Running head: Meloxicam effect after castration + branding

Effect of subcutaneous Meloxicam on indicators of acute pain and distress after castration and branding in 2 mo old beef calves[1]

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ABSTRACT

The aim of this study was to assess knife castration and knife castration + branding in 2-mo old calves, and the effect of a single dose of s.c. meloxicam at mitigating pain indicators. Seventy-one Angus crossbred bull calves (128 ± 18.5 kg of BW) were used in a 3 × 2 factorial design where main factors included procedure: sham (control calves, **CT**; *n* = 23), knife (**KN**; *n* = 24) or knife + branding (**BK**; *n* = 24) and medication: single s.c. administration of lactated ringer solution (**NM**; n = 35) or a single dose of 0.5 mg/kg of s.c. meloxicam (**M**; n = 36). Physiological samples were collected at T0, 60, 90, 120 and 180 min and on d 1, 2, 3 and 7 after procedure, while behavioral observations were evaluated at 2 to 4 h and 1, 2, 3 and 7 days after procedure. A procedure × time effect ($P < 0.01$) was observed for cortisol, where KN and BK calves had greater ($P \leq 0.01$) cortisol concentrations than CT calves 60 min after the procedure, while BK calves had the greatest ($P < 0.05$) cortisol concentrations, followed by KN calves and by CT calves 90, 120 and 180 min after the procedure. A procedure × time effect ($P = 0.01$) was observed for tail flicks, where KN and BK calves had a greater ($P < 0.05$) number of tail flicks than CT calves on d 1 and 3, while BK calves had the greatest number of tail flicks, followed by KN calves, and then by CT calves on d 2. Haptoglobin had a procedure × medication × time interaction ($P = 0.05$), where BK-NM calves had greater haptoglobin concentrations than BK-M, KN-M and CT calves on d 1 and 3, while BK-NM and KN-NM calves had greater haptoglobin concentrations than BK-M, KN-M and CT calves on d 2 after the procedure. Lying duration and tail flicks had a medication effect ($P = 0.04$; $P < 0.01$) where M calves had greater ($P < 0.05$) lying duration and lower ($P < 0.05$) number of tail flicks than NM calves 2 to 4 h after procedure. No medication effects ($P > 0.10$) were observed for salivary cortisol, substance P and scrotal temperature min after the procedure or for cortisol, substance P, serum amyloid A, stride

length or behavioral observations on d after the procedure. Overall, BK calves presented greater physiological and behavioral indicators of acute pain than KN calves, suggesting that the combination of knife castration + branding was more painful. Meloxicam administered s.c. was effective at reducing physiological and behavioral indicators of acute pain associated with knife castration and knife castration + branding.

Key words*:* acute pain, beef, behavior, branding, castration, pain mitigation,

INTRODUCTION

Castration is a common husbandry procedure done in order to reduce aggressive behavior, improve meat quality and increase on farm safety (Jacobs et al., 1977; Stafford and Mellor, 2005). Common castration methods include band, knife and burdizzo castration (Weaver et al., 2008) with knife castration being reported as the most common method conducted by veterinarians in the USA (Coetzee et al., 2010). In addition, multiple procedures such as ear tagging, vaccination, dehorning and branding are typically done in combination with castration in order to reduce the number of times calves must be handled.

Hot-iron branding is a common method of permanent identification in beef cattle. In North America, branding is done to establish ownership and in Canada it is also done to meet the requirements for exporting cattle into the USA (Schwartzkopf-Genswein et al., 2012). A Western Canadian survey reported that over half of the calves (54 %) were branded and only 4 % of the respondents used pain mitigation (Moggy et al., 2017).

Both castration and branding are painful procedures (Schwartzkopf-Genswein et al., 1997a; Schwartzkopf-Genswein et al., 1997b; Stafford and Mellor, 2005; Pang et al., 2006) usually done without the use of analgesia or anesthesia in North America. Meloxicam is a non-

steroidal anti-inflammatory drug (NSAID) and a practical option for producers due to its ease of administration (s.c.) and long lasting half-life (22 ± 3 h) (Coetzee et al., 2012).

Therefore, the aim of this study was to assess acute pain indicators associated with castration alone and the combination of castration + branding, and to assess the effect of meloxicam at mitigating these indicators in 2-mo-old beef calves. Our hypothesis was that the combination of multiple stressors would elicit a greater stress/pain response than castration alone, and that a single s.c. dose of meloxicam would reduce pain indicators due meloxicam's analgesic and anti-inflammatory properties.

MATERIALS AND METHODS

This protocol was approved by the Animal Care Committees of the Lethbridge Research Centre (ACC number 1410) and the University of Calgary (AC14- 0159) and animals were cared for in accordance with the Canadian Council of Animal Care (CCAC, 2009).

Animal Housing and Management

Seventy-one Angus crossbred beef calves (128 ± 18.5 kg of BW, 67-87 d old calves) and their dams were brought to the Lethbridge Research Centre (LRC) from a neighbouring ranch located 30 km from the LRC. Calves were separated into two groups of 36 and 35 calves as animals were castrated on different days 1 week apart. Cow-calf pairs were housed in 6 experimental pens (treatments mixed within pen) containing a calf shelter (2.4 m × 3.6 m × 1.4 m), straw bedding and a centrally located water system. Three of the pens measured 36.7 m × 22.2 m, and three pens measured 40 m × 27 m. Free choice alfalfa grass was available for the cows, while the calves diet consisted of free choice alfalfa grass, milk from suckling and free choice salt blocks and loose minerals containing a coccidiostat (Diluted Rumensin Drug Premix

92 1100 (Medicated), HI-PRO FEEDS, Okotoks, Alberta, Canada) to prevent diarrhea caused by
93 coccidiosis. The experiment took place on June 23rd to July 7th, 2015.
94 Calves were weighed in a portable chute (Pearsons Livestock Equipment, Thedford,
95 Nebraska) and sampled (saliva, blood, scrotal and rectal temperature) while standing in a tipping
96 table (Calf Roper, Ram-Bull Ltd, Barons, Alberta, Canada) with a head lock. All calves were
97 castrated and branded on a tipping table (Hi-Qual Manufacturing Canada Ltd., MB, Canada)
98 while lying on their left side. Castration was performed first and consisted of making an incision
99 in the scrotum with a Newberry knife (Syrvet Inc., Waukee, IA) and crushing and cutting of the
100 cords with an emasculator. All castrations were done by the same experienced veterinarian.
101 Branding was done with the use of an electric hot-iron based on 3 combined marks: a number, a
102 symbol and a letter (3 = M) placed on the right rib cage when calves were tipped. Sham calves
103 were handled in the same way as castrated and branded calves. The testicles were manipulated
104 for a similar amount of time and the same iron used to make the brand but unheated was placed
105 on the calves simulating the pressure exerted with the hot-iron. Branding was done by the same
106 experienced person. Calves were castrated for an average time of 1.1 ± 0.19 min, branded for 0.5
107 ± 0.18 min and sampled for 2.7 ± 2.64 min, for an average restraining time of 3.1 ± 2.75 min.
108 Calves were equally distributed by weight into treatments and pens, and randomly
109 assigned to treatments using a deck of cards.The experiment consisted of a 3 × 2 factorial design
110 where main factors included procedure**:** sham (control calves, **CT**; *n* = 23), knife castration (**KN**;
111 *n* = 24) or branding and knife castration (**BK**; *n* = 24) and medication: single dose of 0.5 mg/kg
112 of s.c. meloxicam (Metacam 20 mg/mL, Boehringer Ingelheim, Burlington, Ontario, Canada)
113 (**M**; n = 36) or the corresponding volume of a single s.c. administration of lactated ringer
114 solution (Lactated Ringer's Irrigation, Baxter Canada, Mississauga, Ontario, Canada) (**NM**; n =

35), to yield: CT-NM (n = 11), CT-M (n = 12), KN-NM (n = 12), KN-M (n = 12), BK-NM (n = 12), BK-M (n = 12). Meloxicam and lactated ringer's was administered immediately prior to the procedure.

Measurements of Acute pain and Sample Collection

Cortisol. Salivary samples were collected 24 h before castration (d -1), immediately before castration (**T0**), 60, 90, 120, 180 min and on d 1, 2, 3 and 7 after castration. Samples collected on d 1, 2, 3 and 7 were collected at the same time of day. Saliva was collected, stored and analyzed as described by Meléndez et al. (2017b). The inter-assay CV was 13.2 % while the intra-assay CV was 9.9 %.

Substance P, Serum Amyloid-A, Haptoglobin and Complete Blood Count. Blood samples were collected from all calves through jugular venipuncture on d -1, immediately before castration (T0), 60, 90, 120, 180 min and on d 1, 2, 3 and 7 after procedure. Samples for substance P were collected, centrifuged for 15 min at 1.5 × *g* at 0 °C, stored and analyzed as previously described by Meléndez et al. (2017b). Briefly, samples were collected into a 6-ml tubes containing EDTA (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ), where benzamidine hydrochloride was added to reduce substance P degradation. 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). The intra-assay CV was 11.9 % and the inter-assay CV was calculated at 24.2 %.

Blood samples for serum amyloid-A (SAA) and haptoglobin were collected on d 1, 2, 3 and 7, stored and analyzed as previously described by Meléndez et al. (2017b). Briefly samples were collected into a 10-ml non-additive tube (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ), centrifuged for 15 min at 1.5 × *g* at 4 °C and the serum was decanted and frozen at -

80 ºC for further analysis. The inter-assay CV for haptoglobin was 7.6 %, while SAA intra-assay and inter-assay CV were 5.7 % and 13.5 %, respectively.

Blood samples for CBC were collected into a 6-ml EDTA tube (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ) on d 1, 2, 3 and 7 and red blood cells (RBC), white blood cells (WBC), platelets (PLT) and neutrophil: lymphocyte ratio were measured using a HemaTrueHematology Analyzer (Heska, Lobeland, Co).

Scrotal Area Temperature (SCT). Images of the area of the scrotum were collected on d -1, immediately before castration (T0), 60, 90, 120, 180 min and on d 1, 2, 3 and 7 after castration. Images were collected and analyzed as previously described by Meléndez et al. (2017b). Briefly, a FLIR i60 infrared camera (FLIR Systems Ltd., Burlington, ON, Canada) was used to take infrared images of the scrotal area and FLIR Tools version 5.1 (FLIR Systems Ltd.) was used to delineate the scrotal area and to record the maximum temperature.

Rectal temperature (Rectal temp). A digital thermometer (M750 Livestock Thermometer, GLA Agricultural Electronics, San Luis Obispo, CA) was used to collect rectal temperature on d -1, immediately before castration (T0), and on d 1, 2, 3 and 7 after the procedure.

Performance. A portable scale (Pearsons Livestock Equipment, Thedford, Nebraska) was used to obtain the initial (average of d -1 and d 0) and final (d 7) BW. The ADG (kg/d) was calculated by subtracting the weights on d 7 from the average of d -1 and 0 and dividing the result by the number of days in the experiment (7 d).

Behavioral frequencies and Visual Analog Scale (VAS). Behavioral scoring during castration was collected as previously described by Meléndez et al. (2017b). Briefly, two experienced observers marked a line along a 10 cm continuum of their perception of the amount

of pain calves were experiencing during castration and recorded the frequency of urination, defecation, leg movement and vocalizations. Due to the experimental setting, observers could not be blind to the treatments.

Electronic reactivity measurements (ERM). The tipping table was equipped with one 3 dimension accelerometer and the three forces were added to obtain an overall force during castration and branding procedures. Analog signals (V) from the accelerometer were sent to a computer at a rate of 100 samples/ s. Data from control calves collected during sham castration and sham branding were used as the baseline for calves that were castrated and branded. Variables included number of peaks between 1 and 2 SD, 2 and 3 SD, and above or below 3 SD above and below the mean (Fig. 1A) and total area between the mean ± 1 SD, mean ± 2 SD, and mean ± 3 SD (Fig. 1B).

Stride length. Stride length was collected as previously described by Meléndez et al. (2017b). Briefly, calves were recorded when walking through an alley on d-1, immediately after castration, 180 min and on d 1, 2, 3 and 7 after the procedure. Pictures of the back legs were taken with GOM player (GOM Lab, Gretech Corporation, Seoul, South Korea), while stride length was measured using Image J (National Institutes of Health Image, Bethesda, MD). Observers were blind to the treatments.

Behavioral observations. Half of the animals of each treatment were recorded for behavioral observations and focal animal sampling from continuous recordings (Martin and Bateson, 2007) were done for frequencies of tail flicks, foot stamping, head turning and lesion licking, and duration of eating, lying, standing and walking as described by Meléndez et al. (2017b). Briefly, the behaviors scored, and their definitions, were: a) eating: suckling from the udder or ingesting hay or straw from the ground or the feeder, b) lying: either lateral (laying with

hip and shoulder on the ground with at least 3 limbs extended) or ventral (laying in sternal recumbency with legs folded under the body or one hind or front leg extended) lying, c) walking: walking forward more than 2 steps, d) standing: standing on all four legs, e) foot stamping: hind legs are lifted and forcefully placed on the ground or kicked outwards while standing, f) head turning: head is turned and touches the side of the calf's body when standing, including head turning to groom, g) tail flicking: forceful tail movement beyond the widest part of the rump when standing, movement to one side is counted as one action, h) lesion licking: head turning to lick the lesion caused by castration while standing.

Two experienced observers scored behavior for a 2 h period on d 0 between 3 to 5 h relative to treatment application and for 4 min every 10 min for a 4 h period on d 1, 2, 3 and 7 for a subset of 6 animals per treatment. Observers were blind to the treatments. Inter-rater and intra-rater reliability were 0.95 and 0.91 respectively.

Standing and lying behavior. Animals were equipped with accelerometers (Hobo pendant G, Onset Computer Corporation, Bourne, MA) in order to measure 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) as previously described by Meléndez et al. (2017b). Briefly, accelerometers were placed on d -1 with Vet Wrap (Professional Preference, Calgary, Canada) and removed on d 7. Only days with 24 h of information were included in the analysis (d 0 to d 6).

Statistical analysis

A power analysis was conducted for the outcomes of salivary cortisol and tail flicking. An α of 0.05, a power of 0.08 and the mean values and SD from a previous study of 2 month old

beef calves under similar experimental conditions (Meléndez et al. 2017b) were used in the power calculation. Mean cortisol values were 3.3, 3.9 and 4.9 nmol/L and a SD of 0.62, while mean tail flicking values were 46.6, 62.6 and 116.6 n and a SD of 5.6. The power analysis indicated that at least 6-12 calves per treatment were necessary to detect expected differences among treatments. Salivary cortisol, substance P, SAA, haptoglobin, CBC, stride length and behavior the days post castration were analyzed using the MIXED procedure in SAS (SAS, version 9.4, SAS Inst. Inc., Cary, NC) to evaluate the effect of procedure, medication and time on all variables. Fixed effect included procedure, medication, time and their interactions, while random effects included pen and calf within pen. Calves were divided into two groups and castrated 1 week apart. All calves in one pen were castrated on the same day and 'group' was used as a covariate. Animals were the experimental unit as treatments were mixed within pen. All data were analyzed using the mixed repeated measures model (Proc Mixed of SAS) as samples were collected at different time points, with the exception of behavior during castration. Behavior during castration (VAS, frequency of leg movement, urination, defecation, vocalizations and ERM) and performance was analyzed as described above without time effect (as there were no repeated measures). Data were tested for normal distribution with PROC UNIVARIATE (SAS, version 9.4, SAS Inst. Inc., Cary, NC) and physiological data that did not follow a normal distribution were log transformed while behavioral data were square root + 1 transformed. The data collected on d-1 were used as a covariate for all physiological parameters and stride length. Electronic reactivity measurements (ERM) collected for sham calves at the time of castration and branding were used as the mean for ERM for KN and BK calves. Urination and defecation were not analyzed as these behaviors were not present during castration or branding. The analysis with the covariance structure (unstructured, compound symmetry and

autoregressive order one) with the lowest Schwarz's Bayesian criterion was selected as the analysis of choice. Data from the day of castration were analyzed separately from the data the days after castration as the time intervals between samples were different. A post-hoc test was run to separate the Least Square means using the PDIFF option in SAS. Effect of procedure, medication and time were statistically significant when $P \leq 0.05$ and considered a tendency when $0.05 < P \leq 0.10$. An intra-class correlation coefficient with a 95 % CI was used to calculate intra and inter observer reliability of two experienced observers using IBM SPSS statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA).

RESULTS AND DISCUSSION

Physiology

Salivary Cortisol. A procedure × time effect ($P < 0.01$) was observed for cortisol (Fig. 2A), where KN and BK calves had greater ($P \leq 0.01$) cortisol concentrations than CT calves 60 min after the procedure. The BK calves had the greatest ($P < 0.05$) cortisol concentrations, KN calves had intermediate, and CT calves had the lowest concentrations 90, 120 and 180 min after the procedure. No medication effect ($P > 0.10$) was observed for cortisol 60, 90, 120 and 180 min (Fig. 2B) or on d 0, 1, 2, 3, and 7 after the procedure, and no procedure effect ($P > 0.10$) was observed d after castration.

Contrary to our findings, previous studies have reported a reduction in plasma cortisol concentrations in calves receiving NSAIDs prior to a painful procedure, such as surgically castrated calves receiving oral meloxicam compared to un-medicated surgically castrated 227 kg calves (Roberts et al., 2015), carprofen, in band castrated compared to un-medicated band castrated 5.5 mo old calves (Pang et al., 2006), burdizzo castrated calves receiving ketoprofen compared to un-medicated burdizzo castrated 11 mo old calves (Ting et al., 2003) and dehorned

253 calves receiving i.m. injection of meloxicam compared to un-medicated dehorned 6 to 12 week
254 old dairy calves (Heinrich at al., 2009). However, in the previous studies, carprofen and
255 ketoprofen were administered intravenously 20 min before castration, i.m. meloxicam was
256 administered 10 min prior to castration, while oral meloxicam was given concurrently to
257 castrated animals as a bolus administered directly into the rumen. Differences in results between
258 our study and the results of Roberts et al. (2015), where meloxicam was administered at the time
259 of castration, could be due to differences between salivary and serum/plasma concentrations.
260 Although a correlation has been observed between plasma and salivary cortisol concentrations in
261 cattle, caution should be taken when comparing these results as there is a 10 minute time lag
262 between peak plasma and salivary cortisol concentrations (Hernandez et al., 2014) and plasma
263 cortisol has been reported to be more sensitive than salivary cortisol to adrenal activity in pigs
264 (Parrott et al., 1989).
265 Differences between studies could also be due to calves being older than the calves in the
266 present study as a greater stress response has been reported in calves castrated after 6 months of
267 age compared to calves castrated at a younger age (Bretschneider et al., 2005). Differences could
268 also be due to timing of meloxicam administration as the compendium for injectable meloxicam
269 recommends the administration of meloxicam 10 to 20 min prior to the procedure for the
270 reduction of pain caused by abdominal surgery. Based on these results, administering meloxicam
271 s.c. immediately prior to castration may limit the analgesic effect of the drug. However, the
272 results from the present study are consistent with the results from a previous study (Meléndez et
273 al., 2017a) where no differences in salivary cortisol were found between animals receiving pre-
274 emptive analgesia with s.c. meloxicam at 6, 3 or 0 h prior to knife castration up to 4 h following

the procedure. Caution should be taken when interpreting these results as there was a lack of a control group that did not receive medication.

Similar to our results, Sutherland et al. (2013) did not see differences in cortisol concentrations between surgically castrated, dehorned, or surgically castrated + dehorned 3 mo old calves 0, 24 and 72 h after treatment. Sutherland et al. (2013) suggested that lack of differences in cortisol concentrations could be due to a potential ceiling effect of the cortisol response to either castration or dehorning, however cortisol AUC in castrated + dehorned calves was greater than only castrated or only dehorned calves up to 6 h after the procedure, providing some evidence that the combination of procedures is more painful. Similar results were reported by Mosher et al. (2013) who found a tendency for cortisol to be greater 60 min after castration in surgically castrated + dehorned 3 to 4 mo old calves than those that were only castrated. Although, different castration methods and painful procedures such as dehorning and branding can cause different physiological responses, both procedures are painful and stressful and therefore likely to increase cortisol concentrations.

Substance P. No procedure or medication effects ($P > 0.10$) were observed for substance P min or d after procedure (Table 1). These findings are similar to results reporting no differences in substance P levels 60 and 120 min and on d 7 after different castration methods (control, band and knife) in 2 mo old calves (Meléndez et al., 2017b), and on d 0, 1 and 7 after band castrated in medicated or un-medicated (oral meloxicam) weaned calves (Repenning et al., 2013). However caution should be taken when comparing results as the age of the calves differ between experiments. In addition, lack of differences could be a result of other factors (alone or in combination) including, high inter-assay CV, high individual animal variation in the measurements taken which could mask treatment effects, sampling times being inadequate to

298 detect differences among treatments, variables collected were not sensitive enough to detect
299 differences among treatments or that no differences in substance P may suggest no pain markers
300 the days following castration and branding.

301 ***Serum Amyloid-A and Haptoglobin.*** A procedure × time interaction ($P < 0.01$) was
302 observed for SAA (Fig. 2C), where KN and BK calves had greater ($P < 0.01$) SAA
303 concentrations than CT calves on d 1, 2 and 3, while no differences ($P > 0.10$) were observed
304 between procedures on d 0 and 7. No medication effects ($P > 0.10$) were observed for SAA the
305 days after procedure (Fig. 2D).

306 A procedure × medication × time effect ($P = 0.05$) was observed for haptoglobin (Fig.
307 3A), where BK-M calves had greater ($P = 0.04$) concentrations than BK-NM calves on d 0 (prior
308 to castration). The BK-NM and the KN-NM calves had greater ($P < 0.05$) concentrations than
309 BK-M, KN-M, and CT calves on d 1 and 2. The BK-NM calves had greater ($P < 0.05$)
310 haptoglobin concentration than BK-M, KN-M and CT calves on d 3, while KN-M calves had
311 greater ($P < 0.05$) haptoglobin concentrations than BK-M calves on d 7.

312 Both haptoglobin and SAA concentrations were above the normal range for healthy
313 bovines (Haptoglobin: <0.1 g/L and SAA: 1.3 ± 0.4 µg/mL) (Ceciliani et al., 2012) and followed
314 the normal acute phase protein response which increases 24 to 48 h after a challenge and returns
315 to baseline levels approximately 4 to 7 d after (Petersen et al., 2004). Medication effects have
316 been previously described for haptoglobin concentrations, where ketoprofen administration
317 reduced haptoglobin concentrations 1 d after burdizzo castration in 13 mo old calves (Ting et al.,
318 2003) and up to 3 d after surgical castration in 5.5 mo old calves (Earley and Crowe, 2002). Oral
319 meloxicam has also been reported to decrease haptoglobin concentrations after surgical
320 castration in calves at weaning weighing between 216 to 228 kg (Brown et al., 2015) and in 227

kg calves (Roberts et al., 2015). In contrast, there is a lack of literature evaluating the response of SAA after castration and pain mitigation. A study in 7 to 8 mo old beef calves reported greater SAA concentrations than baseline levels after surgical castration, but no effect of time of s.c. meloxicam administration (6, 3 and 0 h before castration) on SAA concentrations (Meléndez et al., 2017a). Lack of differences in the previous study could be due to the fact that all treatments received meloxicam, however no medication effect was observed for SAA in the present study which assessed both medicated and un-medicated calves. A possible explanation could be that NSAID do not have the same effect in reducing the production of different APPs, which could explain the medication effect observed for haptoglobin but not for SAA.

Complete Blood Count. A medication × time effect ($P < 0.01$; $P = 0.02$; $P = 0.02$) was observed for WBC, RBC counts and N:L ratio. The NM calves had greater ($P < 0.05$) WBC counts on d 1 and 2 and greater ($P < 0.05$) N:L ratio than M calves on d 2 after procedure, while M calves had greater RBC counts than NM calves on d 7. A procedure × time effect ($P = 0.04$; $P < 0.01$) was observed for WBC counts and N:L ratio, where KN and BK calves had a greater ($P < 0.05$) WBC and N:L ratio on d 1 compared to CT calves, while and KN calves had a greater N:L ratio than CT calves on d 2 (data not shown). No medication or procedure ($P > 0.10$) effects were observed for PLT.

Similar to our findings, Ballou et al. (2013) reported an increase in N:L ratio and total leukocytes in surgically castrated calves compared to non-castrated calves 6 h after castration, and a reduction in leucocytes and N:L ratio following the administration of lidocaine and flunixine meglumin. Total WBC concentrations were lower in calves given lidocaine + flunixine meglumin before dehorning compared to calves dehorned without pain relief, but no differences were observed for calves castrated or castrated + dehorned with or without pain relief

(Sutherland et al., 2013). In contrast, previous studies have reported no effect of NSAIDs on blood parameters after castration (Pang et al., 2006; Moya et al., 2014). Although levels of WBC, RBC and N:L differed between treatments, levels were within the normal range (Smith, 2008) meaning that calves were not immunocompromised by castration or branding.

Scrotal temperature (SCT) and rectal temperature. No procedure or medication effects ($P > 0.10$) were observed for SCT min after procedure (Table 1). A medication effect ($P = 0.04$) was observed for SCT, where M (36.6 ± 0.46 °C) calves had lower ($P < 0.05$) SCT than NM (36.9 ± 0.46 °C) calves on d 1, 2, 3, and 7. A procedure effect ($P = 0.01$) was also observed where BK (36.9 ± 0.46 °C) and KN (36.9 ± 0.46 °C) calves had greater SCT than CT (36.5 ± 0.46 °C) calves on d 1, 2 , 3 and 7. A medication × time interaction ($P = 0.01$) was observed for rectal temperature, where NM (39.4 ± 0.05 °C) calves had greater ($P < 0.05$) rectal temperature than M (39.2 ± 0.05 °C) calves on d 1 after treatment. A procedure × time interaction ($P = 0.03$) was observed for rectal temperature, where KN (39.4 ± 0.06 °C) and BK (39.3 ± 0.06 °C) calves had greater ($P < 0.05$) rectal temperature than CT (39.1 ± 0.06 °C) calves on d 1. No differences ($P > 0.10$) were observed for rectal temperature on d 0, 2 and 3 after treatment.

Some of animals in the present study presented a fever (≥ 39.4° C) (Smith 2008) during the days after castration. NSAIDs are used in veterinary medicine to reduce body temperature in animals with fever (Lees et al., 2004), however, differences in rectal temperature and SCT between M and NM calves and CT, KN and BK calves was so small that differences likely lack biological significance.

Weight and ADG. A procedure × medication interaction ($P = 0.01$) was observed for ADG, where CT-M (1.3 ± 0.07), KN-NM (1.1 ± 0.08) and BK-M (1.3 ± 0.07), calves had greater ($P < 0.05$) ADG than KN-M (0.9 ± 0.07), and BK-NM (0.9 ± 0.08), calves, while CT-NM (1.2 ±

0.08), calves had greater ($P < 0.05$) ADG than BK-NM calves, but no differences ($P > 0.10$) were observed between CT-NM, CT-M, KN-NM and BK-M calves, nor between CT-NM and KN-M calves. No medication or procedure effects ($P > 0.10$) were observed for initial and final BW.

The ADG was greater in CT-NM and CT-M calves as expected as the animals did not experience the trauma associated with surgery or burn. However, the BK-M calves had greater ADG than BK-NM calves, which may be due to the reduced pain which would motivate the calves to get up, walk and suckle, however, we would also expect to see a greater ADG in KN-M calves compared to KN-NM calves. A possible explanation for the greater ADG observed in KN-NM calves compared to KN-M calves could be due to an increase in suckling in KN-NM calves as a way to cope with pain as suckling has been reported to increase oxytocin release (Lupoli et al., 2001) which can increase the nociceptive threshold (Uvnäs-Moberg et al., 1998). However, caution should be taken when interpreting these results as a difference of 0.2 kg/day may lack biological significance. A possible reason for the expected medication effect observed for the BK group but not in the KN group could be due to meloxicam being more effective at alleviating pain caused by branding (somatic pain) than pain caused by knife castration (somatic and visceral pain). However, the application of an NSAID, such as flunixin meglumin, did not have any effect on wound healing or pain response associated with branding (Tucker et al., 2014) and studies in cancer patients show that NSAIDS are effective at mitigating both somatic and visceral pain (Mercadante et al., 1999). Contrary to our findings, a study reported no differences in ADG in calves undergoing multiple painful procedures such as castration, dehorning and castration + dehorning in 3 to 4 mo old dairy calves (Mosher et al., 2013).

Behavior

Behavioral frequencies and VAS. A procedure × medication interaction ($P = 0.04$) was observed for leg movements, where the BK-M calves had a greater ($P < 0.05$) number of leg movements than CT, KN-NM and KN-M calves during the procedures, but no differences ($P > 0.10$) were observed between BK-M and BK-NM calves (Table 2). The KN-M calves had greater ($P < 0.05$) number of leg movements than CT and KN-NM calves, however no differences ($P > 0.10$) were observed between KN-M and BK-NM calves. A procedure effect ($P < 0.01$) was observed for VAS where BK (5.5 ± 0.07 cm) calves had greater ($P < 0.05$) VAS scores, followed by KN (2.6 ± 0.07 cm) calves, and then by CT (0.4 ± 0.07 cm) calves.

These results demonstrate that surgical castration and hot iron branding are painful procedures as observed by greater VAS scores and numerically greater vocalizations compared to CT calves, however, branding elicits more vigorous behavioral responses than surgical castration at the time of the procedure. This could be due to the differences in pain, as somatic pain is localized and allows for rapid motor reflexes, while visceral pain is poorly localized and leads to muscle contraction and autonomic and emotional responses (Gebhart and Ness, 1991). Similar behavioral results for hot-iron branding have been previously reported in a study comparing hot-iron branding and freeze branding, where hot-iron branded calves vocalized more and had greater exertion forces than freeze or sham calves (Schwartzkopf-Genswein et al., 1997b). Greater VAS scores have also been reported in surgically castrated calves compared to band and control calves (Fell et al., 1986; Meléndez et al., 2017b).

Electronic reactivity measurements. During branding, a procedure effect ($P < 0.01$) was observed for number of accelerometer peaks between 2 and 3 SD above and below the mean (baseline of control calves) and greater or lower than 3 SD above or below the mean, where BK calves had a greater number of peaks than KN calves (Fig. 4A). However, no differences ($P >$

413 0.10) were observed for number of peaks above and below the mean between 1 to 2 SD at the
414 time of branding. A procedure effect ($P < 0.05$) was also observed for total area, where BK
415 calves had greater ($P < 0.05$) total area than KN calves between the mean ± 1 SD, the mean ± 2
416 SD and the mean ± 3 SD (Fig. 4B). During castration, no medication or procedure effects ($P >$
417 0.10) were observed for number of peaks between 1 to 2 SD, 2 to 3 SD, and greater or lower than
418 3 SD, and total area between the mean and ± 1 SD, ± 2 SD and ± 3SD above and below the
419 mean.
420 Movement in the chute has been previously measured during branding (Schwartzkopf-
421 Genswein et al., 1997b) and castration (Moya et al., 2014; Meléndez et al., 2017a) in cattle.
422 However, this was the first time that the portable electronic reactivity movement was used on a
423 tip table to quantify movement at the time of castration and branding. As expected no differences
424 were observed for accelerometer movement at the time of castration, as both groups of calves
425 were surgically castrated. However, differences were observed for branding, as one group was
426 branded with a hot-iron while the other group was sham branded. These results are in agreement
427 with the results observed for VAS scores, indicating that BK calves experienced more pain than
428 KN calves.
429 ***Stride length.*** No medication or procedure effects ($P > 0.10$) were observed for stride
430 length immediately after or 180 min after castration. However, a procedure effect ($P < 0.01$) was
431 observed for stride length, where KN (43 ± 1.1 cm) and BK (43 ± 1.0 cm) calves had greater
432 stride length than CT (40 ± 1.0 cm) calves on d 1, 2, 3 and 7. No medication effect ($P > 0.10$)
433 was observed for stride length on d 1, 2, 3, and 7.
434 Similar results were observed by Meléndez et al. (2017b) who reported no differences in
435 stride length immediately after and 120 min after castration in control, band and knife castrated 2

436 mo old calves. Contrary to our findings, control, band and knife castrated calves at 2-mo of age
437 did not present differences in stride length on d 1, 2, 3 and 5 after castration (Meléndez et al.,
438 2017b). This finding is difficult to explain, as we would expect KN and BK calves to have a
439 shorter stride length than CT calves. Currah et al. (2009) suggested shortening of the stride
440 length as a behavioral indicator of pain associated with surgical castration after observing longer
441 stride lengths in 3 mo old calves receiving flunixine meglumin and a lidocaine epidural than
442 calves receiving a lidocaine epidural or no medication. Differences between studies could be due
443 to the time of sampling as differences in the previous study were observed 4 and 8 h after
444 castration, while in the present study calves were sampled immediately after and 4 h after
445 castration. In addition, measurements were done differently between studies which could explain
446 differences observed in results. Differences included different type of software for image
447 analysis and lack of grid background at the time of video recording in the current study.
448 ***Behavioral observations.*** A procedure × medication interaction ($P < 0.01$) was observed
449 for walking duration (Table 2). The BK-NM and KN-NM calves had greater ($P < 0.05$) walking
450 duration than CT, KN-M and BK-M calves 2 to 4 h after treatment. Lying duration had a
451 medication effect ($P = 0.04$) where M (87 ± 0.4 min) calves had greater ($P < 0.05$) lying duration
452 than NM (66 ± 0.4 min) calves 2 to 4 h after treatment. A procedure effect ($P = 0.03$) was also
453 observed for lying duration 2 to 4 h after treatment, the KN (66 ± 0.5 min) and BK (64 ± 0.5
454 min) calves had lower ($P < 0.05$) lying durations than CT (97 ± 0.5 min) calves.
455 A procedure effect ($P = 0.01$; $P < 0.01$) was observed for standing and foot stamping,
456 where the KN (55 ± 0.5 min) and BK (58 ± 0.5 min) calves had greater ($P < 0.05$) standing
457 duration than CT (29 ± 0.5 min) calves and the BK (30 ± 0.5) calves had greater foot stamping
458 than CT (2 ± 0.5) and KN (9 ± 0.5) calves 2 to 4 h after treatment. A procedure × medication ×

time effect ($P = 0.03$) was observed for foot stamping (Fig. 3B), where BK-NM calves had greater ($P < 0.05$) foot stamping than CT-NM, KN-M, and BK-M calves, and tended ($P = 0.06$) to be greater than CT-M calves on d 1 after treatment. On d 2 after treatment, BK-NM calves had greater ($P < 0.05$) foot stamping than CT, KN-NM, KN-M and BK-M calves. No differences ($P > 0.10$) were observed on d 3 and 7 after treatment.

A medication effect ($P < 0.01$) was observed for tail flicks, the NM calves had greater number of tail flicks than M calves 2 to 4 h after treatment (Fig. 5A). A procedure effect ($P < 0.01$) was also observed for tail flicks, the KN (1346 ± 3.0) and BK (1711 ± 3.0) calves had a greater ($P < 0.05$) number of tail flicks than CT (29 ± 3.0) calves 2 to 4 h after treatment. A procedure × time effect ($P = 0.01$) was observed for tail flicks, where KN and BK calves had a greater ($P < 0.05$) number of tail flicks than CT calves on d 1 and 3 after treatment, while BK calves had the greatest number of tail flicks, followed by KN calves, and then by CT calves on d 2 after castration (Fig. 5B).

A procedure effect ($P = 0.08$) was observed for head turning, BK (24 ± 0.6) calves tended to have greater head turning than CT (3 ± 0.6) calves, however, no differences were observed between both groups and KN (12 ± 0.6) calves 2 to 4 h after treatment. A procedure × medication interaction ($P = 0.01$) was observed for head turning, where KN-NM calves had greater ($P < 0.05$) head turns than CT, KN-M and BK-M calves, but no differences were observed between KN-NM and BK-NM calves on d 1, 2, 3, and 7 after castration (Table 3). Head turning was greater ($P < 0.05$) in BK-NM calves than CT-NM and KN-M calves, but no differences ($P > 0.10$) were observed between BK-NM calves and CT-M and BK-M calves. No differences ($P > 0.10$) were observed between CT, KN-M and BK-M. A procedure × time tendency ($P = 0.06$) was observed for head turning (Table 3), where BK (9.7 ± 2.25) calves had

482 greater ($P < 0.05$) head turns and KN (9.3 ± 2.25) calves tended ($P = 0.09$) to have greater head
483 turns than CT (5.0 ± 2.36) calves on d 1. The BK (12.1 ± 1.91) and KN (6.0 ± 1.91) calves had
484 greater ($P < 0.05$) head turns than CT (4.0 ± 2.00) calves on d 2 after castration, while no
485 differences ($P > 0.10$) were observed between treatments on d 3 and 7.

486 These results suggest that branding in combination with castration is more painful than
487 surgical castration alone, as seen by a greater number of tail flicks and foot stamps 2 to 4 h and
488 on d 1 and 2 after the procedure. Although not significant, a previous study reported greater
489 number of tail flicks in knife (191) than band (78) and control (86) 2 mo old calves on d 1, 2, 3
490 and 5 after castration (Meléndez et al., 2017b). Tail flicks were also greater at the time of hot-
491 iron branding than freeze or sham branding in 320 kg calves (Schwartzkopf-Genswein et al.,
492 1997b). Meloxicam reduced pain related behaviors as seen by a reduction in walking, tail
493 flicking and head turning, and an increase in lying duration in M calves compared to NM calves.
494 Similar findings have reported lower tail flick behaviour in ketoprofen-treated cows than saline-
495 treated cows on d 1 after the first stage of fistulation surgery (Newby et al., 2014) and lower ear
496 flicks and head shakes in meloxicam-treated calves than saline-treated calves after dehorning in
497 6 to 12 week old dairy calves (Heinrich et al., 2010). Contrary to our findings, Sutherland et al.
498 (2013) did not see differences in tail flicking or time spent foot stamping between castrated,
499 dehorned and castrated + dehorned 3 mo old calves either receiving pain relief or no pain relief 3
500 h after castration. Discrepancies between studies could be due to the difference in painful
501 procedures (dehorning vs branding), which can elicit different behavioral responses and/or to
502 differences in medication (lidocaine + flunixine meglumin vs meloxicam). Although no
503 differences were observed for tail flicks and head turns between BK and KN calves, BK calves

had numerically greater number of tail flicks and head turns 2 to 4 h after castration, suggesting that BK calves experienced more pain.

No medication or procedure effects ($P > 0.10$) were observed for eating or lesion licking 2 to 4 h after treatment (Table 2). A procedure effect ($P = 0.01$) was observed for eating, where CT (27 ± 0.4 min) calves had greater eating duration than BK (16 ± 0.4 min) calves, however no differences were observed between both groups and KN (21 ± 0.4 min) calves. Although there were no differences between CT and KN calves, it is likely that greater eating duration leads to greater ADG as CT calves had greater ADG than KN and BK calves. However, values for eating could be different if these were scored for 24 h compared to 4 h. Contrary to our results, castrated, dehorned and castrated + dehorned calves receiving lidocaine and meglumin flunixine had greater eating times than un-medicated castrated, dehorned and castrated + dehorned calves (Sutherland et al., 2013). Differences between studies could be due to the added effect of the anesthetic which could temporarily block the pain associated with the procedures and consequently calves would be more likely to eat compared to calves experiencing pain.

Standing and lying behavior. Standing percentage tended (procedure × medication interaction; $P = 0.06$) to be greater while lying percentage tended (procedure × medication interaction; $P = 0.06$) to be lower in BK-NM calves than KN-NM and BK-M calves, however no differences were observed between these groups and CT and KN-M calves. Lying duration was greater (procedure × time interaction; $P < 0.01$) in CT (54 ± 2.3 min; 56 ± 2.3 min; 61 ± 3.0 min) calves than KN (45 ± 2.2 min; 46 ± 2.2 min; 54 ± 3.0 min) and BK (45 ± 2.2 min; 44 ± 2.2 min; 54 ± 2.9 min) calves on d 0, 1 and 2 after treatment. No differences were observed on d 3, 4, 5, or 6 after treatment (data not shown), suggesting that animals in pain lie for less time than animals that are not in pain. This is in agreement with a previous study where knife castrated

calves had greater standing percentage than band castrated and control calves 2 to 4 h and on d 1, 2, 3, and 5 after castration (Meléndez et al., 2017b). Holstein calves receiving oral meloxicam lay down for longer periods of time on d 1, 2, 3, and 4 after dehorning in comparison to un-medicated calves (Theurer et al., 2012) while i.m. meloxicam-treated calves were less active than un-medicated Holstein calves during the 5 h following dehorning (Heinrich et al., 2010).

A procedure × time effect ($P < 0.01$) was observed for standing bouts, where KN and BK calves had greater ($P < 0.05$) standing bouts than CT calves on d 1 and 2, while BK calves had greater ($P < 0.01$) standing bouts than CT calves, and there was a tendency ($P = 0.09$) for KN calves to have greater standing bouts than CT calves on d 0. No differences ($P > 0.10$) were observed on d 3, 4, 5 and 6.

Lying and standing bouts are an indicator of restless behavior which is associated with pain caused by ischemia (Dinniss et al., 1999). A previous study reported a decrease in standing and lying bouts in band castrated 1-wk old calves while, an increase in standing and lying bouts in 4 mo old band castrated calves, but no differences in 2 mo old band castrated calves (Meléndez et al., 2017b). It seems that restlessness is not only linked with pain caused by ischemia but it might be linked with general discomfort as calves that were surgically castrated, and branded + castrated presented greater standing bouts than CT calves.

No medication effects ($P > 0.10$) were observed for walking, standing, lying, eating and lesion licking on d 1, 2, 3, and 7 after castration, neither for standing and lying bouts or standing and lying duration on d 0, 1, 2, 3, 4, 5 and 6 (Table 3). No procedure effects ($P > 0.10$) were observed for walking, standing, lying and lesion licking on d 1, 2, 3, and 7 after the procedure, neither for standing and lying duration on d 0, 1, 2, 3, 4, 5 and 6 after the procedure. Lack of differences in behavioral and physiological parameters could be due to several reasons such as

sample size, high individual variability, lack of sensitivity of parameters collected, or suboptimal sampling time. Although sample size was calculated for salivary cortisol and tail flicks, it is possible that the sample size was too small to observe differences between treatments for other parameters. High individual variability for physiological and behavioral responses could also mask treatment effects. In addition, the parameters collected may not be sensitive to physiological and behavioral changes associated with pain and inadequate sampling times could also be a limiting factor to observe differences between treatments.

Conclusion

Overall, the combination of procedures elicited a greater physiological and behavioral response than performing knife castration alone, suggesting that the pain/discomfort experienced is greater. Meloxicam did not have an effect on salivary cortisol, substance P, SAA, PLT, stride length, standing and lying duration, standing and lying bouts, and behavioral observation for eating and lesion licking. However, meloxicam was effective at reducing the haptoglobin response, RBC and WBC counts, N:L ratio, scrotal and rectal temperature, tail flicks, walking and lying behavior (2 to 4 h after procedure), and head turning and foot stamping (1, 2, 3, and 7 d after procedure). No differences were observed between KN-M and BK-M calves for the previously mentioned parameters, suggesting that meloxicam was equally effective at mitigating pain caused by knife castration alone and the combination of knife castration + branding. Meloxicam administered s.c. can be used as a drug to mitigate pain associated with castration and branding. Further research is needed to better understand the nature of pain associated with castration and branding practices and the best protocols to mitigate this pain to optimize calf health and well-being.

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715

Table 1. Least square means (± SEM) of physiological samples taken after the procedure of non-castrated (CT, *n* = 23), knife (KN, *n* = 24) and branded and knife (BK, *n* = 24) castrated 2-mo-old Angus crossbred calves with (M, *n* = 36) or without (NM, *n* = 35) a single s.c. meloxicam administration[1]

	Treatment (T)[2]									
	CT	KN		BK			*P-Value*			
Item		NM	M	NM	M	*SEM* [3]	PRD	MED	PRD × T	MED × T
Minutes after castration										
Substance P, pg/mL	81.8	80.1	79.4	82.6	78.0	0.06	0.63	0.35	0.54	0.45
SCT, ºC	36.6	36.5	36.5	36.7	36.3	0.24	0.74	0.60	0.42	0.32
Days after castration										
Cortisol, nmol/L	5.1	2.5	3.7	2.9	2.3	0.13	0.17	0.47	0.38	0.87
Substance P, pg/mL	82.2	78.7	75.8	84.5	81.4	0.07	0.25	0.64	0.29	0.15
SCT, ºC	36.5	37.2	36.7	36.9	36.8	0.48	0.01	0.04	0.31	0.66

[1] Values in the table represent the mean of T0, 60, 90 and 120 min after procedure for substance P and scrotal temperature (SCT); and the means of d 1, 2, 3 and 7 after procedure for cortisol, substance P and scrotal temperature (SCT).

[2]CT: sham non-castrated calves; KN: knife castrated calves; BK: branded and knife castrated calves; NM: single s.c. injection of lactated ringer's immediately before procedure; M: single injection of s.c. meloxicam (0.5 mg/kg) immediately before procedure; PRD: procedure effect; MED: medication effect.

[3]The values correspond to nontransformed means; however, the SEM and the *P*-values correspond to ANOVA analysis using log transformed data.

Table 2. Least square means (± SEM) of VAS, leg movement and vocalizations during castration and behavioral observations assessed 2 to 4 h after procedure for a 2 h period of non-castrated (CT, *n* = 23), knife (KN, *n* = 24) and branded and knife (BK, *n* = 24) castrated 2-mo-old Angus crossbred calves with (M, *n* = 36) or without (NM, *n* = 35) a single s.c. meloxicam administration[1]

	Treatment[2]								
	CT	KN		BK			*P-Value*		
Item		NM	M	NM	M	*SEM*[3]	PRD	MED	PRD × MED
VAS, cm	0.4	2.2	2.9	5.1	5.8	0.08	<0.01	0.08	0.37
Leg movement, n	2.3[d]	5.2[c]	7.5[b]	9.1[ab]	10.8[a]	0.13	<0.01	0.03	0.04
Vocalization, n	2.3	2.2	1.5	6.8	9.9	0.17	<0.01	0.29	0.10
Behavioral obs.									
Walking, min	2.5[b]	5.2[a]	2.5[b]	7.0[a]	3.3[b]	0.16	<0.01	<0.01	<0.01
Standing, min	28.5	66.1	43.7	75.3	40.8	0.70	0.01	0.07	0.14
Lying, min	98.0	53.4	82.1	43.4	85.0	0.77	0.03	0.04	0.12
Foot stamping, n	1.6	12.9	5.4	28.3	31.7	0.75	<0.01	0.37	0.64

[a-d]Least square means within a row with differing superscripts differ ($P \leq 0.05$)

[1]Values in the table represent the means of visual analog scale (VAS), leg movement, and vocalizations and behavioral observations.

[2]CT: sham non-castrated calves; KN: knife castrated calves; BK: branded and knife castrated calves; NM: single s.c. injection of lactated ringer's immediately before procedure; M: single injection of s.c. meloxicam (0.5 mg/kg) immediately before procedure; PRD: procedure effect; MED: medication effect.

[3]The values correspond to nontransformed means; however, the SEM and the *P*-values correspond to ANOVA analysis using square root + 1 transformation.

Table 3. Least square means (± SEM) of behavioral observations on d 1, 2, 3, and 7 and standing and lying behavior on d 0, 1, 2, 3, 4, 5, and 6 of non-castrated (CT, *n* = 23), knife (KN, *n* = 24) and branded and knife (BK, *n* = 24) castrated 2-mo-old Angus crossbred calves with (M, *n* = 36) or without (NM, *n* = 35) a single s.c. meloxicam administration

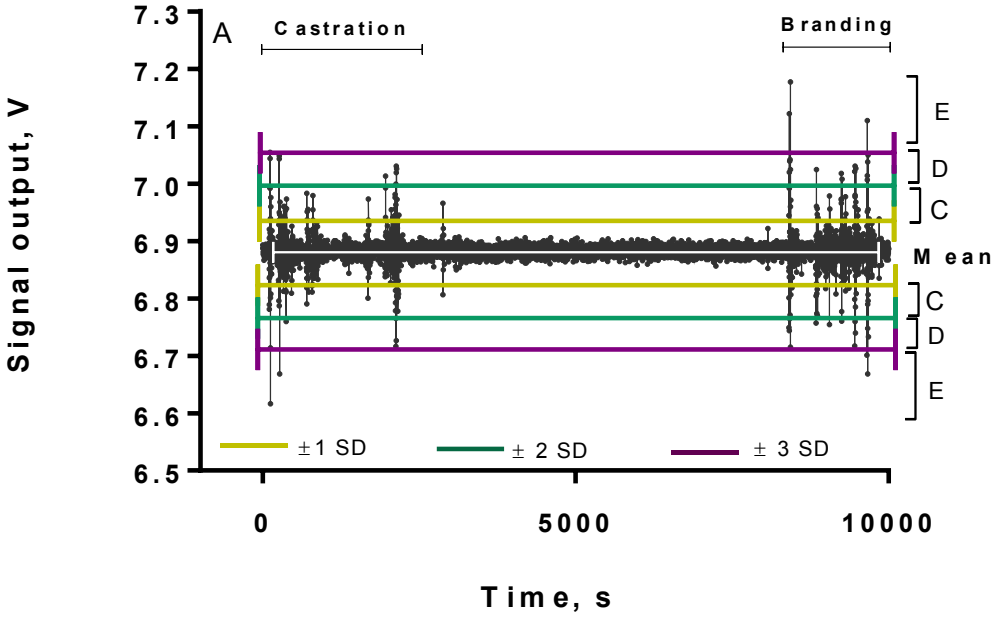
	Treatment (T)[1]									
	CT		KN		BK			*P-Value*		
Item	NM	M	NM	M	NM	M	*SEM*[2]	PRD	MED	PRD × MED
Behavioral obs.										
Walking, min	1.8	2.2	2.0	2.6	1.8	2.0	0.10	0.88	0.24	0.89
Standing, min	29.6	28.7	33.3	29.5	35.5	34.6	0.55	0.53	0.85	0.82
Lying, min	64.5	65.1	60.1	63.9	58.7	59.3	0.42	0.44	0.51	0.96
Eating, min	25.5	29.1	19.7	22.3	14.5	17.6	0.47	0.01	0.16	0.91
Head turning, n	4.1^{c}	6.5^{bc}	11.8^{a}	4.9^{c}	10.7^{ab}	6.9^{bc}	0.26	0.08	0.08	0.01
Lesion licking, n	0.7	0.9	1.6	0.8	1.6	0.8	0.12	0.42	0.11	0.38
Standing and lying beh.										
Standing, %	39.3	39.2	38.6	40.8	41.3	38.8	0.01	0.74	0.86	0.06
Lying, %	60.7	60.8	61.4	59.2	58.7	61.2	0.01	0.74	0.86	0.06
Standing duration, min	41.8	45.0	39.5	42.0	43.3	38.5	0.17	0.39	0.86	0.21
Lying duration, min	59.7	62.8	58.9	58.0	60.1	56.3	0.18	0.39	0.87	0.57
Standing bouts, n	14.4	13.5	15.6	15.2	15.1	15.7	0.09	0.08	0.70	0.57

[a-c]Least square means within a row with differing superscripts differ ($P \leq 0.05$).

[1]CT: sham non-castrated calves; KN: knife castrated calves; BK: branded and knife castrated calves; NM: single s.c. injection of lactated ringer's immediately before procedure; M: single injection of s.c. meloxicam (0.5 mg/kg) immediately before procedure; PRD: procedure effect; MED: medication effect.

[2]The values represented correspond to non-transformed means; however, SEM and *P*-values correspond to ANOVA analysis using square root + 1 transformed data for behavioral observations.

1 Figure 1.



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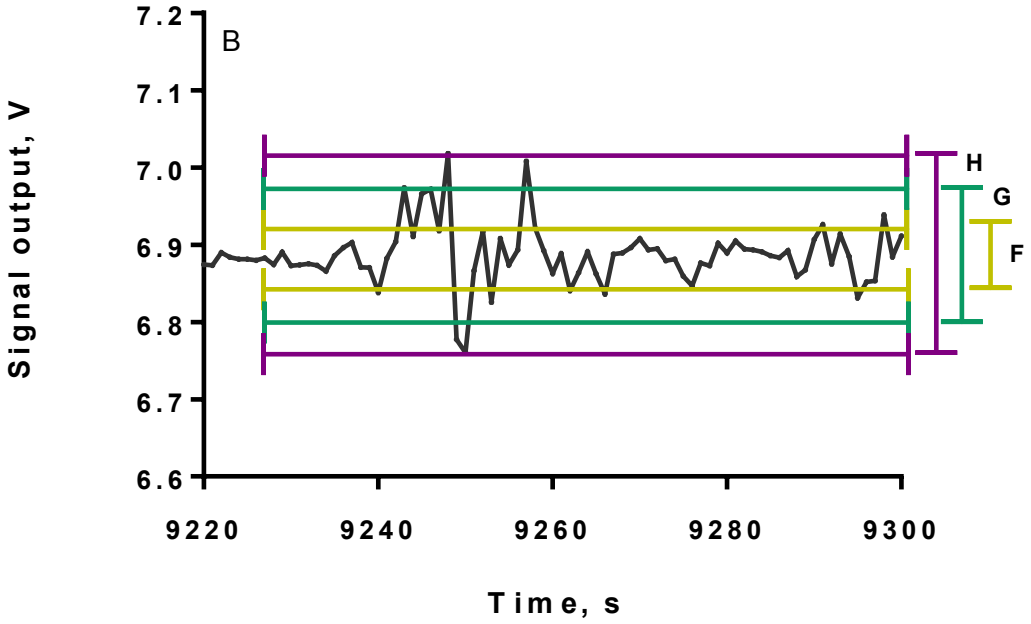

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Figure 1. Signal output in volts of the addition of three forces of a three dimensional accelerometer indicating movement of the tipping table by a calf (#74) during knife castration and branding. (A) C = number of peaks between 1 and 2 SD above and below the mean, D = number of peaks between 2 and 3 SD above and below the mean, and E = number of peaks above or below 3 SD above or below the mean. (B) F= total area between ± 1 SD, G = total area between ± 2 SD and H = total area between ± 3 SD.

Figure 2.

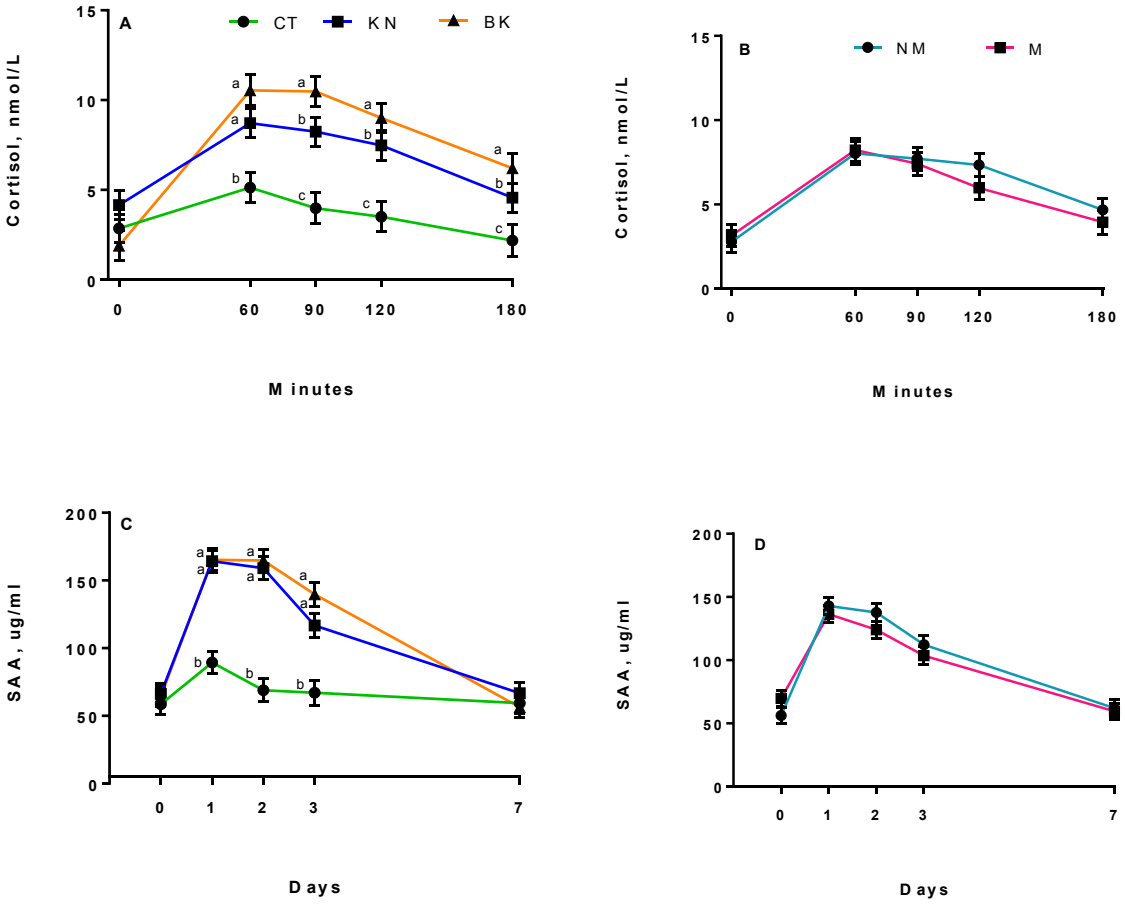
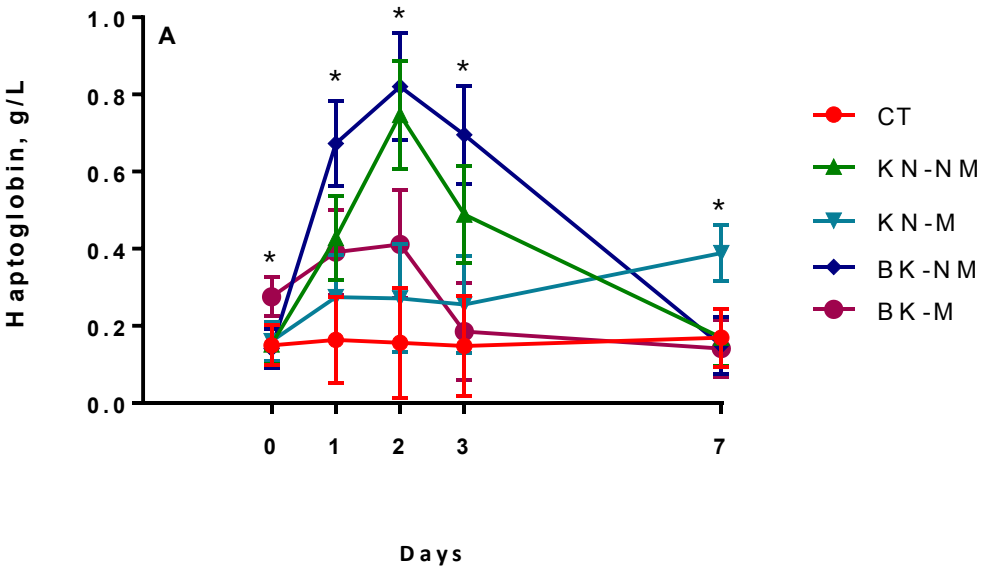

A
CT
KN
BK
Cortisol, nmol/L
Minutes
B
NM
M
Cortisol, nmol/L
Minutes
C
SAA, ug/ml
Days
D
SAA, ug/ml
Days

Figure 2. Least square means and SEM for salivary cortisol (nmol/L) of (A) procedure and (B) medication immediately before treatment (T0), 60, 90, 120 and 180 min after treatment and serum amyloid-A (µg/mL) for (C) procedure and (D) medication on d 0, 1, 2, 3 and 7 after castration of non-castrated (CT, *n* = 23), knife (KN, *n* = 24) and branded and knife (BK, *n* = 24) castrated 2 mo old Angus crossbred calves with (M, *n* = 36) or without (NM, *n* = 35) a single s.c. meloxicam administration. [a-c]Least square means with differing superscripts differ ($P \leq 0.05$).

48 Figure 3.



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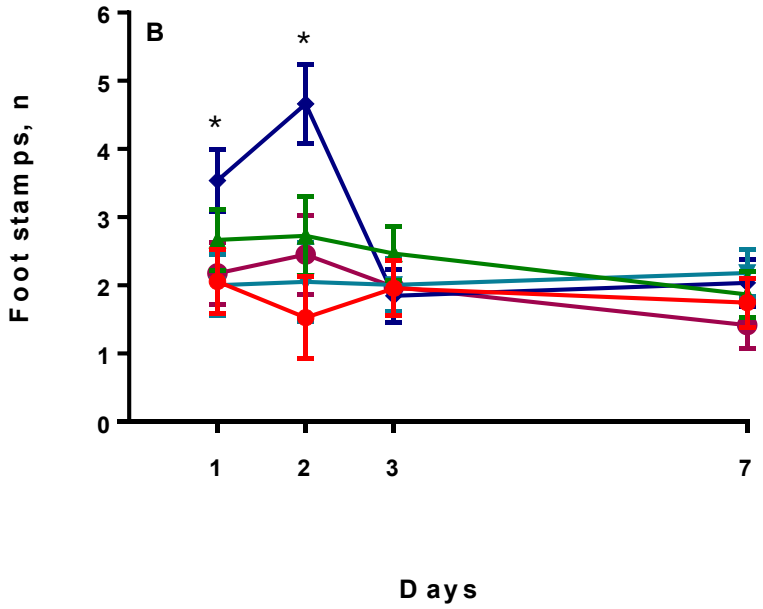

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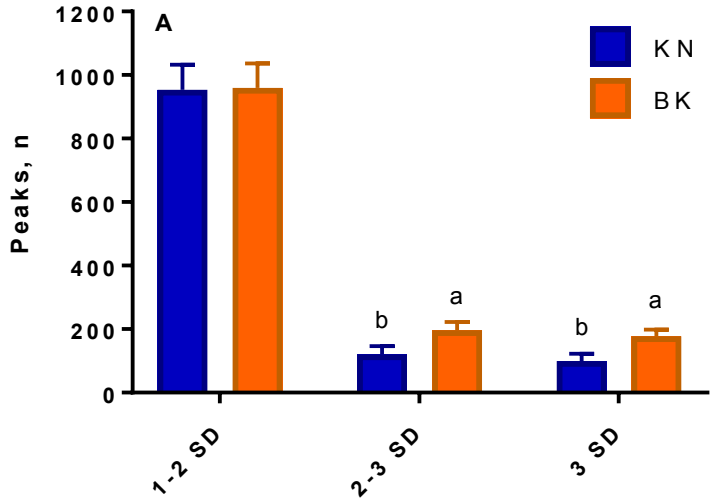
Figure 3. Least square means and SEM for (A) haptoglobin on d 0, 1, 2, 3 and 7 and (B) foot stamps on d 1, 2, 3 and 7 of non-castrated (CT, $n = 23$), knife (KN, $n = 24$) and branded and knife (BK, $n = 24$) castrated 2 mo old Angus crossbred calves with (M, $n = 36$) or without (NM, $n = 35$) a single s.c. meloxicam administration. * $P \leq 0.05$.

Figure 4.


A
1200
1000
800
600
400
200
0
Peaks, n
K N
B K
b
a
b
a
1-2 SD
2-3 SD
3 SD

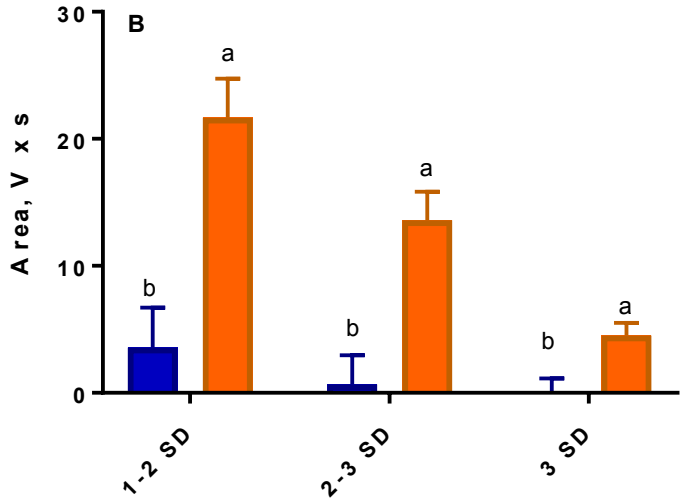
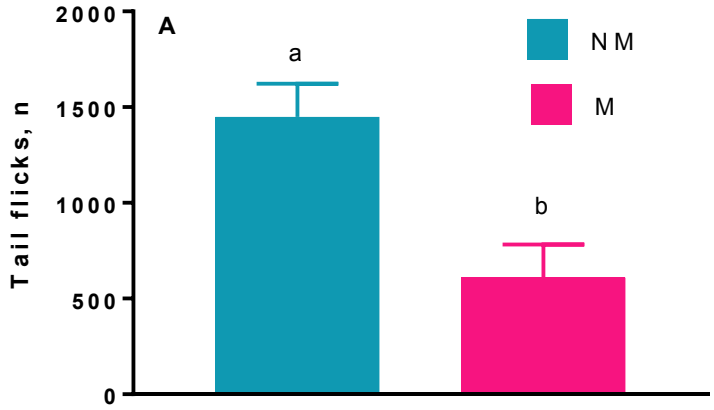

B
30
20
10
0
A rea, V x s
b
a
b
a
b
a
1-2 SD
2-3 SD
3 SD

Figure 4. Least square means and SEM for electronic reactivity measurements (A) peaks (number) and (B) area (V × s) during sham branding (KN, *n* = 24) and hot-iron branding (BK, *n* = 24) of 2 mo old Angus crossbred calves with (M, *n* = 36) or without (NM, *n* = 35) a single s.c. meloxicam administration. [a-b]Least square means with differing superscripts differ ($P \leq 0.05$).

Figure 5.


A
2000
1500
1000
500
0
Tail flicks, n
a
b
N M
M

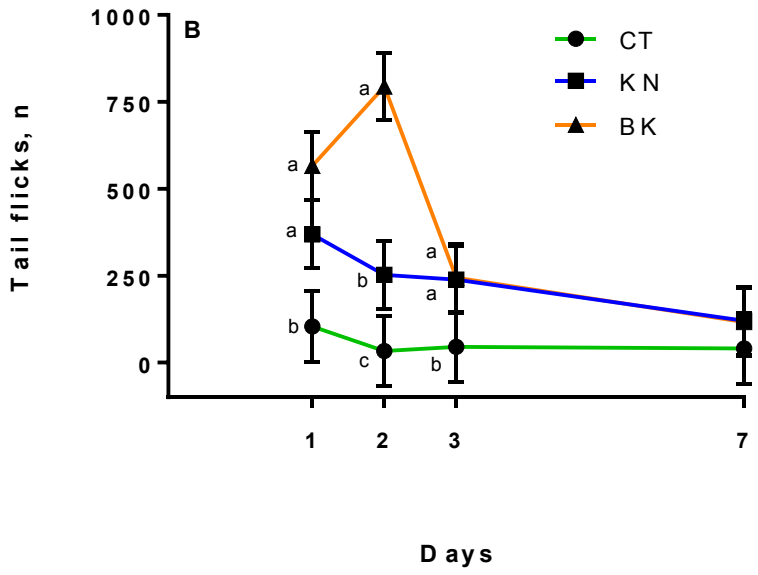

B
1000
750
500
250
0
Tail flicks, n
a
a
b
b
a
a
c
b
CT
K N
B K
1
2
3
7
Days

Figure 5. Least square means and SEM for tail flicks (A) 2 to 4 h after castration and (B) on d 1, 2, 3 and 7 of non-castrated (CT, *n* = 23), knife (KN, *n* = 24) and branded and knife (BK, *n* = 24) castrated 2 mo old Angus crossbred calves with (M, *n* = 36) or without (NM, *n* = 35) a single s.c. meloxicam administration. [a-c]Least square means with differing superscripts differ ($P \leq 0.05$).

Supplementary Material

Figure 6.

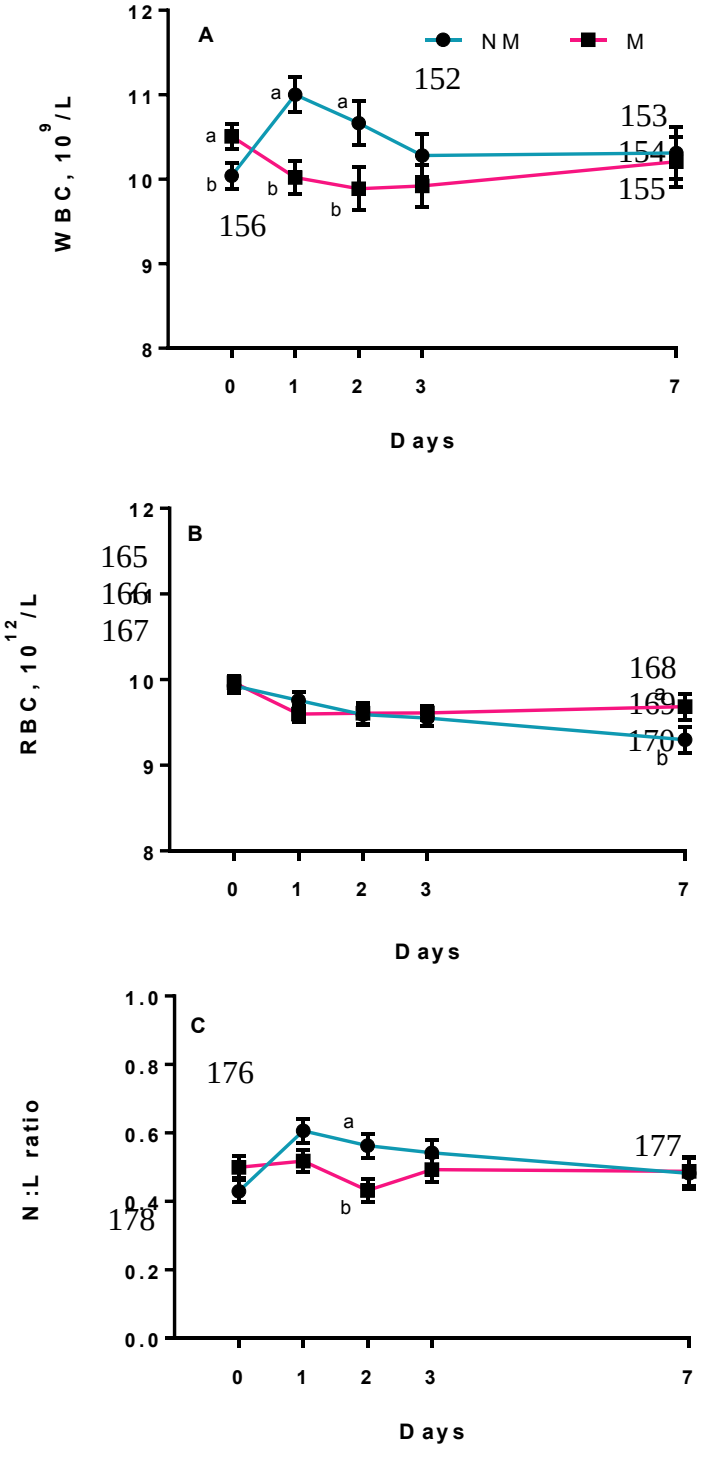
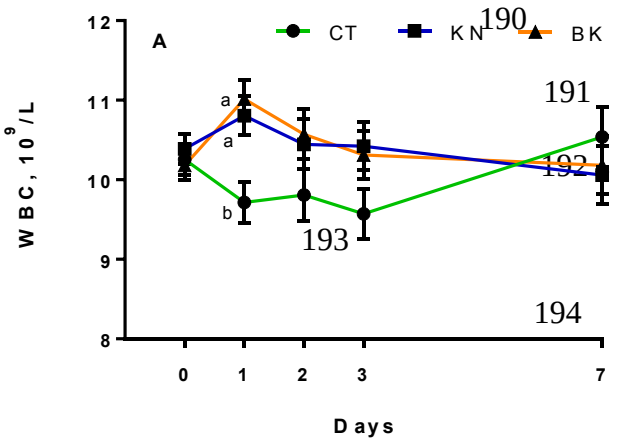


Figure 6. Least square means and SEM for (A) WBC, (B) RBC and (C) N:L ratio on d 1, 2, 3 and 7 of non-castrated (CT, *n* = 23), knife (KN, *n* = 24) and branded and knife (BK, *n* = 24) castrated 2 mo old Angus crossbred calves with (M, *n* = 36) or without (NM, *n* = 35) a single s.c. meloxicam administration. [a-b]Least square means with differing superscripts differ ($P \leq 0.05$).

189 Figure 7.



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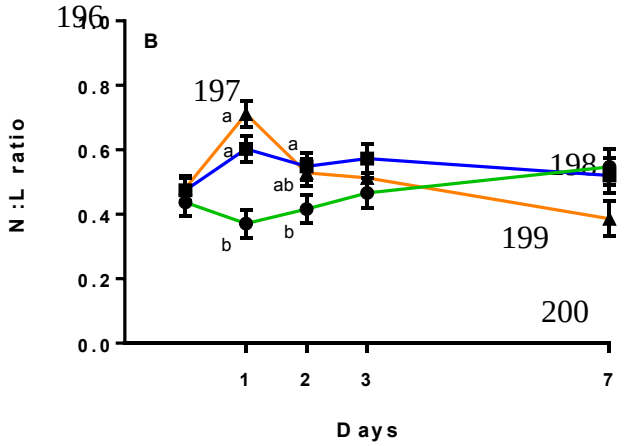

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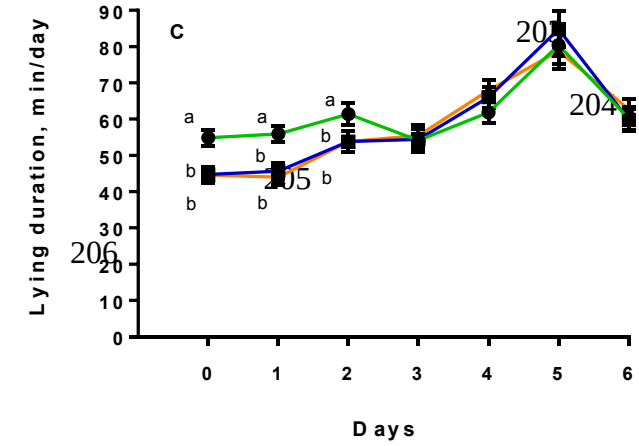

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Figure 7. Least square means and SEM for (A) WBC and (B) N:L ratio on d 1, 2, 3 and 7, and (C) lying duration on d 0, 1, 2, 3, 4, 5, and 6 of non-castrated (CT, n = 23), knife (KN, n = 24) and branded and knife (BK, n = 24) castrated 2 mo old Angus crossbred calves with (M, n = 36) or without (NM, n = 35) a single s.c. meloxicam administration. [a-b]Least square means with differing superscripts differ ($P \leq 0.05$).