

THESIS

EFFECT OF HOT-IRON BRAND SIZE AND MELOXICAM ON BEHAVIOR, HEALTH,
AND PERFORMANCE OF BEEF CALVES IN A PASTURE SETTING

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ABSTRACT

EFFECT OF HOT-IRON BRAND SIZE AND MELOXICAM ON BEHAVIOR, HEALTH, AND PERFORMANCE OF BEEF CALVES IN A PASTURE SETTING

Animal welfare is of growing importance in beef cattle production and therefore there is heightened interest in regularly practiced, painful management practices. Hot-iron branding is a common method of permanent identification of cattle, created by intense thermal damage to the hide. The main objectives of the study were to quantify the effects of hot-iron brand size and meloxicam on pain related behaviors and health and performance parameters of 2-month-old beef calves on a commercial cow calf operation. A total of 126 calves were enrolled in the study (bulls, n=56; heifers, n=70). Calves were hot-iron branded with one of two iron sizes (small (SM), large (LG)) and administered meloxicam (MEL; dosed for a 113-kg calf at 1mg/kg of bodyweight) or a placebo (PLA)), and castrated (CAS) or not castrated (NCAS), if a heifer calf. Following the treatment application, behavioral observation (2 h periods) using instantaneous scan sampling occurred at 6 h and 24 h post branding; these observation periods (OP) represent the pharmacokinetics of oral meloxicam. A logistic regression (PROC GLIMIX) was used to assess the association between castration status, brand size, medication, observation period, as well as any interactions, on the proportional behavioral outcomes. More LG brand calves were observed standing, lying, and displaying event behaviors as compared to the SM brand calves ($P = 0.003$, $P = 0.011$, $P = 0.016$, respectively). OP affected the display of event and state behaviors, with standing observed more at 6 h and event behaviors displayed more at 24 h ($P = 0.0028$). Concurrently, CAS and SM or LG branded calves exhibited more event behaviors and standing

than NCAS calves ($P = 0.022$). No effect of the fixed variables on average daily gain (ADG) or morbidity were observed ($P > 0.05$). MEL did reduce the proportion of branded heifer calves expressing event behaviors as compared to PLA, however it had less of a behavioral impact on the concurrently castrated and branded calves ($P = 0.022$). The results indicate that pain is present within the 24 h following branding and castration and that concurrent castration and branding elicits a greater behavioral response than only branding. This study highlighted both the feasibility of administering an oral analgesic in a commercial setting and that implementing a smaller brand size may be a strategy to reduce pain associated with hot-iron branding.

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CHAPTER I

LITERATURE REVIEW

INTRODUCTION

Painful animal husbandry procedures like castration, branding, and dehorning are regularly practiced in the United States on cattle in production systems. Castration of bull calves, via removal or damage to the testes, is a standard practice in the United States as it reduces aggression and improves meat quality (Bolado-Sarabia et al., 2018; USDA, 2020b). Branding is still a widely used, traditional method of permanent identification of cattle in the United States that lacks practical alternatives. Physiological and behavioral changes associated with pain and stress are evident in cattle during the process of branding (Lay et al., 1992a; Lay et al., 1992b; Schwartzkopf-Genswein et al., 1997b; Schwartzkopf-Genswein et al., 1997a; Tucker et al., 2014b) and castration (Robertson et al., 1994; Molony et al., 1995; Marti et al. 2019), as well as in the hours and days following. Concurrent castration and branding is a common management practice which has been reported to be more painful than procedures performed individually (Meléndez et al., 2018a; Marti et al., 2019). The pain resulting from these procedures is a considerable animal welfare concern in the United States (Schwartzkopf-Genswein et al., 2012; Bir et al., 2020; Kleinhenz et al., 2021). Addressing the pain resulting from these procedures by administering pain relieving drugs is a topic receiving increasing awareness by food animal organization (e.g., American Association of Bovine Practitioners (AABP) and Farmers Assuring Practical Management (FARM)) and those involved in food animal production in the United States. Though analgesic drugs have been shown

to be effective at mitigating pain-related indicators associated with these procedures they are not regularly administered (Fajt et al., 2011; Moggy et al., 2017; Johnstone et al., 2021). Lack of analgesic use is heavily influenced by availability of approved drugs for pain relief resulting from painful husbandry procedures and by the perceived feasibility of incorporating analgesic administration into commercial, on-farm protocols (Johnstone et al., 2021; Robles et al., 2021).

ANALGESIC USE AS A COMPONENT OF ANIMAL WELFARE

Both branding and castration result in acute pain and studies have found pain can persist from branding (Tucker et al., 2014a; Tucker et al., 2014b) and castration (Mintline et al., 2014) for months and weeks following the procedure, respectively. The lack of pain mitigation to reduce acute pain and potential for chronic pain to develop following these procedures is a significant factor associated with an animal's welfare (Molony and Kent, 1997). The principles of animal welfare for livestock raised and kept in "intensive livestock husbandry systems" was defined in 1965 and became the basis for what is now known today as the Five Freedoms (Brambell and Barbour, 1965). Broadly, these freedoms address the basic mental, physical, and behavioral needs of animals which are to be provided by those in charge of the animal's care. (Webster, 2001). The Five Freedoms highlight the moral and ethical obligation that food animal producers, veterinarians, and industry stakeholders have to consider in regards to practicing good husbandry and addressing an animal's rights (Pulina, 2020). The right to "Freedom from Pain, Injury, and Disease" can be satisfied by prevention of pain or by rapid diagnosing and treatment of an animal (Farm Animal Welfare Council, 2009). Freedom from pain, injury, and disease would emphasize the importance of pain prevention (i.e., procedure alternatives) and pain mitigation when performing procedures

like castration and branding. The pain associated with commonly practiced husbandry procedures, like branding and castration, is receiving more awareness by consumers and they favor products or practices in which the animals received pain mitigation for procedures like castration or dehorning (Wolf et al., 2016; Teixeira et al., 2018; Bir et al., 2020). Pain from castration and branding is recognized as adverse and undesirable by various programs in the food animal production industry, like Beef Quality Assurance (BQA), American Association of Bovine Practitioners (AABP), American Veterinary Medical Association (AVMA), and the Farmers Assuring Responsible Management (FARM) dairy program (AABP, 2019; BQA National Manual, 2019; FARM, 2020; AVMA, n.d.). The FARM Program, is a dairy program that requires pain mitigation be provided to calves undergoing castration, dehorning, and branding (FARM, 2020). Besides the FARM program, the other programs have no requirements as to using analgesics for pain mitigation from painful procedures (FARM, 2020). While castration is widely practiced and is an industry standard in the United States, no regulations concerning pain mitigation exist for the procedure. Castration guidelines emphasize seeking guidance from the herd veterinarian on appropriate pain mitigation protocols (AABP, 2019; BQA National Manual, 2019; AVMA, n.d.) and that those performing castration have adequate training and are competent in the procedure (BQA National Manual, 2019; AVMA, n.d.). As for branding, AABP and AVMA recommend the use of alternative permanent identification techniques, but no statement about analgesic use when branding is made (AVMA, 2011). Current guidelines center around communication between the operation management and the veterinarian on proper procedural techniques and development of pain mitigation protocols tailored to the individual operation. Development of a federally approved drug for the purpose of mitigating pain from painful procedures is incredibly pertinent to the increasing focus on food animal welfare.

BRANDING - PURPOSE AND REQUIREMENTS

Branding is long standing, traditional method of identifying cattle and livestock (Lindegaard and Andersen, 2012). Branding methods include the application of a hot iron, heated by electricity or flame, or contrastingly by subzero, liquid nitrogen freeze burns (USDA, 2020b). These marks provide permanent identification to signify ownership, deter theft, differentiate comingled cattle, improve traceability of cattle for disease purposes, and permit interstate shipping in the United States (USDA-APHIS, 2018). The National Beef Quality Audit (NBQA), a benchmarking study of live animal and carcass quality characteristics of fed cattle entering large packing plants, indicated that 25% of cattle have brands, but this percentage does not account for retained animals (i.e. heifers or cows) on commercial operations (Eastwood et al., 2017). A recent USDA-APHIS survey estimated that 26% of all cow-calf operations in the United States brand their cattle, with this equating to roughly 45% of beef cattle and calves being hot-iron branded (USDA, 2020b). Branding, while not necessary in all production settings, becomes an important visible mark to differentiate producers' cattle which are grazing or sharing a community pasture (Moggy et al., 2017). The greater number of branded cattle in the western United States as compared to the eastern region (NAHMS, 1992) may partly be due to the extensive acreage being grazed in the western states and differences in brand laws.

In the United States, branding requirements exist at federal and state levels and the process of registering a brand is done with strict guidelines for brand characteristics (OLSC, 2016; Federal Registrar, 2018; Oklahoma Cattlemen's Association, 2020). Federally, cattle are required to have brands upon entrance into the United States from Mexico and Canada, and all branding methods are accepted (freeze, hot-iron, chemical) (USDA-APHIS, 2018). Recent legislation, requires cattle

entering the United States from Mexico to be branded with a “M” brand between 7.5 cm and 12.5 cm high and wide, with the brand applied to feeder cattle on the right hip and for breeder cattle the right shoulder (Federal Registrar, 2018). This legislation was passed to increase readability, reduce economic loss (hide damage) associated with rebranding for import, and differentiate intact animals from those that are not. State requirements are more extensive, as most states in the United States regulate the size, location, and application method as part of the registration process, although the general size requirements for brands are a minimum of 7.5 cm in height and width (WSDA, 1999; Oklahoma Cattlemen’s Association, 2020; CDFA-AHFSS, 2023; OLSC, 2016). Most states accept hot iron and freeze branding as valid methods, however not all states treat freeze branding as a valid method of identification (CDFA-AHFSS, 2023). To ensure readability of the brands and avoid smudging or “blotching” of the brand, the state of Washington requires adult cattle to receive brands over 7.5 cm tall, while permitting calves to be branded with irons as small as 5.6 cm, as smaller brands applied to adult animals may not be as effective (WSDA, 1999). More surveys of producers are needed to evaluate the true brand sizes used at branding, as although there is a minimum size requirement there is no apparent brand size maximum. Economically, larger brand sizes equate to greater hide damage but this loss in value may not be experienced by the producer who is branding.

Current brand size and location related data is limited, with data reported solely by the NBQA. As of 2016, average brand sizes of cattle entering packing plants were 173.8 cm² for butt brands, 584.1 cm² for side brands, and 226.6² cm for shoulder brands (Eastwood et al., 2017). The audit also reported brand sizes of fed cattle in 2016 as compared to 2011, to have increased in size on the rib and shoulder by nearly 100 cm² and 26 cm², respectively. This increase in brand size at the time of harvest, likely indicates larger brand sizes being used at branding or significantly larger

brands being used on older cattle (Tolleson and Schafer, 2021). The most common sites to brand pre-weaned calves in the United States, as of 2016, are the butt (hip), side (rib), and shoulder, with the rib being least desirable from an economic standpoint due to hide processing and cutting out of the brand (Eastwood et al., 2017). However, regardless of location of the brand, branded hides return less value than non-branded hides (NAHMS, 1992; Federal Registrar, 2018). No brands are placed on the jaw or cheek in the United States (BQA National Manual, 2019; AZDA, 2022), although this is practiced in other countries (Grobler, 2012; Hernandez et al., 2022).

When evaluating the method, brand iron size, or the actual burn surface of the iron (band width), consideration should be given to the subsequent effects on readability and pain resulting from the healing and brand application. A previous study reported that only a third of horses' brand identification (including all numbers and symbols) were able to be correctly and consistently identified by multiple viewers (Aurich et al., 2013). The differences in readability likely were proposed to be related to application of the brand (i.e. temperature and duration) and physiologic factors associated with healing (Aurich et al., 2013). Brand size also likely contributes to readability, but this was not a variable in the Aurich and others (2013) study. The temperature and duration of application of brands (which may be related to brand iron material) and the impacts on pain, wound healing, and readability are all factors which deserve more research. Other challenges regarding efficacy of the brand as it relates to readability, include seasonal changes in hair coats which can obscure the hot-iron brand scar, with this being less of an issue with freeze branding. While reported as less painful (Lay et al. 1992b; Lay et al., 1992a; Schwartzkopf-Genswein et al., 1997b; Schwartzkopf-Genswein et al., 1997a), the main challenge of freeze brands is that their application is limited by coat color as freeze branding destroys the melanocytes while maintaining the integrity of the hair follicles which in turn causes the hair to grow without color, i.e., this

method is not suitable for cattle with light or white coat colors (Hooven, 1968). Recent research demonstrates that branding elicits an intense immediate, pain associated response in cattle (Schwartzkopf-Genswein et al., 1997b; Schwartzkopf-Genswein et al. 1998), but a current gap in knowledge exists about the impacts of components of branding like location and size on pain-related parameters following branding.

CASTRATION – PURPOSE AND REQUIREMENTS

While components of branding are affected by individual state requirements and require registration, castration is a procedure performed with little to no government oversight in the United States. The majority (62.3%) of bull calves on commercial operations are castrated prior to sale (USDA, 2020b), representing upwards of 10 million calves castrated annually (USDA, 2023). Castration inhibits spermatogenesis through methods including hormonal inhibition, chemical damage (Cohen et al., 1990; Oliveira et al., 2017) or physical damage or removal of the testes (e.g. surgical or knife, banding) (Dockweiler et al., 2013; Roberts et al., 2015; Yamada et al., 2021). The purpose of mitigating spermatogenesis is to improve meat quality, control breeding in feedlots, and reduce unwanted aggressive behaviors toward caretakers and conspecifics (Tucker et al., 2014a; Bolado-Sarabia et al., 2018). In the United States, surgical castration, which is the physical damage and removal of the testes from the scrotum (Newman, 2007), is the most common method used (Coetzee et al., 2010). This method is highly effective and efficient but does result in the most pronounced pain response (Cohen et al., 1990; Meléndez et al., 2017; Yamada et al., 2021). Bloodless castration results in ischemia of the tissue followed by necrosis and loss of the testes and scrotum (Becker et al., 2012). The rubber ring (band) method is a common method of bloodless

castration, and is perceived as less painful by producers than surgical castration (Moggy et al., 2017). Immunological castration, which based on the available literature, is not widely practiced (Marti et al., 2015) is achieved by vaccinating an animal against gonadotropin-releasing hormone (GnRH) which is a necessary hormone in the pathway of testosterone release. This vaccine results in antibodies which can bind and inhibit the efficacy of GnRH, subsequently stopping testosterone production (Thompson, 2000). This method of castration has been reported to be less painful than band castration, as seen by reduced cortisol levels and no significant pain associated behavioral response (Marti et al., 2015).

PAIN - WHAT IS IT AND HOW IS IT EXPRESSED

Pain is complex and is the result of “an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage” (Molony and Kent, 1997; IASP, 2017). Pain involves higher order processing within the central nervous system, particularly the cerebral cortex (Themes, 2016). The cerebral cortex has elevated activity during painful procedures, like, castration (Johnson et al., 2009; Lehmann et al., 2017). Following the presence of painful or aversive stimuli are physiological and behavioral changes that serve to “reduce or avoid [tissue] damage and to reduce the likelihood of recurrence and to promote recovery” (Molony and Kent, 1997). Nociception refers to these rapid neurophysiologic and behavioral events following detection of a noxious painful stimulus (i.e., thermal - branding, mechanical - castration, or chemical) (Armstrong and Herr, 2022). These events involve the sensory and motor peripheral nerve fibers, communicating via local reflex arcs and with more complex central nervous system pathways. The neuromotor response initiated by a noxious or

harmful stimulus is near instantaneous in a healthy individual, which is essential in avoiding further tissue damage or injury (Cervero, 2012; Herskin and Di Giminiani, 2018; Meeks et al., 2015). Withdrawal reflexes and escape-avoidance reactions have previously been used to quantify the protective reactions in painful procedure studies (reviewed by Le Bars et al., 2002), such as studies of branding (Lay et al. 1992a; Lay et al. 1992b), and castration (Meléndez et al., 2018b). Outlasting the immediate nociceptive response, pain is categorized by how long it lasts in relation to the duration of the injury healing. These categories are acute and chronic. Acute pain is characterized by the pain from tissue damage occurring shortly after an injury and not outlasting the healing process (Cervero, 2012; Molony and Kent, 1997). The presence of acute pain can coincide with the inflammatory period, which assists with and protects the healing tissue. During this time behavioral and physiological indicators of pain and stress are present. Chronic pain outlasts the healing process and serves no survival advantage (Herskin and Di Giminiani, 2018). Both the immediate and delayed pain from castration and branding is of concern for the welfare of animals, and this concern is heightened when the pain is not addressed or managed.

PHYSIOLOGIC CHANGES ASSOCIATED WITH PAIN AND STRESS

Pain is a type of stressor associated with potential or actual tissue trauma (IASP, 2017), which can activate the hypothalamic-pituitary-adrenocortical (HPA) axis, and elicit physiological and behavioral changes. Stress does not always include the presence of a noxious or adverse stimulus, which is an important consideration when evaluating the physiological and hormonal responses to a stressor. When a stressor is perceived by an organism, such as the case with handling or isolating livestock or performing a painful husbandry procedure, an involuntary physiological

process involving the HPA axis occurs (P et al., 2007; Patricia Gaete, 2016; Watts and Stookey, 1999). Activation of the HPA axis results in the release of hormones including glucocorticoids and catecholamines from the adrenal cortex and adrenal medulla, respectively. The subsequent effects of cortisol, a glucocorticoid, are to mobilize energy stores and alter the immune system's function (Russell, 2012). The release of cortisol, and catecholamines like epinephrine and norepinephrine occur in the minutes and hours following painful procedures like branding, castration, and dehorning (Lay et al. 1992b; Glynn et al., 2013; Sutherland et al., 2013; Petherick et al., 2015; Meléndez et al., 2018a). The involuntary actions that arise from the stimulation of the autonomic nervous system—specifically its sympathetic branch—include the fight, flight, or freeze responses (Molony and Kent, 1997.) From the activation of the sympathetic nervous system, physical and physiological responses occur, including changes in pupil size, cardiovascular rates and cardiovascular output, peripheral perfusion (i.e. vascular changes), and altered metabolic mechanisms (Molony and Kent, 1997; Stewart et al., 2010; Coetzee et al., 2012; Sutherland et al., 2013). Measuring changes in these variables can be of great benefit in understanding animal responses to painful procedures. Inflammation is a result of painful husbandry procedures and is also associated with pain and irritation of the damaged tissue. For example, inflammatory markers such as neutrophil to lymphocyte ratios (Sutherland et al., 2013), white blood cell counts (Meléndez et al., 2019), wound temperature (Moya et al., 2014), and prostaglandin metabolites (Allen et al., 2013; Martin et al., 2022) have been measured to characterize the inflammatory response associated with painful procedures.

BEHAVIORAL CHANGES ASSOCIATED WITH PAIN

Animal pain is complex and can be measured through not only physiological changes but also changes in behavior and motivational states. Monitoring animals for behavioral changes is a non-invasive method, the benefit of which is that there are fewer environmental stimuli (e.g., handling or researcher presence), which may activate the HPA axis, and therefore cause physiological changes and expression of fight, flight, or freeze behavior, which are likely not representative of the pain resulting from a procedure. Animals are unable to directly communicate their pain, and particularly for prey species such as cattle, stoicism can mask outward displays of pain (Carbone, 2020), therefore a comprehensive approach to assessing pain through behavioral and physiological changes is ideal. Behavioral changes in response to pain or stress is highly species dependent (Animals, 2009), and care should be taken when interpreting changes in behavior or identifying abnormal behavior.

Behaviors are generally categorized as states or events, according to their duration; this distinction is important when determining the optimal method of observation to capture behaviors of interest (Bateson and Martin, 2021). In cattle, event behaviors associated with pain often can include tail flicks, foot stamps, kicking, head turns and ear flicks (Sutherland et al., 2013; Van der Saag et al., 2018). State behaviors measured in pain studies often include standing, lying, or ambulation. An important consideration when observing behavior, particularly event behaviors, is that although they are known to be associated with pain in the context of studying painful procedures, they can also be triggered by environmental stimuli, particularly flies (Eicher et al., 2001; Mays, 2013). A previous study reported insect associated behavior changes, whereby

pasture-based dairy cattle had increased tail flicks and foot stamps when not provided with sprinklers (Kendall et al. 2007).

Changes in event and state behaviors are largely procedure dependent and are associated with the region of the animal in which trauma has occurred. Castrated calves have increased incidence of behaviors associated with the rear half of the animals including tail flicking, foot stamping, and head turning (Sutherland et al., 2013; Petherick et al., 2014; Meléndez et al., 2018a; Gellatly et al., 2021). Calves castrated and branded expressed more tail flick, foot stamps, and head turns, compared to only castrated or control calves (Meléndez et al., 2018a). This procedure dependent behavioral change is particularly true when comparing castration and dehorning. Dehorned calves have increased incidence of behavioral changes associated with the head, including ear flicking, head shaking, and head rubbing (Sylvester et al., 2004; Heinrich et al., 2010; Sutherland et al., 2013).

Event behaviors, including kicking, tail flicking, and intense reactions occur at the time of branding, (Lay et al., 1992a; Schwartzkopf-Genswein et al., 1997b). Of the few studies which have recorded behavior in the days and weeks following branding, the majority not measure changes in event behaviors (Tucker et al., 2014b; Tucker-et al., 2014a; Martin et al., 2022; Hernandez et al., 2022). Martin et al. (2022), did capture event behaviors following branding, however they were not directly assessed, rather they were a component of a cumulative visual analog scale which also included other behavioral descriptors. The Martin and others study (2022) not find an effect of branding or meloxicam on event behaviors, but based on elevated prostaglandin metabolites and wound temperature it is likely pain was still present (Martin et al. 2022).

Lying, particularly in cattle, is a behavior which comprises a significant portion of cattle's daily time budget, and therefore changes in lying duration (or inversely, standing) are of interest

following painful procedures (Kilgour et al., 2012). Lying behavior is commonly recorded in castration and or branding studies (Olson et al., 2016; Meléndez et al., 2018b; Marti et al., 2019; Gellatly et al., 2021; Martin et al., 2022), as lying behavior is thought to be a strong indicator of comfort in cattle (Barrier et al., 2014). Duration of standing, bouts of standing, and abnormal hunched posture are thought to be associated with discomfort or pain, as cattle are seen to stand more following surgical castration (Robertson et al., 1994; Molony et al., 1995; Devant et al., 2012; Webster et al. 2013; Petherick et al., 2014; Meléndez et al., 2017). Further, standing is reduced in animals administered pain mitigation (Webster et al., 2013; Small et al., 2014).

Another parameter which is assessed when recording motivational states in the presence of pain or following a painful procedure, is feed consumption, feeding behavior, and average daily gain (ADG). Following castration and or dehorning, calves feed consumption was reduced (Earley and Crowe, 2002; Heinrich et al., 2010; Sutherland et al., 2013) and less time was spent near the feed bunk (Theurer et al., 2012). Additionally, the administration of pain relief in the previously mentioned studies increased time feeding and or time spent near the feed bunks following painful procedures (Heinrich et al., 2010; Theurer et al., 2012; Sutherland et al., 2013)-

Performance parameters like ADG are commonly reduced in the days and or weeks following procedures like castration (Earley and Crowe, 2002; Coetzee et al., 2011a) and dehorning (Coetzee et al., 2012). A study of concurrent castration and branding reported the greatest reduction in ADG and final body weight at the end of 42 days for concurrently processed calves (2 mo.) as compared to only castrated or control calves, with no significant difference in calves which received a single injection of meloxicam (Marti et al., 2019). While no effect of medication was observed in the Marti and others, 2019 study, a multimodal approach of ketoprofen and lidocaine administered prior to castration of older calves (5.5 mo.) did result in the greatest

ADG over the course of a 35-day study (Earley and Crowe, 2002). The differences likely due to age of the calves in the study and the analgesic approach, as older animals have been previously reported to have greater weight loss in the 30 days post castration compared to younger animals (Bretschneider, 2005). Performance outcomes like ADG following painful husbandry procedure studies and analgesic interventions are significant as they are economically relevant to producers, as weight is an important factor in the decision to wean calves (USDA, 2020b) and is an important attribute throughout the beef supply chain. ADG has been recorded to be improved in the post-procedural period when cattle are administered NSAIDs when branded (Tucker et al. 2014b; Berggren, 2018), castrated (Earley and Crowe, 2002), or dehorned (Coetzee, 2012). Behavior is an important component of assessing animal welfare, and adjunct to performance parameters and physiological changes, provides great insight into the wellbeing of animals following painful procedures.

PAIN ASSOCIATED WITH BRANDING

Regardless of branding method (hot iron or freeze), both are considered to be painful as demonstrated by changes in behavioral and physiological pain associated parameters (Lay et al., 1992a; Lay et al., 1992b; Schwartzkopf-Genswein et al., 1997b). Researchers have found that hot-iron branding results in increased heart rates (Valverde, 2005; Lay et al., 1992a) and plasma epinephrine and norepinephrine levels at 30 seconds and 15 minutes post hot-iron brand (Lay et al., 1992b), with these changes being less pronounced in freeze branded cattle. Hot iron brand wounds are more sensitive to pressure than unbranded tissue and remain painful when palpated for up to 10 weeks after branding (Tucker et al., 2014b) and remain significantly warmer than freeze branded or control tissue up to 168 hours following branding (Schwartzkopf-Genswein and

Stookey, 1997c). The latter study indicated greater levels of inflammation from hot-iron branding in the days after brand application. These extreme pain-related behavioral responses are some of the reasons as to why alternatives to branding are so widely sought after and freeze branding is recommended when feasible (Lay Jr. et al., 1992a; Lay et al., 1992b; Schwartzkopf-Genswein et al., 1997b). The immediate and lasting physiological effects of branding are an important consideration when evaluating the pain associated with brand application and the long healing phase of the wound.

Behavior in the days and weeks following brand application is an outcome reported by some branding studies. Few studies have captured behavior in the days and weeks following branding, but those that have recorded behaviors like lying, standing, and ambulation (Grobler, 2012; Tucker et al., 2014b; Marti et al., 2019; Hernandez et al., 2022; Martin et al., 2022). Studies of human burn victims reported that therapy and absence of movement are methods by which to control pain resulting from burn wounds (Latarjet and Choinère, 1995), so it could be hypothesized that branded cattle might also increase the time they spend in a stationary behavior, like lying or standing. Two studies have observed an increase in time lying in the 48 hours after branding for all treatments (Grobler, 2012; Martin et al., 2022), with lying bouts reduced in the meloxicam treated calves in the 12 hours after branding but no major effect of pain mitigation was reported (Martin et al., 2022). Another branding study reported only a slight decrease (0.7 hr) in lying in flunixin meglumine treated cattle and more time lying on day 15 and 26 of the 71 day study (Tucker et al., 2014b). The results of past studies seem to demonstrate that cattle may increase the time they spend in a stationary position, such as when lying down, and that there is little impact of analgesia on behavior. Countries like South Africa (Grobler, 2012) and Brazil (Hernandez et al., 2022), place brands on the jaw, and location of the brand should be considered when comparing

the results of studies performed in the United States. Behaviorally, the Hernandez and others (2022) study reported that calves branded without either local anesthetics or systemic NSAID had greater masticatory muscle tension five days after branding, again this tension might be an effort to reduce movement of the branded hide on the jaw. Based on previous studies, lying, and standing time are behaviors which should be evaluated following branding. Inclusion of more subtle behaviors like shifts or adjustments when standing or lying as well as “tension” scores may also be beneficial (Hernandez et al., 2022; Yamada et al., 2021). Although it is unknown if cattle that are branded on locations other than the cheek would express tension in the face, future research could include tension scores or grimace scales.

The long term effects of branding on behavior and performance are not well understood, but it has been well documented that branding and the subsequent tissue damage produces immediate pain as seen be intense reactions at the time of branding (Lay et al., 1992a; Lay et al., 1992b; Schwartzkopf-Genswein et al., 1997b) and lasting sensitivity of the brand wound (Tucker et al., 2014b; Marti et al., 2019). Brand location and its effect on pain parameters has so far only been studied regarding cheek branding versus hind leg branding (Grobler, 2012). The results of the study reported branding of the cheek to cause increased vocalizations as compared to cattle branded on the rear leg (Grobler, 2012). To the author’s knowledge, no studies have explored manipulating the hot-iron brand itself, specifically the effect of brand size on pain related outcomes.

PAIN ASSOCIATED WITH CASTRATION

Castration has been a common painful procedure studied in cattle, as it is an industry standard in the United States. Changes in pain indices following castration are related to method of castration, analgesic administration, and age at the time of the procedure. Surgical castration elicits a pronounced acute pain response as captured through evaluation of facial expression (Yamada et al., 2021), visual analog scores (VAS) (Marti et al., 2015), blood cortisol (Cohen et al., 1990; Earley and Crowe, 2002; Meléndez et al., 2017), and behavioral changes (Molony et al., 1995; Meléndez et al., 2017; Gellatly et al., 2021). Castration methods entailing the physical removal of the testes by severing the spermatic cord have been reported to cause greater expression of immobile postures, whereas methods of castration such as banding have resulted in greater expression of restlessness and small movements (Molony et al., 1995). Duration of standing, bouts of standing, and abnormal hunched posture are thought to be associated with discomfort or pain, as cattle stand more following surgical castration (Robertson et al., 1994; Molony et al., 1995; Devant et al., 2012; Webster et al., 2013; Meléndez et al., 2017). These changes in durations of stationary behavior may be a method of alleviating agitation of the wound site. Calves surgically castrated, without any topical or systemic pain mitigation also spent more time walking with a stiff gate and had greater foot stamps than sham castrated and medicated calves (Van der Saag et al., 2018). Along with behavioral changes, castration elicits elevations in cortisol, Substance P, and other autonomic responses (Stewart et al., 2010; Dockweiler et al., 2013; Bergamasco et al., 2021).

The age at the time of surgical castration has been reported to produce different pain associated responses (Robertson et al., 1994), with older animals expressing more pain-associated outcomes (Bretschneider, 2005). The majority (56.7%) of bull calves on commercial cow-calf

operation in the United States are castrated before 61 days of age (USDA, 2020b), as earlier castration is associated with less intense pain related responses (Dockweiler et al., 2013). At birth the autonomic nervous system (ANS) is challenged with many new stimuli, and as the ANS develops alongside the limbic system (emotions, memories, and mood regulation), the perception of stimuli can change and may change the response to a painful stimulus (Mulkey and du Plessis, 2019). These physiologic responses are more pronounced in animals which are older at the time of castration as seen by calves of six months of age reported to have produced greater concentrations of Substance P and have cortisol remain elevated nearly twice as long than of the calves of eight weeks of age (Bergamasco et al., 2021; Dockweiler et al., 2013). The Dockweiler et al. (2013) study also reported increased electrodermal activity which can be inferred as increased sympathetic tone associated with distress (Lehmann et al., 2017). The age at which animals are castrated also affects the decision to use pain mitigation, when beef calves over six months of age are castrated, the administration of analgesic drug use doubled (Fajt et al., 2011). Understanding pain responses at different developmental stages is a critical factor in setting guidelines in the United States as to when painful procedures should be performed to minimize pain and maximize animal wellbeing. Other countries enforce pain mitigation for castration in animals over 6 months of age (AHA, 2014), which may reflect integration of these findings into the development of protocols to improve animal wellbeing during and after castration.

CURRENT ANALGESIC USE AND COMMON DRUGS USED

Pain mitigation is only provided to a fraction of cattle in both United States and Canada during branding and castration (Fajt et al., 2011; Moggy et al., 2017; Johnstone et al., 2021) despite

there being effective analgesic drugs permitted for use under veterinary guidance. Branding is perceived by producers and veterinarians as being very painful, although less than 5% use a systemic analgesic for pain relief from branding in the United States (Johnstone et al., 2021; Edwards-Callaway et al., 2023). While considered less painful than branding, the use of systemic or local analgesia by producers and veterinarians occurs less than 50% of the time when castrating cattle (Johnstone et al., 2021; Edwards-Callaway et al., 2023). Similarly, a 2017, Canadian study of cow-calf operations, found that 85% and 72% of respondents believed that branding and castration, respectively, are painful without an analgesic intervention (Moggy et al., 2017). Within this same study, pain mitigation was less frequently provided for animals being branded (4%) than castrated (10%). These studies did not capture the percentage of animals which are concurrently branded and castrated or the percentage of producers which complete these procedures concurrently for bull calves. This information would be valuable as it may promote development of analgesic protocols which can effectively mitigate the increased pain resulting from the simultaneous completion of the procedures.

There are no drugs whose labels' permit their use in livestock for relief from pain associated with painful procedures. As stated within the Animal Medicinal Drug Use Clarification Act (AMDUCA), passed in 1994, extra-label drug use is only permitted by licensed veterinarians when no approved animal drug for an intended purpose is present or if a licensed veterinarian finds an approved medication ineffective for its purpose (AMDUCA, 1994). Currently, all pain mitigating medications are considered extra label, and the accessibility to these medications is governed by access to, and development of a veterinary client patient relationship (VCPR). When analgesics are used for painful procedures, the common classes of drugs prescribed to livestock alone or multimodally, typically in an extra label manner, are non-steroidal anti-inflammatory

drugs (NSAID), local anesthetics, and alpha-2 adrenergic receptor agonists (Coetzee, 2011b; Fajt et al., 2011).

When NSAIDS are administered during castration of cattle, ketoprofen (Earley and Crowe, 2002), flunixin meglumine (Mintline et al., 2014) and meloxicam are the most commonly used drugs. Flunixin meglumine, is one of the only approved NSAID drugs in cattle (beef and dairy) in the United States for relief from pain associated with inflammatory conditions including alleviating pyrexia (fever) associated with bovine respiratory disease, endotoxemia and acute mastitis (Banamine® (Injection), n.d.). A pour-on solution is also approved for use in alleviating pain due to foot rot and fever associated with bovine respiratory disease and mastitis in adult cattle, but is not permitted in beef or dairy calves under two months of age (Banamine® (Transdermal), n.d.). Although Banamine is an approved drug for inflammatory conditions in cattle, as a form of relief for pain resulting from procedures like branding, castration, and dehorning it is permitted only under the guidance of a veterinarian in an “extra label” manner. Limitations of Banamine include intravenous route of administration, as per the label directions, which becomes logistically challenging when processing large herds of cattle. The pour-on solution, while more convenient, is not approved for use in cattle under 2 months of age, which is commonly when castration and branding are performed in the United States (USDA, 2020b) . For both castration and dehorning, pain mitigating protocols including multimodal use of a local anesthetic and systemic analgesics analgesic are most common (Johnstone et al., 2021). Although flunixin is currently the only approved drug for cattle for relief from pain and inflammatory conditions, its label-approved use is limited by both animal age and route of administration. Both of these factors impact its practicality in commercial operations where processing of animals can be both labor and time intensive.

NSAIDs have been reported to be utilized in commercial or production settings by producers and veterinarians when branding (Johnstone et al., 2021). To the author's knowledge, there are limited studies which have implemented analgesic interventions to relieve pain from branding. Past analgesic interventions explored in branding studies, include application of a cooling gel and use of individual or multimodal use of local anesthetic and a systemic NSAID (Hernandez et al., 2022; Tucker et al., 2014b). The cooling gel which was applied at the time of branding and during the healing phase had little effect on pain sensitivity or healing, except for reducing brand temperatures the day of branding (Tucker et al., 2014a). The use of a local anesthetic and or systemic analgesic administered prior to branding was associated with a reduction in facial tension five days after branding on the jaw (Hernandez et al., 2022). Further research is needed as to the effects of different analgesic drugs on pain-related outcomes in the period following branding. Abiding by AMDUCA guidelines, there are acceptable methods of pain relief from castration and hot-iron branding in cattle if they comply with the extra label drug use regulations, routes of administration, and by drug withdrawal times.

MELOXICAM PHARMACOKINETICS

One drug prescribed to manage pain in humans, domestic animals, and seeing increased use in livestock is meloxicam (EMA, 2018). Meloxicam is a selective COX-2 inhibitor of the oxicam class, which acts on inflammatory pathways resulting from induced tissue and cell damage (EMA, 2018). Arachidonic acid is a phospholipid and a component of the phospholipid bilayer or cell wall of body cells. When cell damage is induced (i.e., thermally or mechanically) phospholipase is activated and subsequently arachidonic acid (AA) is released. AA then enters the

cyclo-oxygenase pathway and can either be catalyzed by COX 1 or COX 2, the enzymatic reaction of AA by the COX enzymes results in Prostaglandin E2 (Hannoodee and Nasuruddin, 2022). Prostaglandins resulting from the COX 2 production are associated with inflammation, fever, and pain (Laven et al., 2012). The mechanism of action of meloxicam is to specifically target and inhibit the cyclo-oxygenase 2 enzyme, thus inhibiting the production of Prostaglandin E2 and subsequent inflammatory cascades (EMA, 1999). The routes of administration of meloxicam consist of subcutaneous injection, intramuscular, oral, and recently a transdermal oral gel has been created. Routes which do not involve injection (oral and transdermal) are beneficial as they mitigate the risk for injection site lesions. Oral meloxicam is 100% bioavailable (i.e., the fraction of drug that reaches systemic circulation) in ruminant animals and results in a peak plasma concentration of 11.64 hours (range of 10 to 12 hours) and a half-life of 27.54 hours (range of 19.96 to 43.29 hours) (Coetzee et al., 2009). Oral meloxicam has displayed great potential at being an effective, long-lasting, and easily administered form of pain mitigation when administered before or at the time of husbandry procedures (Allen et al., 2013).

MELOXICAM AND ITS EFFECT ON PAIN RELATED OUTCOMES

Meloxicam has been studied as a systemic analgesic for cattle in studies focused on dystocia and cesarean section (Barrier et al., 2014), castration (Roberts et al., 2015; Lehmann et al., 2017; Meléndez et al., 2018b; Gellatly et al., 2021), dehorning (Allen et al., 2013; Fraccaro et al., 2013; Glynn et al., 2013), branding (Meléndez et al., 2018a; Marti et al., 2019; Martin et al., 2022), induced lameness (Coetzee et al., 2014) and transport associated stress (Van Engen et al., 2014). Based on surveys and Food Animal Residue Avoidance Databank (FARAD) inquiries,

producers have inquired about drug withdrawal times for meloxicam when mitigating pain from dehorning, generalized pain, and lameness (Robles et al., 2021). This indicates a level of interest by producers for using meloxicam when dehorning cattle. Although, the 2017 APHIS-NAHMS study observed that there is a lack of reporting on how often producers are inquiring to veterinarians about pain mitigation for castration and dehorning (USDA, 2020a).

In a recent study examining the effects of surgical castration of calves and meloxicam on inflammatory and pain-related outcomes, meloxicam administration resulted in reductions in physiologic indicators of pain such as salivary cortisol, haptoglobin, white blood cell count (WBC), and scrotal temperature as compared to calves administered a placebo (Meléndez et al., 2018b). Similarly, cortisol (Allen et al., 2013), heart rate (Coetzee et al., 2012) and Substance P (Coetzee et al., 2012; Allen et al., 2013) concentrations were reduced in calves receiving meloxicam as compared to calves that did not receive meloxicam prior to dehorning. Meloxicam has also been associated with reductions in wound temperature following surgical castration (Meléndez-et al., 2018b; Van der Saag et al., 2018) and branding (Martin et al., 2022). Martin et al. (2022) also reported reductions in Prostaglandin E2 metabolites in calves receiving meloxicam at 6, 24, and 48 h post hot-iron branding. Similarly, bulls that were transported to a feedlot and surgically castrated and administered meloxicam had reduced pull rates for illness and reduced incidence of bovine respiratory disease as compared to bulls not administered meloxicam (Coetzee et al., 2011). The previously mentioned studies highlight the potential benefit of meloxicam on reducing the adverse effects of painful husbandry procedures on health and morbidity.

Meloxicam administration has been reported to result in behavioral changes in cattle associated with reduced pain following a variety of procedures (Barrier et al., 2014; Heinrich et al., 2010; Meléndez et al., 2018a; Coetzee, 2014). Several studies have shown that meloxicam

reduced foot stamps following surgical castration (Van der Saag et al., 2018) and increased time spent lying after castration (Olson et al., 2016; Meléndez et al., 2019), branding and or castration (Meléndez et al., 2018a), and dehorning (Theurer et al., 2012). This reduction in foot stamps is likely a result of the anti-inflammatory, and anti-pyretic properties of meloxicam. Prostaglandin production is associated with irritation which could lead to foot stamping and attempts at touching or “itching” the damaged tissue resulting from castration (Gunaydin and Bilge, 2018). As mentioned, prostaglandin levels are reduced following meloxicam administration and could in part cause reductions in behaviors like foot stamping. Meloxicam also reduced tail flicks and increased time spent lying in two month old beef calves castrated or simultaneously castrated and branded calves (Meléndez-et al., 2018a). There are many other examples of the benefits of meloxicam, in addition to the positive behavioral changes. Meloxicam is an effective NSAID when used to mitigate pain from common husbandry procedures and should be further evaluated for this use.

FACTORS IMPACTING ANALGESIC USE

The administration of analgesic drugs for painful procedures is impacted by a host of individual and economic factors. For producers and veterinarians, the major barrier contributing to the acceptance and use of analgesic drugs for livestock undergoing painful husbandry procedures is lack of a Food and Drug Administration (FDA) approved analgesics specifically for the purpose of alleviating pain associated with animal husbandry procedures (Johnstone et al., 2021; Robles et al., 2021). Other factors impacting producer decisions to use pain mitigation include cost and ease of administration (Moggy et al., 2017; Johnstone et al., 2021; Robles et al., 2021), limited understanding of economic benefits (Wagner et al., 2020), and access to accurate

and updated information about pain mitigation practices available for producers (Moggy et al., 2017). Additional factors impacting the use of analgesics are producer and operation demographics (e.g. age and education level), relationship with veterinarians (i.e. accessibility), and attitudes towards pain in livestock (Moggy et al., 2017).

The perception of pain by producers and veterinarians impacts use of analgesia, and perception is impacted by education, demographics, and the procedure being completed (Martin et al., 2019; Robles et al., 2021; Johnstone et al., 2021). Both castration and branding are considered to be painful by producers and veterinarians (Huxley and Whay, 2006; Moggy et al., 2017; Robles et al., 2021; Edwards-Callaway et al., 2023), however the association between the perceived painfulness of a procedure is not reflected in analgesic use. Producers are less likely to consider a procedure painful if it is bloodless and does not result in an open wound, such as the case with band castration versus surgical castration (Moggy et al., 2017). Older producers and veterinarians (over 70 years of age) reported they were not as knowledgeable at recognizing and treating pain as compared to younger producers (41-50 year of age) (Robles et al., 2021). Although less confident in their ability to recognize pain, older producers were actually more sensitive to pain than younger producers (Wikman et al., 2016) and producers over 31 years of age were significantly more likely to perceive abdominal surgery, paste disbudding, and hot-iron branding as painful compared to those under 30 years of age (Edwards-Callaway et al., 2023). Use of analgesic drugs for painful procedures like branding and castration has gradually increased as knowledge and perception of pain has changed. Robles et al., 2021, reported that these changes are related to a change in attitude, increased evidence for analgesic effectiveness, and positive performance and health benefits of livestock (Robles et al., 2021). Increased awareness of animal

welfare by producers is also positively impacting their acceptance of administration of pain mitigation alongside painful husbandry procedures (Martin et al., 2019).

For veterinarians, who play an influential role in the decision to implement pain mitigation (Moggy et al., 2017; Robles et al, 2021), factors such as education level, and age impact both perception of pain and use of analgesic drugs (Moggy et al., 2017). A survey of practitioners reported that younger and more recent veterinary school graduates that assigned higher pain scores to certain procedures were more likely to use analgesics during painful procedures (Huxley and Whay, 2006). Postgraduate training also resulted in a tendency for increased use of analgesic drugs (Huxley and Whay, 2006). Older veterinarians (over 70 years of age) did not feel as knowledgeable at recognizing and treating pain as compared to younger veterinarians (Robles et al., 2021). The veterinarian's education and training therefore directly impact implementation of pain mitigation protocols for painful husbandry procedures. Communication between producers and veterinarians will be a critical component of maintaining good animal husbandry and welfare and will be especially true when it comes to performing painful procedures (Kristensen and Enevoldsen 2008).

There is ample evidence that pain mitigation strategies, such as providing NSAIDS like meloxicam, reduce pain associated with castration and branding. While the implementation of pain mitigating drugs is multifactorial, there is interest in using analgesia and improving animal welfare on commercial operations. There is still much unknown about the pain resulting from hot-iron branding in cattle. Reducing the brand iron size may be a means of both, impacting pain-related changes in behavior, health, and performance and reducing hide loss at harvest.

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CHAPTER 2

EFFECT OF HOT-IRON BRAND SIZE AND MELOXICAM ON BEHAVIOR, HEALTH, AND PERFORMANCE OF BEEF CALVES

SUMMARY

Animal welfare is of growing importance in beef cattle production and therefore there is heightened interest in regularly practiced, painful management practices. Hot-iron branding is a common method of permanent identification of cattle, created by intense thermal damage to the hide. The main objectives of the study were to quantify the effects of hot-iron brand size and meloxicam on pain related behaviors and health and performance parameters of 2-month-old beef calves on a commercial cow calf operation. A total of 126 calves were enrolled in the study (bulls, n=56; heifers, n=70). Calves were hot-iron branded with one of two iron sizes (small (SM), large (LG)) and administered meloxicam (MEL; dosed for a 113-kg calf at 1mg/kg of bodyweight) or a placebo (PLA)), and castrated (CAS) or not castrated (NCAS), if a heifer calf. Following the treatment application, behavioral observation (2 h periods) using instantaneous scan sampling occurred at 6 h and 24 h post branding; these observation periods (OP) represent the pharmacokinetics of oral meloxicam. A logistic regression (PROC GLIMIX) was used to assess the association between castration status, brand size, medication, observation period, as well as any interactions, on the proportional behavioral outcomes. More LG brand calves were observed standing, lying, and displaying event behaviors as compared to the SM brand calves ($P = 0.003$, $P = 0.011$, $P = 0.016$, respectively). OP affected the display of event and state behaviors,

with standing observed more at 6 h and event behaviors displayed more at 24 h ($P = 0.0028$). Concurrently, CAS and SM or LG branded calves exhibited more event behaviors and standing than NCAS calves ($P = 0.022$). No effect of the fixed variables on average daily gain (ADG) or morbidity were observed ($P > 0.05$). MEL did reduce the proportion of branded, heifer calves expressing event behaviors as compared to PLA, however it had less of a behavioral impact on the concurrently castrated and branded calves ($P = 0.022$). The results indicate that pain is present within the 24 h following branding and castration and that concurrent castration and branding elicits a greater behavioral response than only branding. This study highlighted both the feasibility of administering an oral analgesic in a commercial setting and that implementing a smaller brand size may be a strategy to reduce pain associated with hot-iron branding.

INTRODUCTION

Animal welfare is a critical component of livestock production systems (Webster, 2001; Grandin, 2020; Faucitano et al., 2022). There are increasing welfare concerns surrounding painful procedures of livestock in production settings, particularly beef (Fajt et al., 2011; Teixeira et al., 2018) and dairy (Bir et al. 2020). A particular area of interest in the cattle industry is alleviating pain during routine husbandry procedures like branding, castration, and dehorning, with the latter two procedures having been more extensively researched (Coetzee et al., 2010; Coetzee, 2011b; Rault et al., 2011; Coetzee, 2013; Adcock and Tucker 2018; Bartlett, 2021). A national survey published in 2017 indicated that 79% of bull beef calves across operations in the United States are castrated prior to sale and 45% of beef cattle and calves are hot-iron branded (USDA, 2020b). These percentages represent a large population of the total 90 million beef cattle, cows, and calves in the United States (USDA, 2023).

Branding is the most common method of permanent identification (CHAPA, 1993; Eastwood, 2017) in the U.S. cattle industry (USDA, 2020b). Brands are utilized to signify ownership, deter theft, and improve traceability of cattle throughout their lives; each brand is unique and part of an official registry where it is validated by official brand inspection agencies (USDA-APHIS, 2020). Regarding import or export of cattle from the United States, brand sizes and locations are regulated at a federal level (Shea, 2018), but more regulations exist at a state level for acceptable brand size, location, and method of application (i.e. hot iron vs freeze branding) (Oklahoma Cattlemen's Association, 2020; WSDA, 2023). Hot-iron branding is the most common method and entails a fire-heated or electrically heated metal brand applied to the skin causing damage to the hide whereas the other method, freeze branding, utilizes liquid nitrogen which damages the hide via subzero thermal injury and is thought to be less painful than conventional hot-iron branding (Lay et al., 1992a; Schwartzkopf-Genswein et al., 1997b; Schwartzkopf-Genswein et al., 1998). Regardless of location (Grobler, 2012) and method (Lay et al., 1992a; Schwartzkopf-Genswein et al., 1997b) of application, branding results in behavioral and physiologic changes, indicating the procedure is painful (Schwartzkopf-Genswein et al., 1997a; Schwartzkopf-Genswein et al., 1998; Martin et al., 2022).

Surgical castration is the process by which the testes are manipulated and quickly severed from the spermatic cord for the purpose of improving meat quality and reducing aggressive behavior toward conspecifics and animal caretakers (Newman, 2007; Bolado-Sarabia et al., 2018). This procedure is painful, as demonstrated by increases in inflammatory markers (e.g., white blood cells and acute phase proteins), and blood cortisol levels (Earley and Crowe, 2002; Roberts et al., 2015). Acute behavioral impacts of surgical castration have also been reported, such as greater frequencies or observed duration of pain-related behaviors like foot stamps, a stiff gait, and tail

flicks compared to calves that were not castrated (Webster et al., 2013; Van der Saag et al., 2018). Castration and hot-iron branding are often performed concurrently to minimize the number of times animals need to be handled. Calves undergoing the combination of these two procedures, as compared to an individual procedure, experience elevated physiological and behavioral responses associated with stress and pain (Sutherland et al., 2013; Meléndez et al., 2018a; Marti et al., 2019).

Despite the evidence that branding and castration are painful, pain mitigation prior to, during, or post procedure has not been widely adopted for these procedures in the beef industry (Fajt et al., 2011; Edwards-Callaway et al., 2023; Moggy et al., 2017; Johnstone et al., 2021). A survey of veterinarians and producers working in the beef and dairy industries in the United States indicated that less than 50% of producers and veterinarians always or almost always use local or systemic analgesia when castrating (Johnstone et al., 2021). In the United States, less than 5% of veterinarians and producers use systemic analgesics for the purpose of pain relief from branding (Johnstone et al., 2021). There are many identified challenges with providing pain relief including the feasibility of administration and the limited availability of analgesic drugs labeled for alleviating pain associated with husbandry procedures in cattle like castration and branding (Johnstone et al., 2021; Robles et al., 2021). Currently in the United States, no drugs are approved for the purpose of analgesia when performing painful husbandry procedures, but their use is permitted by the American Medicinal Drug Use Clarification Act (AMDCUA) of 1994 under the oversight of a veterinarian in an extra-label drug use (ELDU) manner.

Oral meloxicam, a non-steroidal anti-inflammatory drug (NSAID), has demonstrated potential as a long acting analgesic drug for use in cattle (i.e., oral meloxicam has a half-life of 27 h; Coetzee et al., 2009). Meloxicam is a selective COX-2 inhibitor with analgesic, anti-pyretic, and anti-inflammatory properties. In the European Union (EU) meloxicam is approved for

adjunctive therapy of acute mastitis and for fever and inflammation from acute respiratory disease, while in calves and young cattle it can be administered as supportive therapy for diarrhea. The EU also approved meloxicam as an analgesic for post-operative pain from dehorning in calves (EMA, 2018; Metacam®, n.d.). However, it is still not directly approved for use in common husbandry procedures like castration and branding. Meloxicam has been shown to reduce post-procedural inflammatory markers and reduce pain-related behaviors in cattle post-branding (Martin et al., 2022), castration (Olson et al., 2016), dehorning (Theurer et al., 2012), and caesarean section (Barrier et al., 2014). Meloxicam given orally through a drench or bolus is a practical and efficient method of providing pain mitigation that negates the risk of injection site lesions and is worth further exploration as it pertains to branding and castration. Limited studies exist exploring hot-iron branding and its associated pain response in cattle (Schwartzkopf-Genswein et al., 1997a; Schwartzkopf-Genswein et al., 1997b; Grobler, 2012; Meléndez et al., 2018a; Marti et al., 2019), and even fewer have investigated the administration of an analgesic agent for pain resulting from hot-iron branding (Tucker et al., 2014b; Berggren, 2018; Martin et al., 2022). Although most studies utilizing oral meloxicam are focused on castration and dehorning, oral meloxicam was successful at reducing Prostaglandin E₂ metabolite, a circulating inflammatory mediator, after hot-iron branding (Martin et al., 2022). Research on commercial cow-calf operations is limited, likely due to the accessibility of operations, the variable nature of the operations within the beef sector, and challenges of data collection on expansive grazing and pasture operations. The collection of behavioral data through observation or loggers, is logistically challenging as commercial cow-calf operations often immediately put cattle back onto pasture following processing, resulting in environmental challenges which may impact visibility of the cattle. Additionally, management styles are incredibly diverse amongst operations, which can greatly impact study methodology.

There is limited research on the impacts of brand size (e.g., length, width, total surface area) on pain response in cattle. Therefore, the objective of the current study was to determine the effects of meloxicam and hot-iron brand size on behavior, performance, and health in beef calves after hot-iron branding.

METHODS

This study was approved by the Institutional Animal Care and Use Committee of Colorado State University (Protocol #2322).

Animals and Treatment Subsets:

The study included Black Angus and Black Angus Hereford cross calves (n=126 total; n=56 bulls and n=70 heifers) raised on an extensive pasture setting in Colorado. The average age of calves at the time of the study was 60 ± 10 days. This study was a randomized 2 x 2 factorial trial exploring the factors of brand size (small brand, SM; large brand, LG) and pain mitigation (meloxicam, MEL; placebo, PLA) on behavior, health, and performance of the calves.

Treatments were blocked by sex whereby bulls were castrated (CAS) at the time of branding and heifers were not (NCAS; **Table 1**).

Table 1. The distribution of calves between treatment subsets (n=126).

		Large Brand (LG) n = 62	Small Brand (SM) n = 64	Total n = 126
Bulls Castrated (CAS)	Placebo (PLA)	13	15	28
	Meloxicam (MEL)	14	14	28
Heifers Non-castrated (NCAS)	Placebo (PLA)	19	16	35
	Meloxicam (MEL)	16	19	35

Prior to the day of the branding, calves were randomly assigned to treatment by calf identification number using an online randomizing software (Sealed Envelope Ltd, 2021) to create equal blocks of 15 calves per treatment subset. A unique marking was assigned to each treatment subset using either livestock paint (QuikShot All-Weather, La Co Industries, INC, Elk Grove Village, IL) or heat detection patches (Estroprotect id, Heritage Animal Health, Rockway Inc, Hawarden, IA). These markings served as treatment identification during subsequent behavioral observation.

Processing:

On the first day of the study (July 2021), 126 cow-calf pairs were gathered from pasture and kept in a temporary corral until they were branded. The temporary corral was a fenced portion of pasture (approximately 300 sq meters) and included a centrally located branding pot. The herd was processed as two groups; the first group included 80 cow calf pairs and the second

group consisted of 46 cow calf pairs. Branding for the first group started at 0923 h and ended at 1123 h and branding for the second group began at 1226 h and ended at 1326 h. After branding, cow-calf pairs were moved to two separate pastures to be held through the next day (measuring 92.7 acres.)

Each group was brought into the temporary corral for processing. In accordance with the ranch's existing handling procedures, ranch personnel on horseback roped calves by the neck and rear legs and pulled calves 10 to 20 meters toward the hot iron branding pot. Once in proximity to the brand pot, the ropes were retied around the front and rear hocks to elongate and laterally restrain the calf. While each calf was restrained, they were branded, given either a PLA or MEL bolus, and marked according to treatment subset assignment. All calves were also vaccinated with a subcutaneous 8-way clostridial vaccine (UltraChoice 8, Zoetis Animal Health, Parsipanny, NJ), an intranasal 3-way respiratory vaccine (Inforce, Zoetis Animal Health, Parsipanny, NJ), and a pour-on broad spectrum parasiticide (Ivomec, Boehringer Ingelheim, Burlington, Ontario, Canada). During restraint, bull calves were surgically castrated (method described below). The order of these procedures varied except for marking, which was withheld until after the brand was applied. The time that each calf was branded was recorded to determine when to begin subsequent behavioral observations relative to the last calf in each group being branded.

Branding and Castration:

The SM branding iron had a surface area of 83.82 cm² with a size of 8.25 cm x 10.16 cm and a 0.48 cm thick steel band. The LG branding iron had a surface area of 193.56 cm² with a size of 12.7 cm x 15.24 cm and made with a 0.64 cm thick steel band. The stainless-steel brands were heated over the fire and then applied by the ranch owner to the left rib area of each calf. Brands were applied for 3 to 5 seconds. One member of the ranch staff performed surgical

castration by using a blade to extend and sever the distal (third) of the scrotum. The testicles and spermatic cord were then extended, and the blade was used to scrape the spermatic cord until it separated and retracted back into the abdomen. The incision was sprayed with antibacterial wound spray (Cetrigen, Virbac, Milperra NSW).

Medication – boluses:

During restraint, an oral bolus (MEL or PLA) was administered with a plastic balling gun (JorVet, Loveland, Colorado) based on assigned treatment. Meloxicam (MEL) tablets (10 mg; Zydus Pharmaceuticals Inc., Pennington, NJ) or lactose monohydrate powder (PLA; Thermo Fisher Scientific, Waltham, MA) were encapsulated in gelatin capsules (Torpac Inc., Fairfield, NJ). Based on annual data from previous years, the ranch owner estimated the maximum weight of the calves to be 113-kg at branding. Thus, to provide an adequate analgesic dosage for all calves, meloxicam boluses were all dosed for a 113-kg calf at 1mg/kg of body weight. The attending veterinarian approved the protocol.

Behavioral Observations:

Observation of calf behavior occurred while daylight was available during two periods, approximately 2 h each using instantaneous scan sampling with 5-min intervals (Bateson and Martin 2021). Observation periods (OP) were chosen in an attempt to capture the half-life (27.6 h, range of 19.97 to 43.29 h) of oral meloxicam and due to logistics the peak plasma concentration (11.64 h, range of 10 to 12 h) was not able to be captured (Coetzee et al. 2009). The observation periods occurred at 6 and 24 h relative to when the last calf in each group was branded. The behavioral observations for the first group began at 7 ± 1 h and 24 ± 1 h after treatment administration. The behavioral observations for the second group began at 6.5 ± 0.5 h and 24 ± 0.5 h after treatment administration. For the 6 h post-branding observation period, the

first group was observed from 1740 to 1915 h (95 min total), and due to daylight constraints, the second group was observed from 1945 h to 2055 h (70 min total). For the 24 h post-branding observation period, the first group was observed from 1145 h to 0145 h (120 min total) and the second group was observed from 1415 h to 1615 h (120 min total).

Prior to the study, observers practiced behavioral observations using the ethogram (**Table 2**) to ensure agreement between observers. State behaviors were defined as postural or durational behaviors (Bateson and Martin 2021) and included standing (normal and abnormal), walking, lying (sternal and lateral), nursing, exploratory behavior, play, and social affiliative behavior. Event behaviors were defined as discrete body movements, occurring briefly (Bateson and Martin 2021), and included kicking, tail flicks, head turns, and foot stamps (**Table 2**). During the study period, two pairs of researchers collected behavioral data, with one person in each pair observing behaviors and calling them out and the other recording the verbalized observations. The observer scanned the whole herd (within respective group) and stated the treatment marking (i.e., paint color or patch color) and the observed behavior of each calf at each scan interval. The recorder then tallied all observations within a scan interval for each treatment subset on a data sheet. During observation periods, researchers were positioned at the perimeter of the pastures and used high powered binoculars (Vortex Optics Diamondback HD Binoculars, Vortex Optics, Barneveld, Wisconsin) to view the animals. Researchers maintained a minimum distance of 50 meters from the herd to minimize observer impact on cattle behavior.

Table 2: Description of behaviors evaluated during behavioral observation periods.

	Behavior	Description
<i>State Behaviors</i>	Normal Standing Posture	Standing stationary on all four legs, and not performing another behavior.
	Abnormal Standing Posture	Standing stationary on all four legs, with arched back.
	Walking	The calf makes sequential movement of its limbs in forward, side, or backward direction (Ugwu <i>et al.</i> , 2021).
	Lying Sternal	Lying on the sternum, with all limbs under the body, with one front limb stretched out, or with two front limbs stretched out (Brscic <i>et al.</i> , 2015).
	Lying Lateral	Lying with hind limbs stretched out or on side with all four limbs stretched out (Brscic <i>et al.</i> , 2015).
	Nursing	The calf has its muzzle below the udder making thrusting movements against the udder, making suckling movements and/or ‘wagging’ its tail (Vaarst <i>et al.</i> , 2001).
	Exploratory Behavior	The calf interacts with any non-living object in its environment through sniffing, licking, or by touching object with its nose.
	Play Behavior	<p>Run: This includes trotting (two-beat gait), cantering (three-beat gait) and galloping (four-beat gait) with forwards or sideways movement</p> <p>Jump: Forelegs are lifted off the ground while the front of the body is elevated. Movement is upwards and hind limbs may be lifted off the ground.</p> <p>Heading/head-to-head: Two calves standing front to front, butting head against head or neck</p> <p>(Ugwu <i>et al.</i>, 2021)</p>

	Social-Affiliative Behavior	The calf licks or sniffs another animal; or touches or rubs its head against the body of another animal (Rutherford <i>et al.</i> , 2019).
	Out of Sight	The calf was obscured by environment or unidentifiable and no behavior was recorded.
<i>Event Behaviors</i>	Foot Stamping	Lifting front or hind foot forcefully placing it on the ground (Van der Saag <i>et al.</i> , 2018).
	Kicking	Kicking backward or towards the belly with a hind limb (Van der Saag <i>et al.</i> , 2018).
	Tail-flicking	Sideways movement of the tail from vertical to return from vertical (Van der Saag <i>et al.</i> , 2018).
	Head Turning	Movement of head behind the shoulder to touch rib, flank, or scrotum.
	Out of Sight	The calf was unidentifiable or obscured by the environment and no behavior was recorded.

Measurements collected at pre-weaning processing:

In line with the ranch's pre-weaning processing protocol, ninety-nine days after branding (October 2021) calves were brought in from pasture, restrained in a hydraulic chute (Silencer Commercial Pro, Dubas Equipment, Fullerton, NE) and weighed. The following vaccines were also administered while calves were restrained: a subcutaneous 7-way clostridial vaccine (Bovilis Vision 7 With Spur, Merck & Co., Inc., Kenilworth, NJ), an intranasal 3-way respiratory vaccine (Inforce, Zoetis Animal Health, Parsipanny, NJ), an injectable broad spectrum parasiticide (Dectomax, Zoetis Animal Health, Parsipanny, NJ), and an oral drench (Cellarator Turbo Drench, ADM Animal Nutrition, Quincy, IL)

Health:

Researchers assessed ranch records to determine which calves were treated during the time between branding and weaning. Treatment of sick calves was based on ranch protocols. Medications listed on the ranch treatment sheet were oral drench (Cellarator Turbo Drench, ADM Animal Nutrition, Quincy, IL), injectable respiratory disease antibiotic (Draxxin, tulathromycin, Zoetis Animal Health, Parsipanny, NJ), an injectable, non-steroidal anti-inflammatory drug (Banamine, flunixin meglumine, Merck & Co, Inc, Kenilworth, NJ), and probiotics. Treatment status was defined as either “yes” (1 or more medications received) or “no” (no medication received) during the study period.

Performance:

Birthweights and pre-weaning weights were obtained from ranch records. Average daily gain (ADG) was calculated by taking the difference in pre-weaning weight and birth weight divided by the calf age (in days) at pre-weaning processing.

STATISTICAL ANALYSIS

All analysis was performed in SAS 9.4 (SAS Inst. Inc., Cary, NC) and significance was set at $P < 0.05$. Play, social affiliative behavior, and nursing were observed infrequently (<5%) so were not included in formal analysis. Sternal and lateral lying were combined to create a “lying” category. Abnormal standing and normal standing were combined to create a “total standing” category. The state behaviors explored in the final analysis were exploratory behavior, lying, out-of-sight, total standing, and walking. Tail flick, head turn, kick, and stamp were combined to create a “event behavior” category. The event behaviors explored in the final

analysis were calves “not observed” and “event” behavior. All behaviors were summarized as a proportion (y/n) by treatment subset and by day:

$$y/n = \frac{\textit{number of animals performing a behavior in an observation period}}{\textit{number of animals in a treatment subset} \times \textit{number of scan intervals in an observation period}}$$

A logistic regression (PROC GLIMMIX) with a beta distribution was used to check for any associations between predictor variables and the behavioral outcome of interest. The fixed predictor variables in the model included castration (CAS or NCAS), brand size (LG or SM), medication (MEL or PLA), and observation period (OP) as a repeated measure. First or second group was included as a random effect. Through backwards stepwise removal, the fixed and random predictor variables were evaluated as to whether they resulted in a $\geq 30\%$ change in the outcome of interest (Dohoo, Martin, and Stryhn 2009). If so, they were removed from the model. All possible two-way interactions were checked, and non-significant interactions were not reported. Values in the results are reported as least square means.

A mixed linear regression (PROC MIXED) was used to determine the association between ADG (outcome) and the fixed effects of castration, brand size, and medication. Age at branding was included in the model to determine if there was an association between age at branding and ADG. A logistic regression (PROC GLIMMIX) using a binary distribution was used to determine the association between treatment status during the study period (yes/no) and the fixed effects of castration, brand size, and medication.

RESULTS

Two bull calves (MEL + LG, first group and MEL + SM, second group) were removed from performance and health analyses because they were not castrated when branded. Researchers did not have a mechanism to identify these calves from their castrated counterparts during behavioral observations, therefore they remained in the behavioral data. The prevalence of out-of-sight calves in all subsets, and equal distribution of the bull calves between the first and second group, likely minimized the influence these two calves had on final behavioral outcomes. Five scan intervals for treatment subset (CAS + MEL + LG) were not included in the behavioral analysis due to challenges associated with observation in a pasture setting and movement of calves within the herd, leading to difficulty identifying animals due to their location. Calves were only included in the performance analysis if a birthweight and pre-weaning weight were captured ($n = 93$). A total of 22 calves were categorized as treated and four deaths were recorded by the ranch personnel prior to weaning.

State behaviors (reported as mean \pm SEM):

Brand size was associated with the proportion of calves observed lying ($P = 0.0112$), standing ($P = 0.003$), and out-of-sight ($P = 0.0001$; **Table 3**). There was no effect of medication or castration on lying, walking, exploratory behavior, or the out of sight category ($P > 0.05$). A greater percentage of LG brand calves were observed lying ($24.98\% \pm 5.6\%$) as compared to SM brand calves ($8.56\% \pm 3.3\%$). A greater percentage of LG brand calves ($11.13\% \pm 1.4\%$) were observed standing as compared to SM brand calves ($5.6 \pm 1.0\%$). A greater percentage of SM calves were out of sight ($76.21\% \pm 3.3\%$) as compared with LG branded calves ($46.73\% \pm 3.8\%$; $P = 0.003$).

A two-way interaction was observed between castration status and medication (MEL or PLA) for calves standing ($P = 0.0359$) and out-of-sight ($P = 0.0098$); for NCAS calves, fewer MEL calves were observed standing ($6.48\% \pm 1.4\%$) as compared to PLA calves ($9.15\% \pm 1.8\%$). For CAS calves, more MEL calves were observed standing ($10.7 \pm 1.9\%$) as compared to PLA calves ($6.1 \pm 1.4\%$). For CAS calves fewer MEL calves were categorized as out-of-sight ($50.02\% \pm 5.3\%$) as compared to PLA calves ($71.93 \pm 5.1\%$). For NCAS calves, fewer PLA calves were out of sight ($59.45\% \pm 5.5\%$) as compared to MEL calves ($67.74\% \pm 5.1\%$).

Observation period ($P = 0.0379$) was associated with calves categorized as out-of-sight; SM brand calves were categorized as out-of-sight ($81.25\% \pm 2.9\%$) more than LG brand calves ($73.49\% \pm 3.5\%$). The percentage of calves categorized as out-of-sight was greater for calves at 24 h ($73.04\% \pm 3.5\%$) as compared to 6 h ($81.60\% \pm 3.0\%$). Standing was also impacted by observation period whereby a greater percentage of calves were observed standing during the 6 h OP ($11.14\% \pm 1.4\%$) as compared with the 24 h OP ($5.5\% \pm 1.0\%$; $P = 0.0028$)

Event behaviors (reported as mean \pm SEM):

Brand size was associated with the proportion of calves observed expressing event behaviors ($P = 0.0162$; Table 3). Event behaviors were associated with a medication-castration interaction ($P = 0.0222$). For NCAS calves, fewer MEL calves were observed expressing event behaviors ($16.54\% \pm 2.8\%$) as compared to PLA calves ($20.59\% \pm 3.0\%$). A greater percentage of LG branded calves expressed event behaviors ($23.48\% \pm 2.3\%$) as compared to SM branded calves ($15.83\% \pm 1.9\%$). Additionally, fewer calves demonstrated event behaviors in the 6 h OP ($15.23\% \pm 1.9\%$) as compared to the 24 h OP ($24.31\% \pm 2.3\%$; $P=0.0054$).

For MEL calves, increased event behaviors were observed in CAS calves ($26.13 \pm 3.3\%$) as compared to NCAS calves ($16.54 \pm 2.8\%$). For PLA calves, fewer CAS calves expressed event behaviors ($15.49\% \pm 2.7\%$) as compared to NCAS calves ($20.59 \pm 3.0\%$).

Table 3: Least square means (\pm SEM) of behavioral observations expressed as a percentage of calves performing a behavior during 2hr observation periods at 6 and 24hrs following branding.

Behavior (%)	Brand size			Observation period			Medication * castration ¹				
	SM	LG	p-value	6hr	24hr	p-value	MEL CAS	PLA CAS	MEL NCAS	PLA NCAS	p-value
<i>State</i>											
Exploratory	4.3 \pm 1.1	7.3 \pm 1.5	0.0765	6.2 \pm 1.4	5.1 \pm 1.2	0.5239	-	-	-	-	-
Lying	8.6 \pm 3.3	25.0 \pm 5.6	0.0112	11.1 \pm 3.8	19.9 \pm 5.2	0.1277	-	-	-	-	-
Out-of-sight	76.2 \pm 3.3	46.7 \pm 3.8	0.0001	64.0 \pm 3.8	61.2 \pm 4.0	0.5974	50.0 \pm 5.3	71.9 \pm 5.1	67.7 \pm 5.1	59.5 \pm 5.5	0.0098
Standing	5.6 \pm 1.0	11.1 \pm 1.4	0.0030	11.1 \pm 1.4	5.5 \pm 1.0	0.0028	10.7 \pm 1.9	6.1 \pm 1.4	6.5 \pm 1.5	9.2 \pm 1.8	0.0359
Walking	3.3 \pm 1.0	5.2 \pm 1.4	0.1533	3.5 \pm 1.0	4.9 \pm 1.3	0.2544	-	-	-	-	-
<i>Event</i>											
Not observed	81.3 \pm 2.9	73.5 \pm 3.5	0.0532	81.6 \pm 3.0	73.0 \pm 3.5	0.0379	-	-	-	-	-
Event behaviors	15.8 \pm 1.9	23.5 \pm 2.3	0.0162	15.2 \pm 1.9	24.3 \pm 2.3	0.0054	26.1 \pm 3.3	15.5 \pm 2.7	16.5 \pm 2.8	20.6 \pm 3.0	0.0222

¹ Only the estimates for the interactions which are significant are shown.

Performance:

Brand size ($P = 0.17$) and medication ($P = 0.12$) were not associated with average daily gain. However, there was marginal evidence of an effect of castration status ($P = 0.054$), whereby castrated calves gained $1.14 \pm 0.020\text{kg}$ (mean \pm SE) per day and non-castrated heifer calves gained $1.09 \text{ kg} \pm 0.017\text{kg}$ per day.

Health:

Brand size ($P = 0.6$) and medication ($P = 0.39$) did not influence treatment status. Castration status did not influence treatment status ($P = 0.096$).

DISCUSSION

The objective of this study was to explore the impacts of hot-iron brand size and oral meloxicam treatment as it relates to behavior, performance, and health of beef calves. Although not a primary objective of the research, this study was also able to demonstrate the feasibility of providing pain mitigation on a commercial cow-calf operation. Pain mitigation for painful procedures, such as branding, castration, and dehorning, is a topic of discussion within the cattle industry as concerns for animal welfare are increasing (Robbins et al., 2015; Wolf et al., 2016). Within the United States, hot-iron branding and surgical castration are widely practiced husbandry procedures. Between surveys of producers (USDA, 2020b) and audits of packing plants (Eastwood et al., 2017), it can be estimated upwards of 50% of cattle and calves are hot-iron branded in the United States. Eighty percent of bull calves are castrated prior to weaning in the United States (USDA, 2020b) with surgical castration being adopted as the most common method of castration, as it is a quick and efficient technique (Coetzee et al., 2010). Various

associations recognize that branding and castration are painful (AABP, 2020) (i.e. AABP, AVMA) and the American Veterinary Medical Association (AVMA) advises that “approved or AMDUCA-permissible clinically effective medications” be used to reduce pain and distress when castrating (AVMA, n.d.). Following surgical castration, calves express behavioral indicators of pain and discomfort like increased standing and restlessness (Meléndez et al., 2018b) as well as physiologic indicators like elevated inflammatory markers (Van der Saag et al., 2018). Hot-iron branding results in behavioral indicators of distress like vocalization (Grobler, 2012), forceful escape avoidance reactions, and falling in the chute during brand application (Lay et al., 1992a; Lay et al., 1992b; Schwartzkopf-Genswein et al., 1997b). Studies have also identified physiological changes following hot-iron branding including both elevated heart rates (Lay et al. 1992a; Valverde and Gunkel, 2005) and blood catecholamine levels within the first 15 minutes post branding (Lay et al., 1992a). There is limited research focusing on branding in general but to our knowledge, the present study is the first of its kind to investigate pain associated with brand size in cattle.

In the current study, brand size did impact behavioral outcomes, as seen through an increased percentage of calves expressing event behaviors (tail flick, kick, stamp, and head turn) and standing in the LG branded cattle as compared to the SM branded cattle. As compared to the United States' standard brand size (minimum of 7.6cm x 7.6cm), the SM brand size just exceeds, and the LG brand nearly doubles the minimum standard brand size (Shea, 2018; Lalman et al., 2019; OLSC, 2016). More surveys are warranted to understand the average brand size actually utilized by producers at the time of branding, as brand wound scars increase in size as the animal grows (Tolleson and Schafer, 2021). As seen through anecdotal observation of fed cattle entering large packing plants in 2022, some brands were encompassed the whole side of the torso,

indicating a large initial brand size or implementation of a large permanent mark on potentially older cattle. While the current study applied brands to the left rib, the 2016 National Beef Quality Audit, a benchmarking study performed intermittently within the cattle industry, found the hip to be the most common branding region (Eastwood et al., 2017). To the authors' knowledge, the only study regarding location of branding as it relates to pain is that of Grobler et al., 2012, which found increased vocalizations during branding for cheek branded cattle as compared to upper hind leg branded cattle. The cheek or jaw is no longer utilized as a location of brand placement in the United States (BQA National Manual, 2019; AZDA, 2022). While the effect of location of hot-iron brands as it relates to pain associated parameters deserves more research focus, decreasing the brand size may be a potential approach to reducing pain associated with hot-iron branding.

In the current study neither brand size nor medication were associated with ADG. The lack of treatment effect is likely due to the 14-week period between branding and pre-weaning processing weights which may have diluted any short-term performance impacts. Previous studies have found similar results, perhaps indicating that the acute pain associated with branding does not impact ADG over an extended time frame (Marti et al., 2018; Schwartkopf-Genswein et al., 1997c). A previous comparative branding study (hot-iron vs freeze) of weaned calves also reported no difference in ADG over a 28-day period post branding (Schwartkopf-Genswein et al., 1997c). However other studies have shown that weight gain is negatively impacted following painful husbandry procedures like hot-iron branding (Berggren, 2018) and surgical castration (Marti et al., 2019). Previous studies have shown that treatment with flunixin meglumine IV (Tucker et al., 2014) or meloxicam IM (Berggren, 2018) at the time of branding, and meloxicam IV (Biggs, 2005) at the time of dehorning improved ADG within a brief post-procedural period

(<10 days). Similarly, a multimodal analgesic approach at the time of dehorning of 6-month-old steers increased ADG compared to control calves over a 7-day period (Glynn et al., 2013). While no effect of medication or brand size was captured in the current study, sex was associated with ADG, whereby castrated bull calves had slightly greater (0.05kg) ADG than heifer calves. Similar results were reported in the Martin et al., (2022), study which found that sex of the yearling steers and heifers was the only variable to impact ADG 30 days post branding or meloxicam treatment, with steers gaining 1.1kg more per day than heifers. Future work should continue to include ADG as a performance marker, however it should be captured within a shorter time from the painful procedure (<10 to 15) days) to best capture treatment effects. In addition, studies should recognize the impact which nursing may have on ADG of pre-weaned (i.e., cow-calf operation) and growth compared to calves which are separated from the dam (i.e., dairy), and general sex differences which may exist when utilizing ADG as an effect of treatment.

The treatment status of the calves in the study was based on operation records of whether a calf required any treatment intervention by ranch staff or not. The purpose of examining treatment status was to give insight into the long-term effects (5 months) on the immune system in range beef calves following a painful, high stress event (e.g., handling, branding, castration) and NSAID treatment. Morbidity is undesirable in food animal production as it impacts productivity and animal welfare. Brand size, medication, and castration did not influence treatment status. Only 22 calves were treated over the course of the study, and this may be the reason for lack of differences between treatments and treatment status relating to health. A previous study capturing the effects of castration of 8 to 10 month old bulls on arrival to feedlot, reported that oral meloxicam administered at the time of castration reduced the pull rate for

bovine respiratory disease compared to the bulls given a placebo (Coetzee et al., 2011a). However, this study population was older and likely at greater risk of disease due to long distance shipping, co-mingling, and a new environment. Pain mitigation interventions have been shown to positively impact the time which cattle spend around the feeders (Theurer et al. 2012) and on daily feed intake (Heinrich et al. 2010; Sutherland et al. 2013). Continued motivation to approach feed and eat would positively impact nutrition status which may subsequently impact immune status. While the current study did not capture an effect of brand size, meloxicam, or castration status, the inclusion of health parameters as an effect of painful husbandry procedures in future research will be important when building effective and productive on-farm procedure protocols.

Behavioral changes in cattle often occur following a painful husbandry procedure (Heinrich et al., 2010; Devant et al., 2012; Sutherland et al., 2013), and behavior is a widely used tool to interpret and understand how painful a procedure might be as well as the timeline for which the pain or discomfort lasts. Differences in certain behavior categories between treatment groups were observed in the current study but before discussing these, it should be noted that a considerable proportion of animals were out-of-sight during all data collection periods which likely impacted study outcomes. The pastures where the cow-calf pairs returned post-processing were selected by the ranch operation due to the ample access to grass, forage, and water. The pasture conditions and landscape affected the visibility of the calves, and it was difficult to see all study animals at all times. Lying and standing or being stationary comprises the greatest percentage of a young calves activity budget and these percentages remain relatively consistent between both pasture and individual housing (Kuroda and Sato 1993). The calves categorized as out-of-sight in the current study may have been lying, standing, ambulatory, or obstructed by

other animals, however due to their position within the herd or settling in deep grass, they were not able to be clearly observed.

Lying and standing are two behaviors commonly reported in studies of painful procedures and studies of pain mitigating drugs (Small et al., 2014; Meléndez et al., 2018a; Marti et al., 2019). Concerning surgical castration, the general pattern for changes in these behaviors is an increase in standing and a reduction in lying. Hot iron branding has been most studied with regards to the immediate behavioral reaction of cattle upon placing the hot-iron brand; results of these studies report more exaggerated escape avoidance reactions (Lay et al., 1992b; Lay et al., 1992a) and greater vocalizations at the time of hot-iron brand application as compared to sham or freeze brand application (Lay et al., 1992; Schwartzkopf-Genswein et al., 1998). Few studies have captured behavior in the hours or days post-branding or have examined behavioral differences between calves which have been branded only and those that have not. Martin et al., 2022, captured greater time lying and fewer steps taken 12 to 48 h following branding with an electric iron and Grobler et al., 2012 reported increased lying the day of branding and the next day. Some studies have reported that beyond the 48 hours relative to the time of hot-iron branding, incidence of standing and lying is largely unaffected, even with NSAID administration (Tucker et al., 2014b; Martin et al., 2022). Presumably changes in lying duration or lying position (i.e., lateral or sternal recumbency) may not be greatly impacted if the brand was placed on the hip, rib, or shoulder as limited agitation or environmental stimulus of the brand wound would be occurring when lying (Tucker et al., 2014b).

The large brand size was associated with an increased percentage of calves both standing and lying compared to the small brand calves. In vivo animal models are used to study general physiology of burns (Howard Aulick et al., 1981; Knabl et al., 1999; Gurfinkel et al., 2010), but

the research focusing on the impact of the size of burn as it relates to pain and healing is in human burn victims (Choinière et al., 1989). For example, burn wounds covering over 20% of total body surface area cause both prolonged pruritis and pain as compared to less extensive burns (Ryan et al., 2015). Although brand wounds are not as extensive, this understanding of pain and healing associated with burn surface area may provide insight into how a larger brand iron may negatively impact cattle at the time of branding and during the healing process. Limited research on brand healing exists, but a 2014 study by Tucker et al., found brand wounds took a minimum of 8 weeks to heal and were more sensitive to pressure than non-branded tissue over the duration of the 10 week study (Tucker et al., 2014a). Additionally, application of a topical cooling gel at the time of branding (and additionally at 21 days post branding) did not reduce pain sensitivity or impact rate of healing over the course of the 70-day study. The cooling gel did however significantly reduce wound temperature the day of branding (Tucker et al., 2014b).

Burn victims control their pain by therapy and absence of movement, thus it could be hypothesized that cattle which have experienced a painful husbandry procedure, like hot-iron branding, may also reduce movement (Latarjet and Choinière, 1995). This may help explain the increased incidence of standing and lying observed with larger brands in the current study. Greater occurrence of standing was also captured in castrated bull calves as compared to their branded heifer conspecifics. Similarly greater durations of standing are commonly observed following surgical castration of calves as compared to sham or band castrated calves (Molony et al., 1995; Devant et al., 2012; Webster et al., 2013; Meléndez et al., 2017; Gellatly et al., 2021), potentially because standing reduces agitation and pressure on the site of castration. Comparably, lying time was decreased in surgically castrated animals as compared to their intact counterparts for 5 days post castration (Devant et al., 2012). There is an opportunity for future studies to

explore the impact of brand size on pain parameters. Additionally, there is very limited research exploring the pain response associated with branding in different body locations (i.e., rib versus hip). Although the cattle industry makes recommendations to brand on the shoulder or hip to maximize usable hide by-product, there are no guidelines for branding location based on animal welfare considerations (BQA National Manual, 2019).

There was no overall effect of medication status on the expression of behavior in the current study. However, there was an interaction between pain mitigation received and sex class, which was associated with out-of-sight, standing, and event behaviors. Stomping and tail flicking have been shown to be associated with localized pain resulting from procedures like castration and branding (Schwartzkopf-Genswein et al., 1997b; Petherick et al., 2014; Meléndez et al., 2018a; Gellatly et al. 2021) but it is important to note that they could also be indicative of insect avoidance (Eicher et al., 2001; Kendall et al., 2007). Within the present study, oral meloxicam reduced the display of event behaviors in branded heifer calves as compared to the heifer calves administered a placebo. A recent hot-iron branding study observed no effect of oral meloxicam on frequency behaviors and a limited effect on state behaviors, although oral meloxicam did reduce inflammation as previously noted (Martin et al., 2022). This could be related to the age, sex, and housing environment of the cattle. Martin and others (2022) recorded state behaviors as a summation over the 48-hour study period and pain via a visual analog score at -6-, 6-, 24-, and 48-hour time points. Regardless of medication status, castrated and branded bull calves had greater displays of event behavior compared to branded heifer calves. Meloxicam did not reduce the display of event behaviors in the bull calves as compared to the bull calves administered a placebo. This is difficult to interpret but may be in part due to castrated bull calves being categorized more as out-of-sight, making them unobservable. A recent study of concurrent

branding and castration reported that subcutaneous meloxicam was effective at mitigating pain as seen by reduced tail flicks, increased lying duration, and reduced inflammatory cells and markers in the days following processing (Meléndez et al., 2018a). Observation times, durations, environment, and method of recording behavior varied from that of the current study. Although the present study saw some inconsistencies in behavioral changes as an effect of meloxicam, the positive effects of meloxicam as an ELDU analgesic in livestock has been captured in many studies (EMA, 2018). Studies have reported reductions in prostaglandin metabolites and brand wound temperature post branding (Martin et al., 2022), and displays of pain associated behavior resulting from castration (Meléndez et al., 2018b), dehorning (Heinrich et al., 2010; Allen et al., 2013), cesarean section (Barrier et al., 2014), and induced lameness (Coetzee et al., 2014). The benefit of oral meloxicam is that it has been reported to result in more prolonged behavioral changes (i.e., days) associated with mitigation of pain related to castration (Olson et al., 2016; Meléndez et al., 2019) and branding (Martin et al., 2022). While subcutaneous analgesics also elicit behavioral changes in calves, they are most pronounced in the hours following castration and branding (Marti et al., 2019), and dehorning (Heinrich et al., 2010). These acute behavioral changes are likely associated with the rapid onset of peak plasma meloxicam concentration (Meléndez et al., 2019).

Results of the current study demonstrated that more calves were categorized as standing and out-of-sight at the 6 h post treatment application, while fewer calves were observed expressing event behaviors as compared to the 24 h OP. The 24 h post treatment application OP was chosen to coincide with the half-life of oral meloxicam (range: 19.97 to 43.29 h, mean: 27.54 h; Coetzee et al., 2009). It would have been ideal to also observe behavior at a time representative of the peak concentration (range: 10 to 12 h, mean: 11.64 h) of orally administered

meloxicam but due to daylight constraints that was not possible so the 6 h time point was selected. As previously noted, analgesics can impact behavior; however regardless of pain, cattle behave differently throughout the day, and adult beef cattle on pasture and calves in a feedlot setting had the greatest feeding activity (and proportionally less lying at dawn and dusk) (Robért et al., 2011; Kilgour et al., 2012). The decrease in out of sight calves could in part be attributed to the fact the 24 h OP was captured in the middle of the day and early afternoon and calves may have been more active as compared to the 6 h OP which occurred in the evening (Heinrich et al., 2010). Our results highlighting the time sensitive, analgesic effects of meloxicam are supported by previous studies, which saw decreased expression of abnormal or pain associated behaviors when orally administered meloxicam approaches and reaches peak plasma concentration, as compared to the 24 h timepoint capturing the half-life of meloxicam (Small et al., 2014; Martin et al., 2022). Other studies, where the pharmacokinetics of oral meloxicam were of interest, observation of the animals' behavior or biomarker of interest was done in a more controlled setting (i.e., pens) (Theurer et al., 2012; Small et al., 2014; Olson et al., 2016; Meléndez et al., 2019; Martin et al., 2022) rather than in a pasture setting. Allowing for a greater sampling ability of behavior and other biomarkers, particularly at the peak plasma MEL concentration. Future studies would be sure to observe behavior at the time of peak plasma concentration and half-life of meloxicam to capture peak analgesic effects as well as employ other technologies such as activity loggers. Activity loggers would be able to capture behavioral data through the night, or if calves were in an environment limiting their visibility (i.e., pasture).

CONCLUSION

The results of the current study indicate that hot-iron brand size is a factor which may contribute to pain associated with hot-iron branding. A multimodal analgesic approach may be warranted to combat pain associated with concurrent surgical castration and branding, as oral meloxicam alone did not prove to be effective at reducing event behaviors, which included kicking, foot stamping, head turns, and tail flicks. Areas for future work should further explore the effect of smaller brand sizes, as well as location the brand is placed on the animal (i.e., hip, rib shoulder), and the effect of multiple hot-iron brands used to create one insignia as it relates to the behavior and welfare of cattle. Along with branding related variables, behavioral data collected in a commercial pasture setting will also be important in understanding any impact of environment (i.e., feedlot and pasture) on behavioral changes. The logistics of observing animals in pasture is challenging; to combat this, future behavior studies set in pasture may find success with other treatment marking techniques such as auction patches, breeding patches, ear tags, collars, hair dyes or lighteners, or a combination of treatment marking techniques to reduce any loss of behavior data due to lack of visibility or animal identification in a commercial pasture setting. Although limitations existed in the present study, we were successful in providing pain mitigation in a commercial cow-calf operation which is promising for future research as well as a practical measure in which pain mitigation for castration and branding on commercial operations may be implemented. Brand size should be considered as a potential option to reduce pain associated with branding, but further research is needed on strictly observing the effects of brand size along with a multimodal pain-mitigating approach.

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