Evaluation of pain management products and alternative pain detection techniques to improve cattle welfare

by

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B.S., Kansas State University, 2022

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Anatomy and Physiology
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KANSAS STATE UNIVERSITY
Manhattan, Kansas

2026

Abstract

This dissertation focuses on strategies to improve cattle welfare by evaluating potential pain management products for use during painful management procedures, and exploring pain detection techniques that could aid in reliably evaluating the efficacy of pain management products. The first chapter consists of a systematic literature review that evaluates the reliability of lying and standing behaviors as an indicator of pain in cattle, and describes trends in these behaviors to help future researchers make inferences from their data. The next three chapters evaluate products that could potentially reduce pain during castration and disbudding in calves through an alternative castration device (Chapter 2), maternal appeasing substance analog (Chapter 3), and a lidocaine-infused castration band (Chapter 4). The last chapter (Chapter 5) of this dissertation evaluates the ability of machine learning to detect painful animals through the use of short videos of cattle faces.

Review of 16 relevant articles on lying and standing behavior as an indicator of pain after castration suggests that these outcomes can be a reliable indicator of pain or discomfort. Several studies showed that surgically castrated calves stand for significantly longer than non-castrated control calves within the first 4 days post-castration. Surgically castrated calves may also perform a greater frequency of bouts on the day of castration compared to controls, which could be an indication of restlessness. Regarding band castration, multiple studies showed that band-castrated calves perform a significantly greater frequency of bouts than control calves on the day of castration. In the following weeks, band-castrated calves stood significantly more than control calves. After both surgical and band castration, studies that used accelerometers were the most successful in detecting statistically significant differences in standing and lying behavior between castrated calves and non-castrated control calves. Both studies that analyzed

accelerometer data as a difference from baseline values were able to detect significant treatment differences in standing behavior, highlighting the potential benefits of this method of data analysis. Overall, this review found lying and standing outcomes to be useful indicators of pain or discomfort, and was able to establish trends in these outcomes after surgical and band castration.

A randomized, blinded, controlled pilot study was conducted to determine the viability of an alternative castration device, called the ClipFitter, in cattle and its effects on pain and welfare. Twelve intact male beef-dairy cross calves were assigned to 1 of 3 treatment groups: castration with ClipFitter (CLIP; n=4), castration with rubber ring band (BAND; n=4), or no castration (SHAM; n=4). Calves' plasma cortisol, Substance P, lie/stand behavior, and infrared thermography were assessed at multiple timepoints. No significant differences were detected in plasma cortisol, Substance P, lying and standing behavior, and infrared thermography between CLIP and BAND calves within 7 days post-castration. While the viability of the ClipFitter for castration of calves was demonstrated to be promising, the study was unable to find differences in pain measures that could correlate to a negative state of welfare. Lying and standing outcomes suggest that both ClipFitter and rubber band castration cause acute discomfort or pain, as determined by differences in the average duration of lying and standing bouts. Scrotal IRT outcomes suggest that the ClipFitter reduces blood flow to the scrotum at a level indistinguishable from band castration, implying that the ClipFitter could be potentially viable for calf castration. Overall, the castration effectiveness of the ClipFitter and its implications on chronic pain and animal welfare requires further research that follows calves for a longer period post-castration.

A second randomized blinded controlled trial was conducted to determine if a single topical dose of a maternal appeasing substance analog, alone, or in combination with local anesthesia, with or without systemic analgesia would provide extended pain and stress relief to calves after disbudding and surgical castration. The calves' substance P, plasma cortisol, mechanical nociception threshold, infrared thermography, performance, lie and stand activity, behavior, and gait were evaluated at multiple timepoints. No significant differences in infrared thermography, performance, lie and stand activity, and behavior outcomes were observed between treatments (P>0.05). While Substance P, plasma cortisol, mechanical nociception threshold, and kinetic gait analysis differed amongst some treatment groups, no significant differences were observed between treatment groups that received or did not receive the maternal appeasing substance immediately before castration and disbudding. These results suggest that administering a maternal appeasing substance in addition to other analgesic and anesthetic drugs is neither more or less effective at pain mitigation than lidocaine alone in Holstein calves undergoing surgical castration and cautery disbudding.

The last randomized blinded controlled trial aimed to evaluate pain mitigation and behavior in beef-dairy and Holstein calves castrated with either lidocaine-loaded or standard bands. Calves' average daily gain, lying and standing activity, kinetic gait analysis, plasma cortisol, and potential pain-related behaviors were analyzed over a 28-day post-treatment period. Lying and standing activity outcomes showed a significant effect of breed for the number of standing bouts per day, with the BOD group transitioning from lying to standing more frequently than the HOL group, but this difference was considered biologically insignificant. Gait outcomes showed a treatment × time interaction for gait velocity and gait distance, with the CON group increasing over time. However, no gait outcome differed between LLB and CON groups at any

time point. Neither treatment or breed had an effect on plasma cortisol, performance, or video monitoring behavior outcomes ($P > 0.05$). Overall, this study was unable to find differences in pain outcomes between calves that were castrated with either a lidocaine-loaded or standard band.

Lastly, observational data was collected to evaluate the performance of 2 video-based convolutional neural network architectures using short videos of the face for automated pain detection in beef cattle, and to determine the optimal view of the face by comparing model performance between lateral and front views of cattle faces. A total of 247 short videos of cattle faces, ranging between 1 to 2 seconds, were classified as Pain (n = 143) and No Pain (n = 104) based on castration performed at feedyard arrival. Two convolutional neural network architectures were used to predict pain status among 3 datasets: combined view (front and lateral view videos of the face), front view only, and lateral view only. The front view dataset was able to identify over half of truly painful animals while minimizing false positive classifications, which could have useful clinical applications. Models using the front view dataset showed significantly better discriminatory ability compared to the lateral view dataset, with the greatest ROC-AUC reaching 0.75, indicating that facial expressions visualized from the front may contain more information on pain status. Overall, this study indicated that automated pain detection using facial analysis with machine learning is feasible for feedyard cattle, and could provide value to feedyard operations and improve cattle welfare.

In conclusion, this dissertation could provide valuable information to researchers, veterinarians, and cattle producers on pain management and pain detection strategies to improve cattle welfare. The studies presented in this dissertation advance our knowledge of multiple

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Acknowledgements

First, I would like to thank everyone in the Coetzee Lab for the mentorship, encouragement, and camaraderie. I am especially grateful that we were all able to work as a team to accomplish our goals, and even have some fun along the way. Dr. Coetzee and Dr. Kleinhenz, thank you for believing in me when I applied to be a student in your lab.

To all of the faculty and students in the Beef Cattle Institute, thank you all for accepting me as an honorable student in your lab. I appreciate all the time and effort that the faculty have invested in me. I am thankful for all of the graduate students that were willing to help someone from a different lab (me) with his projects, and for all of the friendships I have made.

To my committee members—thank you for sharing your time, wisdom, and encouragement—I appreciate your leadership that allowed me to navigate through graduate school and that has helped shaped my future.

Lastly, I want to thank my advisor, Dr. Bortoluzzi. I can never say that I am unlucky person, because I was lucky enough to be your student. Thank you for the countless hours you have invested in my growth, both as a researcher and as a person, and for being an unwavering mentor and friend.

Chapter 1 - Lying and standing behaviors as indicators of pain in cattle: A systematic review

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Abstract

Objective and reliable outcomes for pain quantification in cattle are needed to obtain regulatory approval of analgesic and anesthetic drugs. Lying and standing behavior data are commonly collected when evaluating pain status in cattle, however, making inferences from these data may be challenging. The objectives of this systematic review were to evaluate the utility of lying and standing behaviors as objective pain outcomes after surgical or band castration in cattle, and to identify trends in these behaviors that may be caused by castration-related pain. Articles were included in this review if the following criteria were satisfied: 1) original research article published in English and presented in full-text, 2) lying and standing behavior of cattle was evaluated after surgical or band castration, 3) experimental treatments included a group that was castrated with no pain management, and a group that was not castrated (sham) to serve as a comparator. A total of 16 articles were included in the final dataset. Studies using calves less than two months of age at the time of castration found no significant differences in lying or standing outcomes after castration. In studies that evaluated cattle greater than two months of age at the time of castration, results indicated that standing time, standing bouts, and lying bouts can

be useful outcomes for pain detection. For surgical castration, changes in lying and standing occurred within four days post-castration, with calves increasing standing time compared to non-castrated controls. Conversely, band castration caused changes in lying and standing weeks post-castration, which may correspond to the period when the band begins to pierce the tissue. After both surgical and band castration, studies that used accelerometers were the most successful in detecting statistically significant differences in standing and lying behavior between castrated calves and controls. Overall, this review found lying and standing outcomes to be useful indicators of pain or discomfort in cattle two months of age or greater, and trends in these outcomes were identified after surgical and band castration.

Introduction

Flunixin transdermal is currently the only approved drug for pain alleviation for cattle in the United States, which is labeled only for pain associated with foot rot. Extra-label drug use is allowed under the stipulations of the Animal Medicinal Drug Use Clarification Act (AMDUCA), but there are no labeled drugs for controlling pain associated with routine procedures such as castration or disbudding (FDA, 1994). The ability to objectively quantify pain in cattle is vital for obtaining regulatory approval of anesthetic or analgesic drugs (FDA, 2022). Objective outcomes for pain detection are critical for making inferences about cattle pain status, and consequently, evaluating efficacy of pain management drugs.

Lying and standing behavior data are commonly evaluated as an objective indicator of pain in cattle, typically collected by accelerometers (Coetzee, 2011). However, there may be some conflicting evidence in the literature regarding lying and standing behavior after castration in cattle, which could complicate the interpretation of these data. A systematic review of the

relevant literature is needed to evaluate the utility of these data as objective measures of pain, and identify any potential trends in lying and standing behavior after painful experiences. Castration is a painful management procedure that is routinely performed in cattle to reduce unwanted pregnancies and improve carcass quality (Field, 1971). Researchers often use castration as a painful intervention to evaluate the efficacy of anesthetic and analgesic drugs (Stafford et al., 2002; Webster et al., 2013; Daniela M. Meléndez et al., 2018b; Martin et al., 2022). Therefore, this review will focus only on lying and standing behaviors after surgical and band castration with no pain management, using non-castrated control calves as the comparator. The objective of this review is to evaluate the utility of lying and standing behaviors as objective pain outcomes after surgical or band castration in cattle with no pain management, describe the design of studies that detected significant treatment differences for lying and standing outcomes, and evaluate potential trends in lying and standing behavior after surgical and band castration that may be indicative of pain.

Materials and Methods

There were three databases used for this systematic review: PubMed, AGRICOLA, and CABI. The initial search was conducted on August 19, 2026. The search string was identical across all three databases: ((((((((((((cattle) OR ("beef cattle")) OR ("dairy cattle")) OR (bovine)) OR ("bos taurus")) OR ("beef calves")) OR ("beef calf")) OR ("dairy calves")) OR ("dairy calf")) OR ("beef x dairy")) OR ("beef dairy")) OR ("beef dairy cross")) AND (((((((("animal behavior") OR (behavior, animal)) OR (behavior)) OR ("lying behavior")) OR ("standing behavior")) OR (posturing)) OR (accelerometer)) OR ("scan sampling")) OR ("behavioral sampling"))) AND ((((((castration) OR (gonadectomy)) OR (gonadectomies)) OR (banding)) OR ("banding castration")) OR ("band castration")). This search string included

methods of data collection for lying and standing behavior, methods of castration, and multiple cattle breeds. No restrictions were placed on publication date. Studies that met all inclusion criteria were included in the final dataset regardless of the breed or age of the cattle, housing type, data collection method, or length of the sampling period.

Inclusion to this review was considered if the following requirements were met; 1) original research article published in English and presented in full-text, 2) lying and standing behavior of cattle were evaluated after surgical or band castration, 3) experimental treatments included a group that was castrated with no pain management, and a group that was not castrated (sham) to serve as a comparator. Only studies that directly reported lying and standing outcomes (e.g., lying duration, standing bouts) were considered; studies that only reported aggregate behavior scores or "activity" were not considered. Furthermore, studies that only used animals that received concurrent painful interventions (e.g., castration and disbudding) were not considered. After the initial search, abstract screening was conducted by one author. Articles that passed abstract screening were then full-text evaluated by the same author.

Results and Discussion

The initial search yielded a total of 1360 results across the three databases; PubMed yielded 720 results, AGRICOLA yielded 99, and CABI yielded 541. After abstract screening, 64 articles were considered for full-text evaluation. If duplicate articles were yielded across the three databases, only one was included in the full-text evaluation dataset. Many articles that underwent full-text review were excluded because they lacked an experimental group that was castrated with no pain management. Of the 64 articles that were full-text evaluated, 16 articles were included in the final dataset (Figure 1.1). Eight of the 16 articles included in the final

dataset used both surgical and band castration as the painful intervention; therefore, these articles are referenced in multiple sections of this systematic review.

Surgical castration

The search results from this literature review yielded 11 articles that evaluated lying and standing behaviors of surgically castrated cattle with no pain management compared to non-castrated control cattle. One of these articles evaluated cattle of three age groups and was treated as three separate studies (Meléndez et al., 2017), resulting in a total of 13 studies using surgical castration (Table 1.1).

Five of the 13 experiments detected significant treatment differences in lying and standing outcomes after surgical castration. Four out of these five studies found that surgically castrated cattle lie less and stand more compared to non-castrated controls. A study that evaluated lying and standing behaviors after surgical castration in 2-month old beef calves found that surgically castrated calves spent significantly ($P < 0.01$) less time lying and significantly ($P < 0.01$) more time standing from 2 to 4 h post-castration compared to controls, which was assessed through video recorded behavior observations (Meléndez et al., 2017). The calves were also equipped with accelerometers that captured lying and standing data on the day of castration and the following 6 days. Accelerometer data showed that castrated calves spent a greater ($P <0.01$) percentage of time standing and less ($P < 0.01$) time lying compared to non-castrated control calves over the 7-day sampling period (Meléndez et al., 2017).

The same article found similar results in 4 month old beef cattle (Meléndez et al., 2017). A treatment × time interaction was detected for percentage of time standing, with surgically castrated calves spending a significantly ($P < 0.01$) greater percentage of time standing on the day after castration compared to non-castrated controls (Meléndez et al., 2017). For 3 days

following castration, surgically castrated calves also stood for longer periods of time compared to controls, with the authors noting that castrated cattle may have exhibited this behavior to avoid further stimulation of the affected area that may occur while lying (Meléndez et al., 2017). Lastly, castrated cattle performed more standing bouts on the day of castration compared to controls, which can be an indication of restlessness as the cattle change positions more frequently (Dinniss et al., 1999; Meléndez et al., 2017). Overall, these results indicate that 4-month old surgically castrated cattle may have been restless on the day of castration, as the calves transitioned from lying to standing more frequently than controls. Results from the following 3 days after castration suggest that surgically castrated cattle stand for greater periods of time, potentially to limit pressure on the affected area that may be caused by lying (Meléndez et al., 2017).

Corresponding to the results from Meléndez et al. (2017) in 4-month old calves, one study found that 9-month old castrated beef cattle performed increased lying bouts within 4.5 h post-castration compared to controls, assessed using video recordings (Cho et al., 2024). An increased frequency of lying may be indicative of restlessness, as cattle may change position more frequently in an attempt to escape pain or discomfort (Dinniss et al., 1999). Housing and environmental conditions were not reported, making it difficult to compare these results to other studies. Cho et al. (2024) was able to successfully detect differences in lying and standing behavior with a relatively small sample size (n=6) from four hours of video recordings, indicating that 0.5 to 4.5 h post-castration may be an optimal observation period for surgically castrated cattle.

A study that evaluated weaned beef cattle (6 months of age) found that surgically castrated calves stood for significantly more time on the day of castration and the following two

days compared to controls (Roberts et al., 2018). A slightly different approach was taken in this study, as accelerometers were affixed to the cattle and began logging data on day -9 (castration on day 0), which allowed for a relatively long baseline collection period. Cattle arrived to the facility at day -10, leaving only one day of acclimation before baseline data was recorded. The authors did note that baseline behavior data may have not been an accurate reflection of the animals' true baseline due to a short acclimation period (Roberts et al., 2018). Nevertheless, post-castration data was analyzed as a difference from baseline values. This method proved to be successful in detecting significant differences in standing behavior, with surgically castrated cattle standing for over 100 more minutes on days 0, 1, and 2 compared to baseline (Roberts et al., 2018). Standing time on all three of these days was significantly ($P < 0.02$) greater than control animals (Roberts et al., 2018). No significant differences were reported for lying or standing bouts between surgically castrated and control groups ($P > 0.05$). Cattle were group housed in feedlot pens, with pen designated as the experimental unit and a sample size of n=6 (Roberts et al., 2018). This is the only study in the dataset that used pen as the experimental unit, which may have been useful for detecting significant differences in behavior. Cattle housed in the same pen may imitate each other's behavior (Flury and Gygax, 2016), which may reduce potential behavioral differences between treatment groups of cattle housed in the same pen. Although this study had a short acclimation period that may have affected baseline data, this study provides strong evidence that weaned beef calves spend more time standing after surgical castration, particularly in the first three days (Roberts et al., 2018).

Similarly to Roberts et al. (2018), a study assessing lying and standing behaviors in 600 lb (age not reported) weaned beef calves used baseline data to evaluate potential behavioral differences between pre- and post-castration (White et al., 2008). Accelerometers were used to

collect lying and standing data for approximately 18 hours before castration in order to establish a pre-castration baseline, using a sample size of six animals per treatment group. Data was collected for 20 to 25 hours post-castration. When analyzed as a difference from baseline, there was no significant differences ($P = 0.32$) in standing time after castration between surgically castrated and control calves (White et al., 2008). However, when comparisons were made within each treatment group between pre- and post-castration, results showed that surgically castrated calves spent significantly ($P = 0.035$) more time standing after castration compared to before castration (White et al., 2008). This is in contrast to the control group, which showed no difference ($P = 0.11$) in standing time before and after sham castration (White et al., 2008). The authors report that cattle were housed in stalls, but housing is not further described. Other than lying and standing behaviors, no other outcomes collected in this study, which may have limited variation or potential confounders (White et al., 2008). Most other studies evaluating lying and standing behavior also collected physiological outcomes, such as blood or saliva for cortisol concentrations, within the first few days of castration. Cattle typically experience removal from their home pen, restraint in the chute, and human handling during collection of physiological outcomes, which could potentially influence subsequent lying and standing behavior. Overall, the results from White et al. (2008) correspond to the results from Meléndez et al. (2017) and Roberts et al. (2018), showing that surgically castrated cattle spend more time standing for at least 20 hours after castration.

There were several studies that used either live observations or video recorded observations that were unable to detect significant differences in lying and standing behavior after surgical castration (Rust et al., 2007; Sutherland et al., 2013; Van der Saag et al., 2018; Gellatly et al., 2021). The similarity in these studies is that behavior observations were

conducted in multiple short observation periods, rather than one long observation period. Van der Sagg et al. (2018) and Sutherland et al. (2013) each conducted several 5-minute sampling periods only on the day of castration, while Rust et al. (2007) and Gellatly et al. (2021) conducted observations over several days post-castration. Although Gellatly et al. (2021) did use 2-hour sampling periods, the study was conducted over multiple years, which may have introduced additional variation into the data and limited the ability to detect significant differences. Additionally, a very small sample size was used, with only 2 surgically castrated animals being observed (Gellatly et al., 2021). The authors also noted that humans were visible to the calves when video was being recorded, which may have influenced calf behavior (Gellatly et al., 2021). Scoring behavior through live or video recorded observations is laborious; time constraints likely limit the ability to detect significant differences in lying and standing behaviors through these methods. Additionally, scoring live or video recorded behavior can introduce variation through observer bias and fatigue. The use of accelerometers may be a better option for collecting lying and standing behaviors after surgical or band castration in calves, as data collected through accelerometers are more objective and less prone to human error.

Overall, studies that found statistical differences in lying and standing outcomes between treatment groups typically used accelerometers. Given that accelerometers automatically record data continuously, this method allows researchers to collect lying and standing data for much longer periods of time compared to live or video recorded observations, which increases the potential to detect significant differences. With the exception of White et al. (2008), all studies using accelerometers after surgical castration reported lying and standing data for 7 days post-castration, with all significant differences being detected within the first 4 days following surgical castration, including the day of castration (Meléndez et al., 2017; Roberts et al., 2018).

All studies reported similar results, with surgically castrated calves spending more time standing compared to non-castrated controls. Cho et al. (2024) and Meléndez et al. (2017) found that surgically castrated calves performed an increased frequency of lying and standing, respectively, on the day of castration, which is potentially indicative of restlessness. The studies that detected significant differences in lying and standing behavior through video recorded observations used an observation period of 0.5 to 4.5 h, and 2 to 4 h post-castration, respectively (Meléndez et al., 2017; Cho et al., 2024). Lastly, both of the studies that analyzed accelerometer data as a difference from baseline were successful in detecting increases in standing time from pre- to post-castration, indicating that this may be a useful method of analyzing accelerometer data after surgical castration (White et al., 2008; Roberts et al., 2018).

Band castration

The behavior of band-castrated cattle appears to be much different than surgically castrated cattle (Robertson et al., 1994). Consequently, methods of collecting lying and standing data should differ between the two castration methods. It can take up to 9 weeks for scrotums to slough after band castration, but as scrotum size increase, the timeline for scrotal sloughing decreases (Marti et al., 2017). Marti et al. (2017) reported that 1 week old calves at the time of banding sloughed their scrotums at an average of 63 days post-castration, while 4 month old calves sloughed their scrotums at an average of 42 days post-castration (Marti et al., 2017). This could indicate that band-castrated calves experience pain for much longer than surgically castrated calves, and that the size of the calves at the time of band castration could influence the timeline of painful experiences.

Band castration causes acute pain (Robertson et al., 1994), and is thought to cause chronic pain at least until the scrotum is sloughed (Marti et al., 2017; Mancke et al., 2025).

Approximately 2 to 6 weeks post-castration, the band will begin to cut into the scrotum tissue; this may be the onset of chronic pain, as changes in lying and standing behavior have been reported at this time (Mancke et al., 2025). Marti et al. (2017) also showed that 4-month old band castrated calves increased standing time on days 15 to 19 post-castration compared to non-castrated control calves, which correlated with the presence of granulation tissue at the castration site. A potential explanation for these findings is that band-castrated calves stand more at this time to avoid further stimulation of the castration site when lying or changing positions. No changes in lying and standing behavior were observed in 1-week and 2-month old calves, with the authors noting that significant differences may not have been detected due to behavioral changes potentially occurring in these groups after the 28-day sampling period, as smaller scrotum sizes results in delayed presence of granulation tissue (Marti et al., 2017). Therefore, it may be useful for researchers to extend the lying and standing behavior sampling period to 6 to 7 weeks in cattle that are less than 4 months of age at the time of castration in order to capture potential behavioral differences as granulation tissue begins to form.

Garcia et al. (2025) and Ball et al. (2018) reported results synonymous to Marti et al. (2017), with Garcia et al. (2025) finding 8-month old band-castrated beef calves to lie less than control calves between 16 and 19 days post-castration. Similarly, Ball et al. (2018) found that 700 lb band-castrated calves spent more time standing on days 10 and 11 post-castration compared to control animals. The results from these studies reinforces the notion that differences in lying and standing behaviors in larger cattle may be observed earlier in the castration period, which may correlate to the time when the band begins to pierce the scrotum tissue. Both studies used a 28-day sampling period using accelerometers (Ball et al., 2018; Garcia et al., 2025),

252 which proved to be sufficient for detecting statistical differences, given that relatively older cattle
253 were used in the studies.
254 Daniel et al. (2020) found slightly different results when evaluating lying and standing
255 behaviors of 3-month old beef calves housed with their dams in a pasture after band castration.
256 Banded calves spent more time lying on several days between 7 and 27 days post-castration
257 compared to control calves (Daniel et al., 2020), which conflicts with the results from Marti et al.
258 (2017), Garcia et al. (2025), and Ball et al. (2018), that showed banded calves increased standing
259 time and decreased lying within this period. These results may have differed due to differences in
260 housing and environment. Of the studies that found statistical differences in lying and standing
261 behaviors after band castration, Daniel et al. (2020) was the only study that housed cattle in
262 pasture. Calves housed in a pasture may exhibit different lying and standing behaviors;
263 locomotion may be encouraged because they are not confined in smaller pens, or locomotion
264 may be decreased, as it may be more comfortable to lie in grass compared to bare feedlot pens.
265 Additionally, the 3-month old calves in this study were housed with their dams (Daniel et al.,
266 2020), which is approximately the age when calves are most closely paired to the dams
267 (Sawalhah et al., 2024), potentially also influenced lying and standing behavior of the calves.
268 Regarding potential acute pain-related behaviors after band castration, Ball et al. (2018) and
269 Roberts et al. (2018) found that banded cattle performed more lying bouts on the day of
270 castration compared to control calves. The increase in lying bouts indicates restlessness, as the
271 calves are transitioning from lying to standing more frequently, potentially in an attempt to
272 escape the pain or discomfort caused by initial ischemia from the constriction of the band.
273 Schumacher et al. (2026) reported that banded calves stood for shorter periods of time (decreased

stand bout duration) within 7-days post-castration compared to control calves, which could also indicate restlessness caused by acute pain or discomfort.

Bout frequency and bout duration are two different outcomes that are commonly analyzed from accelerometer data, and both can indicate similar biological meaning. An increase in bout frequency corresponds to an increased frequency in transitioning between lying and standing, while a decrease in bout duration corresponds to a decreased time between transitions; both of these outcomes could indicate restlessness. Similarly, an increase in lying bout frequency has similar implications to a decrease in standing bout frequency. Reaching statistical significance in one outcome, such as lying bout frequency, but not in its counterpart (standing bout frequency), could still be a biologically meaningful finding.

Neither Meléndez et al. (2018) or Marti et al. (2017) were able to detect significant differences in lying or standing behavior in 1-week old band-castrated calves when compared to control calves, which were the youngest calves presented in this dataset. From the dataset presented here, 2-month old calves were the youngest in which significant treatment differences in lying and standing behaviors were detected after band castration (Schumacher et al., 2026). Marti et al. (2017) noted that a 28-day sampling period may not be long enough to detect differences in lying and standing behaviors in 1-week old calves due to a delayed onset of tissue granulation. Another potential explanation is that younger calves tend to have smaller scrotums than older calves, implying that the band could be less constrictive on smaller scrotums, therefore inflicting less acute pain or discomfort. Alternatively, younger calves may not express painful behaviors as overtly as older cattle, making it difficult to detect differences in lying and standing behavior.

Gellatly et al. (2021) and Rust et al. (2007) were the only studies evaluating lying and standing behaviors after band castration that did not use accelerometers to collect data. Gellatly et al. (2021) observed calves through video recordings for a total of 16 hours, recorded between 0 and 59 hours post-castration in 2-hour intervals, which may have not been enough time to detect significant differences in lying and standing behavior. Rust et al. (2007) evaluated calf lying behavior for an unspecified period of time, twice daily for 14 days post-castration through live observations, which was also unsuccessful in detecting significant differences in lying behavior between castrates and controls. Accelerometers may be the best option for collecting lying and standing behavior after band castration because it allows for continuous data collection, which could more accurately reflect the animals' true behavior.

Overall, band-castrated calves that received no pain management showed an increased bout frequency on the day of castration compared to non-castrated control calves (Ball et al., 2018; Roberts et al., 2018), which is an indication of restlessness, potentially due to pain or discomfort caused by ischemia. In the weeks following band castration, an increase in standing time was observed in castrated calves compared to control calves (Marti et al., 2017; Ball et al., 2018; Garcia et al., 2025), which potentially correlates to the period when the band begins to pierce the scrotum, causing pain or discomfort. Studies that were able to detect significant differences in lying or standing behavior after band castration used calves that were at least 2 months of age, but often much older (Marti et al., 2017; Ball et al., 2018; Roberts et al., 2018; Daniel et al., 2020; Garcia et al., 2025; Schumacher et al., 2026). Younger band-castrated calves (< 2 months of age) did not exhibit statistically different lying and standing behaviors compared to control calves (Marti et al., 2017; D. M. Meléndez et al., 2018), potentially due to the band having less constriction on the younger calves' smaller scrotum, which could limit the

experienced acute pain and subsequent acute behavioral pain response. Lastly, when evaluating calves less than 2 months of age, it may be beneficial to collect lying and standing behaviors through the use of accelerometers for 6 to 7 weeks post-castration, as younger calves tend to have a delayed onset of granulation tissue at the castration site, which could produce changes in lying and standing behavior (Marti et al., 2017).

Conclusions

Review of the relevant literature suggests that evaluating lying and standing behavior in calves after surgical or band castration can be reliable indicators of pain or discomfort. Calves less than two months of age at the time of castration may not overtly change their lying and standing behaviors after castration, which could explain the lack of significant differences in this population. In studies that evaluated cattle greater than two months of age at the time of castration, results indicated that standing time, standing bouts, and lying bouts can be useful outcomes for pain detection. For surgical castration, changes in lying and standing occurred within four days post-castration. Conversely, band castration caused changes in lying and standing weeks post-castration, which may correspond to the period when the band begins to pierce the tissue. After both surgical and band castration, studies that used accelerometers were the most successful in detecting statistically significant differences in standing and lying behavior between castrated calves and non-castrated control calves. Overall, this review found lying and standing outcomes to be useful indicators of pain or discomfort in cattle two months of age or greater, and trends in these outcomes were identified after surgical and band castration.

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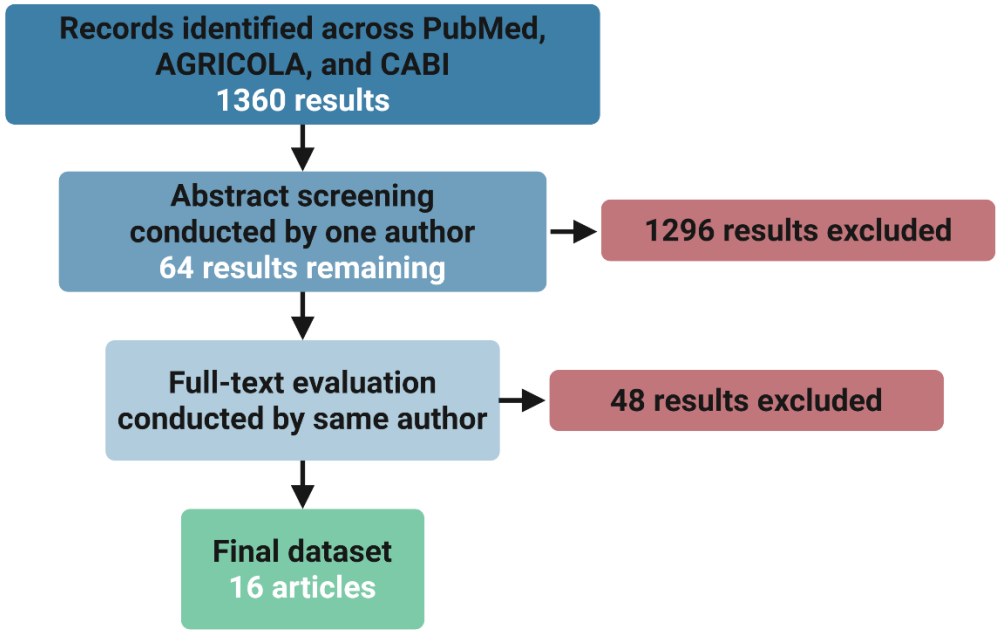


Figure 1.1 Flow chart depicting search results and screening methods used to identify relevant articles for this systematic review.

Table 1.1 Descriptive table of relevant articles that evaluated lying and standing behavior in cattle after surgical castration. Relevant articles included original research articles that evaluated lying and standing behavior of cattle after surgical or band castration, using experimental treatments that included a group that was castrated with no pain management, and a group that was not castrated (sham) to serve as a comparator.

Reference	Study Population	Sample Size	Data Collection Method	Treatment Effect Detected[1]
Surgical castration				
(D. M. Meléndez et al., 2018)	1 week Beef	n=12	Accelerometer	No
(Marti et al., 2019)	2 months Beef	n=11*	Accelerometer	No
(Gellatly et al., 2021)	2 months Beef	n=2*	Video recorded observation	No
(Meléndez et al., 2017)	1 week Beef	n=11*	Accelerometer	No
(Meléndez et al., 2017)	2 months Beef	n=10*	Accelerometer	Yes
(Meléndez et al., 2017)	4 months Beef	n=12	Accelerometer	Yes
(Sutherland et al., 2013)	3 months Holstein	n=10	Live observation	No
(Roberts et al., 2018)	6 months Beef	n=6	Accelerometer	Yes
(Rust et al., 2007)	790 lb Beef	n=10	Live observation	No
(Daniela M. Meléndez et al., 2018a)	2 months Beef	n=11*	Accelerometer	No
(Cho et al., 2024)	9 months Beef	n=6*	Video recorded observation	Yes

(Van der Saag et al., 2018)	2-4 months Beef	n=10	Video recorded observation	No
(White et al., 2008)	612 lb Beef	n=6	Accelerometer	Yes[2]

[1]Significant treatment effect or treatment × time interaction detected ($P < 0.05$) in a lying or standing outcome between surgically castrated calves with no pain management and non-castrated control calves.

[2]Standing outcome was significantly ($P < 0.05$) different pre-castration compared to post-castration in surgically castrated calves, but was not significantly ($P > 0.05$) different than non-castrated control calves.

*If sample size differed between surgically castrated groups with no pain management and non-castrated control groups, the smallest sample size was reported.

Table 1.2 Descriptive table of relevant articles that evaluated lying and standing behavior in cattle after band castration. Relevant articles included original research articles that evaluated lying and standing behavior of cattle after surgical or band castration, using experimental treatments that included a group that was castrated with no pain management, and a group that was not castrated (sham) to serve as a comparator.

Reference	Study Population	Sample Size	Data Collection Method	Treatment Effect Detected[1]
Band castration				
(D. M. Meléndez et al., 2018)	1 week Beef	n=12	Accelerometer	No
(Gellatly et al., 2021)	2 months Beef	n=4	Video recorded observation	No
(Marti et al., 2017)	1 week Beef	n=11	Accelerometer	No
(Marti et al., 2017)	2 months Beef	n=10*	Accelerometer	No
(Marti et al., 2017)	4 months Beef	n=11	Accelerometer	Yes
(Roberts et al., 2018)	6 months Beef	n=6	Accelerometer	Yes
(Rust et al., 2007)	790 lb Beef	n=10	Live observation	No
(Daniel et al., 2020)	3 months Beef	n=6*	Accelerometer	Yes
(Garcia et al., 2025)	8 months Beef	n=16	Accelerometer	Yes
(Schumacher et al., 2026)	2 months Beef-dairy	n=4	Accelerometer	Yes
(Ball et al., 2018)	741 lb Beef	n=18	Accelerometer	Yes

[1]Significant treatment effect or treatment × time interaction detected ($P < 0.05$) in a lying or standing outcome between surgically castrated calves with no pain management and non-castrated control calves.

*If sample size differed between band-castrated groups with no pain management and non-castrated control groups, the smallest sample size was reported.

Chapter 2 - Evaluation of a novel method of castration for calves: A pilot study

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Abstract

Physical castration of male calves is a standard management practice that causes pain. ClipFitter is a novel castration clip that combines the attributes of Burdizzo and band castration, which could reduce failures and improve welfare, respectively. This randomized, blinded, controlled pilot study aimed to determine the viability of the ClipFitter in cattle and its effects on pain and welfare. Twelve intact male beef-dairy cross calves weighing 184 ± 1.8 lb (83.8 ± 0.8 kg) were assigned to 1 of 3 treatment groups: castration with ClipFitter (CLIP; n=4), castration with rubber ring band (BAND; n=4), or no castration (SHAM; n=4). Calves' plasma cortisol, Substance P, lie/stand behavior, and infrared thermography were assessed at multiple timepoints. Infrared thermography images of the scrotum showed a treatment by time interaction, with calves in the CLIP group exhibiting significantly lower scrotal temperature than calves in the SHAM group at 7 d post-castration ($P < 0.05$). Lying and standing outcomes were not significantly different between CLIP and BAND groups ($P > 0.05$). There were no significant differences in plasma cortisol or Substance P concentrations between treatments ($P > 0.05$). The decreased scrotal temperatures observed in the CLIP group at d 7 suggests a decrease in blood flow to the testicles, therefore implying that the ClipFitter device could potentially be a viable

method of castration for cattle. The welfare implications of the ClipFitter device in cattle require further evaluation.

Key Words

castration; pain; welfare; banding; cattle

Introduction

Castration in calves is routinely performed to eliminate unwanted breeding and improve carcass quality.[1] Physical castration is a painful experience regardless of the method.[2] Non-surgical (bloodless) castration methods, such as the Burdizzo or banding with a rubber ring (band), are commonly performed without pain management in calves in the United States.[3] Burdizzo castration is performed by crushing the spermatic cords laterally across the neck of the scrotum with a metal clamp (Burdizzo), just proximal to the testicles. Burdizzo castration results in atrophy of the testicles; however, the scrotum remains intact. Band castration is performed by placing the band in an applicator that allows the band to be placed on the neck of the scrotum, proximal to the testicles. This promotes ischemia to the testicles and distal scrotum, which will typically slough off several weeks following castration.

Band castration is appealing to producers and practitioners because of its effectiveness and ease of application but has been shown to cause pain both acutely and chronically, up to 8 weeks post-castration in calves.[4,5] Previous work has demonstrated band castration to induce a pain response to scrotal palpation within only 3 hours post-castration highlighting the welfare concerns of band castration on acute pain.[5] Approximately 3 weeks post-castration, the band begins to lacerate the scrotum, exposing granulating tissue that appears to induce behavioral changes associated with chronic pain.[6] Therefore, this method potentially causes the most overall pain when compared to other commonly used castration methods.[7–9]

Burdizzo castration has demonstrated welfare benefits in calves by reducing acute and chronic pain when compared to surgical or band castration methods, respectively.[10,11] The Burdizzo method is thought to be effective at reducing chronic pain because of its ability to crush the spermatic nerves, which desensitizes the scrotum distal to the crush location. However, the Burdizzo method carries a high risk of failure, likely due to improper use, which can result in complications that significantly decrease welfare.[4,12] An alternative clamp castration method that is highly effective, unlike the Burdizzo, could potentially improve the welfare of calves during castration.

The ClipFitter[a] is a novel castration device that combines the benefits of the two castration methods, Burdizzo and rubber band. The device consists of a disposable plastic clamp that crushes the nerves and spermatic cords similar to Burdizzo castration. However, like band castration, the ClipFitter remains clipped to the neck of the scrotum until it sloughs off, confirming the castration. Thus, the ClipFitter combines Burdizzo and rubber band castration. The ClipFitter aims to provide the welfare benefits that is observed with the Burdizzo, without the high risk of castration failure or health complications. Nevertheless, the product was designed for castration and tail docking of lambs and has yet to be tested in calf castration. A larger ClipFitter prototype was designed to test this novel castration method in calves. Therefore, the present study aimed to compare castration of beef-dairy cross calves from 8-10 weeks of age using ClipFitter versus a standard rubber band and their effects on lie/stand behaviors, pain biomarkers (substance P (SP) and cortisol), and ocular and scrotal infrared thermography (IRT) outcomes. We hypothesized that the ClipFitter device would reduce the overall pain associated with castration when compared to castration with a standard band in calves.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee at Midwest Veterinary Services (IACUC #23018; December 19th 2023) with concurrence approval from the Kansas State University Institutional Animal Care and Use Committee (IACUC #4979).

Animals and Treatments

This prospective clinical pilot study was conducted at Midwest Veterinary Services in Nebraska in February of 2024. Twelve intact male beef-dairy cross calves weighing 184 ± 1.8 lb (83.8±0.8 kg) and 8-10 weeks of age were enrolled. Calves were sourced from a farm in northern Iowa in December 2023 and were housed on site and given unique identifiers in both ears prior to study commencement. The calves arrived at the study facility weeks prior to the commencement of the study. The calves were then moved to an identical room within the facility 2 days prior to castration for acclimation. Calves were group housed indoors in a climate-controlled BSL-2 facility with a grooved concrete floor spanning 15 m x 8 m that was cleaned of organic matter daily. The floor contained a small drainage pit that was covered by a metal grate, which collected the organic matter. Calves were fed a customized 18% crude protein calf starter diet exceeding the minimum daily nutrient requirements for their weight and given fresh water *ad libitum*.[14]

Calves were randomly allocated to 1 of 3 treatment groups; castration with ClipFitter (CLIP; n=4), castration with standard rubber ring band (BAND; n=4), and no castration (SHAM; n=4). Random assignment was performed by an unblinded individual that was not involved in data collection or analysis using the "rand" function in Microsoft Excel[b]. Study personnel remained blinded to treatments throughout data collection and analysis. Calves were restrained in a hydraulic squeeze chute for treatment administration and all treatments were administered by the same

trained individual. Neither local anesthetics or systemic analgesics were given to any treatment group, as it is routine practice in the United States to perform castration without pain management.[3] Calves in the CLIP group were castrated by applying the ClipFitter laterally to the entire length of the neck of the scrotum using a proprietary applicator, ensuring that the band was placed above both testicles. Application of the ClipFitter took approximately 20 seconds, similar to band application. Calves in the BAND group were castrated by applying a standard castration rubber band to the neck of the scrotum using a commercially available bander,[c] ensuring that the band was placed above testicles. The SHAM group calves were manipulated similarly to the other treatment groups (herded through the chute and testicles were manipulated for 20 seconds); however, calves were not castrated. The time of treatment administration was marked as timepoint 0.

Blood Biomarkers

Blood sample collections occurred 24 h before castration (baseline), and at timepoint 0.5 h, 2 h, 8 h, 24 h, and 168 h post-castration. While calves were restrained in the squeeze chute, using a 10 mL syringe[d] with an 18G × 1" needle,[e] approximately 10 mL of blood was collected from the jugular vein at each timepoint. Five milliliters of blood were immediately transferred to a lithium-heparin and the remaining 5 mL were transferred to an EDTA vacutainer blood tube[f] with benzamidine hydrochloride (1mM final concentration) for plasma cortisol and Substance P (SP) quantification, respectively. The blood tubes were inverted 5 times before placing on ice to ensure proper mixing with anticoagulant components within each tube. The samples were then centrifuged at 1,000× g for 15 minutes at a temperature of 4°C. Plasma was extracted from the centrifuged samples and transferred to cryovials, then stored at –80°C until the conclusion of the

study in Nebraska. Samples were then placed in a cooler with dry ice and transported to Kansas State University where they were stored at –80°C until analysis.

Methods for plasma cortisol determination are described elsewhere.[15] The intra-assay variation for plasma cortisol was calculated at 23.3%, while the inter-assay variation was calculated at 7.8%.

Methods for SP determination are described elsewhere.[6] The intra-assay variation for SP was calculated at 7.01%, while the inter-assay variation was calculated at 9.15%.

Lying and Standing Behavior

Three-axis accelerometers[g] were attached proximal to the right metatarsophalangeal joint with cohesive bandage[h] at -24h timepoint. Methods of accelerometer placement and fastening are described in previous work.[6] Accelerometer angle data were logged every 30 seconds for 24 hours per day during the entirety of the study. The angle data from the loggers were converted into binomial data (stand/lie) using a modified version of a published standard operating procedure by the University of British Columbia.[16] Logged activities included total lying and standing time, number of lying and standing bouts, and duration of lying and standing bouts. A bout was defined as at least one minute (>60 s) of continuously exhibiting the same behavior (either lying or standing). Accelerometers began recording and were placed on the calves' legs just prior to 10:00 a.m. on day -1. Therefore, analysis for activity monitoring was standardized to 12 hours each day, from 10:00 a.m. to 10 p.m., to account for the lack of accelerometer data collection for the full 24 hours on day -1.

Infrared Thermography

An infrared camera[i] was used to capture thermal images of the scrotum and medial canthus of the right eye at baseline (-24h) and 2h, 8h, 24h, and 168h post-castration. All infrared

613 thermography (IRT) images were captured while the calves were restrained in the squeeze chute
614 at a distance of 0.5 m. Images of the scrotum were taken from directly behind the calves, while
615 images of the medial canthus of the right eye were taken at a 90-degree angle from the calves'
616 head. The camera automatically adjusted for changes in ambient temperature and relative
617 humidity. Images were taken within a shaded facility where sun light was not present and therefore
618 did not impact temperature recordings. Images were analyzed and temperatures (minimum,
619 average and maximum) were calculated using research-grade software.[j]

620 ***Statistical Analysis***

621 All statistical analyses were performed with RStudio.[k] Regarding plasma cortisol, the 2 h
622 timepoint was excluded from analysis due to 4 calves testing below the limit of quantification of
623 0.1 ng/mL (3 of these calves were in the SHAM group and 1 calf was in BAND group). All data
624 were evaluated for normality using the Shapiro-Wilk test and a histogram distribution using
625 "shapiro.test" and "histogram" functions, respectively. Linear mixed-effects models were used
626 ('lmer' function of 'lme4' package of R) to test for potential differences in SP, plasma cortisol,
627 activity monitoring, and IRT outcomes. Fixed effects included treatment, time and their
628 interaction, while Calf ID was included as a random effect to account for repeated measures.
629 Residuals were evaluated for normality using "qqnorm", "qqline", and "histogram" functions of
630 R. The comparisons between treatments and time were tested using "emmeans" function (with
631 "emmeans" package) of R. Results were expressed as least-square means and standard error of the
632 mean (SEM) with sidak adjustments to control the family-wise error rate. The significance level
633 for means comparisons was set at $P \leq 0.05$.

Results

Blood Biomarkers

Regardless of treatment group, the highest plasma cortisol concentration was detected at 0.5 h post-castration before decreasing near baseline concentrations 8 h post-castration (timepoint $P = 0.04$). Plasma cortisol did not exhibit a significant treatment × timepoint interaction ($P > 0.05$), and no treatment effect was observed ($P > 0.05$). Substance P concentrations were found to have no significant differences by treatment ($P = 0.39$), timepoint ($P = 0.75$), or its interaction ($P = 0.66$; Table 2.1).

Lying and Standing Behavior

There was an effect of timepoint on average stand bout time, with calves across all treatment groups spending an increasing number of minutes in a stand bout over time ($P < 0.01$; Table 2.2). Compared to baseline, average stand bout time in minutes was statistically higher on days 2, 4, and 5, regardless of treatment. There was a treatment effect for average stand bout time, with BAND calves spending less time standing before switching to a lying position compared to SHAM calves (22.5 min/bout and 27.7 min/bout, SEM = 1.42, respectively; $P = 0.01$; Figure 2.1). Additionally, a treatment effect was found for average lie-bout time, where CLIP calves spent less time lying before switching to a standing position compared to SHAM calves (30.8 min/bout and 40.4 min/bout, SEM = 2.35, respectively; $P < 0.01$; Figure 2.2). Regardless of treatment, calves spent more time in a lie bout on days 3 and 5 post-castration compared to day 0 pre-castration ($P < 0.01$). The average number of lie bouts steadily decreased over time ($P < 0.01$), with calves across all treatment groups performing statistically fewer lie bouts by day 5 compared to baseline (12.1 bouts/day and 16.6 bouts/day, SEM = 1.5, respectively; $P < 0.01$).

Infrared Thermography

A timepoint effect was found to be significant for ocular IRT outcomes ($P = 0.04$; Table 2.3). The minimum temperature of the medial canthus of the eye increased overtime, with the highest temperature at baseline and decreasing temperature at 2 h following castration. When compared to baseline, minimum medial canthus temperatures remained lower at all post-castration timepoints ($P < 0.05$) except for 8 h timepoint in which a small rebound in temperature was observed ($P = 0.09$). Treatment or treatment × timepoint interaction did not have a significant effect on any ocular IRT outcomes ($P > 0.05$). Regarding scrotal IRT outcomes, a significant treatment × timepoint interaction was observed for maximum ($P = 0.03$) and average ($P = 0.04$) scrotal temperature, with CLIP calves exhibiting lower temperatures than SHAM calves at 168 h post-castration (Figure 2.3).

Discussion

Band and Burdizzo castration are commonly used castration methods in cattle. Both methods have appealing characteristics, as band castration is easy to perform and highly effective, while Burdizzo castration has shown to reduce chronic pain when compared to band castration. However, both methods also have adverse effects; band castration creates both acute and chronic pain, and Burdizzo castration is often performed incorrectly which can lead to health complications, ultimately resulting in decreasing welfare of calves. The present study evaluated the use of the ClipFitter, a novel castration clip that combines the attributes of Burdizzo and band castration, versus a standard castration rubber band and a sham group. The aim of the ClipFitter device is to provide a fast, effective, and practical method of castration while potentially improving calf welfare. This study assessed calf pain and welfare through blood biomarkers (plasma cortisol and SP) and behavior (lying and standing activity), while the viability of the ClipFitter was

680 determined through infrared thermography of the scrotum over a 7-day post-castration sampling
681 period.

682 In this study, plasma cortisol was at its greatest concentration at 30 minutes following
683 castration before decreasing over time, however cortisol values were not different between
684 treatment groups. This is unlike a previous study that found plasma cortisol concentrations of
685 Burdizzo-castrated and band-castrated calves to be higher than handled calves within 30 min post-
686 castration.[10] Additionally, Burdizzo castration has been shown to significantly increase plasma
687 cortisol compared to band castration within 30 min post-castration.[9] However, findings presented
688 in this study are in agreement with Melendez et al., in which the authors found no significant
689 cortisol differences between band-castrated and sham calves.[7] Regardless of treatment group,
690 previous work has found cortisol to peak 30 minutes following surgical and sham castration in
691 calves.[17] This implies that, despite not sampling in series, we may have captured peak cortisol
692 concentrations at 0.5 h post-castration.

693 While data regarding the ClipFitter are scarce, a previous study evaluated the concurrent
694 use of a clamp (Burdizzo) and band to castrate lambs and its effect on plasma cortisol.[18] The
695 authors were not able to detect differences in cortisol concentrations on the day of castration
696 between band-castrated lambs and those castrated with both a clamp and band, which is analogous
697 to the findings in this study.

698 Regarding SP, concentrations did not differ between treatment groups, timepoint or their
699 interaction. Lack of SP differences are in accordance with a previous study that found no treatment,
700 timepoint, or treatment × timepoint effects on SP concentrations between band-castrated and non-
701 castrated calves when followed for 7 days post-castration.[7] The results from the present study are
702 also consistent with another study that found no differences in SP concentrations between band-

castrated and non-castrated calves 1 day after castration.[19] A high association between elevated SP concentrations and wound healing has been previously reported, implying that SP may be a better measure of inflammation and wound healing rather than pain.[20,21] Since non-surgical castration does not create wound granulation from the ClipFitter or band lacerating the skin within 7 days post-procedure, this could potentially explain lack of differences in SP concentrations in the present study.

The average duration of stand-bouts was shown to be lower in BAND calves compared to SHAM calves, while the average duration of lie-bouts was shown to be lower in CLIP calves compared to SHAM calves. However, no lying or standing outcomes were significantly different between CLIP and BAND calves. These activity monitoring results suggest that both rubber band and ClipFitter castration cause pain and discomfort. When compared to CLIP and SHAM, BAND calves spent less time standing before changing to a lying position, suggesting that band-castrated calves were not able to tolerate long periods of standing before changing positions. In contrast, CLIP calves spent less time lying before changing to a standing position. This could possibly be explained by the lateral application of the ClipFitter across the neck of the scrotum. The excess plastic on either side of the scrotum may rub on the calves' legs or pull on their scrotums while lying, causing discomfort. No significant differences between BAND and SHAM calves were observed for total lie/stand time or total number of lie/stand bouts. Melendez et al. also found no significant differences ($P > 0.05$) in total lie/stand time or total number of lie/stand bouts between band-castrated calves and non-castrated calves that were 2 months of age.[7]

Regarding ocular IRT of the medial canthus, only a time effect was observed for minimum eye temperature, with temperatures decreasing compared to baseline at 2h post-castration. Painful experiences such as castration are associated with lower ocular temperatures, which can be

attributed to epinephrine release causing changes in sympathetic tone.[22] A previous study found maximum eye temperature to drop quickly (< 5 minutes) after calves were disbudded without local anesthetics, then eye temperature raised above baseline temperatures 5 minutes post-disbudding.[23] This finding highlights the small temporal window required to effectively observe a decrease in eye temperature after a painful experience. Since our first IRT collection was 2 h post-castration, this potentially explains the lack of treatment differences at the medial canthus. For scrotal IRT, a treatment by time interaction was observed for maximum and average scrotum temperature. Both outcomes followed the same pattern, with CLIP calves exhibiting statistically lower scrotum temperatures than SHAM calves at 168h post-castration. However, CLIP and BAND calves did not present differences from one another at this timepoint. Previous work has found band castration to decrease scrotal temperature by 168h post-castration, which is suggestive of blood restriction to the testicles.[24] In the present study, the decreased scrotal temperature observed in CLIP calves at 168 h post-castration suggests that the ClipFitter adequately restricts blood flow to the testicles that is required for castration.

Despite previous researchers' evaluation on the effect of the ClipFitter during lamb castration, to the authors knowledge this is the first study to evaluate the effects of ClipFitter for calf castration.[13] The use of the ClipFitter to castrate young lambs (<1 week of age) did not significantly affect behaviors associated with acute pain compared to non-castrated lambs, as assessed by a Qualitative Behavioral Assessment (QBA). However, when the ClipFitter was evaluated in older lambs (approximately two months of age), they found those castrated with the ClipFitter performed significantly a greater number of negative valanced behaviors indicating acute pain compared to uncastrated. In the present study, calves were castrated approximately at 2 months of age, and lie/stand behavior results suggest that both ClipFitter and rubber band modified

stand- and lie-bout times. Those changes could be associated with pain or discomfort post castration, indicating that both methods alter their normal behaviors. The use of the ClipFitter for castration in younger calves (< 1 week of age) may help to mitigate pain associated with castration, as shown by Dwyer et al. in lambs.[13]

Limitations

Limitations of this study include small sample size, lack of diversity in assessed behaviors, and a small issue regarding the ClipFitter prototype. A small sample size may have hindered our ability to detect differences in physiological measures associated with pain. Assessing these methods on a larger scale may allow us to find significant differences in welfare outcomes between the two methods. Additionally, the lack of behavioral outcomes limited our ability to detect pain following castration. While we did collect lie/stand data, collection of other behavioral outcomes such as abnormal posturing, foot stamping, or licking castration site could have been useful in quantifying pain or discomfort post-castration. Finally, the ClipFitter prototype was slightly too long, as excess plastic on either side of the scrotum may have caused discomfort to the calves' legs as they move, which may have affected the lie/stand outcomes. A smaller ClipFitter size can be developed and tested to confirm this theory.

Conclusions

This study was unable to detect significant differences in plasma cortisol, SP, activity monitoring, and IRT between CLIP and BAND calves within 7 days post-castration. While the viability of the ClipFitter for castration of calves was demonstrated to be promising, we were unable to find differences in pain measures that could correlate to a negative state of welfare. Activity monitoring outcomes suggest that both ClipFitter and rubber band castration cause acute discomfort or pain, as determined by differences in the average duration of lying and standing

bouts. Scrotal IRT outcomes suggest that the ClipFitter reduces blood flow to the scrotum at a level indistinguishable from band castration, implying that the ClipFitter could be potentially viable for calf castration. Overall, the castration effectiveness of the ClipFitter and its implications on chronic pain and animal welfare requires further research that follows calves for a longer period post-castration.

Regarding future studies, evaluating the welfare implications and viability of the ClipFitter until the scrotum sloughs off may provide further insights on potential chronic differences between the ClipFitter and a standard castration band. Additionally, a larger sample size may be useful to detect significant differences, particularly for blood biomarkers, which could be obtained using a power calculation based off data from the present study. Lastly, incorporating additional behavioral outcomes in future studies such as discomfort behaviors (foot stamping, tail swishing, abnormal posturing, etc.) may help determine potential behavioral differences exhibited between the ClipFitter and a standard band.

Endnotes

[a] Eadie Bros & Co Ltd, Selkirk, Scotland

[b] Microsoft Excel, Microsoft Corp., Redmond, WA

[c] XL Bander, S&J Tools, Inc., O'Fallen, MO

[d] Fisherbrand Cat. No. 14955459, Thermo Fisher Scientific, Waltham, MA

[e] Becton, Dickinson and Company, Franklin Lakes, NJ

[f] Vacuette, Greiner Bio-One, Kremsmunster, Austria

[g] HOBO Pendant® G, Onset Corp., Bourne, MA

[h] AmerisourceBergen, Conshohocken, PA, USA

[i] Fluke TIX580, Fluke Corp., Everett, WA

[j] SmartView 4.3, Fluke Corp., Everett, WA

[k] RStudio, Version 2022.02.3, Boston, MA, USA

Disclosures

The authors declare no conflicts of interest. No AI-assisted technologies were used in the generation of this manuscript.

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Table 2.1 Least square means and standard error of blood biomarker outcome variables from day -1 (baseline) to day 7 relative to castration in beef-on-dairy calves that were castrated with a ClipFitter (CLIP; n = 4), standard band (BAND; n = 4) or not castrated (SHAM; n = 4).

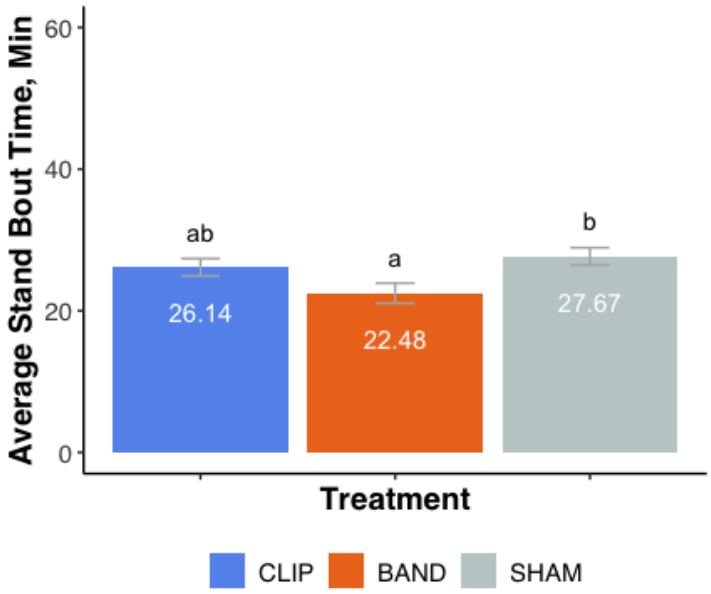
	Treatment				*P*-value		
Variable	CLIP	BAND	SHAM	SEM[1]	Treatment	Time	Treatment x Time
Cortisol, pg/mL	7.97	4.47	7.41	2.67	0.83	0.04	0.79
Substance P, pg/mL	383	432	568	123	0.39	0.75	0.66

[1] Standard error of the mean

Table 2.2 Least square means and standard error of activity monitoring outcome variables obtained via accelerometers on right hind legs that collected data every 30 seconds from day -1 (baseline) to day 7 relative to castration in beef-on-dairy calves that were castrated with a ClipFitter (CLIP; n = 4), standard band (BAND; n = 4) or not castrated (SHAM; n = 4). [a-b] LS-means differ between treatments ($P < 0.05$).

	Treatment				*P*-value		
Variable	**CLIP**	**BAND**	**SHAM**	**SEM[1]**	**Treatment**	**Time**	**Treatment x Time**
Total Lie Time, min	423	471	471	26.1	0.74	0.24	0.88
Total Stand Time, min	292	245	248	26.2	0.75	0.19	0.86
Avg Stand Bouts, n	12.7	13.1	9.5	1.72	0.15	0.10	0.32
Avg Lie Bouts, n	16.4	15.3	12.5	2.51	0.42	<0.01	0.40
Avg Stand Bout Time, min	26.1[ab]	22.5[a]	27.7[b]	1.42	0.01	<0.01	0.56
Avg Lie Bout Time, min	30.8[a]	36[ab]	40.4[b]	2.35	<0.01	<0.01	0.80

[1] Standard error of the mean



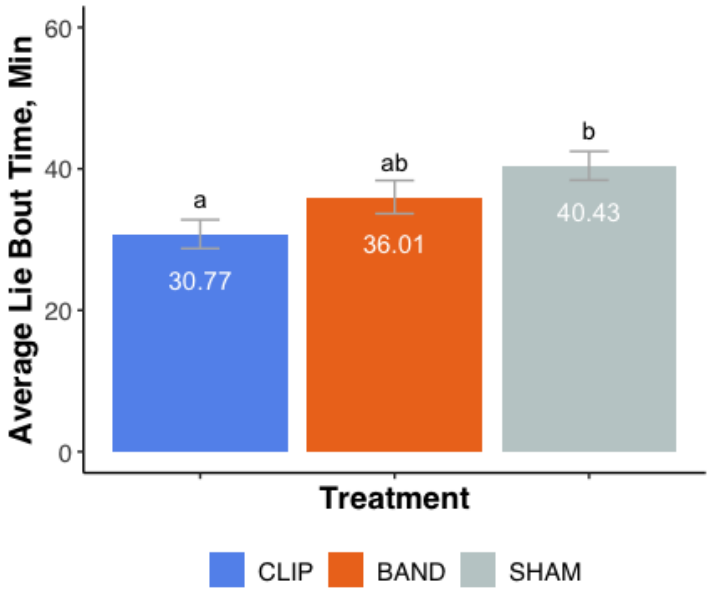
878

879 **Figure 2.1** Average stand bout time in minutes, of beef-on-dairy calves castrated with ClipFitter
880 (CLIP; n = 4), standard band (BAND; n = 4), or not castrated (SHAM; n = 4). a-b LS-means
881 differ (P < 0.05).

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886 **Figure 2.2** Average lie bout time in minutes, of beef-on-dairy calves castrated with ClipFitter
887 (CLIP; n = 4), standard band (BAND; n = 4), or not castrated (SHAM; n = 4). a-b LS-means
888 differ ($P < 0.05$).

889

Table 2.3 Least square means and standard error, in °F (parentheses indicate °C) of infrared thermography (IRT) outcome variables obtained at timepoints -24h (baseline), 2 h, 8 h, 24 h, and 168 h relative to castration in beef-on-dairy calves that were castrated with a ClipFitter (CLIP; n = 4), standard band (BAND; n = 4) or not castrated (SHAM; n = 4).

	Treatment				*P*-value		
Variable	CLIP	BAND	SHAM	SEM[1]	Treatment	Time	Treatment x Time
Ocular Max. IRT, °F (°C)	102.3 (39.1)	102.2 (39.0)	101.4 (38.6)	0.42 (0.23)	0.13	0.29	0.51
Ocular Avg. IRT, °F (°C)	98.9 (37.2)	98.6 (37.0)	98.4 (36.9)	0.44 (0.24)	0.86	0.10	0.95
Ocular Min. IRT, °F (°C)	92.4 (33.6)	91.9 (33.3)	92.8 (33.8)	1.13 (0.62)	0.53	0.04	0.95
Scrotum Max. IRT, °F (°C)	93.3 (34.1)	93.9 (34.4)	97.5 (36.4)	0.85 (0.47)	0.89	<0.01	0.03
Scrotum Avg. IRT, °F (°C)	88.1 (31.2)	89.4 (31.9)	92.3 (33.5)	0.84 (0.46)	0.81	<0.01	0.04
Scrotum Min. IRT, °F (°C)	83.1 (28.4)	84.5 (29.2)	86.0 (30.0)	0.98 (0.54)	0.79	<0.01	0.42

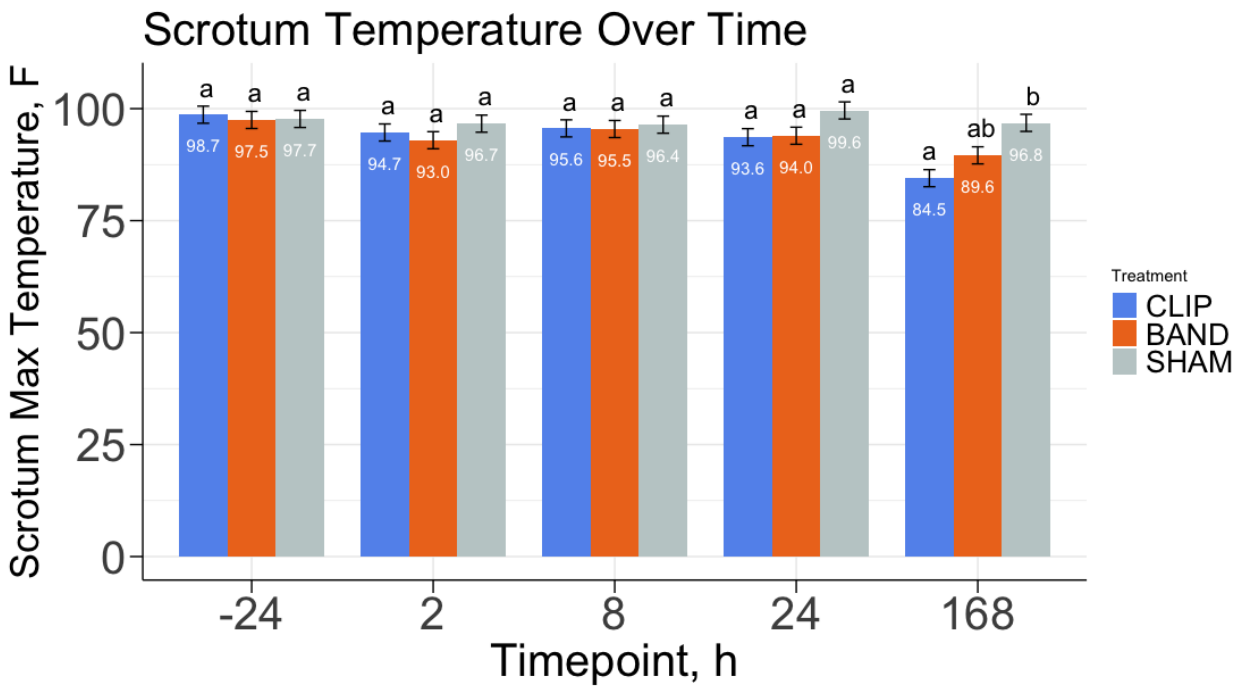


Figure 2.3 Scrotum maximum temperature in °F, measured by infrared thermography, of beef-on-dairy calves castrated with ClipFitter (CLIP; n=4), standard band (BAND; n= 4), or not castrated (SHAM; n = 4). Timepoint represents hour relative to castration. a-b-c LS-means differ ($P < 0.05$).

Chapter 3 - Evaluating the efficacy of maternal bovine appeasing substance on pain outcomes in calves after cautery disbudding and surgical castration

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Simple Summary

Castration and disbudding are common management procedures that cause pain in cattle. The lack of approved drugs for pain relief in cattle presents a challenge in providing adequate pain mitigation during these procedures. In this study, the application of maternal bovine appeasing substance (MBAS), a naturally produced pheromone in cattle, was administered to calves alongside a local anesthetic, with or without a systemic analgesic, and its effects on pain outcomes after castration and disbudding were evaluated. Pain was measured through blood biomarkers of pain and stress, pressure sensitivity tests, infrared imaging of the procedure sites,

behavior observations, and gait changes. Calves that received MBAS and lidocaine before castration and disbudding showed no differences in any pain measure compared to calves that received only lidocaine. These findings suggest that adding MBAS may not enhance pain relief when used with lidocaine during castration and disbudding.

Abstract

Castration and disbudding are routine procedures that cause pain and distress in cattle. Maternal Bovine Appeasing Substance (MBAS) is naturally secreted and thought to alleviate stress in cattle. This randomized blinded controlled trial aimed to determine if a single topical synthetic MBAS analog dose (FerAppease®, FERA Diagnostics & Biologicals, College Station, TX), alone, or in combination with local anesthesia, with or without systemic analgesia would provide extended pain and stress relief to calves after disbudding and surgical castration. Forty-nine unprocessed Holstein bull calves weighing 46.6 ± 7.2 kg were randomly assigned to one of the following treatment groups: lidocaine local anesthesia (LIDO; n=9), lidocaine and meloxicam (LIDO-MEL; n=10), lidocaine and MBAS (LIDO-MBAS; n=10), lidocaine meloxicam and MBAS (COMBO; n=9), sham procedures (SHAM; n=6); and no analgesia (CONTROL; n=5). The calves' substance P, plasma cortisol, mechanical nociception threshold (MNT), infrared thermography, performance, lie and stand activity, behavior, and gait were evaluated at multiple timepoints. Linear mixed models were employed to identify potential associations between outcomes and treatment, post-procedural timing, and their interactions. No significant differences in infrared thermography, performance, lie and stand activity, and behavior outcomes were observed between treatments (P>0.05). Substance P presented a treatment × time interaction, with the CONTROL group exhibiting lower SP concentrations compared to the COMBO group at several post-procedure timepoints (≤192 vs. ≥298 pg/mL,

respectively; SE=31.9; $P<0.01$). A treatment effect was found for plasma cortisol, with the CONTROL group presenting elevated cortisol concentrations ($P<0.01$). A treatment × time interaction was observed for horn bud MNT, with the SHAM group requiring greater exerted pressure to withdraw from the algometer at 72 h compared to all other treatment groups (0.33 vs. ≤ -0.39 KgF, respectively; SE=0.23, $P<0.01$). There was a significant treatment × time interaction on multiple gait outcomes ($P<0.05$). Differences in gait were noted only in CONTROL calves; local and systemic analgesics and MBAS did not differ. These results suggest that administering MBAS in addition to lidocaine, with or without meloxicam, is neither more or less effective at pain mitigation than lidocaine alone in Holstein calves undergoing surgical castration and cautery disbudding.

Key Words

appeasing substance, castration, dehorning, disbudding, pain

List of Abbreviations

FDA, Food and Drug Administration
MBAS, maternal bovine appeasing substance
ADG, average daily gain
GF, feed efficiency
SP, substance P
BW, body weight
FTI, foot-time integral
IRT, infrared thermography
MNT, mechanical nociception threshold

Introduction

Each year, over 15 million dairy and beef calves undergo painful processing procedures such as disbudding and castration in the United States (USDA, 2024), which are performed to prevent unwanted pregnancies in feedyards, reduce the risk of injury to other cattle (Knierim et

al., 2015) and enhance carcass quality (Field, 1971). All physical methods of castration and disbudding invariably cause pain and distress in cattle (Coetzee, 2011). The growing concern regarding the negative effects of practices decreasing animal welfare threatens agricultural sustainability and has contributed to the development of new guidelines that strongly recommend the use of a local anesthetic with a NSAID at time of disbudding and castration (AABP, 2024; AVMA, 2025). Nevertheless, a combination of a local anesthetic and NSAID still may not be sufficient for complete pain alleviation (Sutherland et al., 2013; Martin et al., 2022a). There is evidence that disbudding can cause discomfort for several weeks after the procedure, and NSAIDs commonly used in cattle, such as meloxicam, are thought to be effective for up to 3 days (Coetzee et al., 2009; Adcock and Tucker, 2018).

In view of the current pain mitigation challenges in cattle, adjunctive therapies may help alleviate the pain and distress associated with these procedures. Administering an appeasing pheromone to cattle may help achieve this, as the use of appeasing pheromones has shown to be efficacious in promoting a calming effect on dogs during stressful events (Landsberg et al., 2015; Santos et al., 2020). A pheromone is a chemical signal that allows communication between animals within the same species (Wyatt, 2010). Specifically, an appeasing pheromone is produced by the dam and is thought to have an appeasing effect on both young and adult animals (Pageat and Gaultier, 2003).

The Maternal Bovine Appeasing Substance (MBAS) (FerAppease®) is composed of a fatty acid mixture that aims to mimic the bovine maternal appeasing pheromone naturally secreted by the sebaceous glands of the mammary gland of cows (Cappellozza and Cooke, 2022), which is believed to induce a calming effect in cattle. The MBAS product is applied topically, where it is believed to be assimilated by the vomeronasal gland located in the mucosal

area of the nasal septum, producing central effects that desensitize regions of the brain to threat perception and stress (Cappellozza and Cooke, 2022). A single dose is thought to be effective for up 7 days, with some studies reporting efficacy using a 14-day dosing interval (Bringhenti et al., 2023; Pickett et al., 2024). Previous studies have evaluated the efficacy of MBAS to reduce physiological responses associated with stressful events such as weaning and feedyard entry, with MBAS-treated cattle showing improvements in BW and plasma cortisol compared to control animals (Colombo et al., 2020; Cooke et al., 2020; Vieira et al., 2023). Moreover, a recent study reported increased ADG in MBAS-treated calves 2 weeks after caustic disbudding (Spencer et al., 2025). A potential explanation for this finding is that MBAS alleviated stress associated with disbudding, thereby limiting the production of inflammatory mediators that may influence pain perception (Glaser and Kiecolt-Glaser, 2005; Kawabata, 2011). Therefore, MBAS may have the potential to serve as an adjunctive therapy with local anesthetics and systemic analgesics during painful procedures in cattle.

The objective of this study was to determine if the addition of a single topical dose of MBAS with a local anesthetic, or in combination with a systemic analgesic, would provide extended pain and distress relief to calves after cautery disbudding and surgical castration of 1-2 week old male Holstein calves. We hypothesized that animals treated with MBAS in addition to lidocaine prior to castration and disbudding will experience less stress and greater analgesia than animals treated only with lidocaine. Outcome measures were collected over a 28-day post-procedure period and aimed to assess both acute and chronic pain, which included physiological measures, gait analysis, performance, and animal behavior.

Materials and Methods

All animal care and procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University (IACUC #4844) in accordance with the Guide for Care and Use of Agricultural Animals in Research and Teaching.

Animals and Treatments

A total of 52 Holstein bull calves, 1-2 weeks of age and weighing 46.6 ± 7.2 kg were used in this study. The trial was divided into two cohorts; the first cohort of calves were sourced from a southwest Kansas calf ranch, and the second were sourced from local dairies. This prospective randomized controlled clinical trial took place at Kansas State University Sheep & Meat Goat Unit, September to December 2023. All calves arrived not castrated or disbudded. Upon arrival, all calves received a metaphylactic administration of tulathromycin (Arovyn, Merck Animal Health, Rahway, NJ), and a unique ear tag with their identification number. Additionally, calves received a prophylactic coccidiostat (CORID, Huvepharma, Peachtree City, GA) at a dose of 5 mg/kg administered orally once daily for 7 days. Health assessments were performed by a licensed veterinarian before study commencement, and all calves deemed not healthy were not enrolled in the study. Calves were housed individually in hutches bedded with 10 cm deep wheat straw, with a 1.5 m x 1.5 m fenced-in front yard. Hutches were spaced at least 1 m apart to limit physical contact between calves and were equipped with a water bucket, feed bucket, and a milk bottle holder. Between cohorts, hutches and buckets were cleaned of organic matter and disinfected with a diluted bleach solution. Calves were fed 1.9 liters (17% solids) of milk replacer (22% CP, Herd Maker®, Land O'Lakes, Arden Hills, MN) in accordance with manufacturer directions via milk bottles twice daily at 0630 and 1730 h. All milk bottles and nipples were cleaned and disinfected after each use. Calves were also provided with a

commercially available calf starter ration (89% DM, Calf Grower B-68, Mid-Kansas Cooperative, Manhattan, KS). The calf starter was provided once daily at 700g/45kg of body weight (BW) and was adjusted weekly with increase in BW. Water was provided *ad libitum*.

Calves in cohort 1 and 2 were allowed an acclimation period of 8 and 10 days, respectively, before they were submitted to surgical castration and hot iron disbudding. During the acclimation period, 3 calves were excluded from the study; 2 calves were excluded due to respiratory disease and 1 due to lameness. The remaining 49 calves were randomly assigned to one of the following treatment groups: local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (FerAppease®; LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); and no analgesia or anesthesia (CONTROL; n=5). Review of the relevant literature demonstrated that a sample size of 10 for treatment groups provided a statistical power of 0.80 for the critical outcome of force of the front limbs on gait analysis that will be assessed in this study assuming a sigma of 2.88% and true difference of 3.9% (Kleinhenz et al., 2018; Martin et al., 2022b). A sample size of 6 for the control groups (CONTROL and SHAM) provided a statistical power of 0.80 for the critical outcome of force of the front limbs on gait analysis that will be assessed in this study, assuming a sigma of 2.88% and true difference of 5.2%. Additionally, a small sample size of 6 for the CONTROL group limits the number of calves subjected to castration and disbudding with no analgesia. A rescue analgesic protocol consisting of flunixin meglumine (2.2 mg/kg) was utilized if deemed necessary by a licensed veterinarian. Random assignment was performed prior to enrollment by an unblinded researcher using the “RAND” function of Microsoft Excel (Microsoft Corp., Redmond, WA). Three calves did not present horn buds at the time of

treatment or were genetically polled, and therefore were systematically placed in the SHAM group by substituting calves with similar BW. All study personnel involved in husbandry, data collection, processing laboratory samples, and data analysis were blinded to treatment groups.

Castration, Disbudding, and Treatment Administration

All animals were gently restrained by unblinded individuals during treatment administration, castration, and disbudding. All treatments were administered 10 minutes prior to castration and disbudding by a licensed veterinarian. Apart from the SHAM and CONT groups, all treatment groups received a cornual nerve block using 5 mL of lidocaine/site. Prior to disbudding, a lidocaine injection (Lidocaine 2%) was given halfway between the lateral canthus of the eye and the horn just ventral to the frontal crest on each side of the head. In addition, 2 mL of the local anesthetic was administered subcutaneously as a ring block around each horn bud. Prior to castration, lidocaine was administered into each spermatic cord and infiltrated into the neck of each testicle. Five minutes prior to the procedure, the local anesthetic block was tested by pricking the skin with a hypodermic needle. No animals responded to the needle stick and therefore proceeded to castration and disbud procedures. The SHAM and CONT groups received injections of saline solution (0.9% Sodium Chloride) at the same sites to maintain blindness. The meloxicam (1mg/kg Meloxicam Tablets, USP 15 mg; Zydus Pharmaceuticals Inc. Pennington, NJ), for groups LIDO-MEL and COMBO, and placebo (Whey Protein) capsule, for groups LIDO, LIDO-MBAS, SHAM, and CONTROL, were administered orally using a bolus gun. The MBAS (FerAppease®, FERA Diagnostics & Biologicals, College Station, TX) or a placebo liquid (saline solution with similar food color dye) were administered topically, following manufacturer directions, to both the nuchal skin (2.5 ml) and the muzzle (2.5 ml) using a proprietary applicator at the time of lidocaine administration.

Regarding castration procedures, the scrotum was scrubbed with a disinfectant solution (Chlorhexidine 2%) before the base of the scrotum was incised with a single-use scalpel. The testicles were then removed using an emasculator in accordance with standard industry practices. For the SHAM group, testicles were scrubbed with a disinfectant solution and manipulated for 20 seconds.

Calves were disbudded using a propane dehorner (Express Inline Dehorner, The Coburn Company, Inc., Whitewater, WI) on the surrounding horn bud tissue for 20 seconds, with the buds being subsequently removed. For the SHAM group, a propane dehorner at ambient temperature was applied to the skin for 20 seconds.

Body Weights and Performance

Calves were weighed individually at arrival, and study day –1, 7, 14, 21, 28 (Figure 3.1). The scale was verified prior to each timepoint using multiple certified weights that, when combined, exceeded calf BW. Beginning at 0 kg and after every added weight, the scale was verified within a 2% margin of error. This procedure was repeated when removing the weights from the scale.

The calves' average daily gain (ADG) and feed efficiency (GF) were calculated on a dry-matter basis from study day -1 to 28. A calculated and weighed amount of calf starter was offered every evening and refusals were weighed again 24 hours later to determine feed intake. Following the occurrence of precipitation from inclement weather, calf starter was not included in the intake calculations, which occurred 2 times in the first cohort (day 6 and 27) and 4 times in the second cohort (day 15, 16, 21, and 27). Milk replacer intake was included in the intake calculations on a dry-matter basis.

Blood sample collection

Prior to the first blood sample collection time, the hair over the jugular furrow was clipped. For blood collection, calves were gently restrained in lateral recumbency while blood was collected from the jugular vein using a 10mL syringe (Covidien, Dublin, Ireland) with an 18-gauge needle (BD, Franklin Lakes, NJ). Timepoints for blood collection included –24 h (baseline), 0.5, 1, 2, 4, 8, 24, 48, 72, and 96 h post-procedure. Both plasma cortisol and substance P (SP) were analyzed from each blood collection timepoint. Blood samples were placed on ice immediately following collection until centrifuged at 1,000 g at a temperature of 4°C for 15 minutes. Plasma was extracted from the centrifuged samples and immediately transferred to cryovials (1.8 mL Arcticice Cryogenic Tubes, USA Scientific, Inc., Ocala, FL), then stored at –80°C until analysis.

Plasma Cortisol Determination

Plasma cortisol concentrations were determined with an enzyme-linked immunosorbent assay (ELISA; Cayman Chemical ELISA kit item no. 500360, Ann Arbor, MI, USA). Methods of plasma cortisol determination are described elsewhere (Mancke et al., 2025).

Substance P Determination

A radioimmunoassay (RIA) was used to determine SP concentrations. Methods of SP determination are previously described (Mancke et al., 2025).

Mechanical Nociception Threshold

Mechanical nociception threshold (MNT) was measured in kilograms-force (KgF) using a pressure algometer (Wagner FDX100, Wagner Instruments, Greenwich, CT) at baseline (-24 h) and 2, 4, 8, 24, 48 and 72 h post-procedure. Testing sites were lateral of each horn bud at the horn-hair junction, as previous work has shown lateral of the horn to be the most sensitive following cautery dehorning (Heinrich et al., 2010) and a control site in the center of the

forehead**.** The forehead served as an indicator of referred pain rather than an anatomically remote control site due to its proximity to the horn buds. The 1 cm^2 rubber tip of the algometer was gently pressed to the testing site and pressure was applied at a rate of approximately 1 kg of force per second until the calf withdrew (displayed any overt movement away from the pressure algometer). The individual collecting MNT was blinded to the values (a second individual recorded MNT values) to keep the collection method consistent and prevent bias. The same trained individual collected MNT for the entirety of the sampling period. Each site was tested 3 times per timepoint, and the values of each test site were averaged. The values for left horn bud and right horn bud were averaged to attain a singular horn bud MNT value for each timepoint.

Infrared Thermography

Infrared thermography (IRT) images of the scrotum and disbudding site were collected at baseline (-24 h) and 0.5, 1, 2, 4, 8, 24, 48, and 72 h post procedure using a high-accuracy infrared camera (FLUKE TIX580, Fluke Corp., Everett, WA), which automatically adjusted for changes in ambient temperature and relative humidity. Images of the scrotum were collected from directly behind the calf at a distance of 0.5 m with the calf standing. Images of the horn bud regions were collected from directly above the head at a distance of 0.5 m with the calf standing. The images were analyzed using research-grade software (SmartView 4.3, Fluke Corp., Everett, WA) by placing a circular window over the test location. Within the circular window, the maximum, average, and minimum temperatures were recorded and analyzed for each test location, which included the distal scrotum and both horn buds (Figure 3.2). The horn bud circular window captured the horn buds and 1 cm of surrounding tissue outside of the horn buds. The temperatures of both horn buds were averaged in order to obtain singular maximum, average, and minimum temperature for each calf at each timepoint. A binomial of horn bud

images affected by direct sunlight or not was included in the statistical model as a random effect to account for variation in sunlight exposure.

Lie and Stand Activity

To determine lying and standing activity, three-axis accelerometers (HOBO Pendant G, Onset Corp., Bourne, MA) were attached to the left lateral aspect of the left hind leg proximal to the metatarsophalangeal joint on study day -2 with cohesive bandage and a small amount of glue cement. The accelerometers began logging data at midnight on study day -1 and continued to log data every 30 seconds until day 15, in which the accelerometers were replaced due to limited data storage capacity. The replacement accelerometers began logging at midnight on the day of replacement which allowed the data from the original and replacement accelerometers to be stitched together without data loss. Data from each accelerometer was plotted to visualize the data and ensure that accelerometers did not rotate during the collection period. The axis positions of the accelerometer were converted into binomial data (lying or standing) using a previously validated protocol (UBC AWP, 2013). Data was then summarized into total minutes standing, total minutes lying, total number of standing and lying bouts, and average duration of standing and lying bouts per day. A bout was defined as at least 1 minute of continuously exhibiting the same activity (either lying or standing).

Kinetic Gait Analysis

A floor mat-based gait assessment mat (Strideway, Tekscan, Inc., Norwood, MA) was used to evaluate changes in gait at baseline (-24 h) and 4, 8, 12, 24, and 48 h post-procedure. The mat was calibrated prior to use with verified weights. At least 2 full strides, equaling 8 steps, were captured and analyzed for each timepoint. Gait assessment was repeated for calves that changed gait while walking across the mat. Due to the young age of the calves, they did not have

a sufficient flight zone to walk them across the mat. Thus, study personnel walked in front of the calves with a milk bottle to entice the calves to walk across the mat. Nevertheless, the calves were not touched by study personnel when walking across the mat. Following data collection, a research-grade software (Strideway v.7.7, Tekscan, Inc, Norwood, MA) was used to analyze gait parameters, which determined several gait outcomes including force, stance time, stride length, foot-time integral (FTI), pressure, gait distance, and gait velocity. For each gait outcome (apart from gait distance and velocity), the software calculated values for each limb, which were averaged into "front" and "hind" for statistical analysis.

Behavioral Scoring

Mounted video cameras (Wyze Cam OG, Wyze Labs, Inc., Seattle, WA) were used to collect behavior data. Cameras were mounted inside each calf hutch, pointing towards the entrance, which captured both the inside and small pen area outside of the hutch. The videos were rendered into 30 min segments that captured a pre-procedure baseline (-24 h) and 0.5, 1, 2, 4, 8, 24, 48, 72, and 96 h post-procedure. A specialized behavioral scoring software (The Observer XT, Noldus Information Technology, Leesburg, VA) was used to evaluate behavior by a single trained observer using a detailed ethogram (Table 3.2), adapted from Heinrich et al. (2010).

Statistical Analysis

All data analyses were executed using R (Posit, Boston, MA, version 2022.07.1). Data was evaluated for normality using the Shapiro-Wilk normality test and histogram distribution using 'shapiro.test' and 'histogram' functions of R, respectively. Linear mixed-effects models were used to test performance outcomes (overall ADG and GF) using a fixed effect of treatment and a random effect of cohort. Linear mixed-effects models were used to test plasma cortisol, SP,

IRT, MNT, gait, activity monitoring, BW, and behavior outcomes using fixed effects of treatment, time, and interactions. Regarding outcomes that are reported as a difference from baseline, the baselines values were subtracted from post-procedure values before analysis. For activity monitoring models, data collected on study day 0 was excluded from the data set because of the intensive data collection schedule that required frequent handling of the calves, specifically in the first 8 hours following castration and disbudding. Regarding behavioral outcome models, a binomial of human in pen or not during time of behavioral scoring was included in the statistical model as a random effect to account for variation of human interaction with the calves. Behavior data expressed as counts were transformed to log10 + 1 to achieve normality statistical analysis, then were reported as least squares means and standard error from the non-transformed values while the P-values were reported from the transformed values. Packages used for linear mixed-effect models included "lme4" and "lmerTest". Calf ID was included as a random variable to account for repeated measures, and cohort was included as a random variable to account for variability between cohorts. Residuals were checked for normality using "histogram", "qqnorm", "qqline", and "shapiro.wilk" functions. Results are expressed as LS-means and SE with Sidak adjustment to control the family-wise error rate. The significance level was set at $P \leq 0.05$.

Results

Performance

None of the calves in this study required rescue analgesia. Calf BW at baseline is presented across cohorts and treatment groups in Table 3.1. There were no observed treatment effects (P=0.58) or treatment × time interactions (P=0.99) for BW over time. A time effect was observed for BW with calves across all treatment groups steadily gaining weight throughout the

duration of the study. No treatment effects were observed in overall ADG (P=0.93) or feed efficiency (P=0.92).

Plasma Biomarkers (SP and Cortisol)

Plasma SP concentrations showed a significant treatment × time interaction (P<0.01; Figure 3.3), with the CONTROL group exhibiting lower SP values than the LIDO-MEL group 2 h post-procedure (176 vs. 298 pg/mL, respectively; SE=31.9). Additionally, the CONTROL group presented lower SP values compared to the COMBO group at 8, 24, 48, 72, 96 h post-procedure (≤192 vs. ≥298 pg/mL, respectively; SE=31.9). Apart from the CONTROL group, SP values between all other treatment groups did not appear to differ from one another across all timepoints. The coefficient of variation for intra-assay variability was 6.6% and the inter-assay variability was calculated at 7.8%.

A treatment effect was found for plasma cortisol, with the CONTROL group (3737 pg/mL, SE = 1904) showing overall greater cortisol concentrations compared to LIDO-MBAS and SHAM groups (86.7 and 560 pg/mL, SE=1738, respectively; P<0.01; Figure 3.4) when analyzed as a difference from baseline. Additionally, a time effect was evident, with all treatment groups exhibiting elevated concentrations immediately following castration and disbudding (0.5 h), followed by a gradual decline over time (P<0.01; Figure 3.5). There was no evidence of a treatment × time interaction on plasma cortisol values (P=0.12). The coefficient of variation for intra-assay variability was calculated at 8.0% and the inter-assay variability at 5.4%.

Mechanical Nociceptive Threshold

A treatment × time interaction (P<0.01) was observed for horn bud MNT when analyzed as a difference from baseline, with the SHAM group requiring greater exerted pressure to withdraw from the algometer (i.e., less sensitive) at 72 h compared to all other treatment groups

(0.33 vs. ≤ -0.39 KgF, respectively; SE=0.23; Figure 3.6). Apart from the SHAM group, no other treatment group appeared to differ from one another across all timepoints. There were no observed effects of treatment, time, or their interaction on forehead test site MNT values (P>0.05).

Infrared Thermography

No evidence of significant treatment effects or treatment × time interactions were found for any IRT outcomes (P>0.05). Numerically, maximum horn bud temperature across all timepoints was lowest in the SHAM group (35.2°C; SE=1.49), while all other treatment groups were between 36.2 and 36.7°C (SE=1.52; P=0.73). Similarly, across all timepoints, the SHAM group exhibited the lowest maximum scrotum temperature numerically (33.7°C; SE=0.38), while all other treatment groups were between 34.1 and 34.5°C (SE=0.41; P=0.66). A time effect was found to be significant for maximum horn bud temperature (P<0.05), with temperatures gradually rising from baseline until 8 h post-procedure before plateauing. Similarly, scrotal maximum temperatures were found to differ only by timepoint (P<0.05). All scrotal IRT outcomes followed similar temporal patterns, with scrotal temperatures gradually rising from baseline until peaking 8 h post-procedure.

Gait Analysis

A treatment × time interaction was found for front and hind stance times (P<0.05, Table 3.3). Regarding front stance time, 8 h post-castration the CONTROL group placed their forelimbs on the mat for significantly longer time (0.86 s; SE=0.08) during each stride compared to LIDO, LIDO-MBAS, and COMBO groups (≤0.47 s; SE=0.07; P<0.05; Figure 3.7). At 24 h post-castration, the CONTROL group maintained a longer front stance time compared to LIDO-MEL group (0.85 vs. 0.57 s, respectively; SE=0.07; P=0.03). A similar pattern was observed for

hind stance time, with CONTROL calves at 8 h post-procedure exhibiting increased time with their hind limbs on the mat (0.81 s; SE=0.07) during each stride compared to LIDO, LIDO-MBAS, and COMBO groups (≤0.51 s; SE=0.06; $P<0.05$; Figure 3.8). A significant treatment × time interaction was also observed for hind pressure. At 4 h post-castration, SHAM calves exerted greater hind limb pressure (123.9 kg/cm^2; SE=11.7) compared to LIDO-MEL group (94 kg/cm^2; SE=10.9; $P<0.01$; Figure 3.9). However, at 48 h post-castration, CONTROL calves exhibited greater hind limb pressure (119 kg/cm^2; SE=13.4) compared to the LIDO-MEL group (87.9 kg/cm^2; SE=10.8; $P=0.03$). Aside from differences noted in the SHAM and CONTROL groups, no other treatment groups appeared to differ from one another across timepoints for the evaluated gait patterns.

Behavior

No significant lie and stand activity differences collected using the accelerometers were found between treatment groups ($P>0.05$). However, behaviors such as lying, standing, and number of stand bouts significantly changed overtime for all treatment groups. Regardless of treatment, total lying time per day did not significantly differ from baseline values until 6 days post-procedure, increasing from 1083 to 1128 minutes per day; (SE=40.1; $P<0.01$). Similarly, total standing time did not differ from baseline values until day 9, when it increased from 306 to 369 minutes per day (SE=39.3; $P=0.04$). However, the number of standing bouts per day decreased significantly the day post-procedures (20.4 vs. 15.7 bouts/day; SE=2.36; $P<0.01$). After day 1, the number of standing bouts per day did not appear to significantly differ from baseline values again until 27, 28, and 29 days post-procedure, with the number of standing bouts decreasing from baseline values at these timepoints (20.4 vs. 17.3, 17.1, and 16.7 bouts/day; day -1 vs. 27, 28, and 29, respectively; SE = 2.37; $P<0.05$). In addition, behavioral

scoring through video recording revealed no effects of treatment, time, or treatment × time interactions for any outcome variables ($P>0.05$).

Discussion

Holstein bull calves are routinely subjected to castration and disbudding, causing pain and distress (Coetzee, 2011) that is exacerbated when these procedures are performed concurrently (Sutherland et al., 2013). The lack of FDA-approved analgesic drugs labeled for pain management in cattle hinders the ability to provide pain mitigation during castration and disbudding. This study aimed to evaluate the use of alternative method of stress relief (MBAS) with a local anesthetic, or in combination with a systemic analgesic during castration and disbudding in Holstein calves analyzed over a 28-day post-procedure sampling period.

While the effect of MBAS on performance measures following a stressful experience such as weaning have been explored previously, evaluating MBAS on performance measures following painful management procedures remains largely unexplored. Previous work has found MBAS to increase performance measures including ADG and BW in calves within 15 days post-weaning (Vieira et al., 2023). However, we were unable to detect an increase in performance measures in calves treated with MBAS. Interestingly, we were also unable to find performance differences between the CONTROL and SHAM groups. A previous study found no significant differences in BW 36 days post-castration between surgically castrated and non-castrated calves that were 1 week of age, with the authors suggesting a lack of significant differences due to naturally high variability in feed consumption and growth between calves (Molony et al., 1995), which does align with our findings. It has been reported that younger calves lose significantly less weight following castration compared to older calves, potentially because younger calves may experience less castration-induced stress due to having smaller testicles than older calves

(Bretschneider, 2005), making it more challenging to detect significant BW differences in young calves. Furthermore, a previous study found ADG to be lower in castrated and dehorned calves 7 days following the procedures compared to calves that underwent sham-procedures (Mosher et al., 2013). However, the authors reported that ADG in castrated and disbudded calves rebounded 14-21 days post-procedure. In the present study, final BW and ADG were calculated 28 days post-procedure which may have allowed more time for the CONTROL group to gain weight as pain subsided, potentially attributing to the lack of significant differences for performance measures.

In the present study, we found a significant treatment × time interaction for SP concentrations. Unexpectedly, the CONTROL group (castrated, disbudded, and received no pain management) presented lower SP concentrations than the COMBO group (castrated, disbudded, and received lidocaine, meloxicam, and MBAS at the time of these procedures) at several post-procedure timepoints (8, 24, 48, 72, 96 h post-procedure). Additionally, the SHAM group (not castrated or disbudded) never differed from any other treatment group at any timepoint. These findings are very unexpected because increased SP concentrations have shown to be associated with pain, inflammation, and wound healing (Dunnick et al., 1996; Onuoha and Alpar, 1999). This association has been demonstrated in cattle, as studies have found surgical castration with no pain management to be associated with elevated SP concentrations in calves (Coetzee et al., 2008; Olson et al., 2016). However, the calves in these studies were several months older than the calves in the present study, and age has been shown to influence SP concentrations (Dockweiler et al., 2013; Meléndez et al., 2017). Several studies evaluating SP concentrations following painful procedures in cattle have shown no difference in SP concentrations between castrated and non-castrated animals (Dockweiler et al., 2013; Kleinhenz et al., 2017; Meléndez et

al., 2017; Kleinhenz et al., 2018), but the present study is the first to reported elevated SP concentrations in cattle treated with analgesia relative to untreated cattle after a painful procedures. The SP results presented in this study are unique and further question the utility of this biomarker for pain assessment in cattle.

Plasma cortisol was observed to differ by both treatment and timepoint. The CONTROL group presented statistically greater plasma cortisol concentrations than the LIDO-MBAS and SHAM groups. It was expected to observe the CONTROL group exhibit higher plasma cortisol concentrations than the SHAM group, as previous work has shown concurrent castration and dehorning to significantly increase plasma cortisol concentrations (Mosher et al., 2013; Sutherland et al., 2013). However, the LIDO-MBAS group exhibited statistically lower plasma cortisol concentrations than the CONTROL group while the COMBO group did not. This was an interesting finding considering the COMBO calves received lidocaine, meloxicam, and MBAS at the time of castration and disbudding while the LIDO-MBAS group only received lidocaine and MBAS. Nevertheless, no treatment group differed statistically from one another apart from the CONTROL group. Regardless of treatment, plasma cortisol was measured at its greatest concentration 0.5 h post-procedures before gradually falling and returning to near baseline levels by 4 h post-procedure. This is in accordance with previous work (Coetzee et al., 2008; Mosher et al., 2013; Webster et al., 2013), suggesting that despite not sampling intensively in series, we may have captured peak cortisol concentrations 0.5 h post-procedure.

Horn bud MNT results suggest that the SHAM group was less sensitive around the horn buds at 72 h post-procedure compared to all other treatment groups. This is an expected finding, considering that the SHAM group was not disbudded and therefore not painful around the horn buds. At all timepoints apart from 72 h, SHAM group MNT values remain close to baseline

values, indicating that calves did not become habituated to the algometer before that timepoint. Apart from the SHAM group, no treatment groups appeared to differ from one another at any timepoint. Regardless of treatment group, calves required significantly less pressure to withdraw from the algometer at 8 h compared to 2 h post-procedure, which is in accordance with previous work (Martin et al., 2022c). Apart from the CONTROL group, all calves received a cornual nerve block with 2% lidocaine prior to disbudding. Previous studies report that 2% lidocaine has an onset time of ≤5 min and an effective duration of approximately 1.5 to 2 hours in cattle (Coetzee, 2013; Klein et al., 2024) which potentially explains the decrease in exerted pressure in order for the calves to withdraw from 2 h to 8 h post-procedure, as the calves become more sensitive to pressure as the lidocaine loses effectiveness. Surprisingly, MNT values did not differ between treatment groups at 2 h post-procedure. An increase in MNT values was expected in lidocaine-treated calves at 2 h post-procedure compared to baseline values due to desensitization, but this was not observed. It is possible that by 2 h post-procedure, the lidocaine had lost its analgesic effects and the calves were not desensitized at the horn buds. Another possible explanation for a lack of treatment effects is that the calves were not blindfolded during the MNT procedures, which has been performed in previous MNT methodology in cattle to prevent withdrawal due to visual cues (Stock et al., 2016; Kleinhenz et al., 2017; Martin et al., 2022b).

Treatment appeared to have no effect on horn bud or scrotal IRT outcomes. Past work evaluating horn bud temperature after cautery disbudding has shown sham-disbudded calves to exhibit greater horn bud temperature compared to disbudded calves (Stock et al., 2021). Cautery disbudding produces burned skin around the disbudding site which is reported to decrease skin temperature as the vasculature is disrupted (Hardwicke et al., 2013; Stock et al., 2021). In the present study, the SHAM group horn bud temperatures not found to differ compared to

disbudded calves, which may be due to a small sample size. Regarding scrotal IRT, previous work found surgically castrated calves to exhibit lower scrotal temperatures shortly (<2 h) following castration compared to non-castrated calves (Meléndez et al., 2017), which was not detected in the present study. Similarly to horn bud IRT outcomes, a lack of significant differences between treatment groups for scrotal IRT outcomes may be attributed to a small sample size.

To our knowledge, this study is the first to measure gait after concurrently castrating and disbudding calves. Previous work regarding gait analysis after disbudding in calves found that non-disbudded calves exhibited lower gait distance compared to calves that received either lidocaine, lidocaine and meloxicam, or no pain management prior to disbudding (Martin et al., 2022b) which is unlike the present study where we found no effects of treatment, time, or its interaction on gait distance. Regarding gait analysis after surgical castration in calves, a previous study found that across all timepoints, front stance time was lower in calves that received either lidocaine only or no pain management compared to calves that received lidocaine and meloxicam prior to castration (Martin et al., 2022a). Other gait measures such as stride length have shown to be affected by surgical castration (Currah et al., 2009), however we did not observe any treatment differences for stride length. We found that both front stance time and hind stance time presented a treatment × time interaction, with the CONTROL group exhibiting higher values in multiple post-procedure timepoints compared to multiple other treatment groups. Therefore, in multiple timepoints, the CONTROL group spent more time with their front and hind limbs on the mat per stride, suggesting hesitancy to walk due to pain or discomfort associated with castration and disbudding.

No significant differences between treatment groups were observed for any lie and stand activity outcomes through the use of accelerometers. Previously, Heinrich et al. (2010) found that calves given lidocaine and meloxicam were less active up to 5 h post-disbudding compared to calves that were not disbudded, however the authors were unable to detect differences from 5 h to removal of the accelerometers (24 h post-disbudding). Similar results were observed in a different study in which meloxicam-treated calves spent less time lying at 2 h after disbudding compared to sham-castrated calves, but no differences were observed after that timepoint (Sutherland et al., 2018). Therefore, in the present study, since day 0 data for activity monitoring was excluded from the data set due to rigorous outcome collection and frequent handling of the calves, we may have missed an opportunity to detect differences in lying and standing behaviors between treatment groups. Other studies have found no differences in lying and standing times in Holstein calves that were sham-disbudded compared to calves that were disbudded with lidocaine and a systemic analgesic (Sutherland et al., 2013; Röder et al., 2022), which does represent the findings in the present study. Although calves were individually housed, all calves were within visual and auditory range of each other and may have imitated each other's behavior, which could be a potential explanation for a lack of treatment differences for lying and standing outcomes.

We found no effect of treatment, time, or their interaction on any behavioral outcomes with video monitoring. A previous study observed calves that were castrated and disbudded with no analgesia had a statistically higher frequency of ear flicking and foot stamping up to 3 h post-procedure when compared to calves that were not castrated or disbudded (Sutherland et al., 2013). Another study found ear flicking counts to be significantly higher in calves that received only lidocaine compared to calves that received lidocaine and meloxicam prior to disbudding,

with the greatest difference between the treatment groups occurring 6 h post-disbudding (Heinrich et al., 2010). In the present study, calves were being frequently handled up to 8 h post-procedure to collect data for a multitude of other outcomes including blood collection for SP and cortisol, IRT, MNT, and gait analysis, which may have influenced the calves' ability to express their natural behavior in response to pain associated with castration and disbudding. The intensive data collection regimen in the several hours following castration and disbudding may explain the lack of differences in behavioral outcomes.

A limitation of this study is the frequent collection of multiple outcomes, particularly in the hours immediately following castration and disbudding. Intense handling of the calves within the first 8 hours post-procedure may have influenced outcomes including plasma cortisol, SP, MNT, IRT, gait analysis, activity monitoring, and behavioral scoring. Additionally, 3 calves were genetically polled and therefore systematically placed in the SHAM group, which may have influenced study outcomes. Another limitation of this study was a small sample size, particularly for performance outcomes, which made it difficult to detect potential significant differences between treatment groups for outcomes with high natural variability. This limitation was exacerbated when comparing SHAM and CONTROL groups, as these groups only had 5 and 6 animals, respectively.

Conclusions

The analyses revealed that the CONTROL group experienced considerable stress post-procedure, and all treatment groups apart from SHAM were more sensitive to pressure around their horn buds post-procedure compared to baseline. These findings reinforce the notion that castration and disbudding cause pain and stress in young calves. However, no significant differences were observed between the LIDO, LIDO-MBAS, LIDO-MEL, or COMBO treatment

groups. This suggests that there is no evidence that administering MBAS in addition to lidocaine, or in combination with meloxicam is neither more or less effective at pain mitigation than lidocaine alone in Holstein calves undergoing surgical castration and cautery disbudding.

Author Contributions

J.S., E.B., M.M., R.B., L.F.: data curation. J.S. and E.B.: formal analysis. J.S.: writing – original draft. E.B. and J.C.: investigation. E.B., P.D., J.C.: resources. E.B. and J.C.: supervision and validation. M.K. and J.C.: conceptualization, funding acquisition, methodology, and visualization. All authors: writing – review and editing.

Disclosures

This study was funded by FERA Diagnostics & Biologicals.

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Table 3.1 Descriptive statistics of bodyweight, in kg, of 49 Holstein calves at baseline (day -1) by cohort and treatment group.

	Treatment						
Variable	LIDO	LIDO-MEL	LIDO-MBAS	COMBO	SHAM	CONTROL	SD[1]
Cohort 1	n=5	n=5	n=5	n=4	n=3	n=3	
Bodyweight, kg	43.4	39.5	41.4	40.6	40.8	42.3	3.9
Cohort 2	n=4	n=5	n=5	n=5	n=3	n=2	
Bodyweight, kg	45.7	41.9	43.7	40.8	43.8	44.3	5.2

[1]Standard deviation, largest SD was reported.

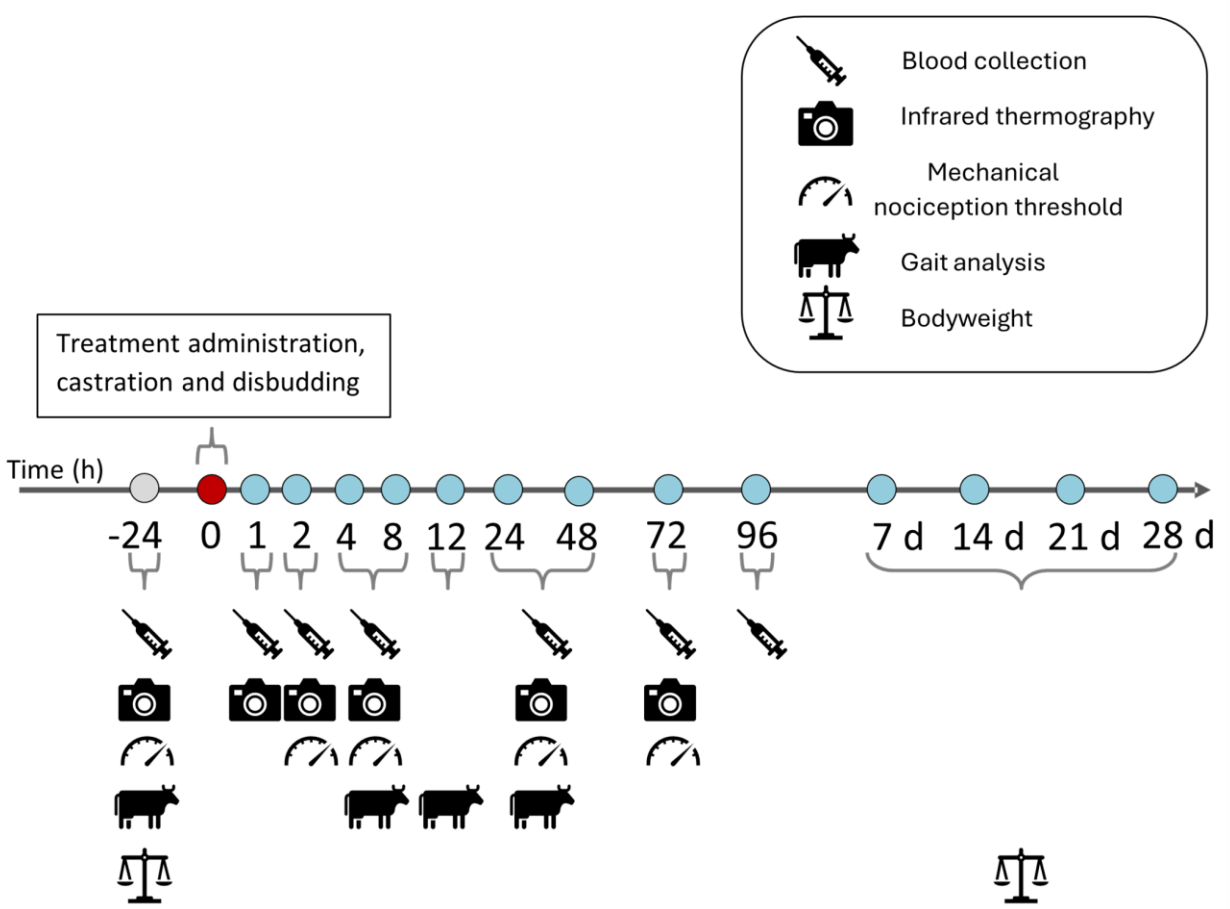


Figure 3.1 Sampling timeline for 49 Holstein calves that were subjected to surgical castration and cautery disbudding and received either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5). Gray dot indicates baseline outcome collection. Blue dots indicate post-procedure outcome collection. Outcomes were collected in order listed under each timepoint from top to bottom.

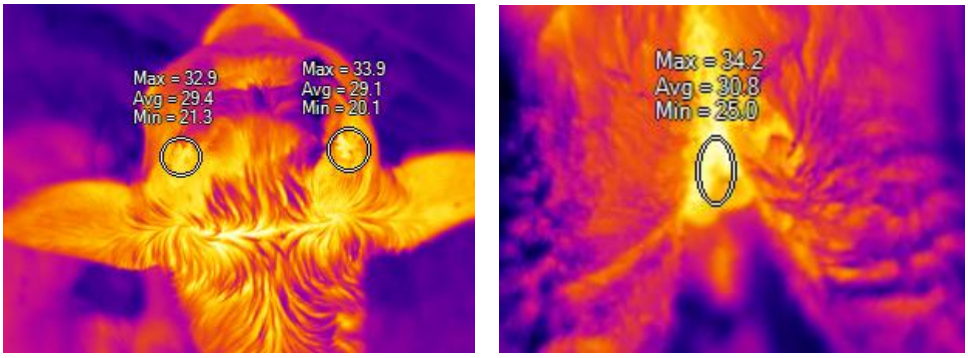


Figure 3.2 Infrared thermography of the disbudding sites (left) and scrotum (right) with a circular analysis window overlay and maximum, average, and minimum temperature in °C. Images were taken post-procedure on 49 Holstein calves that were given either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5).

Table 3.2 Ethogram adapted from Henrich et al. (2010), used to evaluate behavior by video monitoring following surgical castration and cautery disbudding in 49 Holstein calves that received either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (FerAppease®; LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5).

Behavior	Description
Duration	
Inside hutch, %	Calf is in hutch
Outside hutch, %	Calf is outside of hutch
Stand, %	Calf is upright with all 4 hooves in contact with the ground
Lie, %	Calf is in either sternal or lateral recumbency
Count	
Look to flank, n	Number of times the calf turns its head towards the flank while standing
Wound lick, n	Number of times the calf turns to lick the castration site while standing
Ear flick, n	Number of movements of one or both ears in a quick motion independent of head shaking
Tail flick, n	Number of forceful tail movements
Foot stamping, n	Number of times a hind leg was lifted and placed forcefully on the ground or kicked outward while standing
Head shaking or rubbing, n	Repeated movement of the head from side to side in a rapid motion or rubbing top of head against the hutch or fence

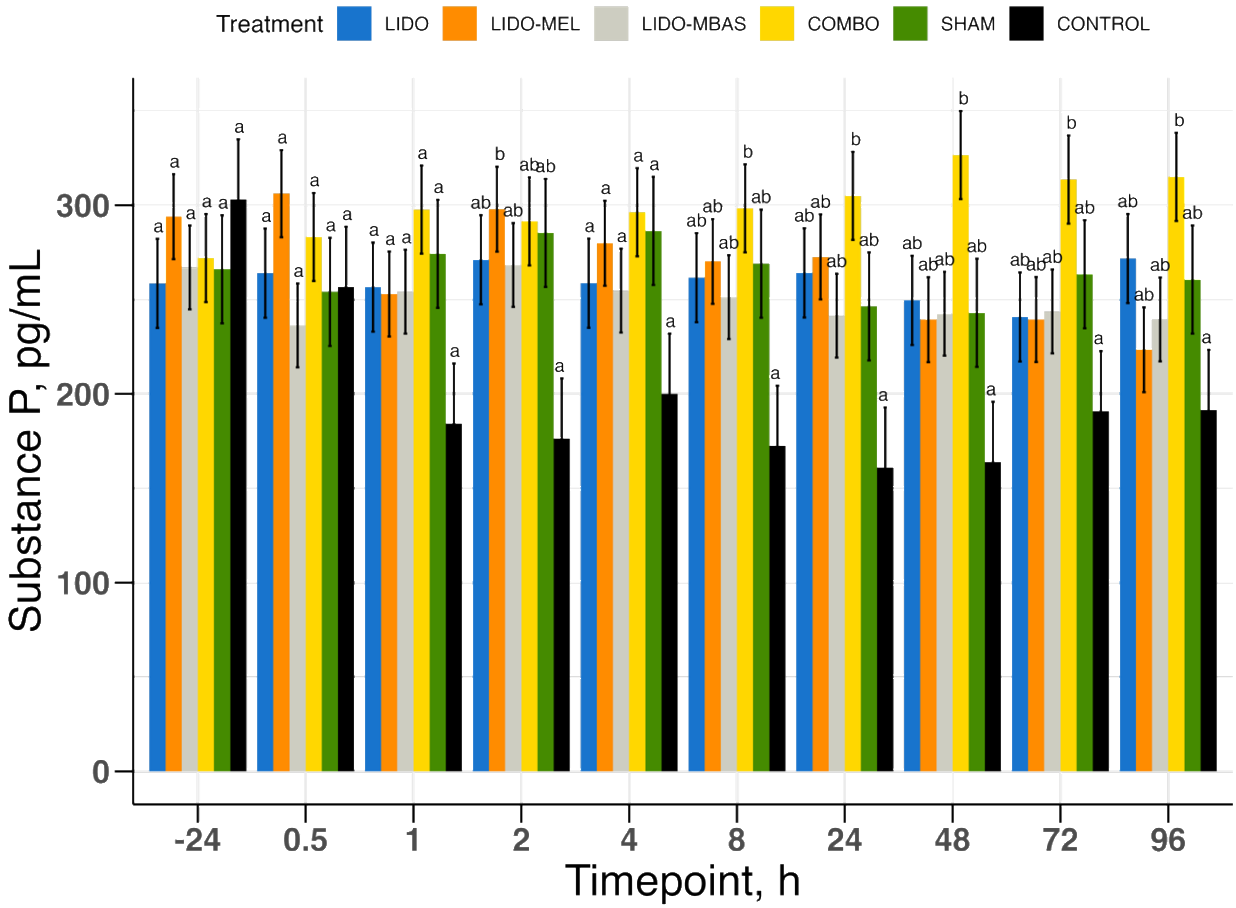


Figure 3.3 Plasma substance P concentrations in pg/mL (±SE), by treatment and timepoint. Data were collected following surgical castration and cautery disbudding of 49 Holstein calves that were given either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5). $^{a\text{-}b}$LS-means differ ($P<0.05$; Sidak adjustment).

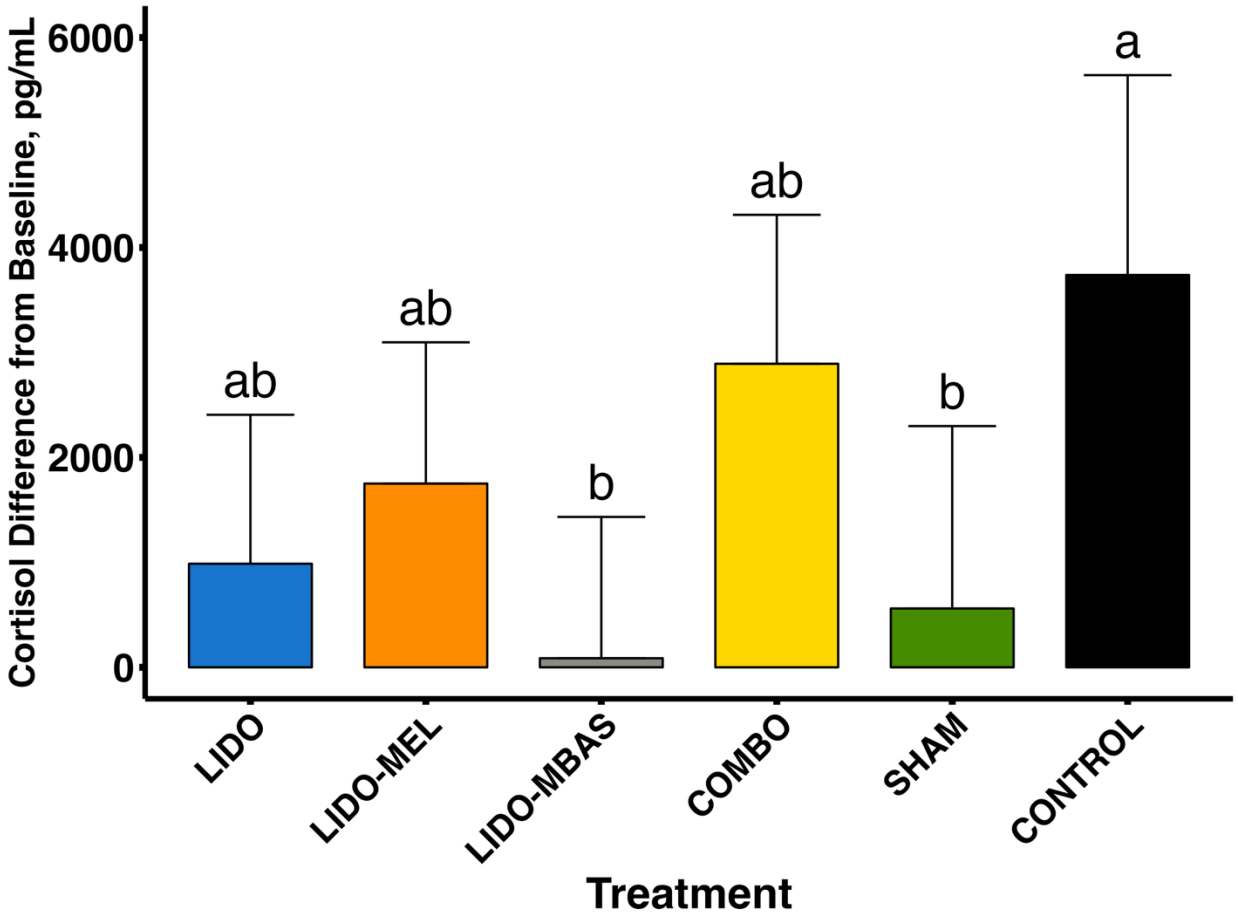


Figure 3.4 Plasma cortisol concentrations in pg/mL (±SE), by treatment group across all post-procedure timepoints and represented as a difference from baseline. Data were collected following surgical castration and cautery disbudding of 49 Holstein calves that were given either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5). $^{a\text{-}b}$LS-means differ ($P<0.05$; Sidak adjustment).

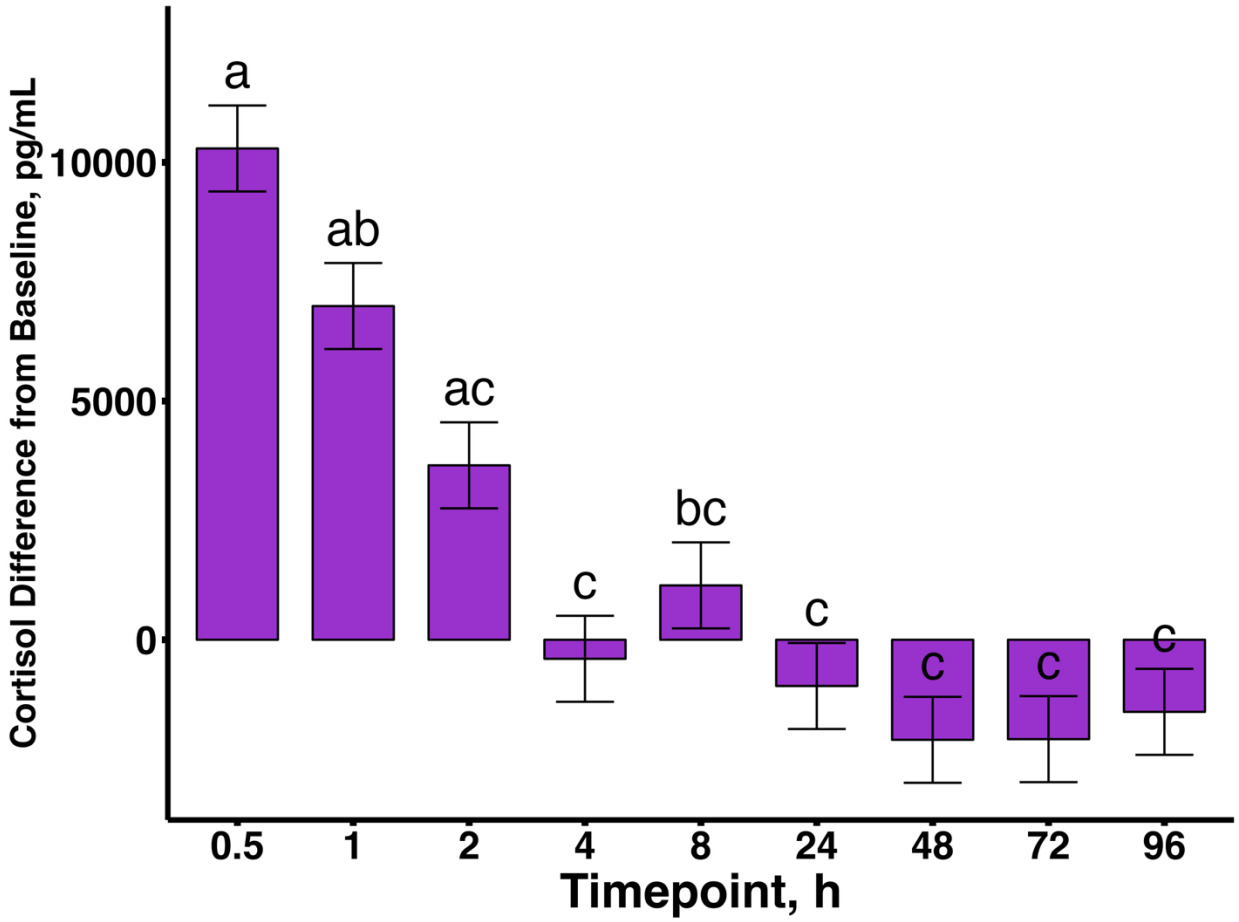


Figure 3.5 Plasma cortisol concentrations in pg/mL (±SE), by timepoint and represented as a difference from baseline. Data were collected following surgical castration and cautery disbudding of 49 Holstein calves that were given either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5). Timepoint represents hour relative to castration and disbudding. [a-b-c] LS-means differ ($P<0.05$; Sidak adjustment).

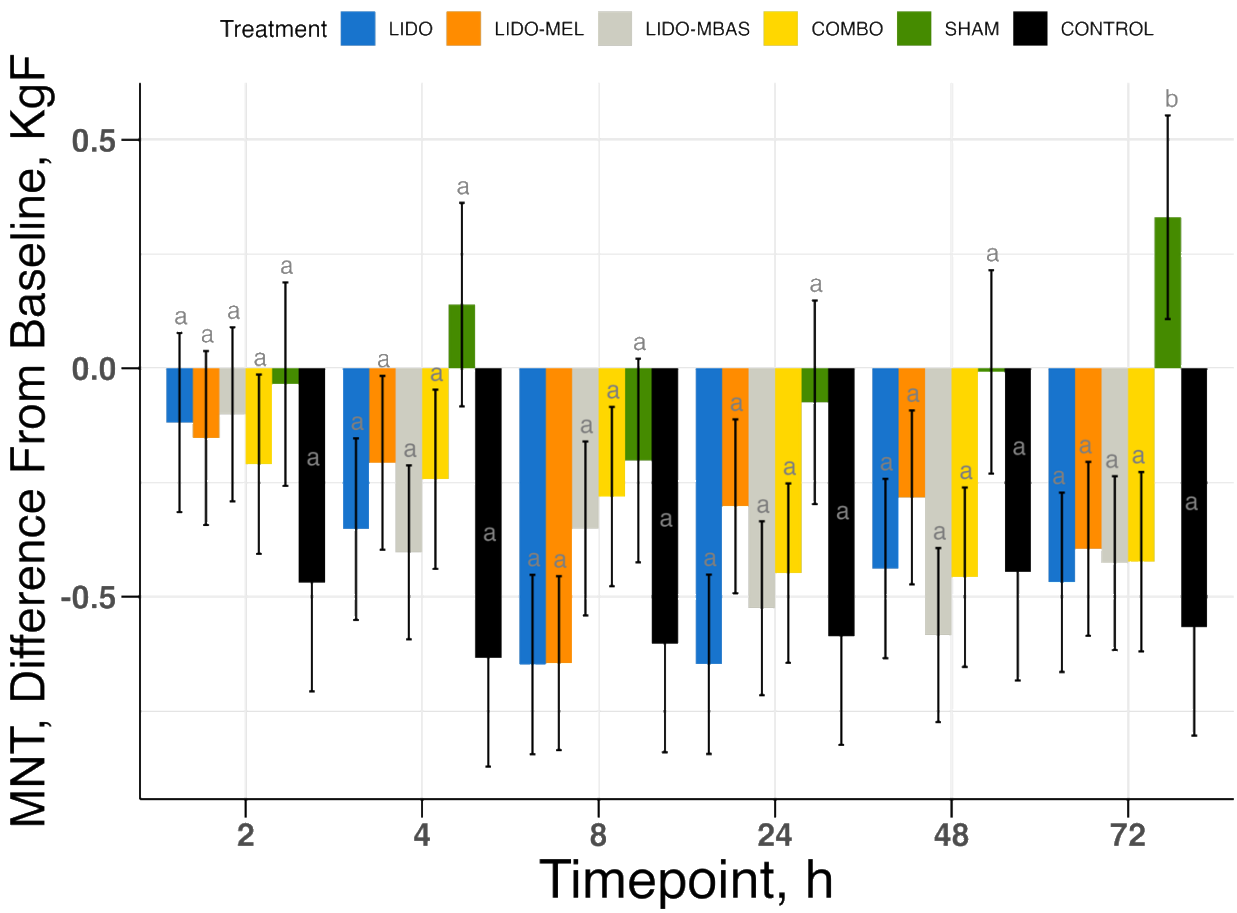


Figure 3.6 Horn bud mechanical nociception threshold in KgF (±SE), by treatment and timepoint, represented as a difference from baseline. Pairwise comparisons were performed by timepoint. Data were collected following surgical castration and cautery disbudding of 49 Holstein calves that were given either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5). Timepoint represents hour relative to castration and disbudding. [a-b] LS-means differ (P<0.05; Sidak adjustment).

Table 3.3 Estimated marginal means of kinetic gait analysis outcomes of 49 Holstein calves submitted to surgical castration and cautery disbudding randomly assigned to receive either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5).

	Treatment							*P*-value		
Variable	LIDO	LIDO-MEL	LIDO-MBAS	COMBO	SHAM	CONTROL	SE[1]	TRT[2]	Time	TRT X Time[3]
Gait Distance, cm	173	171	183	177	178	140	12.7	0.98	0.70	0.53
Gait Velocity, cm/s	82.2	78.8	90.5	88.3	86.0	83.6	6.14	0.24	0.51	0.38
Front Force, kg	38.4	35.9	41.1	37.1	37.9	40.0	6.31	0.24	<0.01	0.83
Hind Force, kg	29.4	28.3	31.0	30.5	33.0	32.2	5.41	0.07	0.06	0.29
Front Stance Time, s	0.6	0.611	0.578	0.576	0.538	0.671	0.04	0.91	0.04	<0.01
Hind Stance Time, s	0.561	0.588	0.553	0.557	0.534	0.663	0.04	0.99	<0.01	0.02
Front Stride Length, cm	79.6	74.6	81.3	81.3	81.1	80.5	3.25	0.08	0.89	0.30
Hind Stride Length, cm	82.5	74.1	81.5	80.4	84.4	80.6	3.62	0.35	0.88	0.52
Front FTI, kg	15.2	14.9	16.4	15.1	12.8	18.7	2.52	0.78	0.22	0.90
Hind FTI, kg	10.8	10.8	11.2	11.2	11	12.5	1.73	0.72	0.67	0.60
Front Pressure, kg/cm^2	114	108	120	112	114	118	11.8	0.10	0.39	0.94
Hind Pressure, kg/cm^2	98.7	88.8	97.1	99.1	103	99.7	10.8	0.07	0.65	0.03

[1]Greatest SE was reported.

[2]TRT, treatment effect.

[3]TRT X Time, interaction between treatment and time effect.

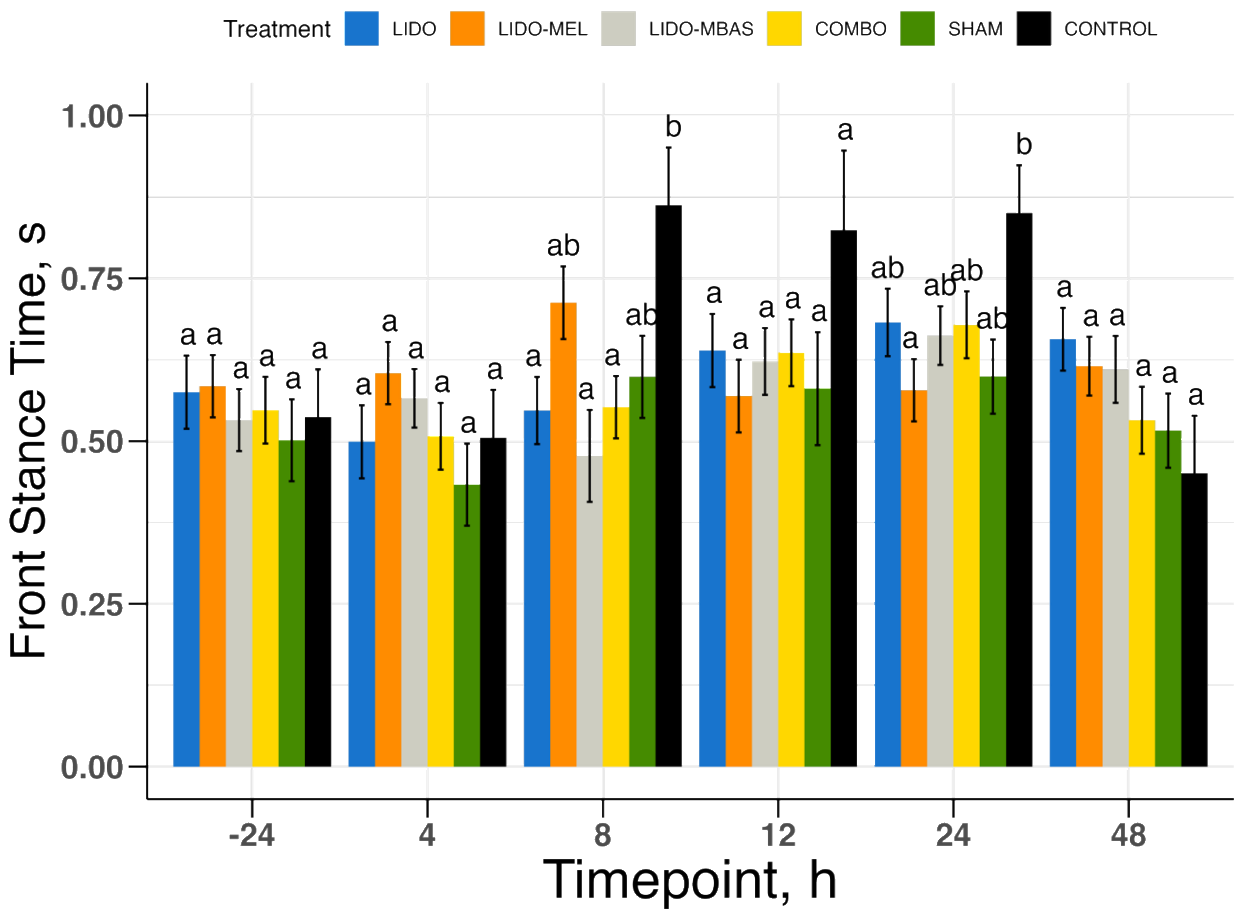


Figure 3.7 Average front stance time (±SE; s) by treatment and timepoint. Pairwise comparisons were performed by timepoint. Data were collected following surgical castration and cautery disbudding of 49 Holstein calves that were given either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5). Hour represents hour relative to castration and disbudding. [a-b] LS-means differ ($P<0.05$; Sidak adjustment).

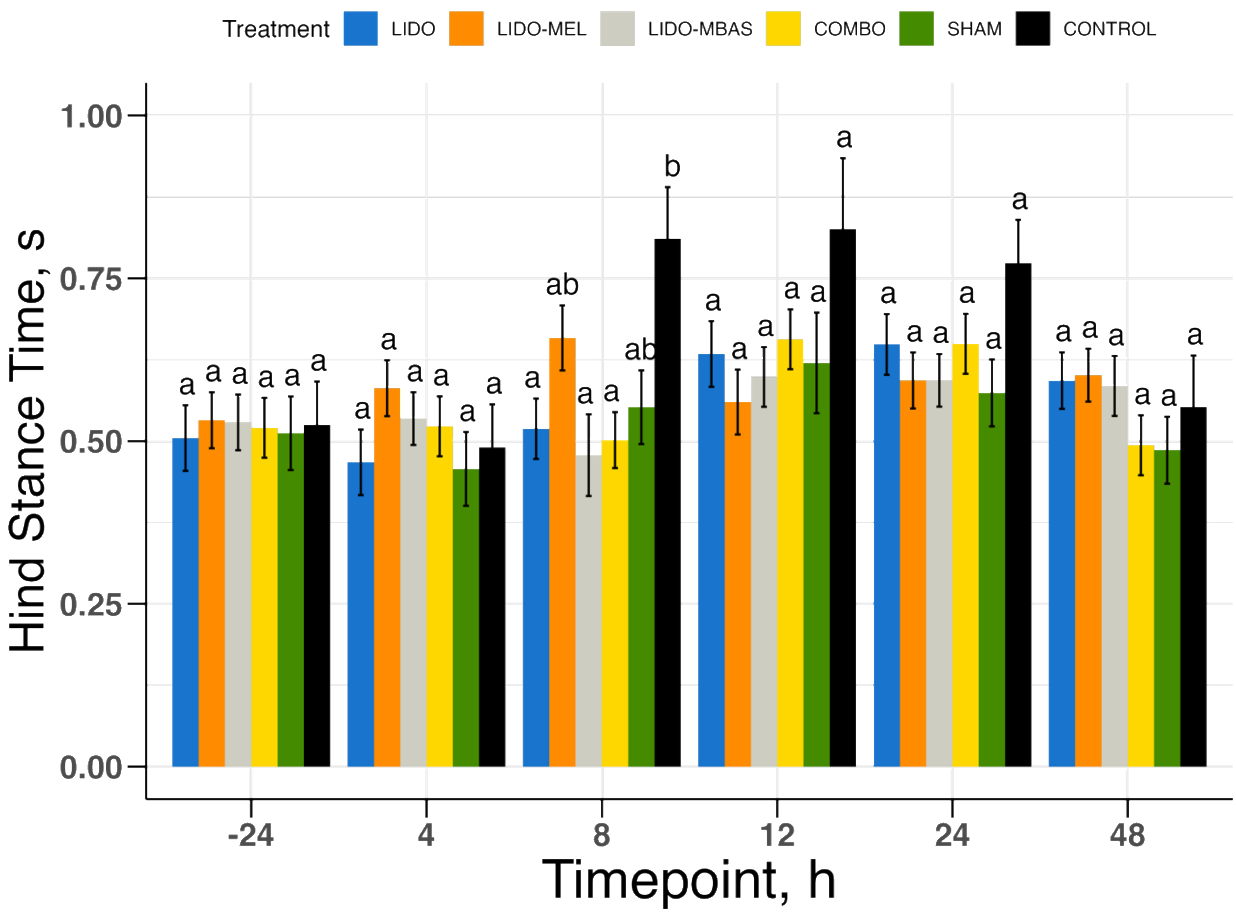


Figure 3.8 Average hind stance time (±SE; s) by treatment and timepoint. Pairwise comparisons were performed by timepoint. Data were collected following surgical castration and cautery disbudding of 49 Holstein calves that were given either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5). Hour represents hour relative to castration and disbudding. [a-b] LS-means differ (P<0.05; Sidak adjustment).

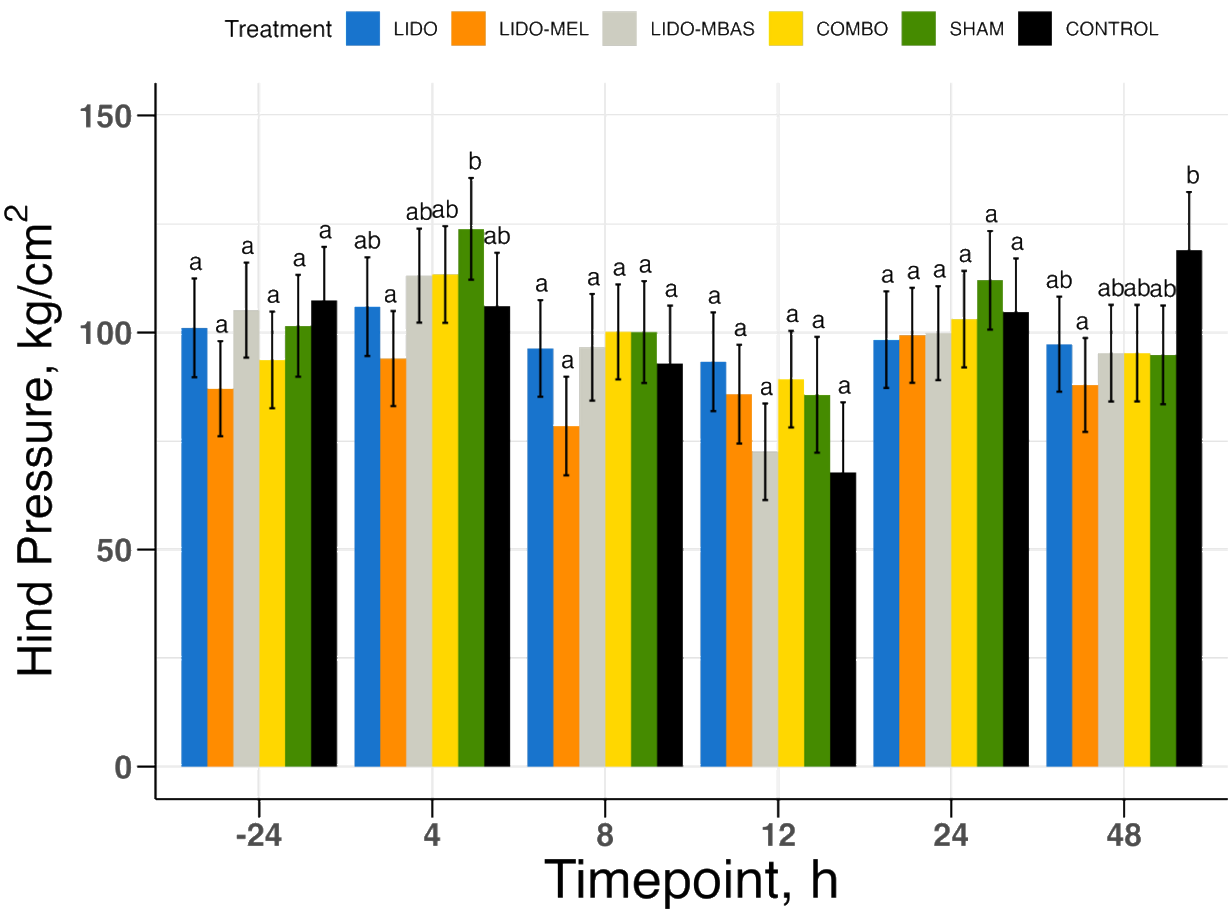


Figure 3.9 Average hind pressure (±SE; kg/cm^2) by treatment and timepoint. Pairwise comparisons were performed by timepoint. Data were collected following surgical castration and cautery disbudding of 49 Holstein calves that were given either local lidocaine anesthesia (LIDO; n=9), local lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), local lidocaine and topical MBAS (LIDO-MBAS; n=10), local lidocaine and systemic meloxicam and topical MBAS (COMBO; n=9), sham procedures (SHAM; n=6); or no analgesia or anesthesia (CONTROL; n=5). Hour represents hour relative to castration and disbudding. [a-b] LS-means differ (P<0.05; Sidak adjustment).

Chapter 4 - Comparing the use of lidocaine-loaded versus standard bands to reduce pain and discomfort in dairy and beef-dairy calves

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Abstract

Introduction of greater number of beef-dairy calves entering the United States beef market continues to highlight the importance of pain mitigation during castration. This randomized blinded controlled trial aimed to evaluate pain mitigation and behavior in beef-dairy (BOD) and Holstein (HOL) calves castrated with either lidocaine-loaded (LLB) or standard bands (CON). Thirty-eight bull calves weighing 183 ± 37 lb were randomly assigned to 1 of the following 2 treatment groups; castrated with lidocaine-loaded bands (BOD-LLB, n=9; HOL-LBB, n=9), or castrated with a standard control band (BOD-CON, n=10; HOL-CON, n=10). Calves' performance (Final BW, ADG), lying and standing activity, kinetic gait analysis, plasma cortisol, and potential pain-related behaviors were analyzed over a 28-day post-treatment period. Linear-mixed effects models were used to determine potential associations between outcomes and treatment, breed, time, and potential interactions. Lying and standing activity outcomes

showed a significant effect of breed for the number of standing bouts per day, with the BOD group transitioning from lying to standing more frequently than the HOL group (18.3 and 17.3 bouts/day, respectively; SEM=0.49; P=0.03). Gait outcomes showed a treatment × time interaction for gait velocity and gait distance, with the CON group increasing over time. However, no gait outcome differed between LLB and CON groups at any time point. Neither treatment or breed had an effect on plasma cortisol, performance, or video monitoring behavior outcomes ($P > 0.05$). Overall, this study was unable to find differences in pain outcomes between calves that were castrated with either a lidocaine-loaded or standard band.

Introduction

Castration of male calves is a routine management practice amongst cattle producers in the United States. The procedure is done to reduce injuries related to aggression and mounting behaviors, especially in confinement operations, avoid unwanted pregnancies in mixed-gender pens, and enhance carcass quality traits.[1,2] The most common castration methods are surgical (incision of the scrotum and removal of testicles) and non-surgical (entrapping or crushing of the spermatic cord, promoting constriction of blood supply to the testicles). Elastrator rubber bands (i.e., banding) were reported as the most common non-surgical castration method for young calves employed by bovine veterinarians in the United States.[3] In addition, the same method is used by producers due to its low cost, efficiency, and practicality.[4] Both surgical and non-surgical methods of castration have been shown to cause pain in livestock.[5–9]

Acute effects of band castration in calves include restlessness, abnormal posturing, and elevated cortisol levels within hours of castration, potentially due to pain and discomfort of initial ischemia from the constriction of the band.[8,10] Weeks following band castration, the band begins to create an open wound as it penetrates the scrotal tissue. This has been reported to be

associated with changes in lying and standing behavior that are indicative of hesitation to change positions, potentially to avoid further stretch or stimulation of the scrotal area.[11] Coinciding with the reported timeline of band penetration into the scrotal tissue, band-castrated calves have exhibited transient deceases in feed intake and ADG between 3 to 4 weeks post-castration, which could be an indicator of chronic pain and compromised welfare.[5]

Band-castrated calves present with mild signs of pain at the time of castration compared to surgical castration; therefore, banding is often perceived as less painful by human observers.[12] However, results from previous studies indicate that banding may cause greater overall detriment to calves over the entire banding period than surgical castration.[13,14] Band castration has been reported to cause an overall reduction in ADG, increased licking of the castration site, and higher, more prolonged cortisol concentrations compared to surgical castration, even when administered with a local anesthetic and a nonsteroidal anti-inflammatory drug.[13,14] These results suggest that band castration continues to cause discomfort and pain for prolonged periods, exceeding the duration of analgesia provided by a single injection of lidocaine or oral meloxicam.

To mitigate pain from non-surgical castrations, a lidocaine-loaded castration band (LLB) (Lidoband™, Solvet, Alberta, CA) has been developed, which releases therapeutic doses of lidocaine into the scrotal tissue. In vitro data showed rapid release of lidocaine within the first 30 minutes, followed by a slow release lasting at least 48 hours, with tissue concentrations ranging from 0.5 to 3.5 mg of lidocaine per gram of tissue and peaking 6 h post-banding. In vivo data from the same study suggested that calves castrated with LLB's showed less pain response to electrostimulation seven days post-treatment than those castrated using standard bands plus

lidocaine injections, indicating that lidocaine from the LLB's was delivered to the tissues for at least seven days.[15]

Regarding the Withdrawal Interval (WDI) recommendations for lidocaine, the current WDI for cattle intended for slaughter is 1 to 4 days, depending on the route of administration.[16] However, the use of the Lidoband™ does not currently carry a WDI recommendation in the United States due to its classification as a medical device rather than a systemic drug.

As beef-dairy cross calves increase steadily throughout the country, more of these animals are expected to enter the beef market.[17] Welfare concerns regarding castration of beef and dairy calves have been previously addressed.[1,18] However, no data are available comparing differences in band castration pain measures between dairy and beef-dairy cross calves utilizing this novel product. Dairy calves are generally more altricial than beef calves, exhibiting delayed suckling behavior compared to the more precocious beef calves.[19] There appear to be other behavioral differences between the breeds, as dairy cattle have been shown to exhibit less defensive behavior and are generally more solitary compared to beef cattle; however, little is known about how dairy and beef calves differently exhibit pain-related behaviors.[20] To accurately assess and manage pain in these animals, it is crucial to understand the differences in pain behaviors between dairy and beef calves. Such data are urgently needed to guide practitioners on the use of lidocaine-loaded bands for analgesia in calves and to determine if effectiveness varies between dairy and beef-dairy cross calves. The objective of this study was to determine whether the use of lidocaine-loaded bands to castrate two different cattle groups (dairy and beef-dairy cross) provides extended pain mitigation, as assessed by pain biomarkers and animal behavior.

Materials & Methods

All animal care and procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University.

A total of 40 intact male calves, 2 to 3 months of age, consisting of two different breeds, Holstein (HOL; n = 20) and Angus-influenced beef-dairy cross (BOD; n = 20), were enrolled in this study. Calves were sourced from a commercial calf ranch in central Wisconsin. This prospective randomized controlled clinical trial, following a 2x2 factorial design including breed (HOL or BOD) and castration band type (lidocaine-loaded band [LLB] or an identical standard band [CON]), was conducted at the Kansas State Stocker Unit from September to October 2024. Upon arrival, calves were weighed and received a metaphylactic dose of tulathromycin (Tulieve, Norbrook Laboratories, Lenexa, KS). An ear tag was placed in each ear with a unique identification number, with ear tags following a color scheme to identify calves by ear tag color. All calves were group-housed in one 18.2 x 15.2 m soil-surfaced pen, 70% bedded with straw, with a concrete apron, and had access to fresh water *ad libitum.* A total mixed ration (14.8% CP and 75.9% DM) was fed at 6.0 kg/head on a DM basis once daily at 8:00 AM. Calves were given 2 days of acclimation before baseline measurements were collected. A trained researcher conducted health observations once daily at approximately 9:00 AM.

Calves were randomly assigned by breed (HOL or BOD) to 1 of 2 treatment groups: castration with a lidocaine-loaded band (HOL-LLB; n = 9 and BOD-LLB; n = 9) or castration with an identical standard band with no pain management (HOL-CON; n = 10 and BOD-CON; n = 10). Random allocation to treatment groups was performed in Microsoft Excel (Microsoft Corp., Redmond, WA) using the “random” function by an unblinded individual who was not involved in animal husbandry, data collection, laboratory sample analysis, or statistical analysis.

The standard bands were attained from the manufacturer of the LLB's; therefore, they did not contain lidocaine but were identical in appearance to the LLB's, which allowed for blinding to treatment group. All study personnel involved in animal husbandry, data collection, laboratory sample analysis, or statistical analysis were blinded to treatment groups.

A hydraulic squeeze chute (SILENCER®, Moly Manufacturing, Lorraine, KS) was used to restrain calves during treatment administration. Treatment administration began at 7:00 AM and was marked as time point 0. Castration bands were applied by a veterinarian using a commercially available banding device, and band retention was evaluated each week during BW collections. Apart from study day 0 (2 h and 8 h time points), outcome variables, including BW, gait analysis, and plasma cortisol, were collected at approximately 7:00 AM on each data collection day before feeding. Animals were removed from their home pen and kept in a holding area while waiting to enter the chute for blood collection. At time points when both blood and gait analyses were collected, blood was drawn before gait analysis to control for potential variation in plasma cortisol concentrations attributable to gait analysis (Figure 4.1). Collection of blood and gait data for all calves took no longer than 2 h total at any given time point. The calves did not have access to feed or water while in the holding area, but was available upon return to their home pen.

Calves were fed a total mixed ration of dry-rolled corn, warm season grass hay, and supplements to meet nutritional needs at an average of 6.0 lb/head once daily on a dry-matter basis throughout the duration of the study. Crude protein was calculated to be 14.8% and total net energy was 1.48 Mcal/lb.

Outcome measurements

Performance

Calves were weighed at arrival (day -2), baseline (day -1), 7-, 14-, 21-, and 28-days post-castration (Figure 4.1) at a stationary scale and chute located near the pens. The accuracy of the scale was assessed using verified weights that exceeded calf BWs by up to 2% before each BW collection. Performance outcomes included both overall and weekly ADG.

Lying and standing activity

Lying and standing activity was recorded using three-axis accelerometers (Hobo Pendant ® G Data logger, Onset Computer Corp., Bourne, MA) that were attached to the right-hind leg (metatarsophalangeal joint) on day -2 using cohesive bandage (Cohesiant™ Wrap, AmerisourceBergen, Conshohocken, PA) and a small amount of tag cement (Kamar Adhesive, Kamar Products, Inc., Zionsville, IN). The accelerometers logged the axis position once every 60 seconds from 12:00 A.M. on day -1 through the 28-day post-castration sampling period. However, lying and standing data from days -1, 0, 7, 14, 21, and 28 were excluded from the analysis because other outcomes were collected that may have affected the natural lying and standing outcomes. The axis position data were converted into a binomial (lying or standing) using a previously validated protocol,[21] which was then summarized as total minutes lying and standing, the number of lying and standing bouts per day, and the average duration of lying and standing bouts per day. A bout was defined as continuously exhibiting the same activity (either lying or standing) for at least 1 minute.

Kinetic gait analysis

A floor-mat-based pressure/gait assessment system (Walkway, Tekscan, Inc., Norwood, MA) was used to evaluate gait prior to castration for baseline measurements (-24 h) and post-

castration on days 0 (8 h), 7, 14, 21, and 28 (Figure 4.1). A minimum of 8 steps, 2 per limb, were collected for each observation. Calves were gently moved across the mat until at least 2 steps per limb were captured, and they maintained a uniform gait. A calm, quiet handling technique was used to move calves across the mat. Calves were slowly approached from the flank with no overt arm movements or noise to guide calves across the mat without physical intervention, aiming to encourage the calves' natural gait. The mat was calibrated prior to use with verified weights by an experienced researcher. Research-grade software (Walkway Research v.7.70-31, Tekscan, Inc.) was used to assess gait outcomes, including stance time, stride length, force, pressure, force-time integral (FTI), gait velocity, and gait distance. As previously described,[22] stance time was determined as the duration when the foot was in contact with the ground, while FTI was determined as the impulse, which is calculated as the area under the curve for force vs. time. Gait distance was determined as the distance between the left front and right front limbs. Gait outcomes were analyzed by front limbs (average of right front and left front) and hind limbs (average of left hind and right hind). Gait outcomes associated with a shift in bodyweight (force, pressure, and FTI) were also analyzed as front-minus-hind limbs (front-hind), as this has previously been successful in detecting bodyweight shifts.[6]

Blood collection and plasma cortisol determination

Blood collection for plasma cortisol concentrations was performed at baseline (-24 h) and 2-, 8-, 24-, 48-, 72-h post-castration (Figure 4.1) by jugular venipuncture using an 18-gauge needle (BD Diagnostics, Franklin Lakes, NJ) attached to a 10 mL syringe (Fisher Scientific, Pittsburgh, PA). Blood was immediately transferred to an EDTA (BD Diagnostics) and lithium-heparin (Greiner Bio-One, Monroe, NC) vacutainer tube for plasma cortisol and plasma lidocaine determination, respectively. The blood samples were stored on ice immediately

following collection. The samples were then centrifuged at 1,500 × g for 10 min at 4°C. Plasma samples were transferred to cryovials and stored at -80°C until analysis.

Plasma cortisol concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) as previously described.[11] The intra-assay variation was calculated to be 9.5%, while the inter-assay variation was calculated to be 8.0%.

Behavior through video monitoring

Mounted video cameras (Wyze Cam OG, Wyze Labs, Inc., Kirkland, WA) fastened to the front and back of the pen, above eye level, were used to collect 30-minute videos to assess animal behavior at baseline (-24 h) and day 0 (2 h), 1, 2, 4, 7, 14, 21, and 28 post-castration (Figure 4.1). A total of 20 calves, 5 calves from each group (HOL-LLB, BOD-LLB, HOL-CON, BOD-CON), were randomly selected for subsampling of behavioral outcomes due to time restrictions, given the laborious demands of video monitoring. The same 20 randomly selected calves were observed at all time points. The videos captured animal behavior after the calves returned to their group pen following collection of other measures, with the videos beginning 15 minutes after the last human interaction. The videos were captured at approximately the same time of day (10:00 AM) for each time point. A total of 4 cameras were used, with each camera capturing a quadrant of the pen. Videos from all 4 cameras were synchronized and rendered together, allowing for a complete view of the pen. Since the calves were group-housed, they received an ear tag in each ear, which followed a color scheme that allowed identification by ear tag color. A specialized behavioral scoring software (The Observer XT, Noldus, Leesburg, VA) was used by trained researchers to analyze animal behavior using a detailed, adapted ethogram (Table 4.1). Maintenance behaviors (head over bunk, grooming) and social behaviors (allogrooming, head rubbing) were collected in addition to pain-related behaviors (foot stamping,

look to flank, lick castration site). Cattle have shown to decrease the frequency of maintenance and social play behaviors when painful, potentially to limit further tissue damage and nociceptive stimulation.[23,24] In contrast, foot stamping, attention to flank, and licking castration site have been reported to increase in frequency following castration.[13,25] Maintenance and social behaviors were automatically scored as a duration percentage (time performing behavior divided by total observation time), while pain-related behaviors were automatically scored as counts. Inter- and intra-rater reliability was determined by scoring 2 randomly selected 30-minute videos of calves that were not included in the subsampling. A total of 2 researchers scored the behavior, with inter- and intra-rater agreement exceeding 95% prior to scoring. Inter- and intra-rater reliability was automatically calculated by the behavioral scoring software (The Observer XT).

Statistical analysis

All statistical analyses were executed through RStudio version 2022.02.3 (Posit, Boston, MA). Regarding lying and standing activity data, days -1, 0, 7, 14, 21, and 28 were excluded from the dataset because gait analysis was conducted on these days, which may have altered the calves' natural lying and standing activity. All data were assessed for normality using the Shapiro-Wilk test and a histogram distribution using the "shapiro.test" and "histogram" functions, respectively. Behavior outcomes through video monitoring were collected as percentages and counts; behaviors collected as percentages were arcsine-transformed to achieve normality, while count data were converted into a binomial and analyzed as a probability of occurrence. Regarding behaviors collected as percentages, this was defined as the time spent performing a specific behavior divided by the total time of the observation period. Behaviors collected as counts were categorized as a binary outcome, present or absent, due to low occurrence of many behaviors during the 30-minute observation periods. Generalized linear

mixed-effects models were used ("glmer function of the "lme4" package) with "family = binomial" to test for potential differences in behavioral outcomes that were collected as counts. Linear mixed-effects models were used ("lmer" function of the "lme4" package) to test for potential differences in performance, lying and standing activity, gait analysis, plasma cortisol, and behavioral outcomes collected as percentages. For all models except for overall ADG and final BW, fixed effects included treatment, breed, and time point, while CalfID was included as a random effect to account for repeated measures. For overall ADG and final BW models, time point was not included as a fixed effect, and CalfID was not included as a random effect since there were no repeated measures. In contrast, arrival BW was included as a covariate in the final BW model. Residuals were evaluated for normality using "qqnorm", "qqline", and "histogram" functions. Results for behavioral outcomes, expressed as counts, were reported as the probability of performing the behavior and the standard error of the mean (SE). Results for performance, lying and standing activity, gait analysis, plasma cortisol, and behavioral outcomes, expressed as percentages, were reported as least-squares means and SEs with Sidak adjustments to control for the family-wise error rate. The arcsine-transformed means and upper/lower SE bounds for percentage behavior outcomes were back-transformed for interpretation. Significance was set at $P \leq 0.05$.

Results

A total of 40 intact male calves were enrolled in this study; however, 2 calves (1 HOL-LLB and 1 BOD-LLB) were excluded from the analysis due to health issues (lameness and respiratory disease) as determined by a veterinarian before treatment administration. Therefore, a total of 38 calves weighing 183 ± 37 lb (83 ± 17 kg) were used in this study.

Performance

Overall ADG showed no significant differences by treatment group (0.93 and 0.84 kg/day, SE = 0.05; LLB and CON, respectively; $P = 0.55$), breed (0.95 and 0.82 kg/day, SE = 0.05; BOD and HOL, respectively; $P = 0.38$), or their interaction ($P = 0.69$). There was no evidence of an effect of treatment, breed, or their interaction on weekly ADG ($P > 0.05$). Weekly ADG differed by time point, with ADG increasing significantly over time (1.08, 1.52, and 3.83 lb/day; SE = 0.13; week 1, weeks 2 and 3, and week 4, respectively; $P < 0.01$), regardless of treatment. Final BW showed no significant differences by treatment group (99.9 and 97.5 kg, SE = 1.16; LLB and CON, respectively; $P = 0.58$), breed (101.0 and 96.0 kg, SE = 1.13; BOD and HOL, respectively; $P = 0.07$), or their interaction ($P = 0.46$).

Lying and standing activity

Lying and standing activity outcomes revealed a significant breed effect for the number of standing bouts per day, with BOD exhibiting a greater number of standing bouts per day than HOL (18.3 and 17.3 stand bouts/day, respectively; SE = 0.49; P = 0.03; Table 4.2). There were no significant breed or treatment effects for any other lying or standing activity outcomes, including total lying and standing time per day, number of lying bouts per day, and average lying and standing bout duration per day ($P > 0.05$).

Kinetic gait analysis

Gait analysis outcomes, represented as differences from baseline, showed several significant differences by treatment, breed, time point, and their interactions ($P < 0.05$; Table 4.3). Front stance time showed a significant treatment × breed × time point interaction, with the BOD-LLB group placing their forelimbs on the mat for a shorter duration per stride compared to the BOD-CON group at 8 h post-castration (-0.13 vs 0.13 s, respectively; SE = 0.06; $P = 0.01$;

Figure 4.2). Hind FTI was shown to be higher in the CON group when compared to the LLB group (0.64 and 0.15 kg/s, respectively; SE = 0.74; $P = 0.03$). Gait velocity showed a significant treatment × time point interaction, with the CON group exhibiting an increased gait velocity from day 0 to day 7 (-9.76 vs. 13.49 cm/s, respectively; SE = 7.52; $P < 0.01$; Figure 4.3) before returning to near baseline levels for the remainder of the 28-day sampling period. Gait distance also presented a significant treatment × time point interaction, with the CON group increasing gait distance from day 0 (8 h post-castration) to day 28 (-12.49 vs. 1.60 cm, respectively; SE = 5.29; $P < 0.01$; Figure 4.4). In contrast, gait distance for the LLB group did not appear to differ significantly across time points ($P > 0.05$), with the largest deviation from baseline being only -3.89 cm at 21 days post-castration. No other gait outcomes showed a significant effect of treatment, breed, or their interaction ($P > 0.05$).

Plasma cortisol

Plasma cortisol concentrations were not shown to have any effects of treatment (7369 and 6644 pg/mL, SE = 550; LLB and CON, respectively; $P = 0.67$), breed (6643 and 7371 pg/mL, SE = 536; BOD and HOL, respectively; $P = 0.76$), or its interaction (6520, 6766, 8219, and 6521 pg/mL, SE = 778; BOD-LLB, BOD-CON, HOL-LLB, and HOL-CON, respectively; $P = 0.42$). However, plasma cortisol differed significantly by time point. Regardless of treatment, a spike in plasma cortisol was observed at the first post-castration time point (2 h), which was significantly higher than baseline (11620 vs. 6823 pg/mL; SE = 699; $P < 0.01$), before gradually falling over time.

Behavior through video monitoring

Behavioral outcomes expressed as percentages, including time spent at the bunk, grooming, and allogrooming, did not show significant effects of treatment, breed, time point, or

their interactions ($P > 0.05$). Additionally, neither of those factors nor their interaction affected behavioral outcomes expressed as probabilities of occurrence, including foot stamping, looking to flank, and licking the castration site ($P > 0.05$).

Discussion

Band castration causes both acute and chronic pain, which may result in greater overall reduction in calf welfare compared to other common castration methods, such as surgical castration.[26,27] Nevertheless, surgical castration is commonly perceived as more painful by human observers compared to banding, as it produces more intense pain-related behaviors (e.g., kicking, vocalization) at the time of castration than banding.[7] A castration band loaded with slow-releasing lidocaine (LLB) was developed to mitigate both acute and chronic pain caused by banding. With the United States experiencing an influx of beef-dairy cross calves, it is pertinent to understand how these calves exhibit pain-related behaviors to effectively assess and manage pain. The objective of this study was to determine if the use of lidocaine-loaded bands to castrate two different cattle breeds (Holstein and beef-dairy cross) provides extended pain mitigation, assessed by pain biomarkers and animal behavior over a 28-day post-castration sampling period.

No significant differences in performance measures, including overall ADG, weekly ADG, and final BW, were detected by treatment group or breed. It was expected to see higher performance measures in the BOD group, as beef cattle generally exhibit greater ADG and feed efficiency than dairy cattle.[28] Thus, a lack of significant performance differences by breed and potentially by treatment group may be due to a relatively short feeding period (28 days) or to the study population exhibiting naturally high variation in growth, as suggested in a previous study on young calves.[10]

The BOD group was observed to perform more standing bouts per day than the HOL group, suggesting that the BOD group transitioned between lying and standing more often. While the pain from band castration may have altered standing-bout behaviors between the BOD and HOL groups, this difference could also be due to natural differences between breeds. As such, the BOD group may have exhibited increased standing bouts due to a greater frequency of bunk visits or general interactions within the pen (i.e., more curious) compared to the HOL group. Regardless, the number of standing bouts, although statistically different between breeds, only differed by 1 per day. Unlike the present study, a recent study found that around 20-days post-castration, LLB-castrated calves spent less time standing per bout compared to calves castrated with a standard band.[11] In the same study, this coincided with the period that the band began to cut into the scrotal tissue, causing wound granulation. This change in lying and standing behavior could be an indication that the calves were hesitant to change positions, potentially to avoid further stimulation of the affected area. One potential reason that these results were not observed in the present study could be differences in housing. In the aforementioned study, calves were housed individually, whereas all calves were group-housed in one large pen in the present study. Previous research has demonstrated that group-housed cattle tend to synchronize lying behaviors, which may have influenced the lack of standing and lying differences between treatment groups in the present study.[29,30] Regarding behavior assessed through video monitoring, there were no observed behavioral differences between treatment groups or breeds, which was likely limited by the short 30-minute observation periods that were recorded at approximately 10:00 AM, when most calves were feeding at the bunk. The results of lying and standing behavior in addition to behavior assessed through video monitoring indicate little difference in behavioral pain responses between beef-dairy and Holstein calves.

Gait analysis revealed decreased front-stance time in the BOD-LLB group compared with the BOD-CON group at 8 h post-castration. This study appears to be the first to assess kinetic gait analysis with a pressure mat system following band castration. Past studies have used a pressure mat system to evaluate gait following surgical castration.[6,31] One of those studies found that analyzing gait outcomes as front minus hind (front-hind) was successful in detecting shifts in body weight, however, front-hind outcomes were statistically insignificant in the present study.[6] This may indicate that band castration may not cause a detectable shift in body weight. A different study found that surgical castration without pain management decreased front stance time compared with calves that were surgically castrated and given a nonsteroidal anti-inflammatory drug, suggesting that decreased front stance time after castration could indicate pain,[31] which does not correspond to the findings in the present study. This may suggest that band- and surgically castrated calves walk differently after castration, and that comparison of gait outcomes between the 2 methods may not be appropriate in future research. Surgical castration immediately results in acute tissue trauma and an open wound, whereas band castration results in ischemia and necrosis of the scrotum caused by prolonged constriction of the band. These fundamental differences between castration methods could influence gait outcomes differently as calves compensate for 2 potentially distinct pain experiences. Nevertheless, in the present study, the BOD-LBB and BOD-CON groups both deviated (in opposite directions) from baseline front stance time values at 8 h post-castration. The BOD-CON group may have exhibited greater front stance time at 8 h post-castration to compensate for advancing the hind legs more slowly, potentially to minimize further stimulation at the castration site. Further research may be warranted to elucidate the implications of front stance time following band castration.

Hind FTI increased in the CON group compared with the LLB group, but this difference did not withstand post hoc testing and should not be considered meaningful. Regarding gait velocity, a significant increase was observed in the CON group from 8 h to 7 days post-castration. A previous study reported an increase in gait velocity after surgical castration, suggesting that increased velocity may indicate pain.[31] Additionally, the same study reported a decrease in gait distance following surgical castration, noting that decreased gait distance may be suggestive of pain.[31] In the present study, gait distance in the CON group was shown to be lower 8 h post-castration when compared to the 28-day time point. It is noteworthy that the CON group changed their gait over time, while the LLB group did not; however, the LLB and CON groups did not differ from one another for any gait outcome at any time point.

No significant treatment or breed effects were observed for plasma cortisol concentrations. This is analogous to a recent study that found no treatment differences between calves that were castrated with either a lidocaine-loaded band or a standard band.[11] Further aligning with our findings, a study comparing band castration with or without an injection of lidocaine reported no significant differences in plasma cortisol concentrations within 2.5 hours following castration,[32] which surpasses the duration of action of a lidocaine injection in cattle.[33] Concerning breed, at no point within the 72 h post-castration blood sampling period did cortisol appear to differ by breed, suggesting that breed (either beef-dairy or Holstein) did not affect the level of acute stress caused by band castration. Regardless of treatment group, plasma cortisol was significantly elevated at the first post-castration time point (2 h) compared to pre-castration baselines. Several previous studies report an increase in plasma cortisol shortly after sham castration, suggesting that the cortisol spike observed at 2 h post-castration in the present study

may be partly attributable to handling and manipulation in the chute, in addition to the discomfort of castration.[9,31,34]

Group housing was noted as a limitation of this study, which may have limited the ability to detect behavioral differences assessed by both accelerometers and video monitoring. Randomly subsampling 5 calves from each treatment group for behavior through video monitoring may have limited the ability to detect significant differences between treatment groups; therefore, a small sample size is also a limitation for this outcome. Additionally, 30-minute behavior observation periods may have been too short to detect treatment or breed differences. Most behaviors were performed too infrequently to allow statistical comparison of count data, which resulted in the transformation of count data into binary outcomes of "behavior present" or "behavior absent". A lack of a negative control group (i.e., a group that was not castrated) was also a limitation of the study, particularly for the kinetic gait analysis and plasma cortisol outcomes. Without a negative control group, we were unable to differentiate true castration effects from potential confounders, such as changes in cortisol or gait that may have been caused by handling or other environmental factors rather than castration. Lastly, a relatively short sampling period of 28 days was considered a limitation of this study, considering that banded calves are thought to experience chronic pain until the scrotum sloughs. By day 28, only 2 calves had sloughed their scrotum, therefore potentially missing opportunities to detect differences in behavioral pain responses.

Conclusions

This study found little difference in behavioral pain responses between beef and dairy calves and Holstein calves during band castration. While the number of standing bouts per day differed statistically between the breeds, the BOD group showed only 1 more standing bout per

day than the HOL group. No other behavioral or physiological differences were observed between beef-dairy and Holstein calves. Regarding band type, kinetic gait analysis showed that the CON group significantly altered their gait over time, whereas the LLB group did not; however, gait outcomes did not differ between the LLB and CON groups at any time point. This study found no evidence that castration with an LLB provides pain relief up to 28 days post-castration compared to a standard band; however, kinetic gait analysis outcomes and their implications on pain associated with band castration may warrant further investigation in order to objectively quantify pain in cattle.

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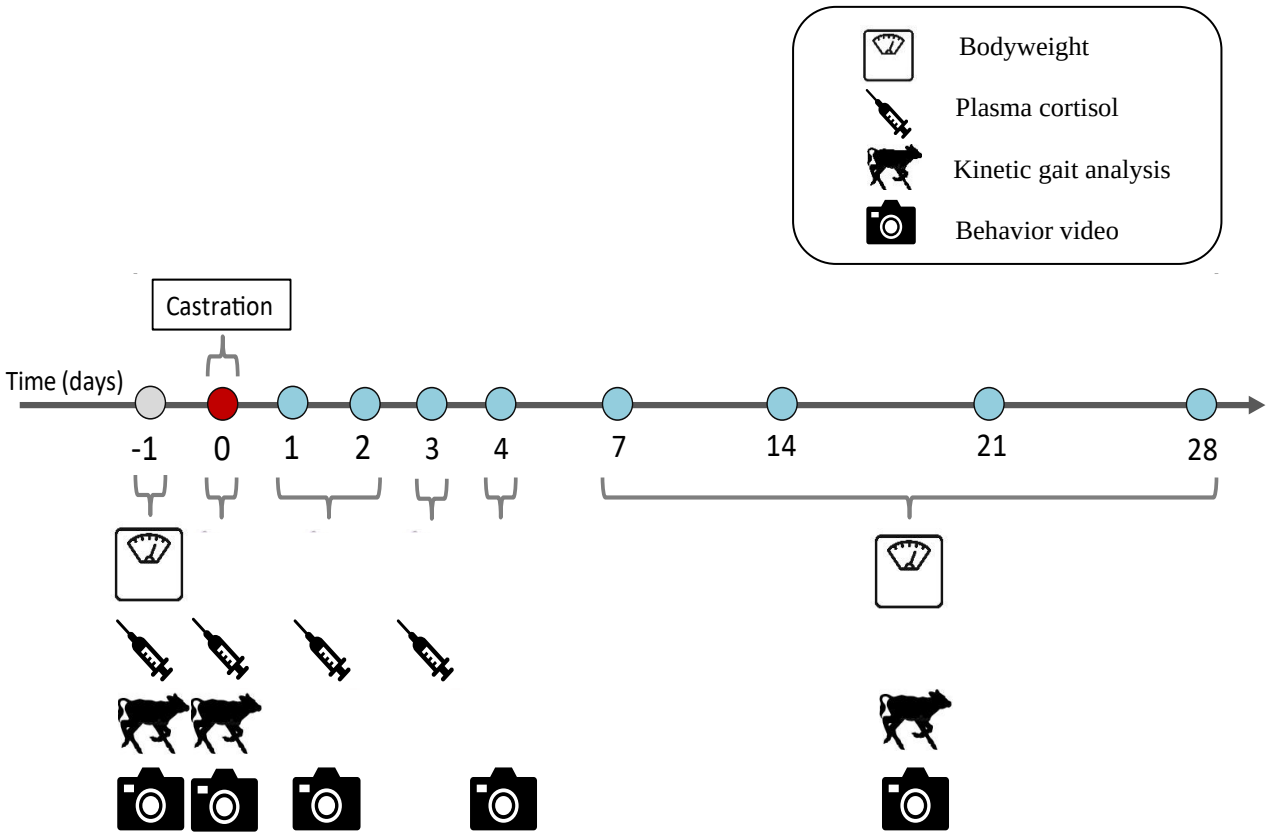


Figure 4.1 Timeline of pain and performance outcomes collected on beef-dairy (BOD) and Holstein (HOL) calves that were castrated using either a lidocaine-loaded band (HOL-LLB; n = 9, BOD-LLB; n = 9) or standard band with no pain management (HOL-CON; n = 10, BOD-CON; n = 10). The outcomes were collected sequentially from top (collected first) to bottom (collected last) of the timeline within each day. Lying and standing behavior outcomes (not pictured) were collected every 60 seconds for the entire study through the use of 3-axis accelerometers. On day 0, outcomes were collected 8 hours following castration.

Table 4.1 Adapted ethogram used to assess behavior associated with castration of beef-dairy (BOD) and Holstein (HOL) calves using either a lidocaine-loaded band (HOL-LLB; n = 5, BOD-LLB; n = 5) or standard band with no pain management (HOL-CON; n = 5, BOD-CON; n = 5) through video capture and assessment with a behavioral scoring software (The Observer XT).

Behavior	**Description**
Duration (%)	
Head over bunk	Front shoulders are against bunk with head through bunk cables
Grooming	Scratching or licking hair
Allogrooming	Scratching or licking hair of another calf
Head rubbing	Rubbing or throwing head toward another calf in a playful manner
Count (n)	
Foot stamping	Hind leg is lifted and forcefully placed on the ground or kicked outward while standing
Look to flank	Turns head around toward flank region
Lick castration site	Hind leg is lifted while the head reaches between hind legs to lick castration site

Table 4.2 Estimated marginal means of lie and stand outcomes of beef-dairy (BOD) and Holstein (HOL) and calves castrated with either a lidocaine-loaded band (BOD-LLB; n = 9, HOL-LLB; n = 9) or a standard control band (BOD-CON; n = 10, HOL-CON; n = 10) using three-axis accelerometers (HOBO Pendant G) attached to the right hind leg.

	Treatment			**Breed**			***P*-value**						
Variable	LLB	CON	SE[1]	BOD	HOL	SE[1]	TRT	Breed	Time	TRT[2] × Breed	TRT[2] × Time	Breed × Time	TRT[2] × Breed × Time
Stand time (min/day)	559	570	7.58	575	554	7.40	0.59	0.49	<0.01	0.91	0.95	0.99	0.98
Lie time (min/day)	873	862	7.59	857	879	7.41	0.52	0.40	<0.01	0.99	0.94	0.99	0.98
Stand bouts (n/day)	18.3	17.3	0.49	18.3	17.2	0.48	0.07	0.03	<0.01	0.07	0.88	0.71	0.50
Lie bouts (n/day)	14.6	13.5	0.61	14.7	13.5	0.59	0.48	0.46	<0.01	0.73	0.39	0.92	0.71
Stand bout time (min/bout)	32.7	35.6	1.11	33.6	34.6	1.09	0.46	0.30	<0.01	0.30	0.79	0.98	0.85
Lie bout time (min/bout)	65.0	70.6	2.22	64.5	71.2	2.16	0.28	0.25	<0.01	0.45	0.84	0.32	0.42

[1]SE, standard error; largest SE was reported

[2]TRT, treatment effect

Table 4.3 Model estimated marginal means of kinetic gait analysis outcomes, represented as difference from baseline, of beef-dairy (BOD) and Holstein (HOL) and calves castrated with either a lidocaine-loaded band (BOD-LLB; n = 9, HOL-LLB; n = 9) or a standard control band (BOD-CON; n = 10, HOL-CON; n = 10) using a mat-based pressure system (Walkway).

	Treatment			Breed			*P*-value						
Variable	LLB	CON	SE[1]	BOD	HOL	SE[1]	TRT	Breed	Time	TRT[2] × Breed	TRT[2] × Time	Breed × Time	TRT[2] × Breed × Time
Front stance time (s)	-0.01	0.03	0.03	-0.01	0.03	0.03	<0.01	0.07	<0.01	<0.01	<0.01	0.08	0.01
Front stride length (cm)	0.96	2.93	1.57	4.96	-1.05	1.54	0.25	0.32	0.73	0.48	0.16	0.81	0.67
Front force (kg)	2.82	2.57	1.28	3.15	2.24	1.26	0.46	0.54	0.15	0.71	0.08	0.84	0.24
Front pressure (kg/cm^2)	0.05	0.02	0.09	0.06	0.01	0.09	0.66	0.90	0.01	0.76	0.38	0.08	0.10
Front FTI[3] (kg/s)	2.15	1.74	0.87	2.06	1.83	0.85	0.23	0.70	<0.01	0.19	0.051	0.26	0.10
Hind stance time (s)	0.00	-0.01	0.03	-0.02	0.02	0.03	0.06	0.06	0.03	0.06	0.06	0.06	0.09
Hind stride length (cm)	-2.91	0.98	1.69	0.59	-2.52	1.66	0.74	0.81	0.12	0.66	0.06	0.49	0.31
Hind force (kg)	-0.03	0.37	1.50	0.36	-0.03	1.47	0.88	0.86	0.10	0.99	0.48	0.80	0.44
Hind pressure (kg/cm^2)	-0.06	0.08	0.09	-0.04	0.06	0.09	0.13	0.07	0.02	0.30	0.15	0.37	0.85
Hind FTI[3] (kg/s)	0.15	0.63	0.74	-0.14	0.93	0.72	0.02	0.07	0.01	0.06	0.33	0.31	0.68
Gait distance (cm)	-0.68	-3.50	4.74	5.26	-9.45	4.65	0.09	0.13	0.23	0.48	<0.01	0.40	0.13
Gait velocity (cm/s)	-6.26	-1.75	6.68	-2.14	-5.87	6.55	0.20	0.95	0.13	0.34	<0.01	0.54	0.13
Front-hind Force (kg)	2.85	2.20	1.59	2.79	2.26	1.56	0.62	0.50	0.94	0.75	0.51	0.95	0.83
Front-hind Pressure (kg/cm^2)	0.12	-0.05	0.11	0.11	-0.04	0.11	0.12	0.17	0.42	0.54	0.81	0.75	0.78
Front-hind FTI (kg/s)	1.99	1.11	0.63	2.19	0.90	0.62	0.33	0.12	0.85	0.68	0.69	0.64	0.56

[1]SE, standard error, largest SE was reported

[2]TRT, treatment effect

[3]FTI, force-time integral

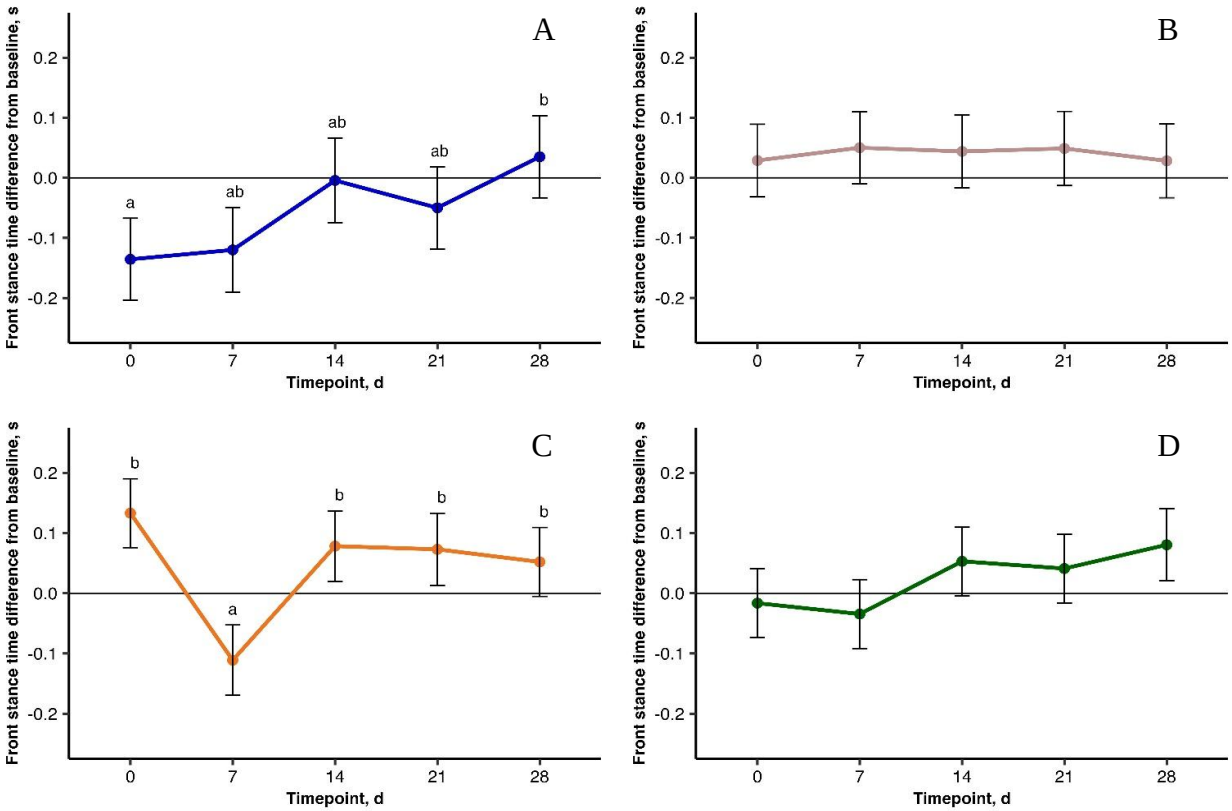


Figure 4.2 Average front stance time (±SE; s) for each breed-treatment combination and represented as a difference from baseline. Pairwise comparisons were performed within each breed-treatment combination across all time points. Kinetic gait analysis was collected from beef-dairy (BOD) and Holstein (HOL) and calves castrated with either a lidocaine-loaded band (BOD-LLB; n = 9; A, HOL-LLB; n = 9; B) or a standard control band (BOD-CON; n = 10; C, HOL-CON; n = 10; D) using a mat-based pressure system (Walkway). Time point represents day relative to castration. Data from the day 0 time point was collected 8 hours following castration. [a-b] LS means differ ($P \leq 0.05$; Sidak adjustment).

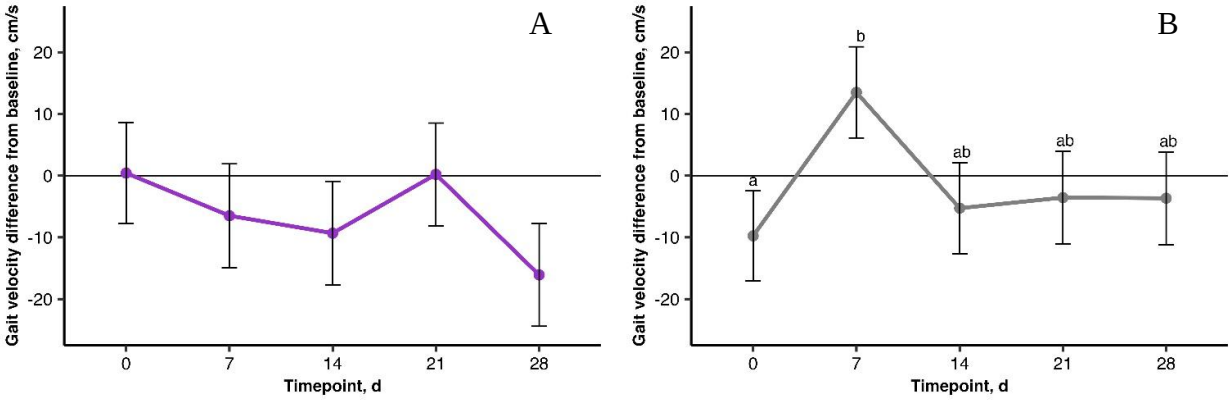


Figure 4.3 Average gait velocity (±SE; cm/s) for each treatment group and represented as a difference from baseline. Pairwise comparisons were performed within each treatment group across all time points. Kinetic gait analysis was collected from beef-dairy and Holstein calves castrated with either a lidocaine-loaded band (LLB; n = 18, A) or a standard control band (CON; n = 20, B) using a mat-based pressure system (Walkway). Time point represents day relative to castration. Data from the day 0 time point was collected 8 hours following castration. [a-b] LS means differ ($P \leq 0.05$; Sidak adjustment).

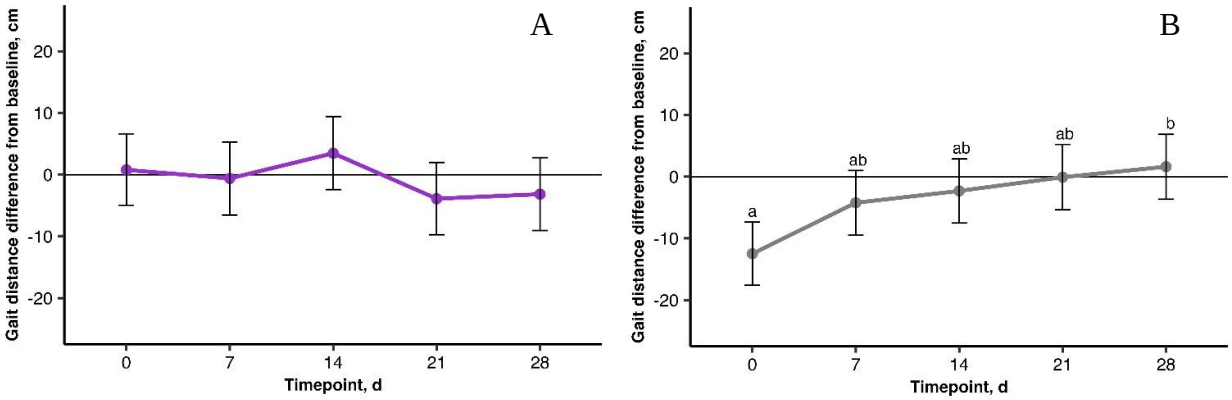


Figure 4.4 Average gait distance (±SE; cm) for each treatment group, represented as a difference from baseline. Pairwise comparisons were performed within each treatment group across all time points. Kinetic gait analysis was collected from beef-dairy and Holstein calves castrated with either a lidocaine-loaded band (LLB; n = 18, A) or a standard control band (CON; n = 20, B) using a mat-based pressure system (Walkway). Time point represents day relative to castration. Data from the day 0 time point was collected 8 hours following castration. [a-b] LS means differ ($P \leq 0.05$; Sidak adjustment).

Chapter 5 - Automated facial analysis for pain detection in feedyard cattle using deep learning video-based models

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Abstract

Timely identification and treatment of pain is essential for maintaining positive animal welfare. Human observers struggle to accurately identify painful cattle, although advancements in machine learning (ML) have allowed for the development of image and video-based models that may automatically identify painful animals. The objectives of this study were to evaluate the performance of 2 video-based convolutional neural network (CNN) architectures using short videos of the face for automated pain detection in beef cattle, and to determine the optimal view of the face by comparing model performance between lateral and front views of cattle faces. A total of 247 short videos of cattle faces, ranging between 1 to 2 seconds, were classified as Pain (n = 143) and No Pain (n = 104) based on castration performed at feedyard arrival. Two CNN architectures, MobileNetV2 and ResNet50, were used to predict pain status among 3 datasets: combined view (front and lateral view videos of the face), front view only, and lateral view only. Model performance was evaluated using area under the receiver operating characteristic curve (ROC-AUC), accuracy, precision, recall, and specificity. MobileNetV2 paired with the front view dataset achieved the greatest ROC-AUC, indicating moderate discriminative ability. MobileNetV2 with the front view dataset also achieved the greatest precision of 87.5% while

maintaining a recall of 58.3%. Overall, results suggest feasibility of accurate pain detection in feedyard cattle using facial analysis through ML, and that front view videos of cattle faces may provide more information on pain status compared to lateral view videos.

Introduction

Cattle are prey animals that mask their pain in an attempt to avoid predation, making it difficult for human observers to accurately assess cattle pain status (Carbone, 2020). Common sources of pain or discomfort in cattle include lameness, mastitis, and respiratory disease, along with routine management procedures such as castration, disbudding, and branding. Untreated pain from these sources has deleterious effects on animal welfare and contributes to a negative public perception of the industry (Ammann et al., 2023; Coetzee, 2013). Timely and accurate detection of pain is needed to properly treat affected animals and maintain positive animal welfare.

In recent years, an influx of research has been conducted regarding the association between pain and facial expressions, leading to the development of facial grimace scales for several species (Dalla Costa et al., 2014; Farghal et al., 2024; Langford et al., 2010; Viscardi et al., 2017). In 2010, a facial grimace scale for mice was developed which aided in pain recognition by scoring changes in several facial landmarks including the nose, cheeks, ears, and eyes (Langford et al., 2010). A beef cattle grimace scale was recently developed, suggesting that cattle may express pain through subtle involuntary facial expressions despite being generally stoic (Farghal et al., 2024). However, scoring pain using a grimace scale, particularly for cattle, is time consuming, subjective, and requires extensive training due to subtle changes in facial expression that are difficult to evaluate. This makes it unlikely that cattle operations in the United States would implement a grimace scale for pain detection. An automated pain

recognition system using facial analysis would require minimal additional labor to detect pain and could potentially be deployed in operations of all sizes.

Advancements in artificial intelligence (AI), specifically machine learning (ML), have allowed for the development of automated pain detection models (Fang et al., 2025). The face is a promising landmark for pain detection because it may reflect pain from a variety of conditions and sources. Consequently, a robust image- or video-based ML model using cattle faces could be effective for pain detection from a variety of pain sources (Mogil et al., 2020; Mota-Rojas et al., 2025).

Video-based models using convolutional neural networks (CNN) may have an advantage over single image-based models by capturing temporal changes (Zhang et al., 2025). Automated video-based facial analysis for pain detection has been researched previously in multiple species with promising results (Chiavaccini et al., 2024; Feighelstein et al., 2023, 2022; Tuttle et al., 2018; Zhang et al., 2025). No published data exist regarding automated facial analysis for pain detection in a cattle feedyard environment. Data are needed to understand the feasibility of an automated system to detect pain in beef cattle in a commercial environment.

Little published data are available regarding the optimal face view for imaging or recording. One previous study using image-based models compared performance between frontal and lateral view datasets in sheep, finding frontal view images to have marginally better performance than lateral view images (Feighelstein et al., 2025). The eye and cheek regions are both important landmarks for pain detection as described by the Calf Grimace Scale, which are observed most clearly by lateral view frames (Farghal et al., 2024). Frames captured with a lateral view of the face may provide more information on pain status compared to front view

frames. Data are needed to establish the most appropriate face view for capturing images intended for automated pain detection in cattle.

The objectives of this study were two-fold; 1) evaluate the performance of 2 different video-based CNN architectures using short videos of the face for automated pain detection in beef cattle, and 2) determine the optimal view of the face by comparing model performance between lateral and front view videos of cattle faces. We hypothesize that both CNN architectures will perform moderately well at detecting pain from short videos of cattle faces, and models with lateral view of cattle faces will perform better than front view models.

Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University prior to study initiation (IACUC #5565). No animals were castrated solely for the purpose of this study.

Animals and intervention

This study was conducted at a collaborating feedyard in eastern Kansas using beef cattle. A total of 9 cohorts were evaluated, with each cohort comprised of both bulls and steers upon feedyard arrival. A cohort was defined as a group of cattle from the same origin that arrived at the feedyard on the same truck, and were housed in the same pen at the feedyard. Cohorts were procured from operations in the central and southeastern United States. Bulls were surgically castrated upon feedyard arrival, which was considered the painful intervention in this study. Bulls at arrival were classified as “Pain” and steers at arrival were classified as “No Pain” based on whether surgical castration was performed at the time of processing, or not, respectively. No anesthetics or analgesics were used during castration, which is standard for this feedyard. This study did not influence any of the feedyard’s management decisions, including cattle

procurement and decisions on the use of pain management. All animals also received a metaphylactic dose of tulathromycin in addition to bovine respiratory disease vaccine, oral dewormer, and a unique identification tag in the left ear during processing. No other painful procedures were performed on any of the animals observed during this study. Animals with visible ailments such as lameness, wounds, and pinkeye were excluded from observation due to potential confounding with pain status. Steers were evaluated for wounds in the scrotal area, and any steer that had been recently castrated as indicated by an open wound or granulation tissue in the scrotal area, was excluded from observation due to potential confounding with pain status. The animals' identification number, sex, weight, and potential ailments were recorded at the time of processing. Each cohort of cattle arrived on the same truck, and all animals were processed in a squeeze chute on the day of arrival before being sent to the same pen for video recording.

Videos were recorded across 9 different days from early February to late May 2026, as videos were recorded on one day per cohort. Castrations began no earlier than 13:00 and all videos were recorded between 14:30 and 18:30 on the same day. Four video cameras (GoPro HERO12 Black, GoPro, Inc., San Mateo, CA) mounted to the pen near the water tank recorded video at 30 frames per second for 3 hours following the processing of the last animal. No humans were in or near the pens while videos were being recorded as to not influence the cattle behavior. Short video clips (between 1 and 2 seconds) of cattle faces were extracted from the raw videos using a video editing software (DaVinci Resolve version 20, Blackmagic Design, Milpitas, CA). Clips were used in the dataset based on the following requirements: 1) animals must be within approximately 3 m of the camera, 2) must have a clear and complete view of the face for the duration of the clip, 3) animal must exhibit minimal head movement with no change in face view (i.e., change from front to lateral view), and 4) animal identification (ear tag) must be clearly

visible. For animals that had multiple clips that met requirements, the first clip that met requirements was included in the dataset to limit observer bias. Clips between 1 and 2 seconds were used to potentially capture temporal changes in facial expression while improving the chances of collecting a sufficient number of clips that meet requirements for use. Short temporal changes in facial expression, deemed micro-expressions, occur within a fraction of a second in horses, and therefore could potentially be captured by a 1 second clip (Tomberg et al., 2023). The background was cropped out of all clips to keep the face of only one animal in the frame, as the raw video often contained multiple cattle in frame (Figure 5.1). All video clips represented a unique animal as verified by their identification tags.

Model training

A deep learning approach was used to classify Pain cattle from No Pain cattle, with ground truths being established by recent castration status; cattle classified as Pain were bulls at feedyard arrival and subsequently surgically castrated, whereas No Pain cattle were steers at arrival, and therefore, castration was not performed. Two CNN architectures, MobileNetV2 and ResNet50, were utilized using ImageNet weights and paired with a 128 unit long short-term memory (LSTM) layer to model temporal information across frames. All models were executed in Python using TensorFlow, NumPy, OpenCV, scikit-learn, and Matplotlib libraries.

Video clips were also categorized by camera view, either front or lateral view of the face, in order to compare model performance between the views. Face color of the cattle was also recorded into three categories: black, red, and white, in order to visualize the proportion of different phenotypes between pain classes, which may bias the models if vastly different.

Up to 60 frames were extracted from each video clip, corresponding to a maximum clip duration of 2 seconds. For clips that contained less than 60 frames, additional blank frames were included

to maintain consistent clip lengths. A masking procedure was applied so that the LSTM excluded the blank frames from analysis. Extracted frames were resized to 224×224 pixels and standardized to a scale between 0 and 1 before model training. Random augmentation was applied to each frame during training but was consistent across all frames in the clip. A sigmoid output layer generated probability of pain at the clip level.

An 80/20 split was used with 80% of data used for training and 20% used for validation with stratified sampling to retain class balance. MobileNetV2 and ResNet50 models were trained using the combined view dataset (front and lateral views), then models were trained using the front view dataset and lateral view dataset separately. All clips were unique animals, with no subject overlap between training and validation sets. Models were trained using the Adam optimizer with a learning rate of 1×10^{-4}, and a batch size of 2 was used with up to 10 epochs. Early stopping was applied based on validation loss using a patience of 3 epochs and the best performing model weights were saved using a checkpoint method.

Model performance was evaluated on the validation dataset using the predicted classification of pain for each clip compared to known outcomes. Area under the curve (AUC) was calculated using receiver operating characteristic (ROC) curves and optimal classification thresholds were determined from the ROC curves by maximizing Youden's J statistic (sensitivity + specificity – 1). Several metrics were calculated to assess model performance including accuracy, precision (positive predictive value), recall (sensitivity), and specificity. Definitions of these metrics are listed in Table 5.1.

Results

Descriptive statistics

There were 981 animals eligible for observation in this study. A total of 250 video clips were rendered that met the criteria for inclusion. Since each video represented a unique animal, a total of 250 animals were used in this study. Three animals died within one week of video recording and were removed from the dataset due to potential confounding with pain status, therefore the final dataset contained 247 video clips of unique animals (Table 5.2). Video clips were categorized by castration status, Pain (n=143) and No Pain (n=104), corresponding to a Pain prevalence of 57.9% and a No Pain prevalence of 42.1%. Ground truth Pain animals weighed 387 ± 60 lb, while ground truth No Pain animals weighed 397 ± 60 lb.

Video clips were also labeled by the camera view of the face, front (n=106) and lateral (n=141). Front camera view represented 42% and 44% of clips in the Pain and No Pain classes, respectively. The proportion of cattle with black, red, and white faces were similar between pain classes (Figure 5.2).

Model Performance

Threshold-independent discriminative ability amongst models is summarized in Table 5.3. Models using the front view dataset achieved the highest ROC-AUC, with MobileNetV2 reaching 0.75. In contrast, MobileNetV2 with the lateral view dataset had the lowest ROC-AUC of 0.51. Model performance metrics using Youden-optimized classification thresholds are also described in Table 5.3. The MobileNetV2 architecture paired with the front view dataset achieved the greatest accuracy of 72.7%, whereas the accuracy of all other CNN-dataset combinations was 55.2 to 64%. The greatest precision (PPV) was also produced by MobileNetV2 with the front view dataset, reaching 87.5%, while achieving the third-best recall

(sensitivity) of all CNN-dataset combinations. This is in contrast to MobileNetV2 with the combined view dataset achieving the greatest recall of 82.8% but producing a precision of 64.9%, which was the lowest precision of all CNN-dataset combinations. Models using the lateral view dataset followed similar trends to the front view dataset, but underperformed in every metric.

Discussion

This study evaluated the predictive capabilities of 2 pre-trained CNN backbones, MobileNetV2 and ResNet50, to classify pain status in feedyard beef cattle from 1 to 2 second video clips recorded within 3 hours of surgical castration. The MobileNetV2 model with the front view dataset reached an AUC of 0.75, indicating moderate discriminatory ability (Swets, 1988). While performance varied across CNN-dataset combinations, results from the MobileNetV2 architecture with the front view dataset indicate that automated facial analysis through ML may be useful for detecting pain in feedyard cattle under natural conditions.

The clinical application of this automated system is to augment the decision to treat animals with pain management drugs, or not, at the individual animal level. If management decisions were made based on this model alone, a false negative would result in a truly painful animal not receiving the pain management that it requires, while a false positive would result in a pain-free animal receiving unnecessary pain management. While misclassifications of any kind are unwanted, false negatives may have more severe implications in this case. The cost of pain management drugs are relatively cheap compared to potential losses in performance that may be caused by pain (Brown et al., 2015), and an abundance of false negatives could lead to a reduction in animal welfare that may negatively affect public perception of the cattle industry. Employing a model with a high recall would allow for greater detection of truly painful animals.

The MobileNetV2 architecture with the combined view dataset showed the highest recall of 82.8%; despite its precision of only 64.9%, this CNN-dataset combination may provide the greatest value to cattle producers and lead to a greater improvement in cattle welfare. In contrast, a model with high precision indicates that a high proportion of animals predicted as Pain are truly painful, which limits false positives (Sokolova and Lapalme, 2009). The performance of the MobileNetV2 architecture with the front view dataset indicated that 87.5% of animals predicted as Pain were truly painful, while 58.3% of truly painful animals were correctly classified as Pain. A model with this combination of high precision and modest recall could still have useful clinical applications by limiting false positives while still detecting pain in over half of truly painful animals. With this approach, it is more likely that treating animals based on positive predictions will alleviate suffering and improve welfare.

The models in this study were evaluated with no subject overlap between training and validation sets. As mentioned in previous literature, this is important to ensure that the model is not learning animal-specific features, which could artificially inflate performance metrics (Feighelstein et al., 2022). The model accuracies presented in this study are consistent with others that evaluated models with no subject overlap between training and validation sets (Andresen et al., 2020; Rashid et al., 2020; Zhang et al., 2025).

We hypothesized that models using the lateral view dataset would perform better than models using the front view dataset. This hypothesis was based on the lateral view better capturing potentially important landmarks such as the eyes and cheeks. However, the findings in this study indicate the front view may provide more information on pain status compared to the lateral view. A previous study reported that models with front view datasets performed slightly better than lateral view datasets in sheep, which is consistent with the results from the present

study (Feighelstein et al., 2025). A different study evaluated beef cattle facial expressions after acute pain, finding the cattle to mainly express pain through the presence of dilated nostrils, open mouth, and raised brows (Müller et al., 2019). All of these expressions can be observed clearly with a front view of the face, with nostril shape being particularly exclusive to the front view. This may explain the increased performance with the front view compared to the lateral view dataset in the present study. The similar proportion of front and lateral views between pain classes indicates that the models were not biased based on camera view alone.

Models using the combined view dataset had lower discriminatory ability for both CNN architectures compared to the front view dataset, despite containing over twice as many clips. One potential explanation is model performance with the combined view dataset was hindered by the poorly-performing lateral view clips, which may have reduced the models' ability to consistently learn painful facial expressions (Chen et al., 2022). Recall and specificity of the combined view datasets was inverse of the front and lateral datasets, which may be a result of an increased diversity of training clips and a larger sample size.

To our knowledge, this study is the first to evaluate the capability of ML to classify pain in beef cattle in a feedyard environment. On standard feedyard operations, cattle will often be housed in a temporary receiving pen upon arrival, which may be a high-risk pen for pain due to recent handling and transportation. An automated pain detection system could be useful for augmenting decisions on whether specific animals should receive pain management, or not. Data collected in this study simulated how cattle could be automatically evaluated for pain upon arrival the feedyard under natural conditions. However, 3 aspects of video collection were constrained: all video was collected between 14:30 and 18:30, and all ground truth Pain cattle were painful from the same source (surgical castration), and the background was removed from

all video clips. Reducing these sources of variation allowed the feasibility of automated pain detection systems to be evaluated under an otherwise uncontrolled environment.

Despite constraining multiple aspects of data collection, the feedyard environment presented some limitations. Harsh natural lighting and excessive cattle movement contributed to the difficulty of collecting quality videos of cattle in a feedyard setting. The natural outdoor lighting often casted shadows on the animals' faces, which likely increased variation across clips and made facial features less distinct. This was especially prominent in black-hided cattle, whose facial features are difficult to visualize even in conditions with controlled lighting (Ginger et al., 2023). This potentially represents a large limitation of facial analysis for pain detection in cattle, as a recent audit reported that 62.3% of fed steers and heifers in the United States were black-hided (Schwartz et al., 2025). Cattle often moved out of frame or turned their heads away from the camera before 2 seconds of quality video could be captured, or simply did not come near the camera, which contributed to video of only 250 cattle being collected out of the 981 eligible. Videos were recorded for 3 hours after processing, which proved to be an insufficient amount of time to capture video of a large proportion of animals in the pen. For clinical use, cameras placed near the waterer could continually surveil the pen which would eventually capture video of all animals when they come to drink.

Conclusions

Results from this study indicate that automated pain detection using facial analysis with ML is feasible for feedyard cattle. The MobileNetV2 architecture with the front view dataset was able to identify over half of truly painful animals while minimizing false positive classifications, which could have useful clinical applications. Models using the front view dataset showed significantly better discriminatory ability compared to the lateral view dataset, indicating that

facial expressions visualized from the front may contain more information on pain status. Lastly, performance between CNN architectures were inconsistent across datasets, highlighting that increased CNN complexity may not translate to increased performance.

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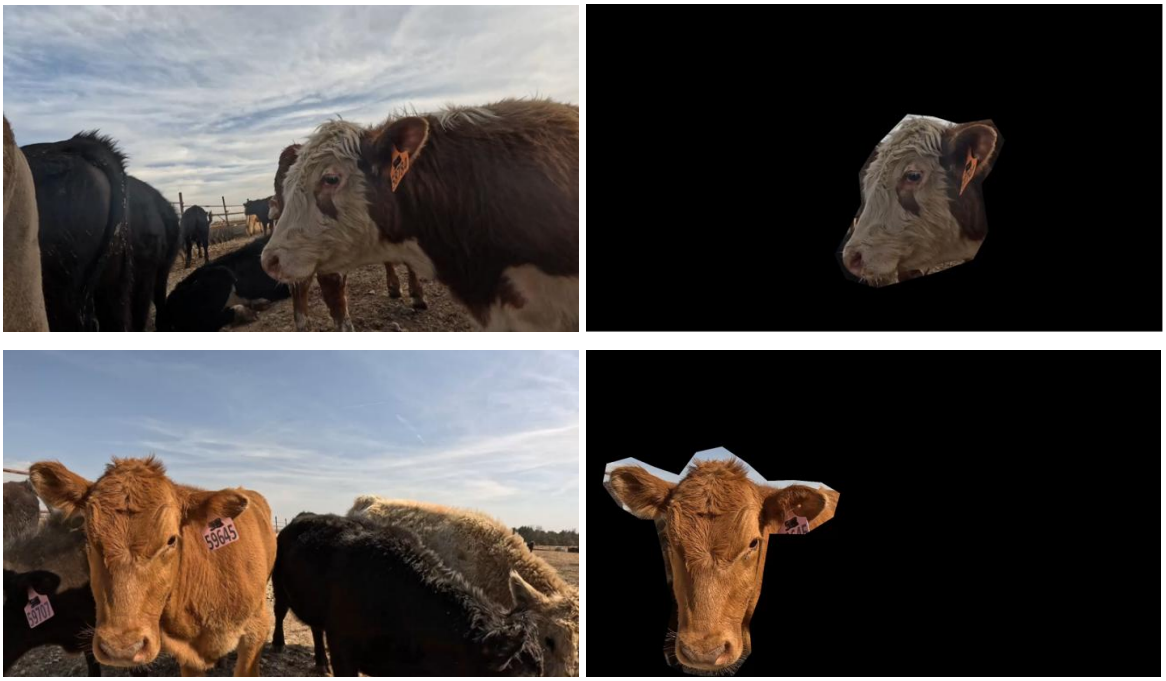


Figure 5.1 Examples of frames extracted from video clips collected by cameras placed near the waterer in a feedyard pen to test the feasibility of an automated pain detection system through machine learning (ML). The top row represents a lateral view, while the bottom row represents a front view before and after background removal.

Table 5.1 Performance metrics and their corresponding definitions that were used to evaluate 6 different convolutional neural network (CNN) and dataset combinations, with datasets consisting of front and lateral views of feedyard cattle faces taken up to 3 hours after surgical castration. Cattle classified as ground truth Pain were subjected to surgical castration shortly before video recording, while ground truth No Pain cattle were not.

Performance Metric	**Definition**
Accuracy	The proportion of all animals that were correctly classified as either Pain or No Pain
Precision (Positive Predictive Value)	The proportion of animals classified as Pain that were truly painful
Recall (Sensitivity)	The proportion of truly painful animals that were correctly classified as Pain
Specificity	The proportion of truly pain-free animals that were correctly classified as No Pain

Table 5.2 Distribution of video clips by camera view of the face, used to test the feasibility of an automated pain detection system through machine learning (ML). Cattle classified as ground truth Pain were subjected to surgical castration shortly before video recording, while ground truth No Pain cattle were not.

Camera View	Pain	No Pain	Total
Front videos (n)	60	46	106
Lateral videos (n)	83	58	141
Total videos (n)	143	104	247

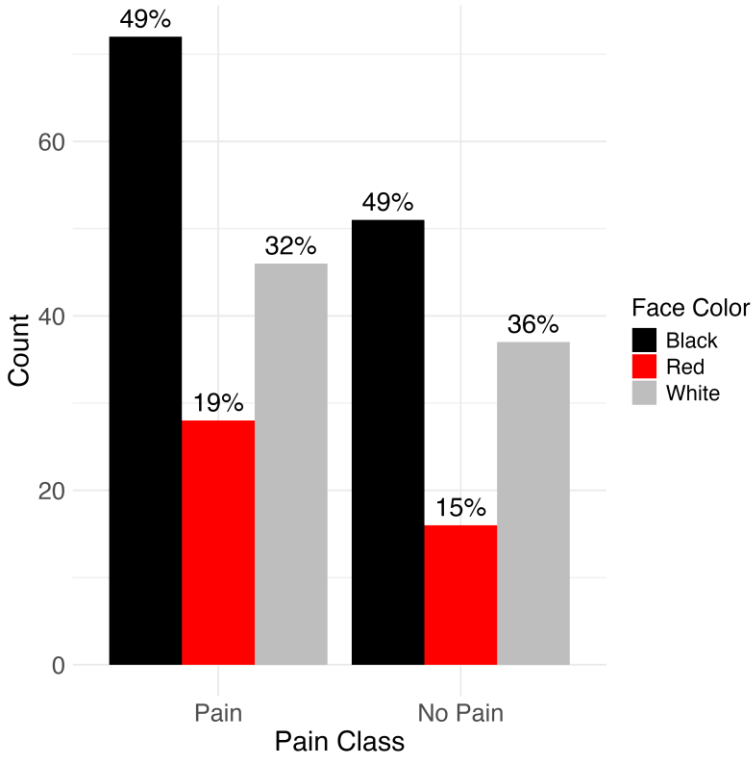


Figure 5.2 Descriptive distribution of animals by face color between pain classes. Video recordings of cattle faces were used to test the feasibility of an automated pain detection system through machine learning (ML). Cattle classified as ground truth Pain were subjected to surgical castration shortly before video recording, while ground truth No Pain cattle were not.

Table 5.3 Threshold-independent discriminative ability and classification performance amongst datasets and convolutional neural networks (CNN) used to test the feasibility of an automated pain detection system through machine learning (ML) in feedyard cattle. Performance metrics were calculated using Youden-optimized classification thresholds.

Model	ROC-AUC	Accuracy (%)	Precision (%)	Recall (%)	Specificity (%)
Combined view					
MobileNetV2	0.527	64.0	64.9	82.8	38.1
ResNet50	0.612	62.0	67.9	65.5	57.1
Front view					
MobileNetV2	0.750	72.7	87.5	58.3	90.0
ResNet50	0.625	63.6	83.3	41.7	90.0
Lateral view					
MobileNetV2	0.510	62.1	75.0	52.9	75.0
ResNet50	0.544	55.2	75.0	35.3	83.3

ROC-AUC = Receiver Operating Characteristic Area Under the Curve