UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO DE CIÊNCIAS AGRÁRIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM AGROECOSSISTEMAS

Zimbábwe Osório Santos

Disbudding pain in calves:
mitigating short-term effects and exploring long-term consequences

Florianópolis
2023

Zimbábwe Osório Santos

Disbudding pain in calves:

mitigating short-term effects and exploring long-term consequences

Dissertação submetida ao Programa de Pós-Graduação em Agroecossistemas da Universidade Federal de Santa Catarina como requisito parcial para a obtenção do título de Mestre em Agroecossistemas.

Orientadora: Profa. Maria José Hötzel, Dra.
Coorientadora: Profa. Marina Andrea Graefin Von Keyserlingk, Dra.

Florianópolis

2023

Osório Santos, Zimbábwe
Disbudding pain in calves: : mitigating short-term effects and exploring long-term consequences / Zimbábwe Osório Santos ; orientador, Maria José Hötzel, coorientador, Marina Andrea Graefin Von Keyserlingk, 2023.
90 p.

Dissertação (mestrado) - Universidade Federal de Santa Catarina, Centro de Ciências Agrárias, Programa de Pós-Graduação em Agroecossistemas, Florianópolis, 2023.

Inclui referências.

1. Agroecossistemas. 2. caustic disbudding. 3. cattle. 4. animal welfare. 5. hot-iron disbudding. I. Hötzel, Maria José . II. Andrea Graefin Von Keyserlingk, Marina . III. Universidade Federal de Santa Catarina. Programa de Pós-Graduação em Agroecossistemas. IV. Título.

Zimbábwe Osório Santos

Disbudding pain in calves:

mitigating short-term effects and exploring long-term consequences

O presente trabalho em nível de Mestrado foi avaliado e aprovado, em 10 de agosto de 2023, pela banca examinadora composta pelos seguintes membros:

Profa. Maria José Hötzel, Dra.
Universidade Federal de Santa Catarina

Prof. Rolnei Ruã Darós, Dr.
Pontifícia Universidade Católica do Paraná

Prof. Matheus Deniz, Dr.
Universidade Estadual Paulista

Profa. Patrizia Ana Bricarello, Dra.
Universidade Federal de Santa Catarina

Certificamos que esta é a versão original e final do trabalho de conclusão que foi julgado adequado para obtenção do título de Mestre em Agroecossistemas.

ICP-Edu
Documento assinado digitalmente
Oscar Jose Rover
Data: 14/09/2023 11:14:35-0300
CPF: ***.116.509-**
Verifique as assinaturas em https://v.ufsc.br

Coordenação do Programa de Pós-Graduação

ICP-Edu
Documento assinado digitalmente
Maria Jose Hotzel
Data: 14/09/2023 10:33:57-0300
CPF: ***.508.720-**
Verifique as assinaturas em https://v.ufsc.br

Profa. Maria José Hötzel, Dra.
Orientadora

Florianópolis, 2023.

RESUMO

Na bovinocultura moderna, os chifres são vistos como um inconveniente, levando à prática de amochamento, que envolve a destruição do tecido originário dos chifres em bovinos não mochos durante primeiros dias de vida. Esta dissertação aborda algumas lacunas de conhecimento relacionadas a este procedimento doloroso. Embora haja uma crença entre produtores e veterinários de que o amochamento caustico é menos doloroso do que o amochamento por cauterização por calor, não há consenso na comunidade cientifica sobre o grau de dor associado a cada método. No Capítulo II exploramos essa questão, através de uma revisão sistemática realizada para reunir a literatura experimental comparando os dois métodos. Encontramos 6 estudos que de alguma forma compararam os dois métodos. Apesar de todos os estudos relatarem diferenças entre os métodos, houve pouca concordância entre os estudos nos sete tipos variáveis que foram relatadas, e muitos estudos tiveram limitações severas que comprometeram seus achados. Consequentemente, a literatura científica atual não fornece evidências suficientes para determinar o método de amochamento menos prejudicial para os bezerros. Apesar do amochamento cáustico ser um procedimento doloroso, o controle da dor muitas vezes é negligenciado, principalmente o uso de bloqueio anestésico local, cuja eficácia ainda é motivo de debate. Portanto, também investigamos a eficácia de diferentes abordagens farmacológicas na mitigação da dor após o amochamento cáustico. Para tal, estudos que examinaram diferentes abordagens farmacológicas também foram incluídos na revisão sistemática apresentada no Capítulo II. As principais descobertas dos estudos foram relatadas narrativamente, mas também usamos uma abordagem meta-analítica para resumir os efeitos gerais das intervenções sobre o cortisol sanguíneo. Nossos resultados indicam que o bloqueio anestésico de lidocaína, isoladamente ou combinado com analgésicos, controla efetivamente os aumentos nos níveis de cortisol e reduz o comportamento da dor no curto período de tempo após o amochamento cáustico, enquanto os analgésicos administrados isoladamente não. No Capítulo III, buscamos responder se um procedimento doloroso no início da vida (especificamente o amochamento cáustico) poderia induzir mudanças no limiar de dor a longo prazo, apesar da implementação das melhores práticas no controle da dor. Para responder esta questão, um ensaio controlado randomizado foi realizado, onde um grupo de bezerros teve um dos botões cornuais removidos aos 10 dias de vida por meio do amochamento cáustico, enquanto outro grupo de bezerros foi submetido a um amochamento simulado. Trinta dias depois, ambos os grupos tiveram o botão contralateral removido por meio de amochamento por cauterização por calor. Medimos o limiar de dor antes e depois de cada procedimento usando um dolorímetro. Nossos resultados sugerem que bezerros previamente expostos a dor podem ser menos sensíveis à dor após um procedimento doloroso subsequente. Em resumo, esta dissertação apontou que ainda não é possível determinar se o amochamento cáustico ou cauterização por calor é o método menos prejudicial para o bem-estar dos bezerros. No entanto, fornecemos evidências de que o uso do bloqueio anestésico com lidocaína em combinação com anti-inflamatório não esteroidais é uma maneira relativamente eficaz de mitigar a dor do amochamento cáustico. Além disso, está dissertação também destaca a necessidade de considerar as consequências a longo prazo de procedimentos dolorosos.

Palavras-chave: amochamento caústico; ferro quente; amochamento químico; bovinos; bem-estar animal.

ABSTRACT

In modern cattle production, the presence of horns is often seen as an inconvenience, leading to the practice of disbudding, which involves the destruction of horn-originating tissue in non-hornless cattle during their early days of life. This dissertation addresses knowledge gaps related to this painful procedure. While there is a common belief among farmers and veterinarians that caustic disbudding is less painful than heat-cautery disbudding, the scientific community lacks consensus on the extent of pain associated with each method. We explored this issue in Chapter II. A systematic review was undertaken to gather the experimental literature comparing these two methods, and studies were comprehensively examined from an animal welfare perspective, a synthesis of their findings and limitations was provided. We have found 6 studies that somehow compared the two methods. Despite all studies reporting differences between the disbudding method, although six studies compared the methods, there were few agreements across the seven types of reported outcomes, and many studies had severe limitations compromising their findings. Consequently, the current scientific literature does not provide sufficient evidence to determine the less detrimental disbudding method. However, caustic disbudding is a painful procedure, and its pain control is often overlooked, especially the use of local anesthetic block, which efficiency is still a subject of debate. Thus, we also investigate the effectiveness of different pharmacological approaches in mitigating pain following caustic disbudding. To address this issue, articles that examined different pharmacological approaches were also included in the systematic review presented in Chapter II. The main findings were narratively reported, but we also build more on this using a meta-analytical approach to summarize the overall intervention's effects on blood cortisol. Our results indicate that lidocaine anesthetic block, either alone or combined with analgesics, effectively controls raises in cortisol levels and reduces pain behavior in the short time after caustic disbudding, while analgesics given alone do not. In Chapter III we seek the answer to whether a painful procedure early in life (e.g., caustic disbudding) could induce long-term threshold changes, despite the implementation of best practices in pain control. To address this question, a randomized controlled trial was conducted where one group of calves had one of the horn buds removed at 10 days of life through caustic disbudding, while another group of calves underwent a sham disbudding. Thirty days later, both groups had the contralateral horn removed through heat-cautery disbudding. We measured pain sensitivity before and after both disbudding procedures using an algometer. Our findings suggest that calves previously exposed to pain may be less sensitive following subsequent painful procedures. In summary, this dissertation pointed out that it is not possible yet to determine whether caustic or heat-cautery disbudding is the less detrimental method for calves' welfare, however, we provided evidence that using lidocaine anesthetic block in combination with non-steroidal anti-inflammatory drugs is a relatively efficient way to mitigate caustic disbudding pain. Moreover, this dissertation also highlights the need to consider the long-term consequences of painful procedures.

Keywords: caustic disbudding; hot-iron; chemical disbudding; cattle; animal welfare.

RESUMO EXPANDIDO

Introdução

A ausência de dor sempre é considerada uma premissa para o bem-estar animal. No entanto, procedimentos dolorosos são frequentemente realizados em animais utilizados na produção de alimentos e matérias-primas, especialmente em animais jovens. A dor durante o período neonatal tem recebido atenção considerável na medicina humana, especialmente porque esta pode alterar o desenvolvimento normal do sistema nervoso, resultando em alterações a longo prazo no limiar da dor. Embora haja uma preocupação crescente sobre as alterações a longo prazo na sensibilidade à dor devido à dor neonatal em humanos, poucas investigações tem sido feitas a respeito deste assunto em animais de criação. Chifres constituídos por um núcleo ósseo envolto por camadas de pele queratinizada, são uma característica encontrada em todos os bovídeos. Entretanto, no contexto da pecuária, o desenvolvimento de chifres dos bovinos é muitas vezes impedido por meio de uma prática dolorosa conhecida como amochamento. O amochamento consiste na destruição do tecido originário do chifre em animais jovens e a redução de acidentes e a redução do espaço por animal, e razoes estéticas e culturais são algumas das justificativas comumente apresentadas pelos produtores para a realização deste procedimento. O amochamento por cauterização por calor e cáustico são os métodos mais comumente usados. Sabe-se, atualmente, que a dor do amochamento por cauterização por calor tem diversas consequências negativas para os bezerros como comportamento relacionados à dor, aumento de cortisol, pessimismo, comportamento antissocial, entre outros. Embora seja menos estudado, o amochamento cáustico também tem sido associado a consequências negativas para os animais. Estudos relataram que existe uma crença comum entre produtores e veterinários de que o amochamento cáustico é menos doloroso do que o amochamento por cauterização por calor, embora não haja consenso na comunidade científica sobre a extensão da dor associada a cada método. Estudos também mostraram que bezerros amochados causticamente recebem menos mitigação da dor, especialmente bloqueio anestésico local, cuja eficiência ainda é objeto de debate.

Objetivos

O Capítulo II desta dissertação teve como objetivos: a) comparar os efeitos do amochamento cáustico e por cauterização por calor no bem-estar dos bezerros; b) investigar a eficácia de diferentes abordagens farmacológicas na mitigação da dor associada ao amochamento cáustico. No Capítulo III procuramos responder se um procedimento doloroso no início da vida (especificamente o amochamento cáustico) poderia induzir alterações de limiar a longo prazo, apesar da implementação das melhores práticas de controlo da dor.

Metodologia

Uma revisão sistemática foi conduzida para alcançar os dois objetivos do Capítulo II. Buscas bibliográficas foram realizadas nas plataformas Google Scholar, Scopus, Web of Science, and PubMed. Estratégias de busca foram construídas visando buscar trabalhos onde o amochamento cáustico foi realizado em bezerro e as buscas foram completadas em 10 de maio de 2023. Triagens foram realizadas visando manter apenas estudos empíricos revisados por pares, escritos em inglês e que compararam os dois métodos de amochamento utilizando variáveis relacionadas ao bem-estar do animal ou examinaram os efeitos de analgésicos, ou do bloqueio anestésico local para a mitigação da dor do amochamento cáustico. Informações gerais dos estudos incluídos na revisão foram extraídas, tais como: informações sobre os animais; sobre o procedimento de amochamento; fármacos utilizados e formas de aplicação; e variáveis medidas. Quando os estudos trouxeram uma comparação estatística entre os métodos de amochamento ou as formas de mitigação da dor, os resultados dessa comparação foram extraídos qualitativamente, informando se determinado tratamento teve frequência/média/mediana maior, menor ou igual em relação aos outros tratamentos. Se nenhuma análise estatística foi realizada, os resultados do estudo foram extraídos de maneira descritiva. Em estudos que comparam estratégias de mitigação da dor e o nível de cortisol sanguíneo foi uma das variáveis reportadas, dados de desvio padrão e media foram extraídos de forma contínua. Meta-analises foram conduzidas para explorar o efeito das diferentes estratégias de mitigação da dor nos níveis de cortisol sanguíneo.

Para alcançar o objetivo do capítulo III, um estudo randomizado controlado foi conduzido para testar se o amochamento de um botão cornual aos 10 dias de vida afetaria o limiar de dor após a amochamento de um segundo botão cornual 30 dias após o primeiro procedimento. Vinte bezerros fêmeas e seis machos da raça holandesa foram distribuídos aleatoriamente para o grupo controle ou tratamento. Aos 9,5±1,8 dias, os bezerros do grupo tratamento tiveram um botão cornual amochado com pasta cáustica, enquanto os bezerros de grupo controle foram submetidos a um amochamento simulado, em ambos os casos foi fornecido controle multimodal da dor (xilazina, bloqueio do nervo cornual com lidocaína e meloxicam). Quatro semanas depois, bezerros de ambos os grupos tiveram o botão cornual contralateral amochado com ferro quente, novamente com controle multimodal da dor. As respostas nociceptivas mecânicas foram avaliadas semanalmente usando um dolorímetro aplicado adjacente a ambos os botões cornuais e na garupa, começando 3 dias antes do primeiro amochamento e terminando 30 dias após o segundo. Mudanças entre o limiar de dor após o segundo amochamento e a média dos dois dias anteriores foram testadas utilizando modelos mistos.

Considerações Finais

Nesta dissertação, tive como objetivo comparar o impacto no bem-estar do amochamento cáustico e do amochamento por cauterização por calor em bezerros e determinar se um dos métodos é menos prejudicial. Minha pesquisa descobriu efeitos negativos no bem-estar para ambos os métodos, e diferenças nas populações experimentais, variáveis, avaliação e métodos de reportar dificultam declarar um dos métodos como menos prejudicial. Eu também comparei diferentes abordagens para mitigar a dor causada pelo amochamento cáustico e descobri que o bloqueio anestésico com lidocaína reduz efetivamente a dor de curto prazo. Além disso, explorei se experiências dolorosas no início da vida, como o amochamento cáustico, influenciam a sensibilidade à dor mais tarde na vida, revelando que tais experiências podem alterar a sensibilidade geral à dor. Atualmente as evidências científicas confirmam que a dor em humanos e animais ocorre de maneira semelhante em termo

fisiológicos e emocionais, apesar deste entendimento, a mitigação da dor continua subutilizada devido a vários fatores, incluindo influências culturais e restrições legais. Uma abordagem multidisciplinar é necessária para abordar estas barreiras e promover a adoção generalizada de práticas de mitigação da dor. Nesta dissertação demostrei que procedimentos dolorosos realizados no início da vida podem ter efeitos duradouros na sensibilidade à dor. Estas descobertas destacam a necessidade de considerar as consequências a longo prazo ao realizar o amochamento. O princípio da precaução deverá orientar-nos, dada a incerteza dos riscos associados a estes procedimentos. Em resumo, esta dissertação não só abordou a mitigação imediata da dor no amochamento cáustico, mas também enfatizou a importância de considerar as consequências a longo prazo e explorar alternativas para substituir práticas dolorosas em bezerros.

Palavras-chave: amochamento cáustico; ferro quente; amochamento químico; bovinos; bem-estar animal.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

BW -body weight

CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CI – confident interval

CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico

CP – Crude Protein

d – Day

DEFRA – Department for Environment, Food & Rural Affairs (United Kingdom)

DMW - Daniel M. Weary

DFMA - Department of Agriculture, Food and the Marine (Ireland)

DN - Dr. Naylor Dehorning Paste

Fig. – Figure

g – Grams

h. – Hour

IASP – International Association for the Study of Pain

ICU – Intensive Care Unit

IM – Intramuscular route

I.N-N – Ihakri NUNC-NFÔONRO

IQR – Interquartile range

kgf – kilogram force

L – Liter

LETA – Laboratório de Etologia Aplicada e Bem-Estar Animal

m – Miter

MAF – Ministry of Agriculture and Forestry (Finland)

mg – Microgram

min. – Minute

MJH – Maria José Hötzel

mL – Milliliter

mm – Millimeter

MNT – Mechanical Nociceptive Threshold

mo. – Month

MvK - Marina Andrea Graefin Von Keyserlingk

NAHMS – National Animal Health Monitoring System

no. – number

NPC – no pain control

NSAIDs – no steroidal anti-inflammatory drug

OIE – World Organization for Animal Health

OR – oral routes

PRIMA-P - Preferred Reporting Items for Systematic Reviews and Meta-Analyses

SC – Subcutaneous routes

SD – Standard Deviation

SE – Standard Error

sec. – Second

SMD – Standardized Mean Difference

UBC – University of British Columbia

UFSC – Universidade Federal de Santa Catarina

USDA – United States Department of Agriculture

wk. - week

y. - Year

Z.O-S – Zimbábwe Osório-Santos

SUMÁRIO

1 CHAPTER I - GENERAL CONSIDERATIONS REGARDING PAIN AND DISBUDDING ON CALVES

1.1 PAIN DEFINITION

According to the International Association for the Study of Pain (IASP) (RAJA et al., 2020), pain can be defined as a sensory and emotionally unpleasant experience associated with potential or actual tissue damage. The IASP also points out that the inability to communicate does not deny the possibility that a human or non-human animal experiences pain (RAJA et al., 2020). From an evolutionary perspective, pain has a protective function, since it allows animals to identify and avoid harmful stimuli and protect injured parts of the body; additionally, pain-related behaviors expression can recruit assistance and care from other animals (RAJA et al., 2020; STEINKOPF, 2016). However, despite pain's adaptive role, it has also adverse effects on animal welfare (RAJA et al., 2020).

Physiologically, pain occurs through three processes: transduction, transmission, and perception (VIÑUELA-FERNÁNDEZ et al., 2007). The transduction occurs when specialized free nerve endings, called nociceptors, translate noxious stimuli (mechanical, thermal, or chemical) into electrophysiological activity. Subsequently, these stimuli are transmitted through a first-order neuron to the dorsal root where a synapse with a second-order neuron occurs. These stimuli are then transmitted to the thalamus (brain) via the spinothalamic tract. The thalamus is considered the main "sorting center", since it is the structure responsible for transmitting stimulus to multiple areas of the cortex. In the cortex, stimuli will be processed, giving meanings in terms of evaluation (chronic or acute), intensity of pain, location, and incorporation of affective aspects, resulting in the perception of pain (DILLWORTH; MENDOZA; JENSEN, 2012).

1.2 TYPES OF PAIN AND MITIGATION STRATEGIES

Pain is usually divided into three types: intraoperative and inflammatory, both of which are physiologic, and neuropathic, which is a pathology (ADCOCK; TUCKER, 2018a; ANDERSON; MUIR, 2005). Intraoperative pain usually occurs soon after the injury happen, due to electrophysiological activity conducted by type A and C-fibers. A-fibers are myelinated and, as a result, they can conduct the electrophysiological activity at high speed, resulting in a sharp pain feeling that leads the animal to promptly move away from the harmful stimulus (BOURNE; MACHADO; NAGEL, 2014). C-fibers are unmyelinated; thus, the electrophysiological activity is transmitted at a low speed, causing a delayed, diffuse, duller pain sensation (BOURNE; MACHADO; NAGEL, 2014). When the surgical procedure is confined to a specific region of the body and the anatomy is favorable, intraoperative pain is mitigated providing a local anesthetic block (BEAUSSIER et al., 2018; LIVINGSTON, 2010). Lidocaine is commonly used for this purpose, and it works by blocking the influx of sodium ions into the membrane nerves, preventing the depolarization which convert the harmful stimulus in electrophysiological activity. Lidocaine just leads to the numbness of the blocked region, which can last for two hours when associated with epinephrine (KRISHNA PRASAD; KHANNA; JAISHREE, 2020), but does not lead to unconsciousness (BEAUSSIER et al., 2018).

Inflammatory pain occurs mainly during the healing process following injury or surgical intervention. During this period, several inflammatory mediators are produced in the injury site and surrounding tissue, including prostaglandins, histamine, substance P bradykinins, cytokines, chemokines, and purines (VIÑUELA-FERNÁNDEZ et al., 2007). This “inflammatory soup” sensitizes the nociceptors, which means that their depolarization occurs at lower-intensity stimuli, resulting in a decrease in the pain threshold in the affected region (primary hyperalgesia) (ANDERSON; MUIR, 2005; VIÑUELA-FERNÁNDEZ et al., 2007). The use of systemic analgesics such as non-steroidal anti-inflammatories drugs (NSAIDs) is one-way to mitigate inflammatory pain, and NSAIDSs are the most commonly used analgesics in farm animals (KLEINHENZ; VISCARDI; COETZEE, 2021; STEAGALL et al., 2021). NSAIDs can inhibit the cyclooxygenase enzymes, which mediate the conversion of arachidonic acid into inflammatory substances and, as a result, they also prevent neuronal sensitization (LIVINGSTON, 2010). These drugs are used to provide some pain relief in the first hours after the procedure; however, there are currently no strategies for managing prolonged inflammatory pain in farm animals (ADCOCK, 2021). For instance, a single dose of NSAIDs can provide pain relief for up to 44 h (Heinrich et al., 2010), although painful procedures can take several weeks to heal completely (ADCOCK; TUCKER, 2018b; DRWENCKE; ADCOCK; TUCKER, 2023).

Neuropathic pain occurs when nerve tissue is damaged. In this case, the unperfect growth of nerve structures can lead to the formation of a neuroma. These structures hurt when stimulated, but also hurt spontaneously (RAJPUT; REDDY;

SHANKAR, 2012). The neuroma's pain is considered a pathology because it lacks adaptive value, can persist even after the injury is fully healed, and eventually become chronic (ADCOCK; TUCKER, 2018a; RAJPUT; REDDY; SHANKAR, 2012). Neuromas have been also found in farm animals after beak trimming (GENTLE, 1986) and tail docking (LARRONDO et al., 2019; SANDERCOCK et al., 2016), but there are still no mitigation strategies for these animals (ADCOCK, 2021; ADCOCK; TUCKER, 2018a).

Pain during the neonatal period has received considerable attention in human medicine (CARTER; BRUNKHORST, 2017). One of the reasons is that it can alter the normal development of the nervous system, resulting in long-term changes in pain threshold (WALKER, 2014). Some studies in humans and rodents have shown that painful experiences early in life result in lower pain thresholds later in life, which is called hyperalgesia. For example, a study in rodents showed that rats that experienced a paw incision at 3 days of life had hyperalgesia in a subsequent incision as adults, compared with those who did not underwent the same procedure (BEGGS et al., 2012). Similarly, circumcised babies cried longer and showed more facial pain expressions when vaccinated 6 months later compared to uncircumcised babies (TADDIO et al., 1997). In another study with school-age children (9-14 years old), a lower nociceptive (thermal) threshold was reported for children who had to stay at the intensive care unit (ICU) as babies in comparison with those who did not (HERMANN et al., 2006).

Some studies show an opposite trend, with early painful experiences leading to an increased pain threshold, i.e., hypoalgesia. For instance, a greater number of painful procedures as a baby was associated with lower cortisol levels at seven years old. (BRUMMELTE et al., 2015). Babies born prematurely and who underwent a high number of painful procedures show a greater pain threshold (assessed by the cold water immersion method) than premature babies who suffered a lower number of painful events (VEDERHUS et al., 2012). There is some evidence that pain control, specially lidocaine during panful procedures can reduce these effects or even prevent hyperalgesia in rodents and humans (TADDIO et al., 1997; WALKER, 2014).

1.3 LONG -TERM CONSEQUENCES OF PAIN IN FARM ANIMAS

The absence of pain has always been considered a premise for animal welfare. For instance, "free from pain, injury and disease" was one of the five freedoms proposed by Britain's Farm Animal Welfare Council in 1965. Absence of pain is still present in the Animal welfare definition adopted by the World Organization for Animal Health, where animals “experience good welfare if [...] they are not suffering from unpleasant states such as pain, fear or stress” (OIE, 2021). Although absence of pain has been considered essential to ensure animal welfare for at least 58 years, painful procedures are often performed on farm animals (PRUNIER et al., 2013; STEAGALL et al., 2021). In dairy cattle, specifically, many painful procedures are performed in the first days of life, such as identification, removal of extra teats, castration, and the focus of this thesis, disbudding (SHIVLEY et al., 2019; VASSEUR et al., 2010).

Although there is growing concern about long-term changes in pain sensitivity due to neonatal pain in humans, little research has been done on farm animals. Findings obtained in human and rat studies cannot be directly generalized to cattle, as the latter are precocial and, as a result, have more developed central nervous system at birth (SCHEIBER et al., 2017). Yet, the few available studies on ruminants suggest that the effects previously reported in humans and rodents are somehow replicable in ruminants. For instance, the three studies that examined the effect of early painful experience in ruminants reported an increase in response to subsequent painful experiences. Lambs castrated at early age were found to show more abnormal postures after tail docking performed 30 days after castration than lambs that were castrated at 10 days of age (MCCRACKEN et al., 2010). Ewes that were tail-docked at 3 d of life showed higher levels of pain behavior when giving birth as adults compared with non-tail-docked animals (CLARK et al., 2014). Heifers dehorned at 5 and 35 days of age showed a higher heart rate when vaccinated at 11 months of age than heifers dehorned at 56 days of age (ADCOCK; TUCKER, 2020a). However, these studies are based on behavioral and physiological outcomes, and the pain threshold was not assessed directly in any of them.

1.4 CATTLE HORNS AND WHY DISBUDDING IS STILL A COMMUM PRATICE

Horns, consisting of a bone core encased by keratinized skin layers, are a trait found in all bovids, with wild cattle possessing them in both sexes (DAVIS; BRAKORA; STILSON, 2014). The benefits of horns include intrasexual competition for mates in males and defense against predators for both males and females, which may explain their persistence throughout evolutionary history (JANIS, 1982; STANKOWICH; CARO, 2009). In cattle the originating horn structure, known as the horn bud, is already present in the fetus around 70 days of gestation (WIENER et al., 2015). As the calf is born, the horn bud is free-floating in the skin layer above the skull, but it gradually attaches to the frontal bone as the calf grows, ultimately connecting the hollow centers of the horn core to the frontal sinus (ROUSSEAU, 2022).

Although the horned phenotype is widespread in wild cattle populations, it is a recessive trait controlled by the polled gene with two alleles (ALDERSEY et al., 2020). Recessive homozygous individuals (*pp*) exhibit a horned phenotype, while heterozygotes (*Pp*) and homozygous (*PP*) display a hornless phenotype, often referred to as polled (ALDERSEY et al., 2020). In some cases, polled animals (genotype *PP* or *Pp*) may develop a structure called scur instead of a fully formed horn. The scur is an appendicular structure similar in shape to a horn and contains a bone core, but it lacks hollowness and fusion to the frontal bone skull. Instead, the scur is attached to the skull by a soft cartilaginous material (CAPITAN et al., 2011). This particular phenotype is believed to be governed by a different gene and influenced by the sex and breed (SCHAFBERG; SWALVE, 2015). However, genetic mechanisms remain not completely elucidated (GROBLER; VAN MARLE-KÖSTER; VISSER, 2021).

In the context of animal farming, the horn bud is often removed to prevent horn growth, a practice known as disbudding (USDA-NAHMS, 2016). This procedure must be performed before the horn bud attaches to the frontal sinus (AABP, 2019). Reducing accidents, reducing required spare, esthetical and cultural reasons are some of the justifications commonly stated by farmers to carry out this procedure (CARDOSO; VON KEYSERLINGK; HÖTZEL, 2016; COZZI et al., 2015). However, disbudding is painful (STILWELL et al., 2009; WINDER et al., 2018a) and deliberately inflicting pain on animals is a practice overwhelming rejected by the public (HÖTZEL et al., 2017; ROBBINS et al., 2015). Consequently, the dairy industry has been seeking new alternatives.

As a result of the simple genetic heritage, the polled trait can be easily spread in the population through selective traditional breeding. One study suggests that introducing polled genetics in dairy herds could be cheaper than disbudding calves using pain mitigation (THOMPSON et al., 2017). However, this alternative raises concerns regarding the potential loss of genetic merit in animals, as polled animals have traditionally been less intensively selected for production traits compared to their horned counterparts (MUELLER et al., 2019). Research indicates that the incorporation of polled genetics may slow down genetic progress and increase inbreeding (MUELLER et al., 2019; SPURLOCK; STOCK; COETZEE, 2014). In the case of beef cattle, polled genetics is not consistently associated with lower genetic merit and may even exhibit positive associations with relevant production traits. However, introducing polled genes can be challenging in *Bos taurus indicus* due to the limited number of available polled bulls (RANDHAWA et al., 2021).

Considering the aforementioned scenario, gene editing has been proposed as an alternative approach to selective breeding. This bioengineering technology allows for the easy incorporation of the naturally occurring polled gene (*P*) into horned dairy cattle (CARLSON et al., 2016). The main advantage over traditional breeding is that gene editing can sustain great annual genetic merits gains and relatively low levels of inbreeding (MUELLER et al., 2019). However, gene editing in farm animals still faces challenges. For instance, the unintended transfer of bacterial genetic material along with the polled gene has been observed by scientists at the US Food and Drug Administration (MIT - TECHNOLOGY REVIEW, 2019). Although gene editing is pointed out as an accurate bioengineer tool by some scientists (THOMAS et al., 2019), unexpected findings are being reported, such as significant on-target (KOSICKI; TOMBERG; BRADLEY, 2018) and off target (FU et al., 2013) mutagenesis. Furthermore, public acceptance plays an important role in the advancement of gene editing. Applications that offer potential animal welfare benefits tend to receive more public support (MCCONNACHIE et al., 2019; YUNES et al., 2021), but perceived risks and uncertainties may undermine their progress (YUNES et al., 2019, 2021).

1.5 CONSIDERATIONS REGARDING DISBUDDING

As a consequence of the challenges in implementing polled genetics, disbudding continues to be a routine procedure in dairy farms (USDA-NAHMS, 2016) and, although less common, also in beef farms (USDA-NAHMS, 2020). Heat-cautery and caustic disbudding are the most commonly used methods, and although heat-cautery disbudding is still the first method used in many countries (HÖTZEL et al., 2014; USDA-NAHMS, 2016; WINDER et al., 2018b) caustic disbudding uses has rapidly increased in the United States (SARACENI et al., 2021a).

It is currently known that heat-cautery disbudding has several negative consequences on calves. For instance, cortisol levels are raised to 2 h (STAFFORD; MELLOR, 2011); pain behaviors are increased up to 24h (FAULKNER; WEARY, 2000); heat-cautery wounds are sensitive through all the healing process, which takes 9 weeks (ADCOCK; TUCKER, 2018b); wound also appears to hurt spontaneously for at least 20 days after the procedure (ADCOCK; TUCKER, 2020b). Additionally, heat cautery disbudding was reported to induce various negative affective states such as pessimism (NEAVE et al., 2013), antisocial behavior (GINGERICH; CHOULET; MILLER-CUSHON, 2020), aversion (EDE; VON KEYSERLINGK; WEARY, 2019) and anhedonia (LECORPS et al., 2019). There is enough evidence to believe that caustic disbudding is also a painful procedure. For instance, caustic disbudding increases pain behaviors expression and cortisol levels (BRAZ et al., 2012; STILWELL et al., 2009), and disbudding wounds remain sensitive for a period even longer than reported for heat cautery disbudding (DRWENCKE; ADCOCK; TUCKER, 2023). However, caustic disbudding pain has been way less studied than heat-cautery disbudding. Several studies have shown that caustic disbudding is considered less painful than heat-cautery disbudding by farmers and veterinarians (ANDRIGHETTO CANOZZI; ROSSI BORGES; JARDIM BARCELLOS, 2020; SARACENI et al., 2021b; SHI et al., 2022), and calves often receives less pain mitigation (COZZI et al., 2015; USDA-NAHMS, 2016), especially local anesthetic bock (SARACENI et al., 2021b; WINDER et al., 2016) than calves undergoing heat-cautery disbudding.

1.6 THIS DISSERTATION AIMS

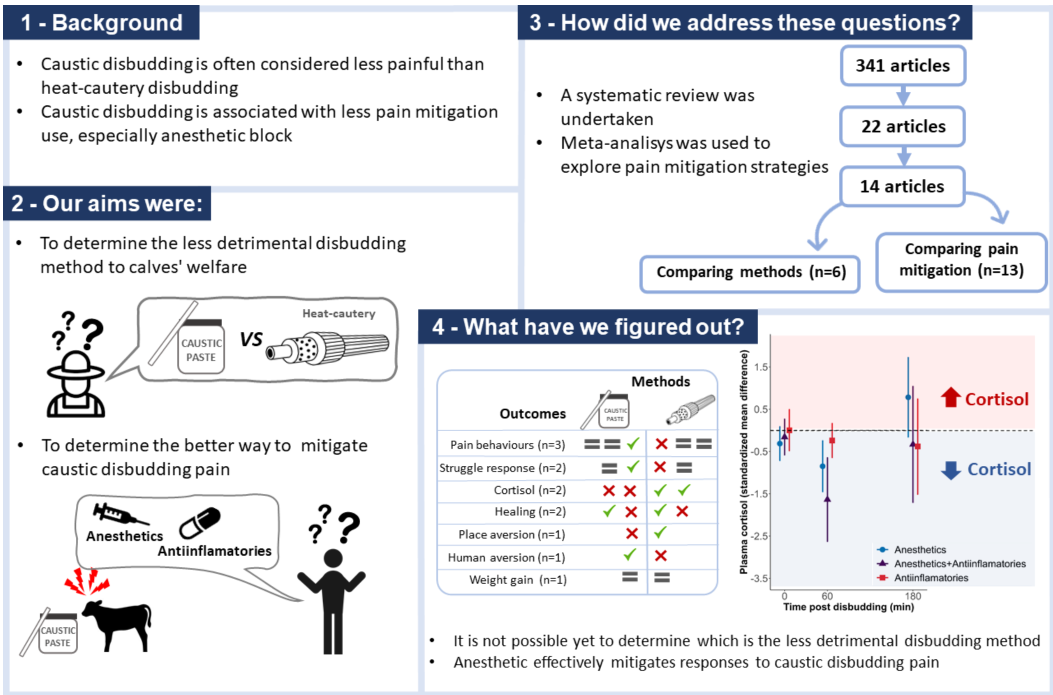
This dissertation seeks to address some existing knowledge gaps regarding disbudding practices. While there is a common belief among farmers and veterinarians that caustic disbudding is less painful than heat-cautery disbudding, there is no consensus in the scientific community on the extent of pain associated with each method. Caustic disbudding pain control is often overlooked, especially the use of local anesthetic block, which efficiency is still a subject of debate. Thus, the primary objective of Chapter II was to compare the effects of heat-cautery and caustic disbudding on calves' welfare. The secondary objective of Chapter II was to investigate the effectiveness of different pharmacological approaches in mitigating pain following caustic disbudding. In Chapter III we sought to answer whether a painful procedure early in life (I.e., caustic disbudding) could induce long-term threshold changes, despite the implementation of best practices in pain control. Lastly, in Chapter IV, we highlighted the main findings of our research, and emphasized the areas where additional efforts should be done to advance animal welfare knowledge in the field of disbudding.

2 CHAPTER II - ON THE HORN DILEMMA: A SYSTEMATIC REVIEW COMPARING HEAT-CAUTERY AND CAUSTIC DISBUDDING AND PAIN MITIGATION STRATEGIES FOR CAUSTIC DISBUDDING PAIN [1]

Zimbábwe Osório-Santos[1]; Maria José Hötzel[1]*

[1]Laboratório de Etologia Aplicada e Bem-Estar Animal (LETA), Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina. Rod. Admar Gonzaga, 1346, Itacorubi, 88034-001, Florianópolis, SC, Brazil
*Correspondence to maria.j.hotzel@ufsc.br

2.1 GRAPHICAL ABSTRACT



2.2 HIGHLIGHTS

- There is not enough evidence in the current literature to recommend heat cautery over caustic disbudding or vice versa;

[1] A version of Chapter II is in preparation for submission. Z. O-S was involved in conceptualization, methodology, conducting the study, data curation, formal analysis, and writing the original draft. MJH supervised the research, reviewed and edited the manuscript.

- Lidocaine alone or combined with non-steroidal anti-inflammatories has a protective short time effect (up to 60 min) on cortisol levels following caustic disbudding;
- A pain rebound effect was observed when lidocaine was provided without NSAIDs;
- Anti-inflammatories mitigating effect on caustic disbudding pain varied across studies;
- Caustic disbudding pain remains a relatively poor studied subject.

2.3 ABSTRACT

Research has shown that caustic disbudding is considered less painful than heat cautery-disbudding by farmers and veterinaries, and often associated with lower pain control uses, especially the lidocaine anesthetic block. This systematic review aimed to: I) compare the effects of heat-cautery and caustic disbudding on calves' welfare; II) explore the effectiveness of systemic analgesics, cornual anesthetic block, and their combination to mitigate pain following caustic disbudding in calves. Articles were identified via searches in the Google Scholar, Scopus, Web of Science, and PubMed databases (n= 483). Only peer-reviewed, empirical studies comparing both methods using welfare-related outcomes or examining the use of analgesics and/or anesthetic block following caustic disbudding were included. Six studies were used to compare heat-cautery and caustic disbudding, the main finding was extracted and described narratively. Thirteen studies were used to explore the pain mitigation effects of pharmacological interventions in comparison with the no-pain control group. Meta-analysis was used to explore pharmakos' effects on cortisol levels, while pain behavior and other welfare-related outcomes were described narratively. Outcomes used to compare heat-cautery and caustic disbudding varied greatly across studies and conflicting findings were present when more than one study reported the same outcome. Furthermore, most studies had severed experimental limitations such as differences in age, pharmacological intervention, and quasi-experiment design. Lidocaine was tested alone or with analgesics on 8/13, and it was the only cornual anesthetic block tested. Ten out of 13 studies tested analgesics, which were mainly non-steroidal anti-inflammatories drugs (NSAIDs; n=9). The outcomes of the meta-analysis demonstrated that lidocaine anesthetic block alone or associated with analgesics reduced plasma cortisol until 1 h post disbudding. Furthermore, reductions

in cortisol and pain behaviors were predominately in our narrative review, however, a pain rebound effect was also reported once lidocaine alone wears off. No pain rebound effect was observed when NSAIDs were combined with lidocaine, although no effect in reducing cortisol was detected in the meta-analysis when analgesics were used alone. Our results showed that the current scientific literature does not provide sufficient evidence to determine which method, caustic disbudding or heat-cautery disbudding, is better in terms of the calf's welfare. But caustic disbudding is indeed painful, and pain mitigation must be used. Caustic disbudding pain is better mitigated when the lidocaine anesthetic block is given, reducing the acute pain immediately following the procedure, and combining it with analgesics (especially NSAIDs) might prevent later pain response when the anesthetic effect wanes.

Keywords: Cortisol, Dehorning, Chemical disbudding, Hot-iron disbudding; Animal welfare; Lidocaine

2.4 INTRODUCTION

Disbudding, is a painful procedure, commonly performed on dairy calves (SARACENI et al., 2021b) in order to prevent horn growth, and is done for practical, cultural, and esthetic reasons (CARDOSO; VON KEYSERLINGK; HÖTZEL, 2016; KLING-EVEILLARD et al., 2015). Heat-cautery and caustic disbudding are the most common methods used to disbud bovine calves (COZZI et al., 2015; HÖTZEL et al., 2014; SARACENI et al., 2021b). Heat-cautery disbudding involves using a heated iron to destroy the horn bud, while caustic disbudding utilizes alkaline substances to cause liquefactive necrosis (LINDÉN et al., 2023). Although heat-cautery is still the most used method among dairy farmers in USA, caustic disbudding uses have increase from 12.2% in 2007 to 32.5% in 2014 (USDA-NAHMS, 2016).

Calves subjected to caustic, compared to heat-cautery disbudding, appear to struggle less during the procedure (STILWELL; LIMA; BROOM, 2007), and pain behavior starts to be more evident only 10 to 15 minutes after the procedure, when farm personal may not be present (STILWELL et al., 2009; WINDER et al., 2017). Perhaps for these reasons, caustic disbudding is often pointed out as less painful than the heat-cautery method, even with no scientific consensus on the degree of pain caused by each method. For example, Chinese dairy industry stakeholders (SHI et al., 2022), Brazilian veterinarians and animal scientists (CANOZZI; BORGES;

BARCELLOS, 2022), USA dairy farmers (SARACENI et al., 2021b), and Canadian dairy farmers and veterinarian (WINDER et al., 2016) perceive caustic disbudding as less painful than heat-cautery disbudding. This belief is reinforced by caustic products advertisers that claim the method to cause "less stress to the animal" (PBS-ANIMAL HEALTH, [s.d.]). However, both procedures are painful, resulting in increases in serum cortisol levels (STILWELL et al., 2009, 2010) and pain related behaviors (BRAZ et al., 2012; WINDER et al., 2018a) in the hours following disbudding, and emotional states associated with fear and pessimism (EDE; VON KEYSERLINGK; WEARY, 2020b; LECORPS et al., 2019) some days after. Therefore, pharmacological approaches are necessary to mitigate some of these adverse effects (HERSKIN; NIELSEN, 2018; STAFFORD; MELLOR, 2011).

The combination of cornual anesthetic bock and non-steroidal anti-inflammatory drugs (NSAIDs) is currently the standard recommendation to control pain in heat cautery disbudding (WINDER et al., 2018a). The anesthetic bock prevents intraoperative pain in the first hours following procedure (STILWELL et al., 2010; WINDER et al., 2017), and NSAIDs prevents the strong pain rebound effect that occurs once the effect of lidocaine wanes (KLEINHENZ; VISCARDI; COETZEE, 2021), extending the pain relief for up to 6 (STILWELL et al., 2012) or 44 h (HEINRICH et al., 2010) depending on the used pharmakos. However, the efficiency of cornual anesthetic bock on caustic disbudding is still under debate. For example, Vickers et al., (2005) did not find a decrease in pain behaviors when lidocaine anesthetic was given in sedated calves in an ring block, while Stilwell et al., (2009) found positive outcomes in cortisol and pain behaviors when lidocaine was used in non-sedated calves. Given this uncertainty, some guidelines exempt caustic disbudding from receiving anesthetic block. For example, the England legislation (DEFRA - UK, 2003), and the Australian animal welfare guidelines exempt farmers from giving pain control in the age at which caustic disbudding is recommended (ANIMAL HEALTH AUSTRALIA, 2016).

The absence of a summary of current scientific evidence on the effects of heat-cautery and caustic disbudding on calves' welfare, as well as the mitigation strategies on caustic disbudding pain might result in legislation and guidelines biased or with weak scientist support (EL BENNI; GROVERMANN; FINGER, 2023). In human health, the evidence-based medicine (EBM) approach has been used to overcome this limitation, and to ensure that medicine practices and guidelines are aligned with the best available scientific evidence (FERNANDEZ et al., 2015) while respecting patients values and preferences (DJULBEGOVIC; GUYATT, 2017). The evidence-based medicine employs methos such as systematic reviews and meta-analyses, that allow to gather the available relevant literature on a given topic to extract the overall effect of a certain intervention.

Our systematic review aims were:

I - To identify and compare welfare related outcomes of heat cautery and caustic disbudding on calves.

II – To investigate the impact of local anesthesia, systemic analgesia and their combination on plasma cortisol levels, pain behavior, and other secondary outcomes in caustic disbudded calves.

2.5 MATERIALS AND METHODS

2.5.1 Protocol

This review was carried out following the PRIMA-P guidelines (PAGE et al., 2021).

2.5.2 Eligibility Criteria

2.5.2.1 Population and Study Design

Our search strategy initially aimed to retrieve information on both bovine calves and goat kids however, only three studies on goat kids were retrieved, thus opted to examine only studies on calves in this review. Thus we target peer-reviewed controlled trial studies, published in English where caustic substances were tested in bovine calves with no concurrent painful procedure. Calf age and breed were not limited. Controlled trial studies were used to address the first aim of this review, and randomized controlled trials were used to address our second aim.

2.5.2.2 Intervention and comparator group

Any retrieved controlled trial study comparing caustic and cautery disbudding was included in the first part of the review, where heat cautery disbudding was considered the comparator group. To be included in the second part of the review, studies had to have at least two of the following group: analgesic alone; analgesic combined with cornual anesthetics block; cornual anesthetics block, and no pain control.

2.5.2.3 Outcomes

Cortisol and pain behavior were chosen as primary outcomes because they are widely accepted as pain and distress indicators (STAFFORD; MELLOR, 2011; TSCHONER, 2021; WINDER et al., 2018a), additionally, they were the variables most frequently reported in our preliminary search. However, searches were not limited by any outcome in our search strategy.

2.5.3 Information Sources and Search Strategy

PubMed, Web of Sciences, Scopus, and Google Scholar databases were used to search for possible eligible studies. Literature searches were completed on May 10th, 2023. Based on pilot research, the authors were aware that the literature regarding this subject is relatively scarce; thus, to avoid loss of any possible relevant material we opted to build a search strategy based only on our target population (caves disbudded using caustic methods), not limiting searches by interventions, comparators groups or outcomes. The search strings used were:

(calf OR calves OR cattle OR goat* OR kid*)
AND
("caustic paste" OR "chemical")
AND
(disbud* OR *horn*)

2.5.4 Selection process

Search results from Pubmed, Web of Sciences, and Scopus were automatically extracted in RIS format and uploaded to the Rayan platform, which is a web used to perform systematic reviews (OUZZANI et al., 2016). Duplicates were automatically pointed by Rayyan (MCKEOWN; MIR, 2021), and were manually checked by Z.O-S. Duplicates that were not automatically detected were removed manually. Due to the impossibility of download Google's retrieved results in a format compatible with Rayyan platform, we manually screened articles on the Google Scholar platform using an Excel sheet. For practical reasons, a simplified screening protocol was used for this particular database, aiming to identify potentially relevant articles that would be included with articles from the other platforms and subjected to a different screening. The Z.O-S opened manually each of the 909 results retrieved on Google Scholar, and the full content available was screened based on the following points:

- Is it a peer-reviewed article with a length of more than 500 words and written in English?
- Does the article describe the use of calves or goat kids in caustic disbudding procedures?
- Does the article aim to test pain mitigation or compare caustic disbudding with cautery disbudding?

Twenty out of the 909 results met the eligibility criteria. These articles were included along with the rest of the articles from Scopus, PubMed, and Web of Science.

2.5.4.1 First Screening

Preliminary article selection, based on title and abstract, was independently conducted by Z.O-S and MJH. The screening process involved assessing the articles based on the following questions:

A) Does the article cover on the topic of caustic disbudding procedures?

B) Does the article involve the use of calves or goat kids?

C) Does the article test pain mitigation or compare caustic disbudding with the cautery method?

The articles were classified independently for each evaluator as “included", "excluded," or "unclear" based on whether they met the criteria or not. Articles rated as "excluded" by both evaluators were excluded from further consideration. Articles were considered eligible for the second screening if they were classified as "included," or “unclear” by both evaluators, or "included” by one and “unclear" by the other. Disagreements between the reviewers regarding articles classified as "excluded/unclear" or "included/excluded" were resolved through discussion and consensus. Articles proceeded to the second screening if an agreement could not be reached on the eligibility.

2.5.4.2 Second screening

Articles eligible from the preliminary screening underwent a second screening. Both reviewers (Z.O-S and MJH) independently analyzed the full text of each article, and assessed them based on the following questions:

D) Is the full text of the article available?

E) Is the full text written in English?

F) Does the full text contain more than 500 words?

G) G.1. Is the full text a primary research article that describes an experimental study comparing caustic and cautery disbudding in any aspect?

OR

G.2. Is the full text a primary research article that describes an experimental study testing pain control to mitigate caustic disbudding pain in calves?

During the second screening, articles were classified as either "included" or "excluded" without the "unclear" option. If both reviewers agreed that an article did not fulfill any of the criteria, it was excluded. Disagreements between the two reviewers were resolved through consensus. In cases where consensus could not be reached, a third person intervened. Articles that successfully fulfilled all the criteria, including G.1, were included in the first part of this review, which aimed to compare the effects of cautery and caustic disbudding on calves’ welfare. Articles that fulfilled criteria G.2 were used to answer the second question of this review, concerning pain management of caustic disbudding. However, before being included, G.2’s articles underwent an additional screening at the study level, as some articles presented more than one study. The additional criteria for this screening were:

- Does the study assess one or both of the following pain management strategies: cornual anesthetic block or systemic analgesics or their combination?

- Is the study a controlled randomized trial?
- Is there a concurrent comparison group, such as a no pain control group or a systemic analgesia group in case the intervention involves anesthetic block + analgesia?

2.5.5 Data collection and data items

Studies approved in the second screening were included in the review, and data from these studies were extracted using a standardized Excel sheet. The sheet was pilot-tested in preliminary searches.

2.5.5.1 General information

The Z.O-S extracted general information about each study, including the country, year, and season of the study, as well as population characteristics such as age, breed, and sex. Disbudding details, such as the type of disbudding (caustic paste/stick or hot-iron), and how it was carried out, were also extracted. For the pharmacological intervention, the extracted data included the drug and dose given, the route or technique of administration, and the timing relative to disbudding. Each treatment groups (no pain control, analgesic alone, anesthetic alone, or anesthetic and analgesic), the number of animals per treatment, and assessed variables were specified for each study.

2.5.5.2 Objective I - Comparing caustic and cautery disbudding

Studies that compared caustic and cautery disbudding were all assessed by Z.O-S. Information on any welfare-related outcome used to compare both methods were extracted. When a statistical comparison was made between disbudding types in the assessed study, the results of this comparison were qualitatively extracted, stating whether caustic disbudding had a greater, lower, or equal frequency/mean compared to cautery disbudding. If no statistical analysis was conducted, descriptive findings from the studies were extracted.

2.5.5.3 Objective II- Pain mitigation of caustic disbudding

Plasma Cortisol and pain behaviors

Z.O-S and a third person (I.N-N) independently extracted the mean and standard deviation of plasma cortisol concentration and each pain behavior for each reported sampling time in the studies. If only the standard error of the mean was reported, it was converted to standard deviation based on the sample size (HIGGINS; LI; DEEKS, 2022). For cortisol, the method of obtaining blood (catheterization or venipuncture), was also extracted. For pain behavior, the type of observation (live or video), the behavior type, and the duration of the observation were recorded. WebPlotDigitizer was used to extract data from graphs (DREVON; FURSA; MALCOLM, 2017). Z.O-S and I.N-N independently extracted the information and discussed discrepant values. If no errors were found, the values were averaged.

Secondary outcomes

Secondary outcomes were extracted using the same approach used for “objective I”, again, all reported outcomes and assessment methods were extracted. Statistical findings were qualitatively extracted, stating whether the intervention (analgesics, anesthetics, or their combination) resulted in no difference, a greater or a lower mean/frequency in comparison with the comparator group (no pain control, or analgesics). When multiple times after disbudding were reported, results were extracted for each time. If no statistical analysis was conducted, findings were extracted descriptively.

Summary measures

Meta-analysis was conducted for cortisol whenever at least two studies reported the same outcome measured at similar times, using similar methods, with corresponding intervention and comparator groups. As studies varied regarding the reported time points, different time points across studies were clustered in one data point (similar time) based on the criteria reported by Winder et al., (2018a). Briefly, cortisol time points were clustered into a similar-time if they did not differ by more than 10 minutes in the first 70 minutes post-disbudding and 20 minutes thereafter. Based on this criteria, similar time “0 min” (including time 0 (n=2) and -5 min (n=4)), and similar time point “60 min” (including 50 min (n=1) and 60 min (n=6)) were created. No cluster was needed for times 15 min (n=3), 90 min (n=2), 120 min (n=2), 180 min (n=4), 240 min (n=2), and 1440 min (n=2).

We a priori opted to use the same criteria used by Winder et al., (2018a) to create similar times for pain behavior. However, this was not implemented because meta-analysis was not conducted for this outcome due to the variability in how it was assessed. Ten out of the 13 studies reported pain behaviors, however, assessment and reporting approaches varied significantly across studies. For example, pain behaviors were sometimes listed separately by types, such as head shakes and head-scratching (WEYL-FEINSTEIN, et al., 2021); in other cases, the behaviors were summed together to create a total behavior score, but this sum did not necessarily include the same behaviors across authors (STILWELL et al., 2009; STILWELL; LIMA; BROOM, 2008; WINDER et al., 2017); one study used an analog scale to rate pain behaviors (BRAZ et al., 2012). Some studies reported pain behaviors at multiple time points following the procedure (STILWELL; LIMA; BROOM, 2008; WEYL-FEINSTEIN, et al., 2021), while others aggregated behaviors across several hours into a single measurement (MORISSE; COTTE; HUONNIC, 1995; VICKERS et al., 2005). In two cases, no variation units were reported behaviors (BRAZ et al., 2012; MORISSE; COTTE; HUONNIC, 1995). Due to this lack of standardization, it was not possible to carry out a meta-analysis for pain behavior data.

2.5.6 Synthesis methods

2.5.6.1 Considerations regarding the intervention and comparator group

Analgesics group

The analgesics group was defined as the group that underwent a caustic disbudding and received some systemic analgesics to mitigate pain responses. The analgesic could be either a non-steroidal anti-inflammatory drug (NSAID) or any other class of analgesics. In studies where multiple analgesics groups were present, we defined the analgesics group as the one receiving a single dose of analgesics closest to the caustic disbudding procedure. This was the group reported in our quantitative and qualitative synthesis. Thus, in Stilwell, Lima and Broom et al.,(2008), flunixin-meglumine injected 5 minutes before the procedure (group F0) was chosen as the analgesics group in this review instead of the injection at 60 min before (group F1). Similarly, in Braz et al., (2012), tramadol (IV) administered 15 min before the procedure was selected as the analgesics group instead of rectal tramadol 90 min before. In Karlen et al., (2021), M1 was considered equivalent to our analgesics group as it received a single dose of oral meloxicam, whereas M2 received a second dose 24 hours later.

Anesthetic (lidocaine) group

Anesthetic group was defined as the group that underwent a caustic disbudding and received a cornual anesthetic block to mitigate pain responses. Since lidocaine was the only anesthetic reported in the included studies, the term "lidocaine group" will be used henceforward.

No pain control group

No pain control group (NPC) was defined as the group that underwent a caustic disbudding but with no pain control. This group could have received a sham injection with saline solution instead of the pain mitigation drug. The only exception is Vickers et al., (2005), where one group received xylazine and the other received xylazine combined with lidocaine. That study was not included in the meta-analysis; however, it was reported in the qualitative synthesis, with the xylazine group being considered as NPC and the xylazine + lidocaine like the lidocaine group.

Anesthetics (lidocaine) + analgesics

In this group, animals underwent a caustic disbudding procedure and anesthetic block and analgesics were given to mitigate pain. This group will be henceforward named lidocaine + analgesics for the aforementioned reason. Yakan, Akan and Duzguner (2018) were included in the qualitative synthesis. In this study xylazine was combined with the other drugs, therefore the xylazine + lidocaine group was treated as the "Lidocaine group", while the group that received xylazine, lidocaine, and flunixin-meglumine was treated as lidocaine + analgesics group.

2.5.7 Data analyses

All statistical analyses were performed using R (R Core Team, 2021). Cortisol levels were measured as a continuous variable using different scales, such as changes from baseline and pre/post-treatment concentrations. Thus, standardized mean difference (SMD) was chose as measure of effect; it was calculated by employing the escalc() function from the metafor package. The inverse of the variance method was used to weight each study, giving more weight to larger studies with smaller standard deviations. We employed a random-effects model to account for differences between studies, and the heterogeneity was incorporated into the study weights using the DerSimonian-Laird estimator. The heterogeneity between studies was quantified using the I^2 statistic. In cases where substantial heterogeneity was observed (I^2 greater than 50%), we explored possible sources of heterogeneity through meta-regression or subgroup analyses, but only if a sufficient number of studies (at least 10) were available for meta-analysis within a specific similar time frame. If the number of studies was insufficient, we assessed meta-analysis's outcomes graphically and highlighted studies that showed deviation from the others. However, these studies were not excluded from the analysis (DEEKS; HIGGINS; ALTMAN, 2022).

2.6 RESULTS

2.6.1 Included studies

Out of the 341 articles screened, 14 articles were included in this systematic review. Six studies from six articles were used to address the first objective of this review, while 13 studies from 10 articles were employed to address the second objective. Figure 1 provides a summary of the number of articles excluded at each screening with the corresponding reasons for their exclusion.

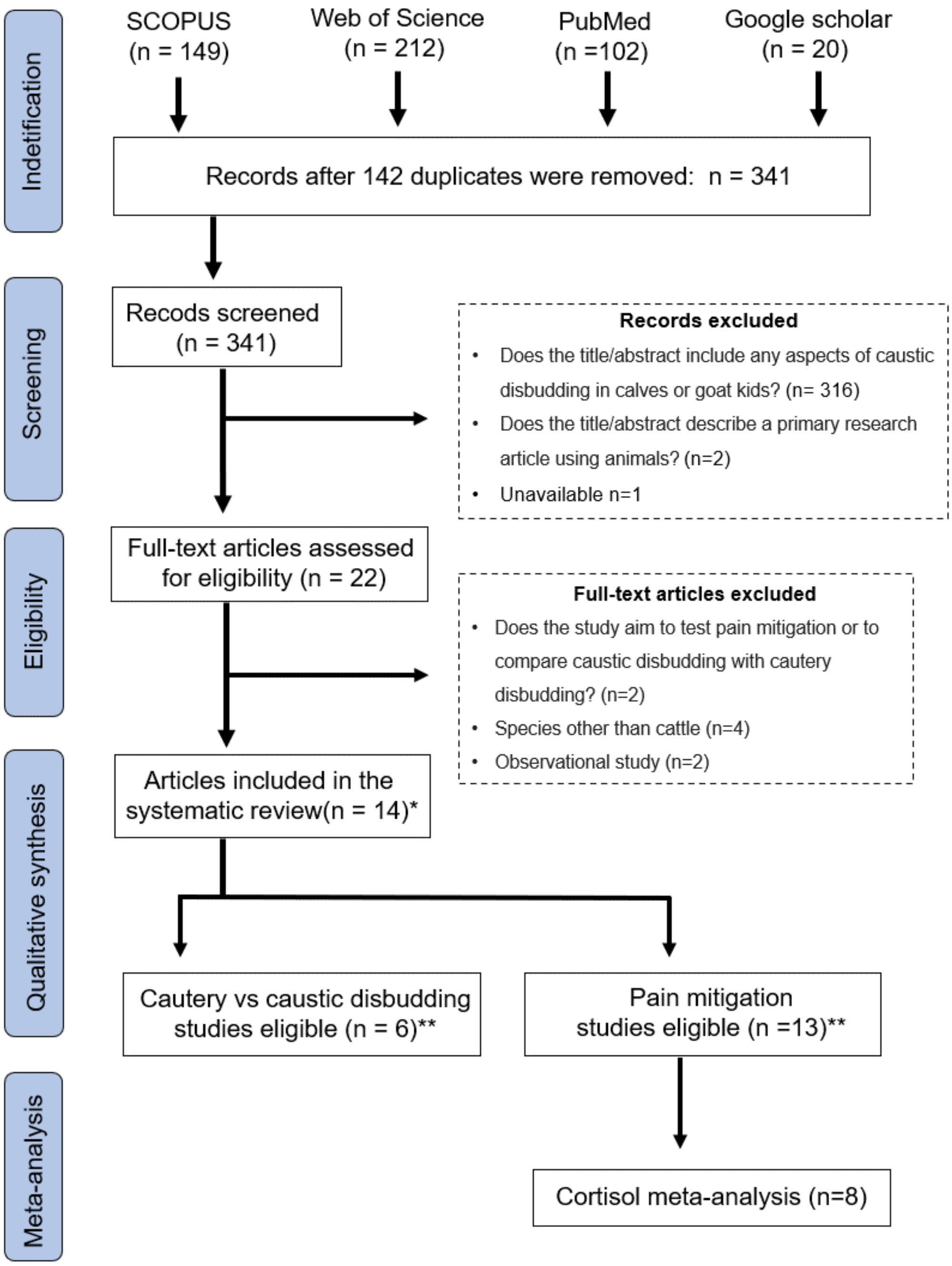


Figure 1: Flow chart of the systematic review process describing the number of articles from each database and the exclusion rates and reasons.
* Some articles reported more than one study.
** Some studies were included in more than one category.

2.6.2 Objective I – Comparing the effect of heat-cautery and caustic disbudding on welfare related outcomes

Table 1 provides an overview of the characteristics of 6 studies that compared heat-cautery and caustic disbudding. Figure 2 summarizes the main findings in 12 comparisons in which was found that there were four comparisons where cautery and caustic disbudding did not differ, four where caustic disbudding was worse than cautery, and four where cautery was worse than caustic disbudding.

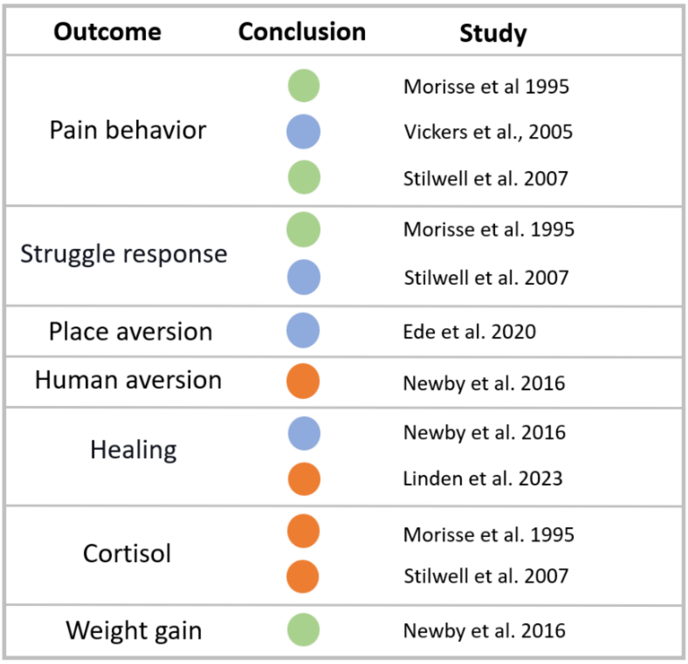


Figure 2: Qualitative synthesis of the findings reported studies comparing caustic and heat-cautery disbudding.
Conclusions could be either caustic disbudding being more negative (indicated by orange marker), cautery being more negative (blue color); and no difference between methods (green color).

Table 1: A summary of the characteristics of the studies included in the systematic review comparing cautery and caustic disbudding methods.

Study	Age	Sex	Breed	Drug	Measured outcomes	Caustic disbudding procedure	Cautery disbudding procedure
Lindén et al. (2023)	6±1 d	Mixed	Predominantly Finnish Ayrshire	Xylazine (0.2 mg/kg - IM) Ketoprofen (0.3 mg/kg - SC) 2% lidocaine (4 ml/horn)	Healing/ Histological	Not reported [DN]	Hot-iron (Gasbuddex, 20 mm of diameter, Albert Kerbl) was heated to ~ 650 ºC and applied for ~ 5 sec.
Ede et al. (2020)	7 ± 2 d	Male	Holstein	Xylazine (0.2 mg/kg - IM) Meloxicam (0.5 mg/kg - SC) 2% lidocaine (5 ml/horn)	Place aversion	~2 cm in diameter and ~1 mm thick calcium hydroxide 24.9%, sodium hydroxide 21.5%[DN]	A preheated hot-iron (X30, 1.3 cm tip, Rhinehart, Spencerville, IN) was applied for ~10 sec.
Newby et al. (2016)	3.6± 2.7 d	Female	Holstein	NPC for the caustic disbudded group; 2% lidocaine (5 ml/horn) for the heat cautery disbudded group	Healing Weight gain Human aversion disbudding effectives	Caustic paste (Dr. Larson's Dehorning) was applied to a ~2 cm area. 2 drops of water were applied in the horn and then the stick (Albert Kerbl HornStick) was rubbed vigorously for 1 min.	Hot-iron (Portasol II) was heated for 10 min and applied for 22.5 Sec.
Stilwell et al. (2007)	25±10 d for caustic disbudded group; 98±15 d for heat cautery disbudded group	Female	Not described for caustic disbudded group; Holstein for the heat cautery disbudded group	NPC	Pain behavior Cortisol Struggle response	A thin layer of sodium or calcium hydroxide was applied.	Hot-iron was heated over 600 ºC, and applied for 30 – 45 sec.
Vickers et al. (2005)	10 to 35 d	Female	Holstein	Xylazine	Pain behavior	Not reported [DN]	Hot-iron at ~ 600°C

				(0.2 mg/kg - IM) for the caustic disbudded group; 2% lidocaine (5 ml/horn) and Xylazine (0.2 mg/kg - IM) For the heat cautery disbudded group			was held on each horn bud for ~ 15 sec.
Morisse et al. (1995)	4 weeks for caustic disbudded group; 8 weeks for heat cautery disbudded group	Males	Montbeliard	2% lidocaine (4 ml/horn)	Pain behavior Cortisol Lying behavior	Stick of potassium hydroxide	Hot-iron electrically heated to 600°C was pressed firmly on each bud for ~ 1 min.

A superscript [DN] indicates were brand “Dr. Naylor Dehorning Paste” was used. Pharmakos could be administrate by intramuscular (IM) or subcutaneous (SC) routes. “NPC” indicates where no pain control was provided.

2.6.2.1 Behavioral responses

Five out of the six studies investigated some behavioral outcome. Vickers et al. (2005) reported a higher median of pain behavior (head shakes) for heat-cautery disbudded calves at the first 4 hours post-procedure and more transitions between laying and standing between 5-12 hours. Accordingly, Stilwell et al., (2007) reported a higher frequency of struggle behaviors for heat-cautery disbudding, though no difference in the total pain behaviors mean (head shake, head rubbing, ear flick, transitions, and inert lying) were seen between groups at observed times: 15, 60, 180, 360 and 1440-min post-procedure. Morisse et al. (1995) did not find any difference in struggle intensity between methods, and lidocaine reduced struggle response regardless of the method. Morisse et al. (1995) did not directly compare the two methods for pain behaviors, but reported that both caustic and cautery disbudded calves exhibited increased pain behaviors in the four hours after the procedure, compared to the four hours before.

Using the conditioned place aversion paradigm Ede et al. (2020) investigated whether calves would avoid more the place associated with caustic or cautery disbudding. Each calf underwent both procedures in rooms with distinct visual cues to allow them to associate each place with one of the disbudding procedures. After 48, 72, and 96 hours, the calves were tested in an apparatus where they could freely move between the rooms associated with cautery and caustic disbudding. Despite having received sedatives, anesthesia, and analgesics during both procedures, calves preferred to spend more time and lay more in the space associated with cautery disbudding at 48 h post the second procedure. This preference suggested that chemical burn is the most aversive. Newby et al., (2016) also studied aversive memories across disbudding methods (caustic paste, caustic stick, and cautery with Lidocaine) exposing calves to the human that performed the disbudding at one and seven days post-procedure. Their findings pointed to the opposite direction, i.e., caustic paste disbudded calves were more likely to approach the human than the other groups, suggesting less fear or aversion.

2.6.2.2 Biological functioning responses

Stilwell et al., (2007) reported a higher cortisol response at 60 min post-procedure for caustic over cautery disbudding. No differences were found in the following sampled times:180, 360, and 1440 min. Similarly, Morisse et al. (1995) reported that caustic disbudding induced a higher cortisol response than heat-cauterization; however, their study did not directly compare both groups regarding cortisol.

Healing outcomes findings reported by Newby et al., (2016) suggested that heat-cautery disbudding wounds tended to heal faster compared to caustic paste disbudding. This was evidenced by the smaller wound diameter observed at 3 weeks post-procedure in the cautery disbudded group. However, the same study found higher odds of redness, purulent discharge, and crust formation in the heat-cautery disbudded group compared to the caustic paste disbudded group. In a study conducted by Lindén et al. (2023), caustic disbudding led to laterally spread of the necrosis beyond the area of paste contact and was associated with worse outcomes in an exploratory histopathological, producing deeper and poorly delimited necrosis than cautery disbudding. In contrast, cautery disbudding appeared to promote faster healing due to the relatively quick formation of granulation tissue. Newby et al., (2016) did not find differences in weight gain in the five weeks post procedure between disbudding methods.

2.6.2.3 Limitations in studies design

Four out of the six studies exhibited design limitations making it challenging to attribute the reported outcomes solely to the disbudding method. Two of the studies (Vickers et al., 2005 and Newby et al., 2016) used different pharmacological approaches in the tested groups, what could influence the observed outcomes. Vickers et al., (2005) employed sedation in the caustic disbudded group, while the heat-cautery disbudded group received sedation combined with a lidocaine cornual block in addition of a ring block around each horn bud. In Newby et al., (2016), the heat-cautery disbudded group received an anesthetic block, whereas the caustic disbudded groups did not receive any pain control. Additionally, in the studies of Morisse et al. (1995) and Stilwell et al., (2007) there were confounding factors related to age, as in both studies the cautery disbudded calves were older. Additionally, Stilwell et al., (2007) did not specify if the claves in the two groups were of the same breed, and the study locations differed between the caustic and cautery groups, with each group originating from different farms (e.g. quasi experiment design). Morisse et al. (1995) conducted their study across three research facilities, but it is unclear if the caustic disbudded group was randomly distributed among these facilities. Differences in struggle behaviors were reported by Stilwell et al., (2007) across heat cautery and caustic disbudding, however behavioral scores differ between disbudding methods. Both scores included open mouth, vocalization, however cautery disbudding score also included lifting front limbs, falling on back limbs, backing. While caustic disbudding scores includes shaking head, stretching back limbs and trying to stand.

2.6.3 Objective II – How to better mitigate caustic disbudding pain

General characteristic of the studies used to address the second aim of this review are summarized in Tables 2.

Table 2: A summary of the characteristics of the studies included in the systematic review exploring the efficiency of lidocaine, analgesics and their combination to mitigate caustic disbudding pain.

Article	Study	Disbudding factors			Breed	Sex	Age	Treatment	Measured outcomes	Drugs	
		Type	Composition	Quantity						Anagesics/sedatives	Anesthetics
Weyl-Feinstein, et al., (2021)	Young group	Paste	24.9% sodium hydroxide and 37.8% calcium hydroxide[DN]	Not reported	Not reported	Not reported	1-7 d	NPC Lidocaine	Cortisol Pain behavior Lying time Activity		2% lidocaine (4 ml/horn)
	Old group	Paste	24.9% sodium hydroxide and 37.8% calcium hydroxide[DN]	Not reported	Not reported	Not reported	8-14 d	NPC Lidocaine Analgesics Lidocaine+Analgesics	Cortisol Pain behavior Lying time Activity	Meloxicam (40 mg - SC)	2% lidocaine (4 ml/horn)
Reedman et al., (2020)		Paste	24.9% sodium hydroxide and 37.8% calcium hydroxide[DN]	Not reported	Holstein	Female	3.7 ± 0.16 d	NPC Lidocaine Analgesic Lidocaine+Anagesics	Cortisol Pain sensitivity Lying time Haptoglobin	Meloxicam (0.5 mg/kg - SC)	2% lidocaine (6 ml/horn)
Stilwell et al., (2008)		Paste	SH-Plus® — Sodium Hydroxide	Not reported	Holstein	Female	10 - 40 d	NPC Analgesic	Cortisol Pain behavior response Lying time	Flunixin-meglumine (2.2 mg/kg - IV)	
Stilwell et al., (2009)	Study 1	Paste	SH-Plus® — Sodium Hydroxide	Not reported	Holstein	Female	27 ± 8 d	NPC Lidocaine Lidocaine + Analgesics	Cortisol Pain behavior	Flunixin-meglumine (2.2 mg/kg - IV)	2% lidocaine (5 ml/horn)
	Study 2	Paste	SH-Plus® — Sodium Hydroxide	Not reported	Holstein	Female	22 ± 4 d	NPC Lidocaine	Cortisol Pain behavior	Flunixin-meglumine (2.2 mg/kg - IV)	2% lidocaine (5 ml/horn)

								Lidocaine + Analgesics			
	Study 3	Paste	SH-Plus® — Sodium Hydroxide	Not reported	Holstein	Female	28 ± 6 d	NPC Lidocaine	Cortisol Pain behavior Lying time		2% lidocaine (5 ml/horn)
Braz et al., (2012)		Paste	24.9% sodium hydroxide and 37.8% calcium hydroxide[DN]	Not reported	Dairy	Not reported	20.72 ± 4.9 d	NPC Analgesic	Pain behaviors	Tramadol hydrochloride (4 mg/kg – IV)	
Winder et al., (2017)		Paste	Not reported[DN]	Not reported	Holstein	Mixed	2 - 32 d	Analgesic Lidocaine+Analgesic	Pain behavior Heart and respiratory rate Feed and play behavior Lying time Grooming Human aversion	Meloxicam (0.5 mg/kg – SC)	2% lidocaine (5 ml/horn)
Karlen et al., (2021)		Paste	mixture of sodium and calcium hydroxide[ND]	Approximately 18 mm in diameter	Holstein	Not reported	3 d	NPC Analgesic	Growth Pain sensitivity Cortisol Substance p Ocular and horn temperature.	Meloxicam (45mg - OR)	
Yakan et al., (2018)		Stick	Calcium hydroxide (REDFORT Boynuz Kalemi)	Not reported	Simental	Mixed	14 ± 2 d	Lidocaine+Anagesics Lidocaine	Heart and respiratory rate Rectal temperature. Cortisol Serum Glucose	Flunixin-meglumine (2.2 mg/kg - IV) Xylazine (0.2 mg/kg - IV)	2% lidocaine (5 ml/horn)

Morisse et al., (1995)	Stick	Potassium hydroxide	Not reported	Montbeliard	Male	4 weeks	NPC Lidocaine	Antioxidative activity Pain behavior Cortisol Struggling Lying time		2% lidocaine (4 ml/horn)
Vickers et al., (2005)	Paste	Not reported[DN]	Approximately 2 cm in diameter	Holstein	Female	10 to 35 d	NPC Lidocaine	Pain behavior	Xylazine (0.2 mg/kg - IV)	1.5 mL Lidocaine + 3 mL lidocaine on ring block

Superscript [DN] indicates were brand “Dr. Naylor Dehorning Paste” was used. Pharmakos could be administrate by intramuscular (IM) or subcutaneous (SC) or oral (OR) routes. “NPC” indicates where no pain control was provided.

2.6.3.1 Quantitative Synthesis

Serum cortisol

Ten out of 13 studies reported blood cortisol levels, and most of the studies reported it in a similar away. The study by Karlen et al., (2021) was not included in the meta-analysis due to the reporting of only a 12-day average. Morisse et al., (1995) compared calves disbudded using lidocaine with the Sham group, which was outside the scope of this analysis and thus not included in the meta-analysis.

Within studies included in the meta-analysis, four studies collected blood through vein puncture (Stilwell et al., 2008; Stilwell et al., 2009 - Study 1, 2, and 3), two studies used catheters (Reedman et al., 2020; Yakan et al., 2018), and two studies did not describe the blood collection method (Weyl-Feinstein, et al., 2021 - old and young groups). We assume that differences in the methods to harvest blood would not interfere with the results.

To assess the cortisol response across different pain control strategies, a meta-analysis was conducted using the data from eight out of the 10 studies that reported cortisol levels. The overall effect measures and confidence interval of the meta-analyzes are presented in Figure 3.

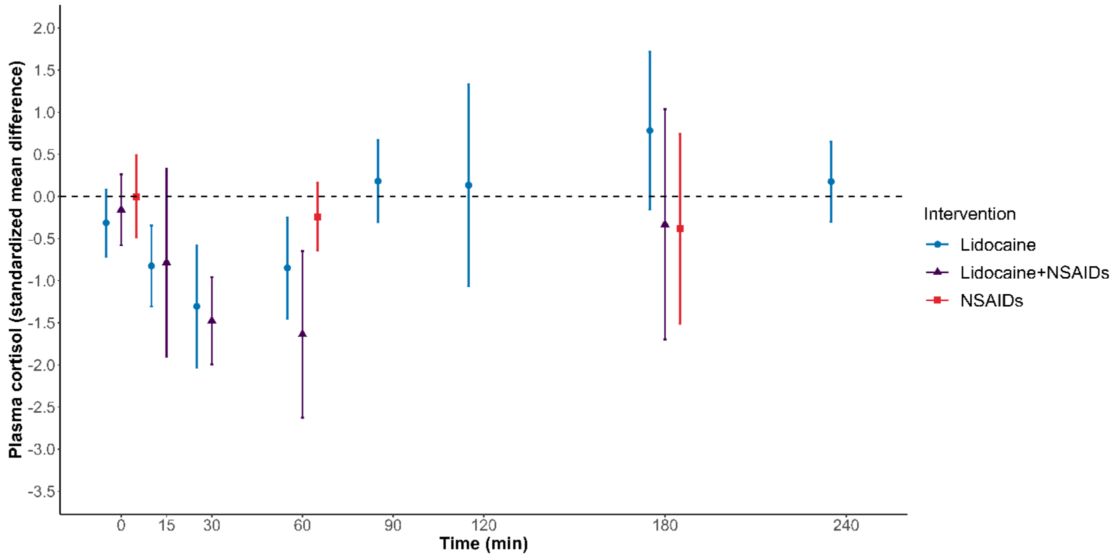


Figure 3: Floret plot of the meta-analyses comparing the effect of different pain control strategies on blood cortisol levels.

Overall effect measures (±95% CI) of random-effects meta-analyses of the effect of lidocaine local anesthesia, compared with no pain control (blue circle); lidocaine combined with non-steroidal anti-inflammatories drugs (NSAIDs) compared with no pain control (purple triangle) and NSAIDs compared with no pain control (red square).

The overall effects were significant when its confidence interval does not include 0 (dashed lines). The number of studies included in each meta-analysis, p-values, and heterogeneity are described in the text.

2.6.3.1.1 Lidocaine alone vs no pain control

This comparison included six studies. Two were reported in each of the following times: 15, 30, 90, 120, and 240 min post procedure; three at time 180 min; four at time 0 min; and five at time 60 min. No effect of lidocaine was found at time zero (SMD estimative and confidence interval: -0.31; CI 95% = -0.71, 0.08; P = 0.12; I^2=0%). A protective effect of lidocaine in cortisol's rise was found at times 15 min (SMD = -0.82; CI 95% = -1.30, -0.34; P < 0.001; I^2= 0%), 30 min (SMD = -1.30; CI 95% = -2.03, -0.58; P < 0.001; I^2= 60%) and 60 min (SMD = -0.85; CI 95% = -1.45, -0.25; P = 0.005; I^2= 66%). No significant effect of lidocaine was detected at 90 min (SMD = 0.18; CI 95% = -0.30, 0.67; P = 0,46; I^2 = 0%), 120 min (SMD = 0.13; CI = -1.06, 1.33; P = 0,82; I^2 = 67%), 180 min (SDM= 0.13; IC 95% = -1.06, 1.33; P = 0,10; I^2 = 65%), and 240 min (SMD = 0.17; CI 95% = -0.30, 0.65; P = 0.56; I^2 = 0%).

2.6.3.1.2 Lidocaine + NSAID vs no pain control

In total, four studies were included in the meta-analyses two studies at times 15, 30 e 180 min, three at 0 min, and four at 60 min. No significant protective effect of lidocaine combined with NSAID was found at 0 min (SMD = -0.16; CI 95% = -0.58, 0.26; P = 0.46; I^2= 0%) and 15 min (SMD = -0.78; CI 95% = -1.90, 0.33; P = 0.16; I^2= 75%). Lidocaine + NSAID had a protective effect at 30 min (SMD = -1.48; CI 95% = -1.99, -0.96; P < 0.0001; I^2 = 0%) and 60 min (SMD = -1.64; CI 95% = -2.62, -0.65; P = 0.0012; I^2 = 78%). No protective effect and high heterogeneity were found at 180 min (SMD = -0.33; CI 95% = -1.7001, 1.0356; P = 0.70; I^2 = 81%).

2.6.3.1.3 NSAID vs No pain control

Only tree studies compared giving NSAIDs to NPC. Although these studies compared two different drugs (flunixin-meglumine and meloxicam) with different routes (IV and SC) we decided to include them in the meta-analyses. Two of these studies were included in the meta-analysis at times 0 and 180 min, and three at time 60 min. No protective effect was found at 0 min (SMD = 0.00; CI 95% = -0.48, 0.49; P = 0.99; I^2 = 0%), 60 min (SMD = -0.24; CI 95% = -0.64, 0.16; P = 0.24; I^2 = 0%), or 180 min (SMD = -0.38; CI 95% = -1.51, 0.74; P = 0.50; I^2 = 62%).

2.6.3.1.4 Addressing heterogeneity

Due to the limited number of studies available for subgroup meta-analysis and meta-regression, these analyses were not conducted. However, we examine the individual studies within each meta-analysis with I^2 greater than 50%.

For the comparison of "lidocaine vs NPC" at 60 minutes, substantial heterogeneity ($I^2 > 60\%$) was identified. All five studies included in this meta-analysis reported a protective effect, with only one study not reaching statistical significance. The magnitudes of the effect varied greatly across studies, ranging from -0.75 to -2.29. For the same comparison at 120 minutes, substantial heterogeneity was observed, with one study reporting a non-significant protective effect and the other study reporting a significant risk effect of increased cortisol in the lidocaine group. Similarly, substantial heterogeneity was again identified at 180 minutes, with two out of three studies reporting a significant risk effect and one study reporting a non-significant protective effect

In the analysis of "lidocaine + NSAID vs NPC," considerable heterogeneity ($I^2 < 75\%$) was found at 15, 60, and 180 minutes. At 15 minutes, the two meta-analyzed studies reported protective effects, but only one reached statistical significance. At 60 minutes, all four studies included in the analysis reported significant protective effects, although the magnitude of the effects varied greatly, ranging from -0.80 to -1.80. At 180 minutes, one study found a significant protective effect, while the other study found a non-significant risk effect. Substantial heterogeneity was also observed in the comparison of "NSAID vs NPC" at 180 minutes, with one study reporting a protective effect and another study reporting a risk effect, but neither of them reached statistical significance.

2.6.3.2 Narrative synthesis

2.6.3.2.1 Lidocaine alone vs No pain control

A total of eight studies were included in this narrative, and notably, seven of them reported at least one positive effect of lidocaine uses compared to no NPC. The timeframes for observing responses varied among the studies, with four assessing responses within the first 180 minutes, three assessing responses from 180 minutes to 24 hours post-procedure, and one study assessing responses between 7- and 12-days post-procedure. Cortisol levels and pain behaviors were measured in seven studies each, while five studies reported additional outcomes.

Cortisol

The cortisol levels in the NPC group were significantly higher than in the lidocaine group at 15 or 30 min post-procedure (REEDMAN et al., 2020; STILWELL et al., 2009 - study 2). Similarly, all the studies that reported cortisol levels at 50 or 60 min post-procedure consistently found higher levels of cortisol in the NPC group (REEDMAN et al., 2020; STILWELL et al., 2009 - study 1 and 2; WEYL-FEINSTEIN, et al., 2021 - old and young group). Although no statistical comparison was made between the lidocaine and NPC groups, Morisse et al., (1995) also found higher levels of cortisol in the NPC group at 60 min. Interestingly, cortisol levels tended to increase in the lidocaine group as the effects of lidocaine waned, while they decreased in the NPC group, leading to both groups reaching similar levels. This convergence in cortisol levels may occur around 90 min (REEDMAN et al., 2020) or 120 min (STILWELL et al., 2009 - study 3). Subsequently, cortisol levels in the lidocaine group either remained similar to the NPC group (STILWELL et al., 2009 - study 1) or surpassed them ((STILWELL et al., 2009 - study 3); (REEDMAN et al., 2020). No significant differences in cortisol levels between the lidocaine and NPC groups were reported after 180 min (MORISSE; COTTE; HUONNIC, 1995; STILWELL et al., 2009 - study 1; WEYL-FEINSTEIN, et al., 2021).

Pain Behaviors

Morisse; Cotte and Huonnic (1995) reported that lidocaine could mitigate behavioral responses during caustic stick disbudding, such as tail flapping, moving back, and ventral falling. However, this study only provided combined values for caustic paste and hot-iron procedures. The earliest reported post-procedure times were at 10 and 15 minutes (STILWELL et al., 2009 - study 1 and 2). In both studies, calves in the NPC group displayed more pain behaviors, including head shaking, ear flicking, head rubbing, and transitions from standing to lying, compared to the lidocaine group. The extent of this effect varied across studies, despite the use of similar populations and the same drug. For example, pain behaviors remained higher in the NPC group at 50 minutes in study 2 from Stilwell et al., (2009), and at 90 minutes in study 3. However, the same author did not find significant difference between the lidocaine and NPC groups at 60 minutes in another (STILWELL et al., 2009 - study 1).

Other studies reported positive effects of lidocaine at 60 minutes. Weyl-Feinstein, et al., (2021) found that lidocaine mitigated head shaking in calves aged between 8-14 days and younger than 8 days. These authors reported that lidocaine decreased head shakes until 120 minutes in older calves (8-14 days), but this effect was only observed in the first 60 minutes in calves until 7 days old (WEYL-FEINSTEIN, et al., 2021). Interestingly, some studies reported what looks like a rebound-like pain effect: lidocaine resulted in more head shaking than the NPC group in calves younger than 8 days from 180 minutes until 540 minutes (WEYL-FEINSTEIN, et al., 2021); in older calves, the head shaking increased at 240 minutes post-procedure and disappeared at 360. Although not statistically significant, a similar rebound effect was observed in the study 1 by Stilwell et al., (2009), whin which lidocaine group display less pain behaviour than NPC at 60 min, but tended to show more pain behaviors than the NPC group at 3 and 6 hours post-treatment.

Total pain and other behaviors, such as self-grooming, rubbing, social interactions, transitions between lying and standing, and head-scratching and shaking did not differ in the 4 hours post-procedure compared to the 4 hours pre-procedure, regardless of the use of lidocaine (MORISSE; COTTE; HUONNIC, 1995). In another study, providing a lidocaine cornual block in addition to a ring block, and sedation with xylazine did not reduce the median number of head rubs, shakes, and transitions in the 4 hours following disbudding compared to only sedated calves (VICKERS et al., 2005).

Other outcomes

In terms of lying time and lying events, no significant effects of lidocaine were found in the 12 hours and 7 days following the procedure, respectively (REEDMAN et al., 2020; WEYL-FEINSTEIN, et al., 2021). Haptoglobin levels were also similar between the lidocaine and NPC groups until 7 days post-procedure (REEDMAN et al., 2020).

Stilwell et al., (2009) reported the occurrence of the behavior laying inert, however, no statistical analyses were conducted on this outcome. In study 3, this behavior was reported to be more frequent in NPC calves during the first 180 minutes, but no major differences were observed between the lidocaine and NPC groups after this time in study 1.

2.6.3.2.2 Analgesics alone vs No pain control

Five studies examined the effects of different analgesics compared to NPC (BRAZ et al., 2012; KARLEN et al., 2021; REEDMAN et al., 2020; STILWELL; LIMA; BROOM, 2008; WEYL-FEINSTEIN, et al., 2021). Among the five studies, two studies reported positive effects of the analgesics. The analgesic drugs tested in these studies were Meloxicam (in three studies), Flunixin-meglumine (in one study), and Tramadol (in one study). The route of administration varied among the studies, with two studies using intravenous administration, two using subcutaneous administration, and one using oral administration. The studies assessed different response periods, including within the first 180 min (n=1), from 180 min to 24 hours (n=2), and between seven and 12 days (n=2). Cortisol levels were assessed in four studies, pain behaviors were assessed in three studies, and other outcomes were assessed in four studies.

Cortisol

In the study of Stilwell et al., (2008), flunixin-meglumine administered intravenously did not result in any significant mitigating effect in cortisol increases at 60 min post-procedure compared to the NPC group. Additionally, there were no differences between the analgesic-treated calves and NPC calves at 180 minutes, neither at 6 and 24 hours. Weyl-Feinstein, et al., (2021) found that calves treated with meloxicam subcutaneously had similar cortisol levels to NPC calves at 60 minutes, 4 hours, and 8 hours post-procedure. Karlen et al., (2021), did not find any differences in cortisol levels over the average of 12 days post-procedure when a single dose of oral meloxicam was administered. On the other hand, Reedman et al., (2020) reported positive outcomes with meloxicam administered intravenously: calves treated with the analgesic and NPC calves had similar cortisol levels at 15 and 30 min post-procedure, which were the peak levels for both groups; however, the decline in cortisol levels in the following minutes was more pronounced in the analgesic-treated group, with statistically lower cortisol levels at 60, 90, and 120 min post-procedure. No significant difference in cortisol levels was found at 180 min in that study.

Pain Behaviors

Flunixin-meglumine administered intravenously did not result in any evident changes in calves' responses to the paste application, such as open mouth, vocalization, head shakes, extending limbs, and standing (STILWELL; LIMA; BROOM, 2008). However, the frequencies of these reactions were only presented descriptively, and no statistical analysis was conducted. Additionally, no differences were found in pain behaviors, including head shake, ear flick, hind-limb scratching head, and transitions from standing to lying and back to standing, at any observed time (60 and 180 min, 6 and 24 h) (STILWELL; LIMA; BROOM, 2008). Weyl-Feinstein, et al., (2021) reported that meloxicam did not result in a reduction of head shakes at 60 min post-procedure. Both the analgesic-treated and NPC groups exhibited peak headshakes at this time. No differences in head shakes were found at 6 or 9 hours. In the study by Braz et al., (2012), intravenous tramadol did not significantly reduce head shakes at any of the observed times within the first 60 minutes, although there was a numerical decrease in the last 30 minutes of observation. However, tramadol did, significantly, decrease ear flicks and head rubs between 45 and 60-minutes post-procedure. Surprisingly, when the degree of pain experienced by the animals was assessed using a numerical rating scale, calves treated with tramadol had higher pain scores than NPC calves in the time between 0 and 15 minutes, and no differences were reported for the remaining time until 60 minutes.

Other outcomes

The laying inert behaviour throughout the 24 hours post-procedure were not altered by the flunixin-meglumine use (STILWELL; LIMA; BROOM, 2008). The use of meloxicam did not alter laying behavior in comparison with the NPC in the first 12 h (WEYL-FEINSTEIN, et al., 2021) or the first 7 days (REEDMAN et al., 2020) following disbudding. Substance P and haptoglobin were not altered in the first 12 and 7 days after disbudding, respectively (KARLEN et al., 2021; REEDMAN et al., 2020). Meloxicam use did not alter average daily gain, body weight, pain sensitivity, maximum ocular temperature, mean horn bud temperature, and the ratio mean horn bud/ ocular temperature in the 12 days following the procedure (KARLEN et al., 2021).

2.6.3.2.3 Lidocaine + analgesics vs No pain control

Four studies examined the effect of combining lidocaine with analgesics compared to NPC (REEDMAN et al., 2020; STILWELL et al., 2009 - Study 1 and 2; WEYL-FEINSTEIN, et al., 2021) and all of them reported at least one positive effect of using lidocaine in combination with analgesics. The analgesics used in the four studies were NSAIDs, with intravenous flunixin-meglumine in two studies and subcutaneous meloxicam in the other two. The observations were made within different time frames, ranging from the first 180 minutes (n=1) to 24 hours (n=2) and between 7 and 12 days (n=1). Cortisol was assessed in all the four studies and pain behaviours in three studies.

Cortisol

Reedman et al., (2020) reported higher cortisol levels in the NPC over the Lidocaine + Analgesics group 15 min post-procedure. Stilwell et al., 2009 (study 2) did not find a difference at 10 min but observed higher cortisol levels in the NPC at 30 min. All the studies consistently found higher cortisol levels in the NPC group compared to the lidocaine + analgesics group at 60 minutes (REEDMAN et al., 2020; STILWELL et al., 2009 - Study 1 and 2; WEYL-FEINSTEIN, et al., 2021). The duration of this effect varied across studies, with Reedman et al., (2020) reporting elevated cortisol levels in the NPC group until 120 minutes, Stilwell et al., 2009 (study 1) observing higher levels until 180 minutes, and Weyl-Feinstein et al., (2021) not finding any effect at 180 minutes. The studies that examined cortisol levels beyond these time points did not report any significant differences between the lidocaine + analgesics and NPC groups (STILWELL et al., 2009 - study 1; WEYL-FEINSTEIN, et al., 2021).

Pain Behaviors

Behavioral responses were higher in the NPC group within the first 10 minutes post-procedure in the study of Stilwell et al. (2009, study 1). Both studies that examined pain behavior found higher levels in the NPC group at 60 minutes (STILWELL et al., 2009 - study 1 and 2; WEYL-FEINSTEIN, et al., 2021). This effect waned at 180 minutes in Stilwell et al. (2009, study 1), but persisted until 240 minutes in Weyl-Feinstein et al. (2021). After these time points, no significant differences in pain behavior were found between the lidocaine + analgesics and NPC groups (STILWELL et al., 2009 - study 1; WEYL-FEINSTEIN, et al., 2021).

Other outcomes

Reedman et al. (2020), found that the Lidocaine + analgesics group exhibited less sensitivity on the horn from 15 minutes to 120 minutes post-procedure, indicating a potential pain-reducing effect. Additionally, in this study the lidocaine + analgesics group showed a tendency towards lower serum haptoglobin levels compared to the NPC group on days 3 and 4 following the procedure; however, no significant differences were observed in standing and lying bouts until 7 days after the procedure. Stilwell et al., 2009, (study 1) found no apparent difference in the number of animals displaying inert lying behavior within the first 1440 minutes (24 hours) post-procedure.

2.7 DISCUSSION

Our study shows that the available literature indicates that caustic disbudding is indeed a painful procedure for calves. Thus, efforts to mitigate pain from caustic disbudding should be at least equal to those employed for cautery disbudding pain management. Furthermore, our research demonstrates that the use of lidocaine cornual nerve blocks effectively control pain responses in the short time following caustic disbudding. This contradicts a common perception expressed by veterinarians and dairy farmers that caustic disbudding would be less painful than cautery disbudding (CANOZZI; BORGES; BARCELLOS, 2022; SARACENI et al., 2021b; SHI et al., 2022); . It also questions a common on farm practice evidenced in studies showing that caustic disbudding is associated with lower usage of pain control (COZZI et al., 2015; USDA-NAHMS, 2016) and, specifically, lower use of anesthetics to block the cornual nerve (SARACENI et al., 2021b; WINDER et al., 2016).

Overall, both disbudding methods have negative effects on welfare, with no clear difference between the two. Inconsistencies were found in three out of the four indicators (pain behavior, struggle, and healing) that included two or more studies. This can be attributed to the limited number of studies, the small sample sizes, differences in assessment methods, reporting practices, and experimental groups. The variations in the procedures across studies could also explain the inconsistencies in the findings across studies. For example, many studies included in our review lacked detailed descriptions of the amount of paste used (LINDÉN et al., 2023; STILWELL; LIMA; BROOM, 2007; VICKERS et al., 2005) or the size of the heat cautery wound (MORISSE; COTTE; HUONNIC, 1995; STILWELL; LIMA; BROOM, 2007; VICKERS et al., 2005). Furthermore, the duration of the heated iron contact with the scalp ranged from 5 to 60 seconds (LINDÉN et al., 2023; MORISSE; COTTE; HUONNIC, 1995). In humans and cattle the duration of the contact with the damaging agent and the extent of the damage are known to influence the degree of pain experienced (COETZEE, 2011; NORMAN; JUDKINS, 2004), highlighting the importance of considering these variables in disbudding studies.

Cortisol was the only indicator with at last two studies pointing to the same direction (MORISSE; COTTE; HUONNIC, 1995; STILWELL; LIMA; BROOM, 2007), with a more pronounced response in the caustic disbudded group. Although both studies that measured cortisol were confounded by age, which might affect cortisol levels (AZEVEDO et al., 2019), the same result was obtained in a study using goats, in which age was balanced across groups (HEMPSTEAD et al., 2018), strengthening our findings. Interestingly, one of the studies included in our review suggests that caustic disbudding causes more severe histological damage than heat cautery disbudding (LINDÉN et al., 2023). Two recently published studies support this finding, showing that caustic paste wounds take twice as long to heal than hot-iron disbudding wounds (ADCOCK; TUCKER, 2018b; DRWENCKE; ADCOCK; TUCKER, 2023). This could be explained by the alkalic burn mechanism, which consists of liquefactive necrosis and saponification, leading deeper penetration of the product into the tissue (SALZMAN; O'MALLEY, 2007).

Our research showed that caustic disbudded calves indisputably benefit from lidocaine cornual nerve block in the first 60 min in terms of cortisol response and that this beneficial effect on the pain behavior response could persist until 180 min. This aligns with the practices used in human medicine, where lidocaine is recommended to control pain during chemical cauterization procedures (ALTINYAZAR et al., 2010) and accidental chemicals and regular burns (FUZAYLOV; FIDKOWSKI, 2009). However, our results do not support the reduced use of lidocaine anesthetic block on caustic disbudding of calves (SARACENI et al., 2021b; WINDER et al., 2017). It has been argued that the absence of an initial reaction could lead farmers to underestimate the pain (REEDMAN et al., 2020). In contrast, our review has shown that caustic disbudded calves can struggle as much as heat-cautery disbudded calves during the disbudding procedure (MORISSE; COTTE; HUONNIC, 1995), and that pain behavior is already higher then NPC at 10 or 15 min post procedure when lidocaine is not given (STILWELL et al., 2009 study 1 and 2). Additionally, lidocaine effectiveness is also questioned due to the differences on the nature of the caustic burn pain (WINDER et al., 2017), which is a legitime concern, given that chemical agents can damage the tissue until it is completely neutralized (PALAO et al., 2010), raising uncertainty regarding the duration of the pain sensation. However, this argument does not consider that lidocaine could provide some relief in the first minutes post-procedure. Only one study did not find any positive effects of the lidocaine cornual nerve block (VICKERS et al., 2005). In that study, using lidocaine plus xylazine lead to higher pain behavior responses then xylazine alone in sham disbudded calves. But it is worth noting that in that study calves received a lidocaine ring block in addition to the cornual nerve block, which could lead to a greater pain response when the effect of lidocaine waned, due to the greater tissue damage associated with the injections. When the same calves underwent an actual caustic disbudding no differences were found between groups. Therefore, caution must be taken before using that study to dismiss the use of lidocaine block, especially because sedation is seldom adopted by farmers (HÖTZEL et al., 2014; USDA-NAHMS, 2016; WINDER et al., 2018b).

Despite the general short-term positive effects of lidocaine alone shown by most studies, a pain rebound-like effect was also noted. Pain rebound is a severe pain when the peripheral nerve block wears off (LAVAND'HOMME, 2018), which in the studies identified in our review was evidenced by the rise in cortisol levels (REEDMAN et al., 2020; STILWELL et al., 2009 - study 3) and pain behavior (WEYL-FEINSTEIN, et al., 2021) above the NPC group after 90 min post-procedure.

Although lidocaine was effective in reducing cortisol levels in all studies included in our meta-analyses, high heterogeneity was detected in several of them, which could limit the generalization of our findings. However, it is important to note that at time 60 min, which represents the higher number of studies, all included studies reported a protective effect of lidocaine. This suggests that heterogeneity at this time is likely due to the variation in the degree of the protective effect, but does not call into question de effect itself. One possible reason for this heterogeneity is the variation in the population traits across studies, especially sex, and age. For example, male calves were reported to have a lower cortisol response in comparison with females (MARTIN et al., 2022). Cortisol basal levels is higher in young dogs (MONGILLO et al., 2014) and mongoose (AZEVEDO et al., 2019) and decrease in older animals. Van Reenen et al., (2005) reported that cortisol levels vary individually between calves, and this variation is stable over time. Additionally, the relatively small sample size per group adopted by the included studies (ranging between 4-28 calves) could have resulted in more bias (KAPLAN; CHAMBERS; GLASGOW, 2014).

The rebound-like effect discussed above was not observed in the studies that combined analgesics (NSAIDs) with lidocaine. This is probably due to the NSAID's ability to inhibit cyclooxygenase, an enzyme that converts arachidonic acid into pro-inflammatory molecules such as prostaglandins and leukotrienes, preventing inflammatory pain (HERSH et al., 2020). However, analgesics affect varied across studies in our narrative review; only one study showed the advantages of using an NSAID in comparison with the NPC group (REEDMAN et al., 2020), and other studies showed the benefits of using an opioid analgesic (BRAZ et al., 2012). In our meta-analysis, NSAIDs alone did not prevent cortisol raises nor extend the protective effect when associated with lidocaine. The fact that it was not possible to conduct any meta-analysis investigating NSAIDs after the 180 min post-procedure could be one of the reasons we failed to find a positive effect, given that those anti-inflammatories typically take a long time to take effect (KLEINHENZ; VISCARDI; COETZEE, 2021). Accordingly, Winder et al., (2018b) only reported positive effects of NSAIDs +lidocaine in comparison to lidocaine alone in the meta-analysis conducted at time 240 min post-procedure. A variation in NSAID effects was also reported by a systematic review conducted by Wagner et al., (2021), where no more than 50% of the studies reported positive effects when meloxicam. Another possible explanation for the variation in the effect of the analgesics is the different molecules and routes used. For example, oral meloxicam reaches the maximum serum concentration at 24 h post-administration, while it occurs at 4 h when the subcutaneous route is used (MELÉNDEZ et al., 2019). Besides, intravenous flunixin and subcutaneous and intravenous meloxicam were reported to have different pharmacokinetic profiles (WAGNER et al., 2021). was given following castration or disbudding.

Our review showed that the animal welfare outcomes of caustic disbudding and the use of pain mitigation have received less attention in comparison to cautery disbudding. For instance, a similar systematic review investigating pain relief following heat-cautery disbudding was able to include twice as much studies as we did (WINDER et al., 2018a) though heat-cautery remains the most commonly used method in various countries such as the USA (USDA-NAHMS, 2016), Canada (WINDER et al., 2018b), Brazil (HÖTZEL et al., 2014), and several European countries (COZZI et al., 2015), caustic disbudding adoption has been rapidly increasing. According to the USA - National Dairy Study, the percentage of caustic disbudded heifers rose from 12.2% in 2007 to 32.5% (HEINRICHS et al., 1994; USDA-NAHMS, 2016). In specific regions such as Wisconsin (USA), caustic disbudding was reported as the preferred option by 61% of farmers (SARACENI et al., 2021b). In this scenario, caustic disbudding is becoming an increasing animal welfare issue.

Taking an evidence-based policy approach, policy decisions should be grounded in scientifically established evidence, as emphasized by El Benni et al., (2023). Overall, our study provides policymakers, veterinarians, and farmers with a comprehensive summary of the existing evidence comparing the effects of caustic and heat-cautery and pain mitigation in caustic disbudding to date. This can allow decisions to be taken based on empirical information, rather than relying solely on personal opinions or biases. For instance, our findings conflict with some of the currently guidelines, in Australia (ANIMAL HEALTH AUSTRALIA, 2016) and the UK (DEFRA - UK, 2003), where caustic disbudding is exempted from receiving anesthetic blocks. Furthermore, countries like Ireland (DAFM - IE, 2014) and Finland (MAF - FI, 2010), have prohibited caustic disbudding, allowing only heat-cauterization methods; however, our findings do not support recommending one method over the other in terms of welfare implications. Yet, it is important to note that robust evidence alone does not guarantee changes in the political sphere, given its complex environment, where social actors' values, knowledge, beliefs, and perceived barriers play a significant role (CAIRNEY; OLIVER, 2017). For example, farmers have acknowledged that the challenges associated with administering anesthetic blocks is an important barrier to implementing pain control measures (SARACENI et al., 2022). In some places anesthetic block must be administered by a licensed veterinarian, which could limit its adoption (CARDOSO; VON KEYSERLINGK; HÖTZEL, 2016; GOTTARDO et al., 2011). A recent study showed that the absence of registered drugs is an important gap to the adoption of pain control (ROBLES et al., 2021). These cases illustrate that some barriers to adoption may have a more substantial impact on the uptake of pain mitigation practices than the scientific certainty itself.

More research has shown that painful procedures carried out without pain mitigation are overwhelmingly rejected by the public (CONNOR; COWAN, 2020; HÖTZEL et al., 2020; ROBBINS et al., 2015), Moreover, there is a strong expectation from the public that farmers must mitigate pain because, among other reasons, it is the more “humane” and “right” thing to do, and “nothing should needlessly suffer” (ROBBINS et al., 2015). On the other hand, veterinarians and farmers also demand proof of the efficacy of pain mitigation drugs to recommend and adopt them (ANDRIGHETTO CANOZZI; ROSSI BORGES; JARDIM BARCELLOS, 2020; HAMBLETON; GIBSON, 2017). Although the scientific community has been doing a substantial effort to address pain mitigation, it is worth noting that the studies included in our research varied greatly regarding the population used, measured outcomes, reporting practices, assessed times, and in many studies caustics disbudding procedures were poorly described. Pronounced design dissimilarities and the absence of standardized outcome measurements might reduce the generalization and certainty of discovered evidences (BAYSINGER et al., 2021; WAGNER et al., 2021). From an ethical standpoint, the scientific community bears a moral responsibility to overcome these limitations and generate more robust and generalizable evidence to support adequate pain control measures. This would not only satisfy public expectations but also ensure that veterinarians and farmers have access to reliable information for decision-making.

2.8 CONCLUSION

The current scientific literature does not provide sufficient evidence to determine which method, caustic disbudding or heat cautery disbudding, has a greater negative impact on the welfare of calves. Since both methods are inherently painful, it is essential to implement pain mitigation measures. Our findings demonstrate that lidocaine cornual block is an effective method for caustic disbudding pain control, at least within the first 60 minutes following the procedure. The use of non-steroidal anti-inflammatory drugs (NSAIDs) appears to prevent pain rebound effects once the lidocaine wears off; however, the positive effects of NSAIDs may vary across studies, potentially compromising animal welfare. Therefore, we emphasize the need for further research on the efficacy of analgesics to maximize their positive effects. Nevertheless, it is crucial to always administer lidocaine local anesthesia as a standard practice.

2.9 ACKNOWLEDGMENTS

We are especially grateful for the help of Ihakri NUNC-NFÔONRO for the support with data collection. Zimbábwe Osório-Santos was awarded a Masters scholarship by CAPES (Ministry of Education, Brazil). Dr Maria J. Hötzel acknowledge support from CNPq (National Council for Scientific and Technological Development, Brazil). The funders had no role in this study design; data collection, analysis, and reporting; and in the decision to write and to publish this article. The authors declare that they have no conflict of interest.

3 CHAPTER III - THE EFFECT OF EARLY PAIN EXPERIENCE IN DAIRY CALVES ON PAIN SENSITIVITY LATER IN LIFE [2]

Zimbábwe Osório-Santos[1,2], Thomas Ede[1,3], Maria José Hötzel[2], Daniel M. Weary [1] and Marina A.G. von Keyserlingk[1]*

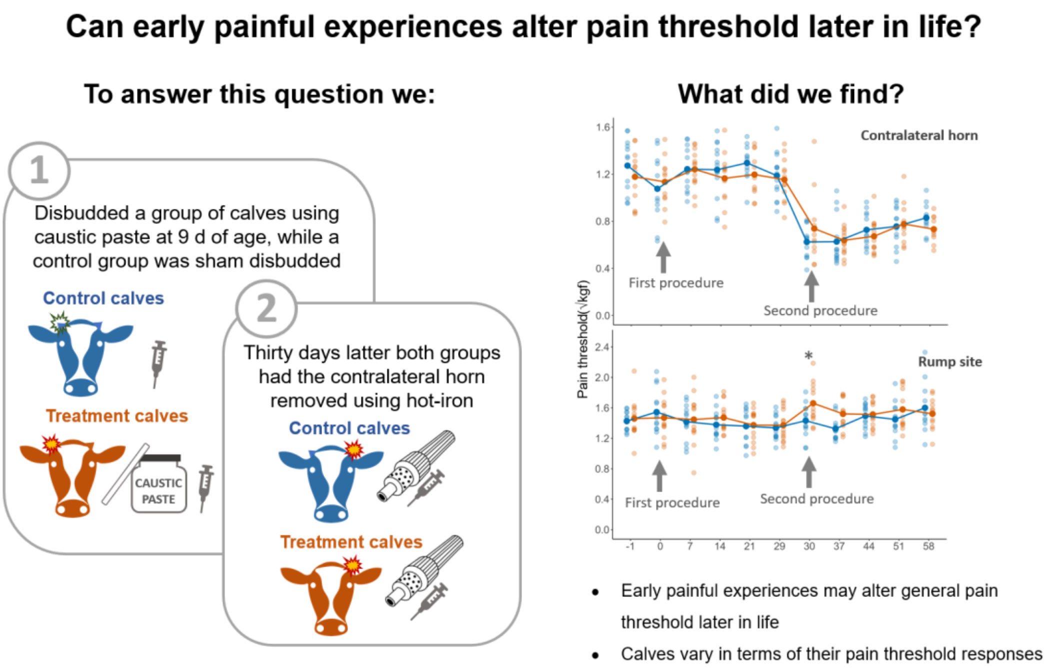
[1] Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, 9 Vancouver, B.C., Canada

[2] Laboratório de Etologia Aplicada e Bem-Estar Animal, Universidade Federal de Santa Catarina, Rod. Admar Gonzaga 1346, Itacorubi, Florianópolis 88034-001, SC, Brazil

[3] Swine Teaching and Research Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, United States of America

*Correspondence to marina.vonkeyserlingk@ubc.ca

3.1 GRAPHICAL ABSTRACT



[2] A version of Chapter III has been submitted for publication in the Journal of Dairy Science Communications. Z. O-S was involved in conducting the experiment, data curation, formal analysis, and writing of the original draft. MvK supervised the research, and Thomas Ede, MJH and DMW co-supervised it. All authors were involved in conceptualization, methodology and reviewed and edited the manuscript.

3.2 HIGHLIGHTS

- Early painful experiences may alter general nociceptive responses later in life in calves
- Our findings do not support our hypothesis of hyperalgesia due to early painful experiences
- Calves vary in terms of their nociceptive responses

3.3 ABSTRACT

In humans, early painful experiences can increase pain sensitivity later in life, but little is known regarding this phenomenon in cattle. This study assessed if a painful event early in life affects later pain sensitivity in 40-d old calves. Twenty female and six male Holstein calves were pseudo-randomly assigned to control or treatment conditions. At 9.5±1.9 d old, treatment calves had one horn bud removed using caustic paste while control calves had one horn sham disbudded, in both cases, multimodal pain control was provided (xylazine, lidocaine cornual nerve block, and meloxicam). Four weeks later all calves had the contralateral horn bud disbudded using a hot-iron, again with multimodal pain control. Mechanical nociceptive responses were assessed weekly using an algometer applied adjacent to both horn buds and on the rump, beginning 3 d before the first disbudding and ending 30 d after the second disbudding. Following the second disbudding, both groups of calves showed evidence of increased sensitivity (i.e. responded to a lower pressure from the algometer, from 1.18±0.04√kgf to 0.68±0.04√kgf) on the contralateral bud, with no evidence of difference between the control group and the treatment group which had experienced a previous pain event. An interaction between treatment and time, likely driven by treatment differences was found on the rump when tested 5 h after the second disbudding event. These responses are not consistent with the hypothesis that an early pain experience results in increased sensitivity to later painful experiences.
Key words: central sensitization, disbudding, caustic paste, welfare

3.4 INTRODUCTION

Disbudding is a routine management practice on dairy farms (USDA, 2014), typically performed in the first few weeks of life (SHIVLEY et al., 2019). Early painful experiences can result in changes in the peripheral and central nervous system, such as sprouting and sensitization of the peripheral nerves, sensitization of the dorsal horn nociceptive circuits, and alteration of the brainstem descending pain control (SCHWALLER; FITZGERALD, 2014). These disruptions help explain pain thresholds shifts in both humans (BEGGS et al., 2012) and rodents (Williams and Lascelles, 2020). Specifically, painful experiences in early life increase pain sensitivity (i.e. decreasing pain thresholds), an effect that can persist for months or years (Brummelte et al., 2015; Taddio et al., 1997).

Previous studies on farm animals have explored the effect of early pain on general sensorial sensitivity (ADCOCK; TUCKER, 2018b; CLARK et al., 2014) and behavioral responses (ADCOCK; TUCKER, 2020a; CLARK et al., 2014; MCCRACKEN et al., 2010). For example, early painful experiences were found to cause a reduction in the overall pain threshold (ADCOCK; TUCKER, 2018b; MIRRA et al., 2018), and an increased behavioral response following a second painful procedure in heifers (ADCOCK; TUCKER, 2020a) and lambs (MCCRACKEN et al., 2010). However, to our knowledge, the effects of early painful experiences on pain thresholds following later painful experiences in calves has not been explored.

3.5 MATERIAL AND METHODS

In the current study, we assessed if an early painful experience, originating from the removal of one horn bud, would affect calf responses to the removal of the second horn bud 4 weeks later. We predicted that animals who experienced early disbudding would show a more pronounced response to the subsequent disbudding event.

This study took place at The University of British Columbia Dairy Education and Research Centre (Agassiz, British Columbia, Canada) between September 2021 - February 2022. The project was approved by The University of British Columbia's Animal Care Committee (# A16-0310).

We undertook a power analysis based on previous studies investigating pain sensitivity following disbudding in dairy calves (i.e., Mintline et al., 2013; Mirra et al., 2018). Based upon this analysis, 26 Holstein calves (20 female and 6 male) were enrolled for the study (mean ± SD BW = 40.22 ± 5.57 kg). Calves were kept in individual pens measuring 1 × 1.5 m, bedded with fresh sawdust for the first 5 d of life. On d 4, all calves were fitted with a single ear tag in each ear using an ear tagging device that punctured the ear (Allflex, Universal Total Tagger). Calves were then moved to a 35 m^2 group pen on d 5 and housed in groups of 10 animals. Fresh sawdust was added weekly. Calves had access to 12 L/d of pasteurized whole milk using automated feeders (CF 1000 CS Combi; Delaval Inc., Tumba, SDR, Sweden) equipped with 1 teat and a raceway (0.4 × 1.5 m) that restricted access to a single calf at a time. Milk allowances accumulated at a rate of 5% of the daily allowance every hour from midnight to 2000 h. The milk feeder delivered a minimum of 0.5 L and a maximum of 9.5 L per visit. Approximately 0.5 m adjacent to the automated milk feeder was a starter feeder (CF 1000 feeder, Delaval, Inc., Tumba, SDR, Sweden), also equipped with a barrier (0.4 × 1.0 m) to allow access to a single calf at a time. All calves had *ad libitum* access to the calf starter (20% CP texturized and consisting of 31.2% flake barley, 15.3% canola meal, 15.0% flaked corn, 12.3% soybean meal, 8.7% wheat, 6.5% molasses; Richie Smith Feeds, Inc. Abbotsford, BC, Canada). Calves were also provided *ad libitum* access to water and hay via automated Insentec feeders (RIC; Insentec B.V.).

At birth, calves were pseudo-randomly allocated to control or treatment conditions, balancing for sex and BW. Each group underwent two procedures. At 9.5±1.8 d old, treatment calves had one horn bud disbudded using caustic paste and control calves underwent a sham procedure (both interventions are described later, and henceforth referred to as the 'first procedure'). Right and left horn buds were balanced within treatment. Thirty days later, calves from both groups had the contra lateral horn bud removed by hot-iron (henceforth referred to as the 'second procedure').

During the first procedure, one calf from each treatment was gently moved to a separate pen (2.0 m x 2.0 m), and both were sedated using a subcutaneous injection of xylazine (0.2 mg/kg, Rompun 20 mg/mL, Bayer, Leverkusen, Germany). After sedation, 1 of the horn buds was pseudo-randomly selected for removal and an anesthetic block (5 mL of 2% lidocaine, 1:100,000 epinephrine, Lido-2, Rafter8, Calgary, AB, Canada) was applied between the lateral *cantus* and the horn bud. After waiting 10 min for the anesthetic to take effect, the region around the horn bud was shaved and desensitization of the cornual nerve was confirmed by the absence of reaction to a needle prick. Caustic paste (calcium hydroxide 24.9%, sodium hydroxide 21.5%, Dr. Naylor Morris, NY, United States) was applied to the area on and around the horn bud (~17 ± 2.2 mm in diameter) of the treatment calves. The amount of paste used per horn bud area was estimated to be 0.30 ± 0.10 g/bud based upon pre-treatment training using 6 non-experimental calves. A ring of petroleum jelly (Original

Vaseline, Unilever, Toronto, ON, Canada) was applied around the paste area to prevent spread of the caustic paste. Control calves underwent a sham disbudding which included all aspects described above with the exception of the caustic paste application. Immediately following the application of the petroleum jelly all calves received a subcutaneous injection of a non-steroidal anti-inflammatory drug (0.5 mg/kg meloxicam; Metacam, 20 mg/mL, Boehringer Ingelheim). Following the first procedure all calves were placed in sternal recumbency and left to recover for 5 h, and then returned to their home pen.

Calves from both groups were subjected to the second procedure (i.e., disbudding of the contralateral horn bud) 30 d later. The disbudding procedure was identical to that described previously, but instead of using caustic paste the horn bud was removed with a hot-iron (X30 1/2" Tip, Rhinehart, Spencerville, IN), pre-heated for 10 min to approximately 500°C. The hot-iron was applied using minimal pressure, rotated gently back and forth for approximately 15 s, until a homogeneous copper-colored ring was formed around the bud. During the 5 h recovery period following both, the first and second, procedures, pain behaviors were recorded: 1) head rub, defined as head in contact with and moving against either a hind leg or against a wall of the pen; 2) ear flicks, defined as ears moving at least once back and forth in rapid succession; and 3) head shake defined as the head moving rapidly from side to side at least once. Behaviors were scored as either zero, when not observed, or the specific frequency when observed. The selected behaviors were chosen given that they have been previously associated with pain following caustic paste and hot-iron disbudding (FAULKNER; WEARY, 2000; WINDER et al., 2017). Two trained observers, 1 of whom was blind to treatment, scored videos continuously for 1 min every 5 min. The sums of all pain behaviours (no.) per observed minute from the 2 observers were highly correlated ($r_{243} = 0.95$, $P < 0.001$).

Mechanical nociceptive threshold (MNT) was assessed using a portable algometer with a 1 cm rubber tip (Wagner Force, One FDIX, Wagner Instruments, Greenwich, CT). Just before each assessment calves were blindfolded and gently restrained to reduce variation introduced by calf movements during measurement (see Frahm et al. 2020). MNT measurements were taken at d -3, -2, -1 before the first procedure, 5 h after and then, twice per week on d 7, 8, 14, 15, 21, 22, 28, and 29 following the first procedure. On d 30, 1 measurement was taken 5 h after the second procedure, and on d 38, 39, 45, 46, 52, 53, 59, and 60, relative to the date of the first procedure. We opted to take the first measure at 5 h to avoid interferences in the behavioral observations described earlier.

The nociceptive threshold of the horn bud was tested first laterally, second superior from the bud, and last at the point between the sacral vertebrae and the femur joint on both sides of the rump (see Figure 4). The order of the left or right sides horn buds and rump measurements were balanced between treatments and sex. For each measure a hand was lightly placed on the region to be assessed and kept there until the calf stopped moving. The hand was then replaced with the algometer rubber tip. The holder then applied pressure at a constant intensity of ~1 kgf/s until the animal moved its head away from the equipment (for nociceptive threshold in the horn bud) or lifted 1 of its legs (nociceptive threshold in the rump). Due to the visibility of the wound, it was not possible to blind the operator of the algometer to treatment groups.

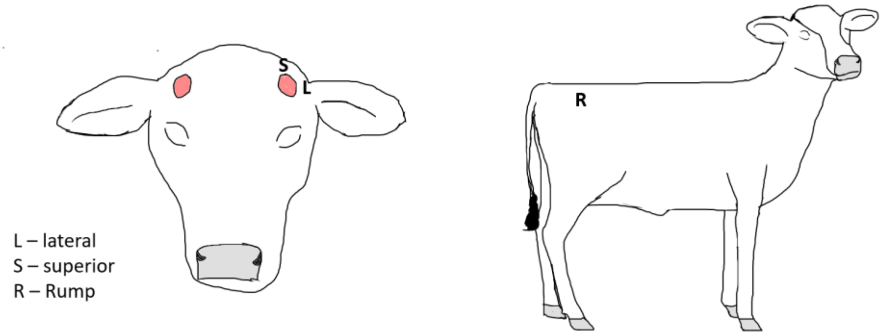


Figure 4: Locations where nociceptive pain threshold was collected.

Calves were treated as the experimental unit in all analyses. The final sample included 26 calves, 13 per treatment. All analyses were performed using R (R Core Team, 2021). To detect and examine outliers we adopted a mixed approach: firstly, each experimental day was screened graphically, with the intention of identifying any between-animal outliers. Using the IQR method (YANG; RAHARDJA; FRÄNTI, 2019) 24 outliers were identified. We then used a within-animal approach, where data from each animal was divided into 3 different time periods: baseline (d -3, -2, -1); between the first and second procedure (d 7, 8,14,15, 21,22, 28, 29); and after the second procedure (d 38, 39, 45, 46, 52, 53, 59, and 60). Mean and standard deviation (SD) were generated for each of these three moments, and the outliers identified using the first approach were again assessed using z-scores. Since no data point surpassed the 2.5 SD threshold, no outliers were removed from the final analysis.

Considering that we predicted that treatment calves would show a lower nociceptive threshold than control calves following the second procedure, we opted to build only 1 model for each body region, comparing the measurements taken in the days before the second procedure and the measurement taken 5 h after. A mixed model (function lmer, package lme4) was used to explore differences between and within treatment groups over time. Time, treatment, and their interactions were set as fixed effects, animal was set as a random effect, and time was treated as a continuous variable. Normality and homoscedasticity of residuals were checked graphically, and data were square-root transformed to improve model fit. We also report all significant interactions (P-value < 0.05), and interactions that were not significant were removed from the final model. Measurements taken at the lateral and superior sites were averaged across the first and second procedures, and also across the left and right sides of the rump. Results are presented in the square root transformed scale.

Since calves exhibited a very low frequency of some pain behaviors, we aggregated the three pain behaviors (ear flicks, head rubs, and head shakes) as described in previous articles on pain from disbudding (EDE; VON KEYSERLINGK; WEARY, 2020a; WINDER et al., 2017). A simple t-test was used to explore possible differences between groups in terms of the aggregated pain behaviors exhibited in the 5 h following the first procedure, and again following the second procedure, with results were presented as means ± SE.

3.6 RESULTS

As expected, only treatment calves showed a decline in MNT at the first procedure site after receiving the intervention, and MNT values remained lower in the subsequent weeks (Figure 5). Given this initial painful experience, we had predicted that these calves would show lower MNT than control calves following their second procedure at the site of the second procedure, as well as at the site of the rump. However, none of our results were consistent with these predictions. We found no evidence of a treatment different in the decline in MNT at the site of the second procedure (Treatment - Control = 0.03 ± 0.09; $t_{1, 50} = 0.32$; $P = 0.75$) (Figure 5.b.). For the rump, we noted an interaction between treatment and time (Interaction estimative = 0.17 ± 0.08; $t_{1, 24} = 2.03$, $P = 0.05$) (Figure 5 c) driven by treatment calves showing elevated rump MTN measures over the first two days after the second procedure. Treatment calves showed lower nociceptive pain thresholds at the first procedure site after receiving the second procedure in the contralateral horn (Treatment - Control = -0.17 ± 0.08; $t_{1, 24}$ = - 2.20, *P* = 0.03) (Figure 5.a).

We found no evidence of differences between treatments in the number of pain behavior 5 h after either the first or second procedure. Across both treatments, calves averaged 6.54 ± 0.88 behaviors/5 h after the first procedure and 5.30 ± 0.95 behaviors/5 h after the second procedure.

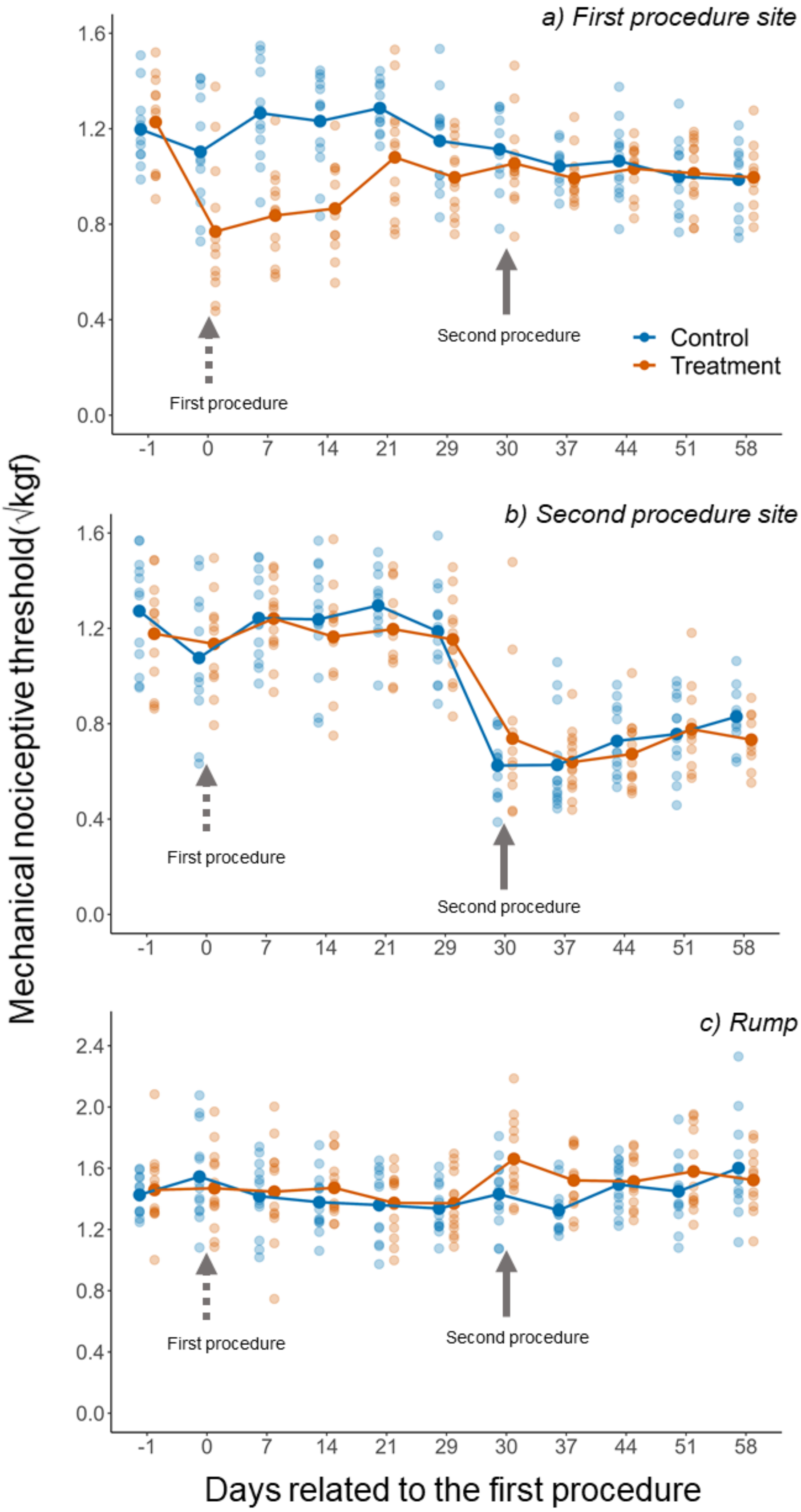


Figure 5: Mechanical nociceptive threshold across the three body regions.
Mechanical nociceptive threshold measured at (a) the horn bud subjected to the first procedure (either sham disbudded for control calves, or caustic paste disbudded for

treatment calves), (b) the horn bud subjected to the second procedure (hot-iron disbudding for all calves), and (c) the rump. Day -1 shows the average of the measurements taken on d -2 and -1 before the first procure. Days 7, 14, 21, 29, 38, 45, 52, and 59 are the average of the two weekly values. The dashed arrow at d 0 and solid arrow at d 30 indicate the measurements taken 5-h after the first and the second procedure, respectively. Values are square root transformed, and lower values indicate higher pain sensitivity. Semitransparent dots show values from individual calves, and solid dots show treatment means.

3.7 DISCUSSION

Contrary to our expectations, a previous painful experience did not lead to a decrease in the nociceptive threshold at the rump and the second procedure site. Unexpectedly, we discovered an interaction, likely driven by treatment differences at the rump site. Interestingly, the nociceptive threshold tended to increase following the second procedure in treatment calves. These findings provide preliminary evidence of hypoalgesia in cattle associated with exposure to a previous painful event. Hypoalgesia induced by early painful experiences has been described in humans. Children aged 9 to 14 y who had been treated for at least 3 d as an infant in a neonatal intensive care unit (NICU), where repeated painful procedures were performed, showed a higher thermal nociceptive threshold compared to children who had not been in the NICU (HERMANN et al., 2006). Moreover, another study showed that an increase in the number of painful procedures during the NICU stay resulted in a lower response to sensory stimuli (in this case immersion in cold water; VEDERHUS et al., 2012).

Contrary to our hypothesis, we did not find evidence of a lower pain threshold at the injury site following the second pain procedure in calves who had previously experienced pain. Previous work has shown that painful experiences in early life can amplify later painful experiences in humans and other animals. For example, ewes that experienced tail-docking during the first week of life showed more pain-related behaviors during parturition than did control animals (CLARK et al., 2014). In humans, children who suffered severe or moderate burns during their first 2 y of life showed a lower nociceptive thermal thresholds when tested at 9 to 16 y. of age (WOLLGARTEN-HADAMEK et al., 2009). In rats, a lower pain threshold was observed after a paw incision in individuals that had undergone a previous incision in the contralateral paw 14 d before (WALKER; TOCHIKI; FITZGERALD, 2009).

Adcock (2021) argued that the age at which the injury occurred and the age when pain sensitivity was assessed may affect the results obtained. For example, lambs castrated on their first day of life showed more pain-related behaviors after being tail-docked approximately 30 d later compared to lambs castrated at 10 d of life (MCCRACKEN et al., 2010). In a study investigating the effect of different ages of hot-iron disbudding on systemic pain thresholds, Adcock and Tucker (2018) found that calves disbudded at 4 d of life had a lower mechanical nociceptive threshold in the rump area than calves disbudded at 40 d. Other work by these authors reported that calves disbudded at 3 or 35 d of age showed a higher heart rate responses to vaccination at 11 mo. of age than calves disbudded at 56 d (ADCOCK; TUCKER, 2020a). In rats, a plantar incision at 3 and 6 d of life resulted in hyperalgesia following a second incision 14 d later; however, hyperalgesia was not observed when the first incision was made at 10, 21, or 40 d of life (WALKER; TOCHIKI; FITZGERALD, 2009). Previous work has speculated that in calves the period before 35 d of age may be particularly sensitive to pain amplification later in life (ADCOCK; TUCKER, 2020a); based on this work we chose to perform the first painful procedure when calves were ~ 10 d old.

One of the potential reasons for the differences in findings between the current study and this earlier work (ADCOCK; TUCKER, 2020a) is the shorter time interval between the two procedures adopted in our study. We used a 30-d gap between procedures, but both procedures took place within the first 60 d of life. Our results may also reflect the efficacy of the multimodal pain management protocol applied to all calves in our study. Multimodal pain control is reportedly effective in mitigating pain induced by both hot-iron and caustic paste disbudding (STEWART et al., 2009; WINDER et al., 2018a). The use of pain control can prevent hyperalgesia. For example, Taddio et al. (1997) reported that human male babies circumcised at 20 d of age displayed more crying and facial expressions associated with pain when vaccinated at 4 or 6 mo., but the use of an anesthetic cream before circumcision reduced this effect.

The type of stimulus used to assess MNT may also have influenced our results. In humans, childhood burns resulted in changes in the thermal, but not mechanical nociceptive response in 11 y old children (WALKER; TOCHIKI; FITZGERALD, 2009). Although mechanical and thermal stimuli have been used to assess nociceptive threshold in cows and calves (PETERS; SILVEIRA; FISCHER, 2015; PINHEIRO

MACHADO F°; HURNIK; BURTON, 1997), mechanochemical have been widely utilized to assess pain threshold following disbudding. We thus opted for a mechanical stimulus in the current study, but future work should consider using more relevant stimuli.

High variability has been reported as a challenge in studies examining mechanical nociceptive thresholds in calves and cows (RAUNDAL et al., 2014; WILLIAMS et al., 2020). However, few studies have investigated the underlying reasons for this variability. In an effort to minimize variation, mechanical nociceptive threshold assessments were conducted by the same trained individual throughout the trial, and measurements were taken at two different locations per site twice a week, allowing for the use of averaged values. Despite these measures, we still found considerable variation among calves; we encourage future research to explore the sources of variability in mechanical nociceptive threshold assessments. One limitation of our study is that all calves were ear tagged when they were 4 d of age. This was done to allow the use of automatic feeders, and to comply with Canadian legal requirements to ensure traceability. Ear tagging is also associated with pain behaviors, including headshaking, ear scratching, tail wagging, foot-stamping, and vocalization (LOMAX et al., 2017; SCHNAIDER et al., 2022; TURNER et al., 2020). This early pain experience may have affected our results.

3.8 CONCLUSION

In conclusion, we found no evidence that a painful procedure, performed at 10 d of age, results in hyperalgesia in response to hot-iron disbudding 30 d later. Indeed, our results are more consistent with hypoalgesia induced by an early painful procedure.

Although pain is an unpleasant experience, and it should be prevented and mitigated, pain also plays an important adaptive role for individuals that experience it, given that it helps to avoid harmful stimuli, and protect any injured part of the body. Besides that, pain responses in farm animals may be also important in farm management, since it allows humans to identify injured or sick individuals, and give them proper veterinarian support, including pain relief. Thus, a decrease in general pain threshold might impair animals' mechanisms to detect respond appropriately to painful events through their lives such as infections, lesions, calving, and social agonistic interactions. Furthermore, it could also reduce the ability of humans to detect animals that are experiencing poor welfare.

3.9 ACKNOWLEDGMENTS

We thank the staff and students at the UBC Dairy Education and Research Centre for their help and support. We are especially grateful for the help of the UBC Animal Welfare program members with data collection (in alphabetical order): Marthe Bourg, Viviane Codognoto, Jana Denissen, Ravleen Gill, Yasamin (Yas) Ranjbar, Phoebe Lam, Caily Mellott, Seonpil Yoo. We also thank Dr Sarah Adcock from the University of Wisconsin-Madison, Ph.D. student Alycia Drwencke and Dr Cassandra B. Tucker from the University of California, Davis for their advice during the planning stages of this study. Zimbábwe Osório-Santos was awarded a Masters scholarship by CAPES (Ministry of Education, Brazil) and the Emerging Leaders in the Americas Program (ELAP), he was also supported by the Animal Welfare Program (AWP - UBC) during his stay at UBC. Dr Maria J. Hötzel acknowledge support from CNPq (National Council for Scientific and Technological Development, Brazil). General funding for the UBC Animal Welfare Program is provided by the NSERC Industrial Research Chair in Dairy Cattle Welfare awarded to MvK and DMW together with our industrial partners the Dairy Farmers of Canada (Ottawa, ON, Canada), Saputo Inc. (Montreal, QC, Canada), British Columbia Dairy Association (Burnaby, BC Canada), Alberta Milk (Edmonton, AB, Canada), Intervet Canada Corporation (Kirkland, QC, Canada), Boehringer Ingelheim Animal Health (Burlington, ON, Canada), BC Cattle Industry Development Fund (Kamloops, BC, Canada), The Semex Alliance (Guelph, ON, Canada), Lactanet (Sainte-Anne-de-Bellevue, QC, Canada), Dairy Farmers of Manitoba (Winnipeg, MB, Canada), and the Saskatchewan Milk Marketing Board (Regina, SK, Canada). The authors have not stated any conflicts of interest. The authors declare no conflict of interest. The funders had no role in this study design; data collection, analysis, and reporting; and in the decision to write and to publish this article.

4 CHAPTER IV - FINAL THOUGHTS

In this dissertation, my primary objective was to compare the impact of caustic disbudding and heat-cautery disbudding in calves' welfare and determine if one method was less detrimental than the other. Through a literature review, I discovered that both methods have negative effects on welfare, and at present it is not possible to determine the "lesser of two evils." However, caustic disbudding is indeed painful, and I proceeded to investigate the most effective pharmacological approach to alleviate its pain. My findings revealed that lidocaine anesthetic block is effective in reducing short-term pain following caustic disbudding and should always be administered. Lastly, I assessed whether a painful procedure early in life, such as caustic disbudding, could impact pain sensitivity during a subsequent procedure later in life. Surprisingly, my research indicated that early painful experiences can diminish general pain sensitivity.

Historically, animals have been denied the ability to experience pain, using claims like “their struggles and avoidance behaviors are not driven by pain, it is just autonomous responses”. Similar arguments were made regarding infants and their ability to feel pain just some decades ago. However, we now have robust evidence that pain is physiologically and neurologically similar across vertebrates and emotional consequences are also similarly observed. Despite this understanding, proper pain mitigation is still underutilized. A couple of decades ago, one could argue that the underutilization was due to a lack of scientific evidence supporting the efficacy of pain control methods. The scientific community, as I just did in this dissertation, has tackled this issue since then, spending significant resources to produce knowledge on how to effectively mitigate pain in a variety of contexts. However, the lack of pain control adoption cannot solely be attributed to the absence of scientific evidence. Various factors contribute to this issue, including cognitive dissonance, the absence of legal requirements or the presence of legal restrictions, cultural influences, practical considerations, and more. I believe a multidisciplinary approach is necessary to address these barriers and achieve what could be considered a med-term goal of 100% adoption of pain control practices.

In chapter III I showed that early painful procedures can have long-lasting effects on the pain sensitivity threshold, especially to subsequent painful events. This dissertation is part of a growing body of studies that sheds light on the long-term consequences of painful procedures. For example, recent findings have shown that caustic disbudding wounds remain sensitive for almost 19 weeks, and heat-cautery wounds can spontaneously hurt for at least three weeks. However, none of these long-term effects are currently being considered when disbudding is performed. At present, there is no available alternative to effectively mitigate long-term pain in farm animals, and the current disbudding protocols do not account for potential long-term threshold changes. I recommend prioritizing further research to address these issues. Furthermore, it is prudent to adopt the precautionary principle, which states that, If the potential risks are significant but uncertain, cautionary measures should be implemented, or the activity itself should be reconsidered. The fact that we are quickly figuring out more unexpected long-term negative effects of pain events highlights the uncertainty of the risk we are taking when these procedures are executed. It is worth noting that genetic hornless cattle, which carry a polled gene, offer a promising alternative that could greatly reduce the need for disbudding procedures, similar to the example seen in beef cattle. Therefore, I recommend more efforts should be directed towards the implementation of this alternative as a way to replace disbudding and avoid associated long-term consequences.

In summary, this dissertation provided evidence to address the immediate question of how to mitigate caustic disbudding pain. However, it has also brought evidence that highlights the need to consider the long-term consequences of these procedures and explore alternatives to mitigate these consequences or completely replace painful practices.

REFERÊNCIAS

AABP. **Dehorning guidelines**. , 2019. Disponível em: <https://www.aabp.org/Resources/AABP_Guidelines/Dehorning-2019.pdf>. Acesso em: 12 nov. 2023

ADCOCK, S. J. J. Early Life Painful Procedures: Long-Term Consequences and Implications for Farm Animal Welfare. **Frontiers in Animal Science**, v. 2, p. 759522, 10 dez. 2021.

ADCOCK, S. J. J.; TUCKER, C. B. Painful procedures. Em: **Advances in Cattle Welfare**. [s.l.] Elsevier, 2018a. p. 157–198.

ADCOCK, S. J. J.; TUCKER, C. B. The effect of disbudding age on healing and pain sensitivity in dairy calves. **Journal of Dairy Science**, v. 101, n. 11, p. 10361–10373, nov. 2018b.

ADCOCK, S. J. J.; TUCKER, C. B. The effect of early burn injury on sensitivity to future painful stimuli in dairy heifers. **PLOS ONE**, v. 15, n. 6, p. e0233711, 3 jun. 2020a.

ADCOCK, S. J. J.; TUCKER, C. B. Conditioned place preference reveals ongoing pain in calves 3 weeks after disbudding. **Scientific Reports**, v. 10, n. 1, p. 3849, dez. 2020b.

ALDERSEY, J. E. et al. Understanding the effects of the bovine POLLED variants. **Animal Genetics**, v. 51, n. 2, p. 166–176, mar. 2020.

ALTINYAZAR, C. H. et al. Digital Block With and Without Epinephrine During Chemical Matricectomy with Phenol. **Dermatologic Surgery**, v. 36, n. 10, p. 1568–1571, out. 2010.

ANDERSON, D. E.; MUIR, W. W. Pain management in ruminants. **Veterinary Clinics of North America: Food Animal Practice**, v. 21, n. 1, p. 19–31, mar. 2005.

ANDRIGHETTO CANOZZI, M. E.; ROSSI BORGES, J. A.; JARDIM BARCELLOS, J. O. Attitudes of cattle veterinarians and animal scientists to pain and painful procedures in Brazil. **Preventive Veterinary Medicine**, v. 177, p. 104909, abr. 2020.

ANIMAL HEALTH AUSTRALIA. The Australian Animal Welfare Standards and Guidelines for Cattle. . 2016.

AZEVEDO, A. et al. Age, sex and storage time influence hair cortisol levels in a wild mammal population. **PLOS ONE**, v. 14, n. 8, p. e0221124, 9 ago. 2019.

BAYSINGER, A. et al. Proposed multidimensional pain outcome methodology to demonstrate analgesic drug efficacy and facilitate future drug approval for piglet castration. **Animal Health Research Reviews**, v. 22, n. 2, p. 163–176, dez. 2021.

BEAUSSIER, M. et al. Perioperative Use of Intravenous Lidocaine. **Drugs**, v. 78, n. 12, p. 1229–1246, ago. 2018.

BEGGS, S. et al. Priming of adult pain responses by neonatal pain experience: maintenance by central neuroimmune activity. **Brain**, v. 135, n. 2, p. 404–417, 1 fev. 2012.

BOURNE, S.; MACHADO, A. G.; NAGEL, S. J. Basic Anatomy and Physiology of Pain Pathways. **Neurosurgery Clinics of North America**, v. 25, n. 4, p. 629–638, out. 2014.

BRAZ, M. et al. Effect of rectal or intravenous tramadol on the incidence of pain-related behaviour after disbudding calves with caustic paste. **Applied Animal Behaviour Science**, v. 136, n. 1, p. 20–25, jan. 2012.

BRUMMELTE, S. et al. Cortisol levels in former preterm children at school age are predicted by neonatal procedural pain-related stress. **Psychoneuroendocrinology**, v. 51, p. 151–163, jan. 2015.

CAIRNEY, P.; OLIVER, K. Evidence-based policymaking is not like evidence-based medicine, so how far should you go to bridge the divide between evidence and policy? **Health Research Policy and Systems**, v. 15, n. 1, p. 35, s12961- 017- 0192– x, dez. 2017.

CANOZZI, M. E. A.; BORGES, J. A. R.; BARCELLOS, J. O. J. Which factors can influence the perception of pain by veterinarians and animal scientists from Brazil? **Journal of Veterinary Behavior**, v. 47, p. 59–69, jan. 2022.

CAPITAN, A. et al. A Newly Described Bovine Type 2 Scurs Syndrome Segregates with a Frame-Shift Mutation in TWIST1. **PLoS ONE**, v. 6, n. 7, p. e22242, 21 jul. 2011.

CARDOSO, C. S.; VON KEYSERLINGK, M. A. G.; HÖTZEL, M. J. Trading off animal welfare and production goals: Brazilian dairy farmers' perspectives on calf dehorning. **Livestock Science**, v. 187, p. 102–108, maio 2016.

CARLSON, D. F. et al. Production of hornless dairy cattle from genome-edited cell lines. **Nature Biotechnology**, v. 34, n. 5, p. 479–481, maio 2016.

CARTER, B. S.; BRUNKHORST, J. Neonatal pain management. **Seminars in Perinatology**, v. 41, n. 2, p. 111–116, mar. 2017.

CLARK, C. et al. Long-term and trans-generational effects of neonatal experience on sheep behaviour. **Biology Letters**, v. 10, n. 7, p. 20140273, jul. 2014.

COETZEE, J. F. A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the United States. **Applied Animal Behaviour Science**, v. 135, n. 3, p. 192–213, dez. 2011.

CONNOR, M.; COWAN, S. L. Consumer evaluation of farm animal mutilations. **Research in Veterinary Science**, v. 128, p. 35–42, fev. 2020.

COZZI, G. et al. Dehorning of cattle in the EU Member States: A quantitative survey of the current practices. **Livestock Science**, v. 179, p. 4–11, set. 2015.

DAFM - IE. 127. Animal Health and Welfare (Operations and Procedures). . 2014.

DAVIS, E. B.; BRAKORA, K. A.; STILSON, K. T. Evolution, development and functional role of horns in cattle. Em: MELLETTI, M.; BURTON, J. (Eds.). **Ecology, Evolution and Behaviour of Wild Cattle**. 1. ed. [s.l.] Cambridge University Press, 2014. p. 72–82.

DEEKS, J.; HIGGINS, J. P.; ALTMAN, D. G. Chapter 10: Analysing data and undertaking meta-analyses. Em: **Cochrane Handbook for Systematic Reviews of Interventions**. 6.3 ed. [s.l: s.n.].

DEFRA - UK. Code of Recommendations for the Welfare of Livestock. . 2003.

DILLWORTH, T.; MENDOZA, M. E.; JENSEN, M. P. Neurophysiology of pain and hypnosis for chronic pain. **Translational Behavioral Medicine**, v. 2, n. 1, p. 65–72, mar. 2012.

DJULBEGOVIC, B.; GUYATT, G. H. Progress in evidence-based medicine: a quarter century on. **The Lancet**, v. 390, n. 10092, p. 415–423, jul. 2017.

DREVON, D.; FURSA, S. R.; MALCOLM, A. L. Intercoder Reliability and Validity of WebPlotDigitizer in Extracting Graphed Data. **Behavior Modification**, v. 41, n. 2, p. 323–339, mar. 2017.

DRWENCKE, A. M.; ADCOCK, S. J. J.; TUCKER, C. B. Wound healing and pain sensitivity following caustic paste disbudding in dairy calves. **Journal of Dairy Science**, p. S0022030223003156, jun. 2023.

EDE, T.; VON KEYSERLINGK, M. A. G.; WEARY, D. M. Assessing the affective component of pain, and the efficacy of pain control, using conditioned place aversion in calves. **Biology Letters**, v. 15, n. 10, p. 20190642, out. 2019.

EDE, T.; VON KEYSERLINGK, M. A. G.; WEARY, D. M. Social approach and place aversion in relation to conspecific pain in dairy calves. **PLOS ONE**, v. 15, n. 5, p. e0232897, 14 maio 2020a.

EDE, T.; VON KEYSERLINGK, M. A. G.; WEARY, D. M. Conditioned place aversion of caustic paste and hot-iron disbudding in dairy calves. **Journal of Dairy Science**, v. 103, n. 12, p. 11653–11658, dez. 2020b.

EL BENNI, N.; GROVERMANN, C.; FINGER, R. Towards more evidence-based agricultural and food policies. **Q Open**, p. qoad003, 22 fev. 2023.

FAULKNER, P. M.; WEARY, D. M. Reducing Pain After Dehorning in Dairy Calves. **Journal of Dairy Science**, v. 83, n. 9, p. 2037–2041, set. 2000.

FERNANDEZ, A. et al. Evidence-based medicine: is it a bridge too far? **Health Research Policy and Systems**, v. 13, n. 1, p. 66, dez. 2015.

FRAHM, S. et al. Nociceptive Threshold of Calves and Goat Kids Undergoing Injection of Clove Oil or Isoeugenol for Disbudding. **Animals**, v. 10, n. 7, p. 1228, 20 jul. 2020.

FU, Y. et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. **Nature Biotechnology**, v. 31, n. 9, p. 822–826, set. 2013.

FUZAYLOV, G.; FIDKOWSKI, C. W. Anesthetic considerations for major burn injury in pediatric patients. **Pediatric Anesthesia**, v. 19, n. 3, p. 202–211, mar. 2009.

GENTLE, M. J. Neuroma formation following partial beak amputation (beak trimming) in the chicken. **Research in Veterinary Science**, v. 41, n. 3, p. 383–385, nov. 1986.

GINGERICH, K. N.; CHOULET, V.; MILLER-CUSHON, E. K. Disbudding affects use of a shelter provided to group-housed dairy calves. **Journal of Dairy Science**, v. 103, n. 11, p. 10519–10529, nov. 2020.

GOTTARDO, F. et al. The dehorning of dairy calves: Practices and opinions of 639 farmers. **Journal of Dairy Science**, v. 94, n. 11, p. 5724–5734, nov. 2011.

GROBLER, R.; VAN MARLE-KÖSTER, E.; VISSER, C. Challenges in selection and breeding of polled and scur phenotypes in beef cattle. **Livestock Science**, v. 247, p. 104479, maio 2021.

HAMBLETON, S.; GIBSON, T. Study investigating the attitudes and opinions of cattle farmers and veterinarians in the UK on the use of non-steroidal anti-inflammatory drugs (NSAIDs) for post-disbudding analgesia of calves. **Animal Welfare**, v. 26, n. 3, p. 323–334, ago. 2017.

HEINRICH, A. et al. The effect of meloxicam on behavior and pain sensitivity of dairy calves following cautery dehorning with a local anesthetic. **Journal of Dairy Science**, v. 93, n. 6, p. 2450–2457, jun. 2010.

HEINRICHS, A. J. et al. The National Dairy Heifer Evaluation Project: A Profile of Heifer Management Practices in the United States. **Journal of Dairy Science**, v. 77, n. 6, p. 1548–1555, jun. 1994.

HEMPSTEAD, M. N. et al. Evaluation of alternatives to cautery disbudding of dairy goat kids using physiological measures of immediate and longer-term pain. **Journal of Dairy Science**, v. 101, n. 6, p. 5374–5387, jun. 2018.

HERMANN, C. et al. Long-term alteration of pain sensitivity in school-aged children with early pain experiences. **Pain**, v. 125, n. 3, p. 278–285, dez. 2006.

HERSH, E. V. et al. Nonsteroidal Anti-Inflammatory Drugs and Opioids in Postsurgical Dental Pain. **Journal of Dental Research**, v. 99, n. 7, p. 777–786, jul. 2020.

HERSKIN, M. S.; NIELSEN, B. H. Welfare Effects of the Use of a Combination of Local Anesthesia and NSAID for Disbudding Analgesia in Dairy Calves—Reviewed Across Different Welfare Concerns. **Frontiers in Veterinary Science**, v. 5, p. 117, 5 jun. 2018.

HIGGINS, J. P.; LI, T.; DEEKS, J. Choosing effect measures and computing estimates of effect. Em: **Cochrane Handbook for Systematic Reviews of Interventions**. 6.3 ed. [s.l.] Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, 2022.

HÖTZEL, M. J. et al. A Survey of Management Practices That Influence Performance and Welfare of Dairy Calves Reared in Southern Brazil. **PLoS ONE**, v. 9, n. 12, p. e114995, 15 dez. 2014.

HÖTZEL, M. J. et al. Citizens' views on the practices of zero-grazing and cow-calf separation in the dairy industry: Does providing information increase acceptability? **Journal of Dairy Science**, v. 100, n. 5, p. 4150–4160, maio 2017.

HÖTZEL, M. J. et al. On the Road to End Pig Pain: Knowledge and Attitudes of Brazilian Citizens Regarding Castration. **Animals**, v. 10, n. 10, p. 1826, 8 out. 2020.

JANIS, C. EVOLUTION OF HORNS IN UNGULATES: ECOLOGY AND PALEOECOLOGY. **Biological Reviews**, v. 57, n. 2, p. 261–318, maio 1982.

KAPLAN, R. M.; CHAMBERS, D. A.; GLASGOW, R. E. Big Data and Large Sample Size: A Cautionary Note on the Potential for Bias: B IAS IN B IG D ATA. **Clinical and Translational Science**, v. 7, n. 4, p. 342–346, ago. 2014.

KARLEN, K. J. et al. Efficacy of oral meloxicam as primary pain mitigation following caustic paste disbudding of three day old Holstein calves. **Translational Animal Science**, v. 5, n. 2, p. txz151, 1 abr. 2021.

KLEINHENZ, M. D.; VISCARDI, A. V.; COETZEE, J. F. Invited Review: On-farm pain management of food production animals. **Applied Animal Science**, v. 37, n. 1, p. 77–87, fev. 2021.

KLING-EVEILLARD, F. et al. Attitudes of farmers towards cattle dehorning. **Livestock Science**, v. 179, p. 12–21, set. 2015.

KOSICKI, M.; TOMBERG, K.; BRADLEY, A. Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. **Nature Biotechnology**, v. 36, n. 8, p. 765–771, set. 2018.

KRISHNA PRASAD, G.; KHANNA, S.; JAISHREE, S. Review of adjuvants to local anesthetics in peripheral nerve blocks: Current and future trends. **Saudi Journal of Anaesthesia**, v. 14, n. 1, p. 77, 2020.

LARRONDO, C. et al. Long-term hyperalgesia and traumatic neuroma formation in tail-docked lambs. **Animal Welfare**, v. 28, n. 4, p. 443–454, nov. 2019.

LAVAND'HOMME, P. Rebound pain after regional anesthesia in the ambulatory patient. **Current Opinion in Anaesthesiology**, v. 31, n. 6, p. 679–684, dez. 2018.

LECORPS, B. et al. Pain-Induced Pessimism and Anhedonia: Evidence From a Novel Probability-Based Judgment Bias Test. **Frontiers in Behavioral Neuroscience**, v. 13, p. 54, 20 mar. 2019.

LINDÉN, J. et al. Histopathological findings in a pilot study of dairy calves disbudded with hot cauterization or caustic paste. **Journal of Comparative Pathology**, v. 201, p. 118–122, fev. 2023.

LIVINGSTON, A. Pain and Analgesia in Domestic Animals. Em: CUNNINGHAM, F.; ELLIOTT, J.; LEES, P. (Eds.). **Comparative and Veterinary Pharmacology**. Handbook of Experimental Pharmacology. Berlin, Heidelberg: Springer Berlin Heidelberg, 2010. v. 199p. 159–189.

LOMAX, S. et al. Effect of topical vapocoolant spray on perioperative pain response of unweaned calves to ear tagging and ear notching. **Veterinary Anaesthesia and Analgesia**, v. 44, n. 1, p. 163–172, jan. 2017.

MAF - FI. 592. Government decree on the protection of cattle. . 2010.

MARTIN, M. S. et al. Comparison of lidocaine alone or in combination with a local nerve block of ethanol, bupivacaine liposome suspension, or oral meloxicam to extend analgesia after scoop dehorning in Holstein calves. **JDS Communications**, v. 3, n. 3, p. 189–194, maio 2022.

MCCONNACHIE, E. et al. Public attitudes towards genetically modified polled cattle. **PLOS ONE**, v. 14, n. 5, p. e0216542, 10 maio 2019.

MCCRACKEN, L. et al. Effect of age at castration on behavioural response to subsequent tail docking in lambs. **Veterinary Anaesthesia and Analgesia**, v. 37, n. 4, p. 375–381, jul. 2010.

MCKEOWN, S.; MIR, Z. M. Considerations for conducting systematic reviews: evaluating the performance of different methods for de-duplicating references. **Systematic Reviews**, v. 10, n. 1, p. 38, dez. 2021.

MELÉNDEZ, D. M. et al. Pharmacokinetics of oral and subcutaneous meloxicam: Effect on indicators of pain and inflammation after knife castration in weaned beef calves. **PLOS ONE**, v. 14, n. 5, p. e0217518, 24 maio 2019.

MINTLINE, E. M. et al. Play behavior as an indicator of animal welfare: Disbudding in dairy calves. **Applied Animal Behaviour Science**, v. 144, n. 1–2, p. 22–30, fev. 2013.

MIRRA, A. et al. Acute pain and peripheral sensitization following cautery disbudding in 1- and 4-week-old calves. **Physiology & Behavior**, v. 184, p. 248–260, fev. 2018.

MIT - TECHNOLOGY REVIEW. **Gene-edited cattle have a major screwup in their DNA**. , 29 ago. 2019. Disponível em: <https://www.technologyreview.com/2019/08/29/65364/recombinetics-gene-edited-hornless-cattle-major-dna-screwup/#Echobox=1567519481>. Acesso em: 11 jul. 2023

MONGILLO, P. et al. Effect of age and sex on plasma cortisol and dehydroepiandrosterone concentrations in the dog (Canis familiaris). **Research in Veterinary Science**, v. 96, n. 1, p. 33–38, fev. 2014.

MORISSE, J. P.; COTTE, J. P.; HUONNIC, D. Effect of dehorning on behaviour and plasma cortisol responses in young calves. **Applied Animal Behaviour Science**, v. 43, n. 4, p. 239–247, jul. 1995.

MUELLER, M. L. et al. Comparison of gene editing versus conventional breeding to introgress the POLLED allele into the US dairy cattle population. **Journal of Dairy Science**, v. 102, n. 5, p. 4215–4226, maio 2019.

NEAVE, H. W. et al. Pain and Pessimism: Dairy Calves Exhibit Negative Judgement Bias following Hot-Iron Disbudding. **PLoS ONE**, v. 8, n. 12, p. e80556, 4 dez. 2013.

NEWBY, N. et al. Comparing caustic paste vs caustic stick vs hot- iron+lidocaine disbudding in young dairy calves: behavioral response following disbudding and healing. **The Bovine Practitioner**, p. 196–201, 1 jun. 2016.

NORMAN, A. T.; JUDKINS, K. C. Pain in the patient with burns. **Continuing Education in Anaesthesia Critical Care & Pain**, v. 4, n. 2, p. 57–61, abr. 2004.

OUZZANI, M. et al. Rayyan—a web and mobile app for systematic reviews. **Systematic Reviews**, v. 5, n. 1, p. 210, dez. 2016.

PAGE, M. J. et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. **BMJ**, p. n71, 29 mar. 2021.

PALAO, R. et al. Chemical burns: Pathophysiology and treatment. **Burns**, v. 36, n. 3, p. 295–304, maio 2010.

PBS-ANIMAL HEALTH. **Dr. Larson's Dehorning Paste**. **PBS-Animal Health**, [s.d.]. Disponível em: <https://www.pbsanimalhealth.com/products/dr-larsons-dehorning-paste?gclid=Cj0KCQjw7PCjBhDwARIsANo7CgIo1nLhHEusz5uhBzTDEnGrtwrbwLjYJB6wnBo1RUD3yDVme68sJeoaAkfuEALw_wcB>. Acesso em: 4 jun. 2023

PETERS, M. D. P.; SILVEIRA, I. D. B.; FISCHER, V. Impact of subclinical and clinical mastitis on sensitivity to pain of dairy cows. **Animal**, v. 9, n. 12, p. 2024–2028, 2015.

PINHEIRO MACHADO F°, L. C.; HURNIK, J. F.; BURTON, J. H. The Effect of Amniotic Fluid Ingestion on the Nociception of Cows. **Physiology & Behavior**, v. 62, n. 6, p. 1339–1344, dez. 1997.

PRUNIER, A. et al. Identifying and monitoring pain in farm animals: a review. **Animal**, v. 7, n. 6, p. 998–1010, 2013.

RAJA, S. N. et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. **Pain**, v. 161, n. 9, p. 1976–1982, set. 2020.

RAJPUT, K.; REDDY, S.; SHANKAR, H. Painful Neuromas. **The Clinical Journal of Pain**, v. 28, n. 7, p. 639–645, set. 2012.

RANDHAWA, I. A. S. et al. Comparison of Genetic Merit for Weight and Meat Traits between the Polled and Horned Cattle in Multiple Beef Breeds. **Animals**, v. 11, n. 3, p. 870, 18 mar. 2021.

RAUNDAL, P. M. et al. Handheld mechanical nociceptive threshold testing in dairy cows – intra-individual variation, inter-observer agreement and variation over time. **Veterinary Anaesthesia and Analgesia**, v. 41, n. 6, p. 660–669, nov. 2014.

REEDMAN, C. N. et al. Randomized control trial assessing the efficacy of pain control strategies for caustic paste disbudding in dairy calves younger than 9 days of age. **Journal of Dairy Science**, v. 103, n. 8, p. 7339–7350, ago. 2020.

ROBBINS, J. et al. Stakeholder views on treating pain due to dehorning dairy calves. **Animal Welfare**, v. 24, n. 4, p. 399–406, 14 nov. 2015.

ROBLES, I. et al. Producer and Veterinarian Perspectives towards Pain Management Practices in the US Cattle Industry. **Animals**, v. 11, n. 1, p. 209, 16 jan. 2021.

ROUSSEAU, M. Dehorning. Em: **Comparative Veterinary Anatomy**. [s.l.] Elsevier, 2022. p. 1020–1029.

SALZMAN, M.; O'MALLEY, R. N. Updates on the Evaluation and Management of Caustic Exposures. **Emergency Medicine Clinics of North America**, v. 25, n. 2, p. 459–476, maio 2007.

SANDERCOCK, D. A. et al. Histopathological Characterization of Tail Injury and Traumatic Neuroma Development after Tail Docking in Piglets. **Journal of Comparative Pathology**, v. 155, n. 1, p. 40–49, jul. 2016.

SARACENI, J. et al. Disbudding and dehorning practices for preweaned dairy calves by farmers in Wisconsin, USA. **Journal of Dairy Science**, v. 104, n. 11, p. 11995–12008, nov. 2021a.

SARACENI, J. et al. Disbudding and dehorning practices for preweaned dairy calves by farmers in Wisconsin, USA. **Journal of Dairy Science**, v. 104, n. 11, p. 11995–12008, nov. 2021b.

SARACENI, J. et al. Ontario Dairy Producers' Perceived Barriers and Motivations to the Use of Pain Control for Disbudding and Dehorning Calves: A Qualitative Study. **Animals**, v. 12, n. 8, p. 973, 9 abr. 2022.

SCHAFBERG, R.; SWALVE, H. H. The history of breeding for polled cattle. **Livestock Science**, v. 179, p. 54–70, set. 2015.

SCHEIBER, I. B. R. et al. The importance of the altricial – precocial spectrum for social complexity in mammals and birds – a review. **Frontiers in Zoology**, v. 14, n. 1, p. 3, dez. 2017.

SCHNAIDER, M. A. et al. Vocalization and other behaviors indicating pain in beef calves during the ear tagging procedure. **Journal of Veterinary Behavior**, v. 47, p. 93–98, jan. 2022.

SCHWALLER, F.; FITZGERALD, M. The consequences of pain in early life: injury-induced plasticity in developing pain pathways. **European Journal of Neuroscience**, v. 39, n. 3, p. 344–352, fev. 2014.

SHI, R. et al. Current Attitudes of Chinese Dairy Practitioners to Pain and Its Management in Intensively Raised Dairy Cattle. **Animals**, v. 12, n. 22, p. 3140, 14 nov. 2022.

SHIVLEY, C. B. et al. Management of preweaned bull calves on dairy operations in the United States. **Journal of Dairy Science**, v. 102, n. 5, p. 4489–4497, maio 2019.

SPURLOCK, D. M.; STOCK, M. L.; COETZEE, J. F. The impact of 3 strategies for incorporating polled genetics into a dairy cattle breeding program on the overall herd genetic merit. **Journal of Dairy Science**, v. 97, n. 8, p. 5265–5274, ago. 2014.

STAFFORD, K. J.; MELLOR, D. J. Addressing the pain associated with disbudding and dehorning in cattle. **Applied Animal Behaviour Science**, v. 135, n. 3, p. 226–231, dez. 2011.

STANKOWICH, T.; CARO, T. Evolution of weaponry in female bovids. **Proceedings of the Royal Society B: Biological Sciences**, v. 276, n. 1677, p. 4329–4334, 22 dez. 2009.

STEAGALL, P. V. et al. Pain Management in Farm Animals: Focus on Cattle, Sheep and Pigs. **Animals**, v. 11, n. 6, p. 1483, 21 maio 2021.

STEINKOPF, L. An Evolutionary Perspective on Pain Communication. **Evolutionary Psychology**, v. 14, n. 2, p. 147470491665396, 1 jun. 2016.

STEWART, M. et al. Effects of local anesthetic and a nonsteroidal antiinflammatory drug on pain responses of dairy calves to hot-iron dehorning. **Journal of Dairy Science**, v. 92, n. 4, p. 1512–1519, abr. 2009.

STILWELL, G. et al. Effect of caustic paste disbudding, using local anaesthesia with and without analgesia, on behaviour and cortisol of calves. **Applied Animal Behaviour Science**, v. 116, n. 1, p. 35–44, jan. 2009.

STILWELL, G. et al. Effect of hot-iron disbudding on behaviour and plasma cortisol of calves sedated with xylazine. **Research in Veterinary Science**, v. 88, n. 1, p. 188–193, fev. 2010.

STILWELL, G. et al. Effects of hot-iron disbudding, using regional anaesthesia with and without carprofen, on cortisol and behaviour of calves. **Research in Veterinary Science**, v. 92, n. 2, p. 338–341, abr. 2012.

STILWELL, G.; LIMA, M.; BROOM, D. Comparing the effect of three different disbudding methods on behaviourand plasma cortisol of calves. **Revista Portuguesa de Ciências Veterinárias**, p. 281–288, out. 2007.

STILWELL, G.; LIMA, M. S.; BROOM, D. M. Comparing plasma cortisol and behaviour of calves dehorned with caustic paste after non-steroidal-anti-inflammatory analgesia. **Livestock Science**, v. 119, n. 1–3, p. 63–69, dez. 2008.

TADDIO, A. et al. Effect of neonatal circumcision on pain response during subsequent routine vaccination. **The Lancet**, v. 349, n. 9052, p. 599–603, mar. 1997.

THOMAS, M. et al. Collateral damage and CRISPR genome editing. **PLOS Genetics**, v. 15, n. 3, p. e1007994, 14 mar. 2019.

THOMPSON, N. M. et al. Economic considerations of breeding for polled dairy cows versus dehorning in the United States. **Journal of Dairy Science**, v. 100, n. 6, p. 4941–4952, jun. 2017.

TSCHONER, T. Methods for Pain Assessment in Calves and Their Use for the Evaluation of Pain during Different Procedures—A Review. **Animals**, v. 11, n. 5, p. 1235, 25 abr. 2021.

TURNER, S. P. et al. The Effect of Behavioural Indicators of Calf Discomfort Following Routine Procedures on Cow Maternal Care. **Animals**, v. 10, n. 1, p. 87, 6 jan. 2020.

USDA-NAHMS. **Dairy 2014 - Dairy Cattle Management Practices in the United States, 2014**. , 2014. Disponível em: <https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy14/Dairy14_dr_PartI_1.pdf>

USDA-NAHMS. **Dairy 2014: Dairy cattle management practices in the United States**. [s.l: s.n.].

USDA-NAHMS. **Beef Cow-calf Management Practices in the United States - 2017**. [s.l: s.n.]. Disponível em: <https://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef2017/Beef2017_dr_PartI.pdf>. Acesso em: 11 jul. 2023.

VAN REENEN, C. G. et al. Responses of calves to acute stress: Individual consistency and relations between behavioral and physiological measures. **Physiology & Behavior**, v. 85, n. 5, p. 557–570, ago. 2005.

VASSEUR, E. et al. A survey of dairy calf management practices in Canada that affect animal welfare. **Journal of Dairy Science**, v. 93, n. 3, p. 1307–1316, mar. 2010.

VEDERHUS, B. J. et al. Pain Tolerance and Pain Perception in Adolescents Born Extremely Preterm. **The Journal of Pain**, v. 13, n. 10, p. 978–987, out. 2012.

VICKERS, K. J. et al. Calf Response to Caustic Paste and Hot-Iron Dehorning Using Sedation With and Without Local Anesthetic. **Journal of Dairy Science**, v. 88, n. 4, p. 1454–1459, abr. 2005.

VIÑUELA-FERNÁNDEZ, I. et al. Pain mechanisms and their implication for the management of pain in farm and companion animals. **The Veterinary Journal**, v. 174, n. 2, p. 227–239, set. 2007.

WAGNER, B. K. et al. Non-Steroidal Anti-Inflammatory Drugs: Pharmacokinetics and Mitigation of Procedural-Pain in Cattle. **Animals**, v. 11, n. 2, p. 282, 22 jan. 2021.

WALKER, S. M. Neonatal pain. **Pediatric Anesthesia**, v. 24, n. 1, p. 39–48, jan. 2014.

WALKER, S. M.; TOCHIKI, K. K.; FITZGERALD, M. Hindpaw incision in early life increases the hyperalgesic response to repeat surgical injury: Critical period and dependence on initial afferent activity. **Pain**, v. 147, n. 1, p. 99–106, dez. 2009.

WEYL-FEINSTEIN, S. et al. Assessing the Effect of Pain Relieving Methods During Caustic Disbudding Procedure on Dairy Calf Welfare. **Israel Journal of Veterinary Medicine**, v. 76, n. 3, p. 96–107, set. 2021.

WIENER, D. J. et al. Novel Features of the Prenatal Horn Bud Development in Cattle (Bos taurus). **PLOS ONE**, v. 10, n. 5, p. e0127691, 20 maio 2015.

WILLIAMS, H. J. et al. Repeatability and Feasibility of Pressure Algometry for Quantifying Mechanical Nociceptive Threshold in the Thoracic Region of Calves. **Frontiers in Veterinary Science**, v. 7, p. 442, 31 jul. 2020.

WILLIAMS, M. D.; LASCELLES, B. D. X. Early Neonatal Pain—A Review of Clinical and Experimental Implications on Painful Conditions Later in Life. **Frontiers in Pediatrics**, v. 8, p. 30, 7 fev. 2020.

WINDER, C. B. et al. Practices for the disbudding and dehorning of dairy calves by veterinarians and dairy producers in Ontario, Canada. **Journal of Dairy Science**, v. 99, n. 12, p. 10161–10173, dez. 2016.

WINDER, C. B. et al. Clinical trial of local anesthetic protocols for acute pain associated with caustic paste disbudding in dairy calves. **Journal of Dairy Science**, v. 100, n. 8, p. 6429–6441, ago. 2017.

WINDER, C. B. et al. Effects of local anesthetic or systemic analgesia on pain associated with cautery disbudding in calves: A systematic review and meta-analysis. **Journal of Dairy Science**, v. 101, n. 6, p. 5411–5427, jun. 2018a.

WINDER, C. B. et al. Canadian National Dairy Study: Heifer calf management. **Journal of Dairy Science**, v. 101, n. 11, p. 10565–10579, nov. 2018b.

WOLLGARTEN-HADAMEK, I. et al. Do burn injuries during infancy affect pain and sensory sensitivity in later childhood? **Pain**, v. 141, n. 1, p. 165–172, jan. 2009.

YAKAN, S.; DUZGUNER, V.; AKSOY, O. Effects of Flunixin Meglumine on Oxidant and Antioxidant System after Disbudding with Caustic Paste in Calves. **Acta Scientiae Veterinariae**, v. 46, n. 1, p. 9, 27 out. 2018.

YANG, J.; RAHARDJA, S.; FRÄNTI, P. **Outlier detection: how to threshold outlier scores?** Proceedings of the International Conference on Artificial Intelligence, Information Processing and Cloud Computing - AIIPCC '19. **Anais**... Em: THE INTERNATIONAL CONFERENCE. Sanya, China: ACM Press, 2019. Disponível em: <http://dl.acm.org/citation.cfm?doid=3371425.3371427>. Acesso em: 4 dez. 2022

YUNES, M. C. et al. Is gene editing an acceptable alternative to castration in pigs? **PLOS ONE**, v. 14, n. 6, p. e0218176, 24 jun. 2019.

YUNES, M. C. et al. Gene Editing for Improved Animal Welfare and Production Traits in Cattle: Will This Technology Be Embraced or Rejected by the Public? **Sustainability**, v. 13, n. 9, p. 4966, 28 abr. 2021.