Effect of a single dose of meloxicam prior to band or knife castration in 1-wk-old beef calves: I. Acute pain

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ABSTRACT: In Western Canada, approximately half of the calves produced are castrated before 1 wk of age. Therefore, it is important to identify effective analgesic drugs to mitigate pain associated with castration and consequently improve animal welfare. The aim of this study was to assess the efficacy of a single s.c. dose of meloxicam at mitigating pain associated with knife and band castration in 1-wk-old calves. Seventy-two Angus crossbred bull calves (47.3 ± 6.70 kg of body weight (BW), 1 wk old) were used in a 3×2 factorial design where main factors included castration method—sham (CT), band (BA) or knife (KN) castration— and medication—lactate ringer (NM) or 0.5 mg/kg BW of meloxicam (M). Measurements included different physiological and behavioral parameters. Samples were collected on day -1, immediately before castration (T0); and 60, 90, and 120 min and 1, 2, 3, and 7 d after castration except for visual analog scale (VAS) which was collected at the time of castration. The salivary cortisol concentrations were greater ($P = 0.04$) in KN and BA calves than CT calves 60 min after castration, while 90 min after castration BA had greater concentrations than CT

calves. Substance P concentrations were greater ($P = 0.04$) in NM calves than M calves on d 3 and 7 after castration. The serum amyloid-A (SAA) concentrations were greater ($P = 0.05$) in KN calves than BA and CT calves on days 0, 2, and 3, while BA calves had greater SAA concentrations on day 7 than KN and CT calves. The visual analog scores were greater ($P < 0.01$) in KN calves than BA, and in BA compared to CT calves. The KN calves tail flicked more ($P < 0.01$) than BA and CT calves, and NM calves tail flicked more ($P = 0.03$) than M calves. No castration or medication effect ($P > 0.10$) was observed for stride length, walking, standing, lying ventral, eating, foot stamping, head turning, lying and standing percentage, performance, platelets, or body temperature. Overall, knife castrated calves exhibited a greater acute pain response than band castrated calves. Meloxicam was able to reduce substance P concentrations, white blood cell counts and number of tail flicks after castration, suggesting that the drug was able to mitigate acute pain to some extent. However, meloxicam did not have an effect on the other physiological and behavioral parameters assessed.

Key words: acute pain, beef calves, castration, pain mitigation, welfare

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J. Anim. Sci. 2018.96:1268–1280
doi: 10.1093/jas/sky034

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This is Lethbridge Research Centre contribution # 38717053.

Received October 6, 2017.

Accepted February 15, 2018.

INTRODUCTION

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Castration is a painful husbandry procedure reported to have a negative impact on growth performance, especially when performed at older ages, therefore recommendations have been

made to castrate calves as young as possible in order to reduce tissue damage and consequently reduce stress (Bretschneider, 2005). Nevertheless, independent of the method of castration, beef calves experience pain and discomfort at any age (Schwartzkopf-Genswein et al., 2012), as demonstrated by physiological and behavioral changes in calves as young as 1 wk of age (Robertson et al., 1994; Molony et al., 1995).

In Western Canada, approximately 53% of calves in cow-calf operations are castrated before 1 wk of age (Moggy et al., 2017); therefore, it is of great importance to identify practical and effective pain mitigation strategies for this age group. Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID), approved for use in cattle in the EU and Canada, which has been reported to be effective at mitigating physiological indicators of pain after painful husbandry procedures such as dehorning and castration (Stewart et al., 2009; Roberts et al., 2015). Meloxicam is a practical analgesic option for producers due to its ease of administration (s.c) and long half-life (22 ± 3 h) (Stock and Coetzee, 2015), which makes it an attractive one time administration analgesic. Although meloxicam is labeled for pain relief following debudding, it is not labeled for pain relief after castration.

To our knowledge, there are no studies assessing the efficacy of injectable s.c. meloxicam in 1-wk-old calves. Therefore, the objective of this project was to assess acute pain associated with different castration methods, and the efficacy of s.c. meloxicam administered immediately prior to castration in 1-wk-old beef calves. We hypothesize that knife castration will have greater pain indicators and that meloxicam will be effective at reducing behavioral and physiological indicators of acute pain associated with castration.

MATERIALS AND METHODS

This protocol was approved by Lethbridge Research Centre (ACC # 1410) and the University of Calgary (AC14-0159). All animals were cared for according to the Canadian Council of Animal Care guidelines (CCAC, 2009).

Animal Housing and Treatments

Seventy-two Angus crossbred bull calves (47.3 ± 6.70 kg of BW and 7 to 8 d old) were used in a 7-d experiment at the Agriculture and Agri-Food Canada Lethbridge Research Centre (LRC) (AB, Canada). Cow-calf pairs were transported

from the ranch of origin to the LRC for 30 km in two separate groups of 36 calves each, as groups were castrated at 7 to 8 d of age on separate days 1 wk apart. Upon arrival, animals were weighed and a small area ($5 \text{ cm} \times 5 \text{ cm}$) was shaved on the neck of each calf in order to locate the jugular vein. The seventy-two cow-calf pairs were housed in six experimental pens (12 pairs per pen) three of which measured $36.7 \text{ m} \times 22.2 \text{ m}$, and the remaining three measured $40 \text{ m} \times 27 \text{ m}$. Each pen contained a calf shelter ($2.4 \text{ m} \times 3.6 \text{ m} \times 1.4 \text{ m}$), a centrally located water system and straw bedding. The cows' diet consisted of free choice alfalfa grass while the calves' diet consisted of milk from suckling and free choice salt blocks and loose minerals containing a coccidiostat (Diluted Rumensin Drug Premix 1100 (Medicated), HI-PRO FEEDS, Okotoks, AB, Canada) for the prevention of diarrhea caused by coccidiosis.

Calves were equally distributed by weight into pens and randomly assigned to treatments. Calves were the experimental unit as treatments were mixed within pens with two calves per treatment per pen. The experiment consisted of a 3×2 factorial design where main factors included castration method: sham (control calves, CT; $n = 24$), band (BA; $n = 24$), or knife (KN; $n = 24$) castration; and medication: single s.c. administration of lactated Ringer's (Lactated Ringer's Irrigation, Baxter Canada, Mississauga, Ontario, Canada) (NM; $n = 36$) or a single dose of 0.5 mg/kg of s.c. meloxicam (Metacam 20 mg/mL, Boehringer Ingelheim, Burlington, Ontario, Canada) (M; $n = 36$). A valid Veterinarian-Client-Patient relationship (VCPR) was established prior to the extra-label drug use of meloxicam, as this drug is not labeled for pain mitigation associated with castration. Calves were weighed in a portable chute and restrained, sampled and castrated on a tip table (Calf Roper, Ram-Bull Ltd, Barons, Alberta, Canada). Calves were castrated for a period of 2 to 3 min while tipped on the tip table and sampled for a period of 3 to 5 min while standing in the tip table. All calves were castrated between 7 and 8 d of age by the same experienced veterinarian. Band castration was performed by placing a band (Elastrator Pliers and Rings, Kane Veterinary Suppliers Ltd., Edmonton) using an elastrator on the neck of the scrotum above the testicles. Knife castration consisted of making a latero-lateral incision in the scrotum with a Newberry castration knife (Syrvet Inc., Waukegan, IA) in order to externalize the testicles while crushing and cutting of the spermatic cords was

done using an emasculator. Sham-castrated calves were handled the same way as knife and band castrated calves and the testicles were manipulated for a similar amount of time.

Measurements of Acute Pain and Sample Collection

Samples were collected during the first 7 d after castration, which was defined as the period of acute pain as previously reported by [Meléndez et al. \(2017\)](#).

Physiological parameters.

Salivary cortisol. Samples were collected on day -1, and immediately before castration (T0), 60, 90, 120 min and 1, 2, 3, and 7 d after castration. Saliva was collected by swabbing the oral cavity with a cotton swab placed in a plastic tube and frozen at -20°C for subsequent analysis. Samples were analyzed using an enzyme immunoassay kit (Salimetrics, LLC State College, PA). Interassay and intra-assay coefficient of variability (CV) values were 16.1% and 8.4%, respectively.

Blood samples. Blood samples were collected on day -1, and T0, 60, 90, 120 min and on 1, 2, 3, and 7 d after castration. Samples were obtained via jugular venipuncture into vacuum tubes (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ) for further analysis.

Samples for substance P were collected into 4-mL tubes containing ethylenediaminetetraacetate (EDTA) (BD vacutainer; Becton Dickinson Co), where benzamidine hydrochloride was added to reduce substance P degradation. The sample was centrifuged for 15 min at $1.5 \times g$ at 0°C , and the serum was decanted and frozen at -80°C . Samples were analyzed at Iowa State University, College of Veterinary Medicine (Ames, IA) with some modifications from the previously described procedure by [Van Engen et al. \(2014\)](#). Nonextracted plasma samples were analyzed in duplicate with a double antibody radioimmunoassay using a purchased primary antibody [Substance-P (3-11) Antibody for Immunohistochemistry Human, Rat, Mouse, Phoenix Pharmaceutical #H-061-05]. The range of detection for substance P was between 5 and 320 pg/mL, with an average $R^2 = 0.99$. The coefficient of variation for intra-assay variability was 8.1% and the interassay variability was 15.3%. The limit of detection was 10 pg/mL and the limit of quantitation was 20 pg/mL.

Blood samples for haptoglobin and serum amyloid-A (SAA) were collected into 6-mL nonadditive tubes (BD vacutainer; Becton Dickinson Co),

centrifuged for 15 min at $1.5 \times g$ and the serum was decanted and frozen at -20°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 two levels of in-house controls (bovine serum pools) daily and two levels of Tridelta controls weekly. The inter-assay CV for haptoglobin was 7.6%. SAA concentrations were analyzed using an enzyme-linked immunosorbent assay (Tridelta Phase range SAA kit, TP 807, Tridelta development LTd, Maynooth, Ireland). The intra-assay and interassay CV were 13.7% and 7.5%, respectively.

Blood samples for complete blood count (CBC) were collected into 4-mL EDTA tubes (BD vacutainer; Becton Dickinson Co) and a HemaTrueHematology Analyzer (Heska, Lobeland, Co) was used to measure red blood cells (RBC), white blood cells (WBC), and platelet counts. Hematocrit, hemoglobin, lymphocytes, monocytes, and granulocytes were also analyzed (data not shown).

Scrotal and base temperatures (Scrotal temp and Base temp). Images of the scrotal area and the base of the scrotum were collected on day -1, T0 (before castration), 60, 90, 120 min and on days 1, 2, 3, and 7 after castration. Images were taken from behind the calves, approximately 1 m from the scrotum with a FLIR i60 infrared camera (FLIR Systems Ltd Burlington, Ontario, Canada) and analyzed with FLIR Tools v.5.1 (FLIR Systems Ltd Burlington, Ontario, Canada) using an emissivity coefficient of 0.98 to measure Scrotal temp and Base temp as indicators of inflammation.

Rectal temperature (Rectal temp). Rectal temperature was obtained by inserting a digital thermometer (GLA M750 Livestock Thermometer, San Luis Obispo, CA) into the rectum of calves on day -1, T0 (before castration), 1, 2, 3, and 7 d after castration.

Performance. Animals were weighed on day -1, T0, and 1, 2, 3, and 7 d after castration in a portable scale. Weights collected on day -1 and day 7 were used as initial and final body weight (BW). Average daily gain (ADG) was calculated by dividing the difference in weight from day -1 to day 7 by the number of days between weights (9).

Behavioral parameters during castration.

Two experienced observers recorded visual analog scale (VAS), urination, leg movement, and

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vocalization frequency during castration. The VAS consisted of placing a mark along a 10 cm continuum which served as an indicator of the observer's perception of the amount of discomfort that the animal experienced during castration. The mark was measured to the closest 0.5 cm and the measurements were analyzed. Frequency of urination, leg movement, and vocalization were also recorded at the time of castration. Due to the experimental setting, observers could not be blind to the treatments.

Behavioral parameters after castration.

Behavioral observations. To identify individual calves, penning tags were glued to the back of each calf on day -1 using tag cement (Livestock Identification tag cement, W.J. Ruscoe Company, Akron, OH). Behavior was recorded with two cameras (2.0MP HD IR Bullet Camera, Avigilon, Vancouver, BC, Canada) which were mounted on 6 m poles on the north and the south side of each experimental pen. Observer XT (Noldus Information Technology, Wageningen, The Netherlands) was used by two experienced observers (blind to treatments) for scoring focal animal sampling from continuous recordings (Martin and Bateson, 2007) of 36 calves (6/treatment) to document the duration of standing, walking, lying, and suckling behavior, and frequency of tail flicking, head turning, lesion licking and foot stamping as previously described by Meléndez et al. (2017). Behavioral observations were done continuously for a period of 2 h from 2 to 4 h after castration, and for 4 min every 10 min for a 3-h period 1, 2, 3, and 7 d after castration. Inter-rater and intra-rater reliability were 0.90 and 0.91, respectively.

Stride length. Stride length (SL) measurements were collected on day -1, immediately after castration and 120 min after castration. Stride length was videoed when the animals walked through a 1-m wide and 3-m long alley built with panels and placed immediately after the tip table. Stride length was collected and measured with some modifications from the previously described method of Currah et al. (2009). Modifications included a different type of software for image analysis and no use of grid background at the time of video recording. GOM Player (GOM Lab, Gretech Corporation, Seoul, South Korea) was used to take two pictures of the calves when both of their hind legs were placed on the ground, and ImageJ (Bethesda, MD) was used to measure the distance (cm) between the left and the right hind leg as they walked through the 3-m alley.

Standing and lying behavior. Standing and lying behavior was collected using hobo pendant G data loggers (Onset Computer Corporation, Bourne, MA). Hobo data loggers were covered in plastic film to protect them from moisture and wrapped in foam to protect the device from damage and reduce irritation due to rubbing on the legs of the calves. The data loggers were attached on day -1 to the left hind leg using a 4.6-m long latex flexible cohesive bandage (Latex Flexible Cohesive Bandage, Professional Preference, Calgary, AB, Canada). Data were used from days which had 24 h of data; therefore days -1 and 7 were excluded from the analysis. The data loggers were used to collect standing and lying bouts (number/day), total standing and lying duration (min/day) which was converted to a percentage (%), and mean standing and lying bout duration (min/day) (UBC AWP, 2013).

Calculation and Statistical Analysis

Data were analyzed using Mixed Models (SAS, version 9.4, SAS Inst. Inc., Cary, NC) to evaluate the effect of castration and medication on behavioral and physiological parameters. Fixed effects included castration, medication, time and their interactions, while random effects included pen and calf nested within pen. Data were analyzed for normality using Proc Univariate (SAS, version 9.4, SAS Inst. Inc., Cary, NC) and if data was not normally distributed it was transformed. All physiological parameters were transformed using Naiperian log except for scrotal temperature, rectal temperature, total blood cell count, and weights. All behavioral parameters were square root + 1 transformed except for SL, and percentage data was arc-sin transformed. All data except for VAS, ADG, initial and final BW were analyzed using the repeated measure model (Proc Mixed of SAS). Covariance structures included compound symmetry, autoregressive order 1 and unstructured. The covariance structure with the lowest Schwarz's Bayesian criterion was selected as the analysis of preference. Physiological data from T0, 60, 90, and 120 min and behavioral data from 2 to 4 h after castration were analyzed separately from those obtained on days 1, 2, 3, and 7. Significance was established at $P \leq 0.05$ and a tendency between $0.05 < P \leq 0.10$. A post-hoc test was run when interactions were significant ($P \leq 0.05$) to separate the LS means using the PDIFF option in SAS. An intra-class correlation coefficient with a 95% CI was used to determine inter-rater and intra-rater reliability for behavior using IBM SPSS statistics for Windows, version 22.0 (IBM Corp., Armonk, NY).

RESULTS

Physiology

Minutes after castration. Calves that received meloxicam tended ($P = 0.10$; Table 1) to have greater cortisol concentrations than NM calves (6.1 ± 0.66 vs. 5.1 ± 0.66 nmol/L, respectively) 60 min after castration, however no differences ($P > 0.10$) were seen 90 and 120 min after castration. A castration $\times$ time interaction ($P = 0.04$) was observed for cortisol concentrations, where KN and BA calves had greater ($P = 0.01$; $P = 0.04$) cortisol concentrations than CT calves 60 min after castration, while 90 min after castration only BA had greater ($P = 0.01$) cortisol concentrations than CT calves (Fig. 1A). No differences ($P > 0.10$) in cortisol concentrations were observed between BA and CT calves, compared to KN calves 90 min after castration and

no differences ($P > 0.10$; $P > 0.10$) were observed between castration groups at T0 and 120 min after castration. Substance P tended to be greater ($P = 0.09$) in NM calves (102.7 ± 4.33 pg/mL) than M calves (94.2 ± 4.29 pg/mL) 120 min after castration. Base temp tended to be greater ($P = 0.08$) in BA ($35.5 \pm 0.57^\circ\text{C}$) calves than KN ($34.4 \pm 0.57^\circ\text{C}$) and CT ($34.4 \pm 0.57^\circ\text{C}$) calves 90 min after castration, while BA ($35.3 \pm 0.55^\circ\text{C}$) and CT ($35.3 \pm 0.58^\circ\text{C}$) calves tended to have greater ($P = 0.08$) base temp than KN ($33.5 \pm 0.59^\circ\text{C}$) calves 120 min after castration. Scrotal temp decreased (time effect; $P < 0.01$) over time in all treatments (Table 1).

Days After Castration. Cortisol concentrations had a time effect ($P < 0.01$), where greater ($P \leq 0.05$) concentrations were observed on days 1, 2, and 3 compared to day 7, and there was a tendency ($P \geq 0.06$) for concentrations to be greater on day 1 and 3 compared to day 2 after castration. Substance P

Table 1. Least square means ($\pm$ SEM) of samples taken at T0, 60, 90, and 120 min after castration for cortisol, substance P, base scrotal temperature (Base temp), and scrotal temperature (Scrotal temp) and on days 1, 2, 3, and 7 after castration of cortisol, substance P, haptoglobin, SAA, complete blood count (CBC), base scrotal temperature (Base temp), scrotal temperature (Scrotal temp), and rectal temperature (Rectal Temp) of non-castrated (CT), band (BA) and knife (KN) castrated 1-wk-old Angus crossbred bull calves with (M) or without (NM) a single s.c. meloxicam administration¹

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Item	Treatment ²						SEM ⁴	P-value ³					MED ×T
	CT		BA		KN			CAS	MED	T	CAS ×T		
	NM	M	NM	M	NM	M							
<i>Minutes after castration</i>													
Cortisol, nmol/L	3.9	4.5	4.8	6.0	6.2	4.6	0.12	0.08	0.62	<0.01	0.04	0.10	
Substance P, pg/mL	97.1	103.1	101.7	93.4	102.7	99.5	0.04	0.90	0.78	0.02	0.18	0.09	
Base temp, °C	34.4	35.2	35.5	35.7	34.5	34.7	0.55	0.04	0.29	<0.01	0.08	0.93	
Scrotal temp, °C	34.5	34.7	34.1	34.1	33.7	34.0	0.59	0.18	0.58	<0.01	0.67	0.49	
<i>Days after castration</i>													
Cortisol, nmol/L	4.2	4.0	3.3	3.8	3.5	3.2	0.11	0.78	0.84	<0.01	0.98	0.56	
Substance P, pg/mL	92.3	99.1	100.2	91.7	99.1	90.1	0.05	0.83	0.14	<0.01	0.68	0.04	
Haptoglobin, g/L	0.3	0.2	0.2	0.3	0.3	0.3	0.10	0.31	0.92	0.02	0.72	0.19	
SAA, µg/mL	102.6 ^c	102.8 ^{b,c}	103.1 ^{b,c}	114.0 ^{a,b}	148.7 ^a	108.2 ^b	0.14	0.01	0.80	<0.01	0.05	0.57	
<i>CBC</i>													
WBC, × 10 ⁹ /L	11.4	9.8	10.3	9.9	11.4	9.8	0.54	0.59	0.01	0.09	0.62	0.19	
RBC, × 10 ¹² /L	8.0	7.8	8.1	8.0	8.0	8.2	0.09	0.04	0.64	<0.01	0.30	0.46	
Platelets, × 10 ⁹ /L	521.6	496.3	486.9	480.5	467.3	481.2	23.24	0.31	0.76	0.01	0.83	0.84	
Base temp, °C	33.7	33.9	33.9	33.9	34.3	33.8	0.42	0.83	0.76	<0.01	0.13	0.50	
Scrotal temp, °C	32.7	33.1	31.1	31.4	34.0	33.3	0.42	<0.01	0.96	<0.01	<0.01	0.32	
Rectal temp, °C	39.4	39.4	39.3	39.5	39.4	39.4	0.11	0.71	0.26	<0.01	0.97	0.26	

^{a-c}Least square means within a row with different superscripts differ ($P < 0.05$).

¹Values represent the mean of T0, 60, 90, and 120 min and days 1, 2, 3, and 7 after castration.

²CT = Sham noncastrated calves; BA = Band castrated calves; KN = Knife castrated calves; NM = single s.c. injection of lactated Ringer's immediately before castration; M: single injection of s.c. meloxicam (0.5 mg/Kg) immediately before castration.

³CAS = Castration effect; MED = Medication effect, T = Time effect.

⁴The values presented correspond to non-transformed means, however SEM and P-values correspond to ANOVA analysis using log or square root + 1 transformed data.

Figure 1. Least square means and SEM for (A) cortisol (nmol/L) concentrations of T0, 60, 90, and 120 min and (B) scrotal temperature (°C) of days 1, 2, 3, and 7 after band and knife castration of 1-wk-old Angus crossbred bull calves. Least square means with differing super-scripts differ ($P \leq 0.05$).

concentrations were greater ($P = 0.04$) in NM calves than M calves 7 d after castration and tended to be greater ($P = 0.08$) on day 3 after castration (Fig. 2). Substance P concentrations also tended ($P = 0.06$) to be greater in CT-M, BA-NM, and KN-NM, calves than BA-M, and KN-M, while no differences were observed between CT-NM, and CT-M, BA-NM, BA-M, KN-NM, and KN-M calves. Haptoglobin concentrations increased (time effect; $P = 0.02$) up to day 2 and then returned to similar baseline levels on day 7 after castration. The SAA concentrations were greater ($P = 0.04$) in KN-NM, calves compared to CT-NM, CT-M, BA-NM, and KN-M calves, while BA-M calves had greater ($P < 0.05$) SAA concentrations than CT-NM calves, however, no differences ($P > 0.10$) were observed between K-NM and B-M calves. Also, SAA

Figure 2. Least square means and SEM for substance P (pg/mL) concentrations of days 1, 2, 3, and 7 after band and knife castration of medicated and nonmedicated 1-wk-old Angus crossbred bull calves. * $P \leq 0.10$; ** $P \leq 0.05$.

concentrations were greater ($P = 0.05$) in KN calves than BA and CT calves on days 0, 2, and 3, while BA ($93 \pm 12.5 \mu\text{g/mL}$) calves had greater ($P \leq 0.05$) SAA concentrations than KN ($64 \pm 12.5 \mu\text{g/mL}$) and CT ($65 \pm 11.9 \mu\text{g/mL}$) calves on day 7.

White blood cell count was greater ($P = 0.01$; Table 1) in NM ($11.0 \pm 0.32 \times 10^9/\text{L}$) calves than M ($9.8 \pm 0.30 \times 10^9/\text{L}$) calves. The RBC count was greater ($P = 0.04$) in KN ($8.1 \pm 0.07 \times 10^{12}/\text{L}$) calves and BA ($8.1 \pm 0.07 \times 10^{12}/\text{L}$) calves than CT ($7.9 \pm 0.06 \times 10^{12}/\text{L}$) calves. Platelets increased (time effect; $P = 0.01$) over time. Scrotal temp was lower ($P < 0.01$) in BA calves compared to CT and KN calves on days 2, 3, and 7 after castration (Fig. 1B). Base temp decreased (time effect; $P < 0.01$) during days 1, 2, and 3, while rectal temp increased (time effect; $P < 0.01$) and decreased over time (Table 1).

No castration $\times$ medication effect was observed for any of the physiological parameters with the exception of SAA, and no castration $\times$ medication $\times$ time effect was observed for any of the physiological parameters. No medication or castration differences ($P > 0.10$) were observed for initial and final BW or ADG (Table 2).

Behavior

A castration effect ($P < 0.01$) was observed for VAS scores, where KN ($1.5 \pm 0.06 \text{ cm}$) calves had the greatest scores, followed by BA ($1.3 \pm 0.06 \text{ cm}$), and then by CT ($1.1 \pm 0.06 \text{ cm}$) calves (Table 3). Leg movements were greater ($P < 0.01$) in KN (5.1 ± 0.16) and BA (4.2 ± 0.16) calves than CT (1.4 ± 0.16) calves, while KN (1.2 ± 0.04) calves vocalized more ($P = 0.02$) than BA (1.0 ± 0.04) and CT (1.0 ± 0.04) calves. Between 2 and 4 h after

Table 2. Least square means ($\pm$ SEM) of performance (initial and final BW and ADG) of the first week postcastration of non-castrated (CT), band (BA) and knife (KN) castrated 1-wk-old Angus crossbred bull calves with (M) or without (NM) a single s.c. meloxicam administration

Item	Treatment ¹						SEM ³	<i>P</i> -value ²		
	CT		BA		KN			CAS	MED	CAS × MED
	NM	M	NM	M	NM	M				
Performance										
Initial BW (day −1), kg	46.2	46.7	46.9	47.2	49.0	47.8	2.09	0.65	0.95	0.91
Final BW (day 7), kg	55.2	56.1	55.4	57.2	58.1	56.6	2.41	0.78	0.84	0.77
ADG, kg/d	1.0	1.0	0.9	1.1	1.0	1.0	0.10	0.93	0.32	0.46

¹CT = Sham noncastrated calves; BA = Band castrated calves; KN = Knife castrated calves; NM = Single s.c. injection of lactated Ringer's immediately before castration; M = Single injection of s.c. meloxicam (0.5 mg/Kg) immediately before castration.

²CAS = Castration effect; MED = Medication effect.

³The values presented correspond to non-transformed means, SEM and *P*-values correspond to ANOVA analysis.

castration, suckling duration was greater (castration $\times$ medication interaction; $P = 0.02$) in KN-NM calves than CT-NM and BA-M calves, while CT-M calves had greater suckling duration than CT-NM and BA-M calves. The NM calves tail flicked more ($P = 0.03$) than M calves. The KN (148 ± 2.0) calves tail flicked more ($P < 0.01$) than BA (40 ± 2.0) and CT (3 ± 2.0) calves. The KN-NM calves tended ($P = 0.06$) to lie down less than CT-NM and BA-M calves, while KN calves tended ($P = 0.09$) to walk more than BA and CT calves. The KN-NM calves tended ($P = 0.10$) to have greater standing duration than CT-NM, BA-M, and KN-M calves, however no differences were observed between these groups and CT-M and BA-NM calves.

Standing bouts on day 0 were greater ($P = 0.02$) in BA-M (30 ± 1.7) calves than CT-NM (25 ± 1.7), CT-M (23 ± 1.9), BA-NM (26 ± 1.8), KN-NM (27 ± 1.8), and KN-M (22 ± 1.6) calves, in KN-NM calves than CT-M and KN-M calves, and in CT-NM and BA-NM than KN-M calves. On day 1 after castration, CT-NM (23 ± 1.2), BA-NM (24 ± 1.3), BA-M (23.2 ± 1.2), and KN-NM (24 ± 1.3) calves had greater ($P < 0.05$) standing bouts than CT-M (18 ± 1.4) calves, while on d 4 BA-M (21 ± 1.1) calves had greater ($P < 0.05$) standing bouts than CT-NM (18 ± 1.1), BA-NM (17 ± 1.1), and KN-M (17 ± 1.0) calves. The NM (24 ± 0.7) calves tended ($P = 0.08$) to have greater standing bouts than M (21 ± 0.7) calves on day 1 after castration, while lying bouts tended to be greater ($P = 0.10$) in M (24 ± 1.3) calves compared to NM (21 ± 1.3) calves on day 3 after castration. Lying duration showed a medication effect ($P = 0.03$), where M calves tended to have greater ($P = 0.07$; $P = 0.08$) lying duration than NM calves on days 3, 4, and 5 after castration (Fig. 3A). The NM (1.9 ± 0.01 min) calves had greater ($P = 0.03$) lateral lying than M (0.7 ± 0.01 min) calves, and NM (3 ± 0.8 min)

calves tended ($P = 0.07$) to have greater lateral lying than M (0 ± 0.8 min) calves on day 3 after castration. Tail flicks were greater ($P = 0.03$) in M calves than NM calves on day 7 after castration (Fig. 3B). Lying lateral was greater ($P = 0.01$) in KN calves compared to BA calves on day 3, but no differences ($P > 0.10$) were observed between both KN and BA compared to CT calves, while KN calves had greater lateral lying duration than BA and CT calves on day 7 (Fig. 3C). No castration $\times$ medication or castration $\times$ medication $\times$ time effects were observed for stride length, standing and lying behavior or for behavioral observations days after castration (Table 4).

DISCUSSION

Physiology

The M calves had lower substance P concentrations than NM calves on days 3 and 7 after castration. Meloxicam is an NSAID which works by inhibiting COX enzymes which convert arachidonic acid into prostaglandins, which are proinflammatory substances (Ricciotti and FitzGerald, 2011). Prostaglandin E₂ (PGE₂) has the ability to activate calcium channels in the sensory neurons which in turn stimulates the release of neurotransmitters, such as substance P (Nicol et al., 1992), a neuroactive peptide found across the central nervous system associated with pain, stress, and anxiety (DeVane, 2001). Meloxicam administration has been previously reported to lower substance P concentrations in cases of acute synovitis in the horse and dehorning in cattle (Grauw et al., 2009; Coetzee et al., 2012). Surprisingly, differences in substance P concentrations in the present study were observed after the duration of action of meloxicam which has a half-life of 22 ± 3 h (Stock

Table 3. Least square means ($\pm$ SEM) at the time of castration for VAS scores and frequencies of urination, leg movement and vocalization and for a 2 h period 2 to 4 h after castration of behavioral observations of non-castrated (CT), band (BA) and knife (KN) castrated 1-wk-old Angus crossbred bull calves with (M) or without (NM) a single s.c. meloxicam administration¹

Item	Treatment ²						SEM	<i>P</i> -value ³		
	CT		BA		KN			CAS	MED	CAS × MED
	NM	M	NM	M	NM	M				
VAS ⁴ , cm	1.1 ^c	1.1 ^c	1.2 ^b	1.4 ^b	1.6 ^a	1.5 ^a	0.08	<0.01	0.54	0.23
Urination ⁴ , <i>n</i>	1.0	1.0	1.0	1.0	1.0	1.0	0.03	0.55	0.41	0.56
Leg movement ⁴ , <i>n</i>	1.5 ^b	1.2 ^b	3.1 ^a	5.4 ^a	5.0 ^a	5.3 ^a	0.23	<0.01	0.49	0.44
Vocalization ⁴ , <i>n</i>	1.0 ^b	1.0 ^b	1.1 ^a	1.0 ^a	1.2 ^a	1.1 ^a	0.05	0.02	0.49	0.59
Behavioral obs. ⁵										
Walking, min	2.0	5.2	8.3	2.3	11.81	8.8	0.61	0.09	0.25	0.33
Standing, min	22.3	43.7	39.5	21.5	61.6	35.9	0.96	0.14	0.24	0.10
Lying, min	95.8	71.4	70.6	96.1	46.2	74.5	1.41	0.15	0.55	0.06
Suckling, min	3.0 ^b	34.6 ^a	13.6 ^{a,b}	6.5 ^b	38.0 ^a	12.3 ^{a,b}	1.12	0.36	0.77	0.02
Tail flick, <i>n</i>	14.5	0.0	55.3	26.7	209.6	88.0	2.31	<0.01	0.03	0.25
Foot stamping, <i>n</i>	1.6	6.5	11.2	2.5	27.2	6.6	0.90	0.24	0.26	0.33
Head turning, <i>n</i>	2.0	8.8	11.1	2.6	12.0	3.8	0.59	0.73	0.39	0.13

^{a-c}Least square means within a row with differing superscripts differ ($P < 0.05$).

¹Values represent the mean of time 2 to 4 h after castration.

²CT = Sham non-castrated calves; BA = Band castrated calves; KN = Knife castrated calves; NM = Single s.c. injection of lactated Ringer's immediately before castration; M = Single injection of s.c. meloxicam (0.5 mg/Kg) immediately before castration.

³CAS = Castration effect; MED = Medication effect; T = Time effect.

⁴The values presented correspond to transformed means, SEM and P -value using square root +1 transformation.

⁵The values presented correspond to non-transformed means; however SEM and P -values correspond to ANOVA analysis using square root + 1 transformation.

and Coetzee, 2015). Similar findings were reported by Allen et al. (2013) in 8- to 10-wk-old cautery dehorned calves receiving oral meloxicam (half-life 27 h) (Stock and Coetzee, 2015), which showed reduced substance P levels 120 h after dehorning compared to calves receiving a placebo. Based on these findings, meloxicam is effective at decreasing substance P concentrations; however differences were only detectable well after the expected duration of the pharmacokinetic effect of meloxicam (up to 44 h postadministration). Limiting sampling times to the specific duration of action of drugs could lead to inaccurate conclusions, as present and previous studies have detected differences after this period of time.

Meloxicam did not have an effect on cortisol concentrations, contrary to previous studies which have shown a cortisol reduction in castrated (Roberts et al., 2015) and dehorned (Allen et al., 2013) calves receiving oral meloxicam compared to un-medicated calves. Differences in cortisol concentrations between studies could be due to differences in dose, as the dose for s.c. meloxicam is 0.5 mg/kg of BW, while oral meloxicam is 1 mg/kg of BW, due to injectable meloxicam not being labeled for castration (oral meloxicam is labeled for castration in Canada), or due to age as calves from the previously

mentioned castration study were older calves weighing 227 kg. However, the castration effect observed in the present study is in accordance with previous findings in 1-wk-old calves which reported greater cortisol concentrations in castrated compared to uncastrated calves (Robertson et al., 1994; Molony et al., 1995). A possible reason why differences were observed for substance P but not for cortisol concentrations could be because cortisol is an indicator of acute stress; therefore concentrations are likely to return to baseline levels the day of castration. Previous castration studies in 1-wk-old calves have reported cortisol concentrations returning to baseline 2 h after castration (Robertson et al., 1994; Molony et al., 1995). Differences observed in substance P concentrations on day 3 and day 7 could have been the result of meloxicam's inhibitory effect on prostaglandin production during the first 2 d after castration, which could have consequently reduced PGE₂ concentrations and therefore reduced substance P concentration as previously mentioned.

Acute phase proteins (APP) increase in response to trauma, infection or inflammation (Hughes et al., 2014). In the present study, haptoglobin was not affected by castration or medication, which is contrary to the findings by Roberts et al. (2015) who reported a reduction in haptoglobin concentrations

AQ7

AQ8

Figure 3. Least square means and SEM for (A) lying duration (min/day) of days 1, 2, 3, 4, 5, and 6, (B) tail flicks (n/observation period) of days 1, 2, 3, and 7 and for (C) lateral lying (min/observation period) days 1, 2, 3, and 7 after band and knife castration of medicated and nonmedicated 1-wk-old Angus crossbred bull calves. Least square means with differing superscripts differ ($P \leq 0.05$). * $P \leq 0.10$; ** $P \leq 0.05$; *** $P \leq 0.01$.

after surgical castration in 227 kg calves receiving oral meloxicam compared to non-medicated calves. Lack of differences in haptoglobin concentrations could be explained by the difference

in age at castration between the studies. Similar to our results, Brown et al. (2015), reported differences in haptoglobin concentrations between medicated (oral meloxicam) and nonmedicated calves castrated near weaning but no differences were observed between medicated and nonmedicated calves castrated near birth. Lack of differences in new born calves could be due to castration being an insufficient stimulus to initiate a haptoglobin response (Werling et al., 1996) and/or due to greater haptoglobin concentrations near birth (Orro et al., 2008) which have reached a “ceiling effect.”

Serum amyloid-A has been previously suggested to be a better indicator of acute inflammation than haptoglobin (Horadagoda et al., 1999), and could be one reason why castration methods affected SAA concentrations but not haptoglobin concentrations. An unexpected finding was greater SAA concentrations observed in KN calves at T0, a sample collected immediately before castration. A possible explanation could be that a number of calves in the KN group could have had subclinical inflammation (Karreman et al., 2000) during the first days after birth which could have increased SAA concentrations. However, this is unlikely as no differences were observed between castration groups on day -1 and on day 1 after castration. The KN calves also had greater SAA concentrations than all other groups on days 2 and 3 after castration which could be associated with surgical trauma (Murata et al., 2004) and the subsequent inflammation caused by the scrotal incision and cutting and crushing of the cords after knife castration. In addition, greater SAA concentrations in BA calves on day 7 could be associated with tissue damage and inflammation caused by the band breaking through the skin which in turn can lead to secondary infections. It is common for APP concentrations to increase after a given stimulus (Petersen et al., 2004); however, in the present study, SAA concentrations decreased after castration. This could be due to greater APP concentrations near birth, possibly associated with parturition and/or colostrum intake, and the gradual reduction of concentrations during the first 3 wk after birth (Orro et al., 2008).

Medicated calves had reduced WBC counts than nonmedicated calves. Roberts et al. (201) reported reduced concentrations in total WBC, neutrophils, eosinophils, monocytes, and RBC in castrated calves receiving oral meloxicam (1 mg/kg BW) compared to nonmedicated calves. In the present study, castrated calves had greater RBC concentrations than uncastrated calves. Despite hematological differences between treatments, all

Table 4. Least square means ($\pm$ SEM) of samples taken on days 1, 2, 3, and 7 for stride length and behavioral observations, and for samples taken on days 0, 1, 2, 3, 4, 5, and 6 of standing and lying behavior of non-castrated (CT), band (BA) and knife (KN) castrated 1-wk-old Angus crossbred calves with (M) or without (NM) a single s.c. meloxicam administration

Item	Treatment ¹							<i>P</i> -value ²				
	CT		BA		KN		SEM ³					
	NM	M	NM	M	NM	M		CAS	MED	T	CAS × T	MED × T
Stride length ⁴ , cm	37.0	38.6	36.5	35.6	38.7	38.1	1.48	0.27	0.97	0.83	0.77	0.31
Standing and lying												
Standing, %	27.8	26.1	28.0	27.2	27.7	28.0	1.05	0.80	0.39	< 0.01	0.67	0.90
Lying, %	72.2	74.0	72.0	72.8	72.3	72.0	1.06	0.70	0.39	<0.01	0.64	0.90
Standing duration, min	20.7	21.2	21.1	19.3	20.4	22.2	1.30	0.68	0.85	<0.01	0.54	0.39
Lying duration, min	45.0	46.8	48.0	41.0	45.6	44.5	2.51	0.84	0.31	<0.01	0.35	0.03
Standing bouts, <i>n</i>	20.4	18.7	20.1	21.5	20.6	19.1	0.70	0.19	0.30	<0.01	0.15	0.08
Lying bouts, <i>n</i>	24.1	23.7	23.2	28.1	25.4	25.5	1.73	0.58	0.30	<0.01	0.34	0.10
Behavioral ob.												
Walking, min	2.8	2.4	2.3	3.4	2.7	2.5	0.16	0.99	0.97	0.23	0.73	0.30
Standing, min	29.7	29.7	24.1	34.1	23.6	26.4	0.42	0.43	0.31	0.28	0.66	0.69
Lying Lateral, min	0.9	0.7	1.1	0.0	3.7	1.3	0.15	<0.01	0.03	0.04	0.01	0.07
Lying Ventral, min	39.5	43.3	45.2	35.2	42.6	43.9	0.50	0.70	0.48	0.36	0.67	0.67
Eating, min	7.9	9.6	6.1	8.0	8.9	8.6	0.35	0.68	0.71	0.18	0.22	0.28
Tail flick, <i>n</i>	42.5	55.2	49.0	29.8	44.5	75.5	1.10	0.49	0.56	<0.01	0.24	0.03
Foot stamp, <i>n</i>	5.4	0.1	3.2	2.8	1.0	2.9	0.33	0.80	0.43	0.04	0.55	0.43
Head turning, <i>n</i>	6.3	9.8	4.7	4.6	7.3	6.9	0.33	0.28	0.64	0.83	0.23	0.94

¹CT = Sham noncastrated calves; BA = Band castrated calves; KN = Knife castrated calves; NM = Single s.c. injection of lactated Ringer's immediately before castration; M = Single injection of s.c. meloxicam (0.5 mg/Kg) immediately before castration.

²CAS = Castration effect; MED = Medication effect; T = Time effect.

³The values presented correspond to non-transformed means, however SEM and *P*-values correspond to ANOVA analysis using square root + 1 transformed data.

⁴Stride length: stride length mean of T0, and 120 min after castration.

calves had WBC and RBC concentrations within the normal range ($4\text{--}12 \times 10^3/\mu\text{L}$ and $5\text{--}10 \times 10^6/\text{L}$, respectively) (Smith, 2008). It is possible that the effect observed with oral meloxicam was due to a greater dose and a different route of administration. Oral meloxicam has a prolonged analgesic and anti-inflammatory effect compared to s.c. meloxicam as it is absorbed and excreted from the body at a slower rate than s.c. meloxicam. It is possible that the injectable meloxicam (0.5 mg/kg BW), used in the present study, is not labeled for castration because it is not as efficacious as oral meloxicam (1.0 mg/kg BW), which is labeled for its use to mitigate pain associated with castration. However, in a previous study, Brown et al. (2015) suggested that calves castrated near birth did not benefit from oral meloxicam based on a reduced effect on inflammation between medicated and nonmedicated calves. Similar results were observed in the present study, in which substance P and WBC were the only physiological parameters influenced by meloxicam administration in 7-d-old calves.

Behavior

As expected KN calves had the highest VAS scores (1.5 cm), leg movement, and vocalization frequency at the time of castration, indicating that the scrotal incision and crushing and cutting of the cords was a noxious stimuli of sufficient intensity to produce nociceptive pain and behavioral changes (Woolf, 2010). Banded calves had high VAS scores (1.3 cm) and leg movement frequencies, likely due to the manipulation of the testicles in order to place the band; however this was not a stimuli of sufficient intensity to produce vocalizations. These findings are in accordance with previous findings in which knife calves reacted more actively to knife castration followed by banded calves and uncastrated control calves (Fell et al., 1986; Meléndez et al., 2017). Meloxicam did not have an effect on VAS which can be explained by the fact that NSAIDs do not have an effect on stimuli of high intensity (Malmberg and Yaksh, 1991), but work by decreasing peripheral and central sensitization by reducing prostaglandin production (Burian and Geisslinger, 2005).

Tail flicking is important as it was the only parameter that presented a castration and medication effect. An increase in tail flick number in KN calves could be due to pain/discomfort caused by an increase in the number of action potentials from the nociceptors surrounding the affected areas in the scrotum and spermatic chords, as inflammatory substances can reduce the pain threshold therefore increasing hyperalgesia and allodynia (Tranquilli et al., 2013). An increased number of tail flicks has been previously reported as an acute pain related behavior in farm animals (Molony and Kent, 1997). The M calves had a decreased number of tail flicks compared to NM calves 2 to 4 h after castration. Although not statistically significant, M calves had a lower number of tail flicks than NM calves on day 1 and 3 after castration, however the M calves had a significantly greater number of tail flicks than NM calves on day 7. Increase in the number of tail flicks on day 7 was an unexpected finding; however differences in tail flicks observed on day 7 are likely due to the effect of meloxicam wearing off. These findings are of great importance as they show that meloxicam is effective 2 to 4 h after castration, however contrary to substance P results, the effect of meloxicam did not last beyond the duration of action period. Contrary to our results, Theurer et al. (2012) reported behavioral changes after the duration of action of oral meloxicam, where greater lying times were reported in medicated than un-medicated calves on days 0, 2, 3, and 4 after dehorning.

Lateral lying and restless behavior have been previously reported as pain-related behaviors. Molony et al. (1993) hypothesized that lambs in pain spent more time in a lateral lying position in response to castration and tail docking, while increases in restlessness have been associated with pain/distress caused by ischemia after rubber ring castration in lambs (Dinniss et al., 1999). Interestingly, a study assessing different castration methods in calves at different ages did not find differences in lateral lying in 1-wk-old calves (Molony et al., 1995), while Robertson et al. (1994) reported that 6-d-old calves spent significantly more time lateral lying than 21- and 42-d-old calves. In the present study, KN calves spent more time in a lateral lying position than BA and CT calves on day 3 and day 7 after castration. Increased lateral lying could be a consequence of pain associated with wound inflammation in KN calves, and along with greater scrotal temp in KN and CT calves than BA calves on days 2, 3, and 7 after castration, indicates inflammation of the scrotal area for KN calves. The M calves spent less time in

a lateral lying position than NM calves which could be due to the anti-inflammatory effects of meloxicam. In addition, the BA-M calves had a greater number of standing bouts which is in accordance with previous findings where 1-wk-old band castrated calves had greater standing and lying bouts than knife castrated calves (Meléndez et al., 2017). If restlessness is associated with ischemic pain, we would expect not only BA-M but also B-NM calves to have a greater number of restless bouts. Caution should be taken when interpreting both standing bouts and lateral lying as the numerical differences are small and may not be biologically significant.

Suckling duration was greater for KN-NM and CT-M calves the day of castration, which is contrary to expected. Interestingly, the CT-M calves had greater suckling durations than CT-NM calves. A possible explanation could be that meloxicam worked as an appetite stimulant, however Todd et al. (2010) found that meloxicam does not act as an appetite stimulant in healthy calves, only in calves with diarrhea. More surprising was the increase in suckling duration in KN-NM calves, as we would expect M calves to benefit from the analgesic and anti-inflammatory properties of meloxicam, and consequently calves would be more likely to get up, walk and suckle compared to NM calves. A possible explanation for this finding is that calves increase suckling as a way of coping with pain, as suckling behavior increases oxytocin release (Lupoli et al., 2001), which has an anti-stress effect and the potential to increase nociceptive thresholds (Uvnäs-Moberg et al., 1998).

The NM calves tended to have greater substance P concentrations, lateral lying duration, lower number of lying bouts and greater number of standing bouts. The KN calves tended to walk more, while the KN-NM calves tended to stand more and lie less. These results are as expected and indicate that KN-NM calves experienced more acute pain/stress compared to other treatments. On the other hand, unexpected tendencies included salivary cortisol being greater in M calves 60 min after castration compared to NM calves, and BA-M calves presenting greater number of foot stamps than BA-NM calves. Although tendencies could be due to small sample size, small differences between treatments or high individual variability, we believe that the tendencies reported are relevant and that significance was not achieved mainly due to the small sample size.

Conclusion

Overall, SAA, scrotal temperature, VAS, tail flicks, and lateral lying had a castration effect,

where KN castration was the method which produced more pain related indicators. This could be due to the activation of the mechanical nociceptors at the time of the incision and cutting and crushing of the spermatic chords (VAS). Consequently, the tissue damage caused by knife castration leads to an inflammatory reaction surrounding the affected area (SAA and scrotal temperature). Inflammation can also lead to pain related behaviors, such as tail flicking, which has been previously associated with skin irritation in cattle (Kiley-Worthington, 1976) and lateral lying which has been associated with pain in lambs after castration (Molony et al., 1993).

In addition, a medication effect was observed for substance P, WBC, and tail flicks. It is likely that meloxicam was effective at reducing these variables due to the association between these parameters and prostaglandins, as prostaglandins have the ability to reduce the pain threshold (Tranquilli et al., 2013), release substance P (Nicol et al., 1992), and modulate immune cells (Tilley et al., 2001). However, meloxicam did not have an effect on the other physiological and behavioral parameters measured in this study.

Conflict of interest statement. None declared.

ACKNOWLEDGMENTS

The authors appreciate the invaluable help of Agriculture and Agri-Food Canada research feedlot staff and beef welfare technicians Randy Wilde and Fiona Brown. We are very thankful for the funding provided by Agriculture and Agri-Food Canada and the Beef Cattle Research Council through the Canadian Beef Cattle Industry Science Cluster. We would also like to thank all the students that helped with data collection and behavioral scoring: Jonathan Low, Louise Theron, Ashley Adams, Andrea Lippa, Nicole Desautels, Allecia Gheysens, Chantel deBeurs, Nita Hynes, and Teryn Gilmet.

The co author Sonia Marti was supported by the CERCA program from Generalitat de Catalunya.

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