool. Stud. 7, 4–9. https://www.entomoljournal.com/archives/?year=2019&vol=7&issue=2&ArticleId=4911. Accessed 15 October 2020.

Moggy, M.A., Pajor, E.A., Thurston, W.E., Parker, S., Greter, A.M., Schwartzkopf-Genswein, K.S., Campbell, J.R., Windeyer, M.C., 2017. Management practices associated with pain in cattle on western Canadian cow–calf operations: A mixed methods study. J. Anim. Sci. 95, 958–969. https://doi.org/10.2527/jas.2016.0949.

Molony, V., Kent, J., Robertson, I., 1995. Assessment of acute and chronic pain after different methods of castration of calves. Appl. Anim. Beh. Sci. 46, 33–48. https://doi.org/10.1016/0168-1591(95)00635-4.

Moya, D., González, L.A., Janzen, E., Caulkett, N.A., Fireheller, E., Schwartzkopf-Genswein, K.S., 2014. Effects of castration method and frequency of intramuscular injections of ketoprofen on behavioral and physiological indicators of pain in beef cattle. J. Anim. Sci. 92, 1686–1697. https://doi.org/10.2527/jas.2013-7298.

Olson, M.E., Ralston, B., Burwash, L., Matheson-Bird, H., Allan, N.D., 2016. Efficacy of oral meloxicam suspension for prevention of pain and inflammation following band and surgical castration in calves. BMC Vet Res 12, 102. https://doi.org/10.1186/s12917-016-0735-3.

Pang, W.Y, Earley, B., Sweeney, T., Crowe, M.A., 2006. Effect of carprofen administration during banding or burdizzo castration of bulls on plasma cortisol, in vitro interferon-gamma production, acute-phase proteins, feed intake, and growth. J. Anim. Sci. 84, 351–359. https://doi.org/10.2527/2006.842351x.

Pebesma, E.J., Bivand, R.S., 2005. Classes and methods for spatial data in R. R News 5 (2). https://cran.r-project.org/doc/Rnews/. accessed 14 May 2020.

Petherick, J., Small, A., Reid, D., Colditz, I., Ferguson, D., 2015. Welfare outcomes for 3- and 6-month-old beef calves in a tropical environment castrated surgically or by applying rubber rings. Appl. Anim. Behav. Sci. 171, 47–57. https://doi.org/10.1016/j.applanim.2015.08.018.

R Core Team, 2011. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria. http://www.R-project.org (accessed 14 May 2020).

Stafford, K.J., Mellor, D.J, Todd, S.E., Bruce, R.A, Ward, R.N., 2002. Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. Res. Vet. Sci. 73, 61–70. https://doi.org/10.1016/S0034-5288(02)00045-0.

Stafford, K., 2007. Alleviating the pain caused by the castration of cattle. The Vet. J. 173, 245–247. https://doi.org/10.1016/j.tvjl.2005.11.002.

Ting, S.T.L., Earley, B., Crowe, M.A., 2003. Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growth, and behavior. J. Anim. Sci. 81, 1253–1264. https://doi.org/10.2527/2003.8151253x.

Turner, S.P., McIlvaney, K., Donbavand, J., Turner, M.J., 2020. The Effect of Behavioural Indicators of Calf Discomfort Following Routine Procedures on Cow Maternal Care. Animals 10, 87. https://doi.org/10.3390/ani10010087.

Valente, E.E.L., Paulino, M.F., Detmann, E., Valadares Filho, S.C., Cardenas, J.E.G., Dias, I.F.T., 2013. Requirement of energy and protein of beef cattle on tropical pasture. Acta Scientiarum. Animal Sciences 35 (4), 417–424. https://doi.org/10.4025/actascianimsci.v35i4.21143.

Van der Saag, D., White, P., Ingram, L., Manning, J., Windsor, P., Thomson, P., Lomax, S., 2018. Effects of Topical Anaesthetic and Buccal Meloxicam Treatments on Concurrent Castration and Dehorning of Beef Calves. Animals: an open access journal from MDPI 8 (3), 35. https://doi.org/10.3390/ani8030035.

Walker, C-D., Kudreikis, K., Sherrard, A., Johnston, C.C., 2003. Repeated neonatal pain influences maternal behavior, but not stress responsiveness in rat offspring. Dev. Brain. Res. 140, 253–261. https://doi.org/10.1016/s0165-3806(02)00611-9.

Weary, D.M., Niel, L., Flower, F.C., Fraser, D., 2006. Identifying and preventing pain in animals. Appl. Anim. Behav. Sci. 100, 64–76. https://doi.org/10.1016/j.applanim.2006.04.013.