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Impact of analgesia on health status and productivity of calves during hot-iron branding in Mato Grosso, Brazil

Betydelsen av smärtlindring för hälsa och tillväxt hos kalvar i samband med brännmärkning i Mato Grosso, Brasilien

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SUMMARY

There is not much written in literature regarding hot-iron branding of cattle, despite it being a common husbandry practice in many parts of the world. Hot-iron branding is mostly done without analgesia. An experiment trying to investigate the effects of hot-iron branding on the health and weight gain of heifer calves was made on a commercial farm in Mato Grosso, Brazil. Thirty-two four-month-old heifers were branded on the cheek with a hot iron. The heifers were divided into four groups; the first group was given an anti-inflammatory drug (meloxicam), the second group was given local anaesthesia (lidocain and bupivacain), the third group was given both the anti-inflammatory drug and local anaesthetics and the fourth group was a control group branded without treatment. Health parameters and the weight of the heifers were recorded before the branding and five days after the branding. The results of the study show that there was a treatment effect of local anaesthesia on the incidence of inflammation at the site of the branding (p=0.033). The branding did not affect the heifers' general health according to the parameters that were examined. Five days after the branding calves given NSAID had a higher weight gain (p=0.034). The group given both treatments (NSAID and local anaesthetics) had a higher weight gain five days after the branding. Further experiments with a larger group of cattle and during a longer period of time would make the results more reliable. If possible, it would be beneficial to use one additional control group that was to be sham branded, as this might generate further important information. Observing this additional control group would give insights regarding the difference between the stress caused by the pain from the branding and the stress caused by just being handled. In conclusion, the results from the study indicate an effect of treatment with local anaesthetics on the occurrence of inflammation at the site of the branding. The results also indicate that the use of NSAID has a positive effect on weight development during the five days after branding. Furthermore, the study illustrates the difficulties conducting an experimental study at a commercial farm and the many factors affecting the welfare of production animals when performing husbandry practices, not just the pain from the specific procedure.

SAMMANFATTNING

Det finns inte många studier som rör brännmärkning av nötkreatur, trots att detta pågår i stor utsträckning i stora delar av världen. Detta ingrepp utförs nästan uteslutande utan någon smärtlindring. Ett försök för att undersöka brännmärkningens effekter på hälsa och tillväxt gjordes på en kommersiell gård i Mato Grosso, Brasilien. Trettiotvå stycken fyra månader gamla kvigor brännmärktes i ansiktet. Kvigorna delades in i fyra grupper; den första gruppen fick anti-inflammatoriskt läkemedel (meloxicam), den andra fick lokal anestesi (lidokain samt bupivacaine), den tredje fick både anti-inflammatoriskt läkemedel samt lokal anestesi och den fjärde gruppen var en kontrollgrupp som inte fick någon behandling innan brännmärkningen. Hälsoparametrar samt kvigornas vikt registrerades innan brännmärkningen samt fem dagar efter brännmärkningen. Resultatet av studien visar att det finns en korrelation mellan behandlingen med lokal anestesi och inflammation vid bränningstället i ansiktet (p=0,0325). Brännmärkningen påverkade i övrigt inte kvigornas hälsa enligt de faktorer som undersöktes. Resultaten rörande kalvarnas viktutveckling fem dagar efter brännmärkningen tyder på att det anti-inflammatoriska läkemedlet gör att kalvarna ökar mer i vikt efter brännmärkningen (p=0,0337). Gruppen som fick både anti-inflammatoriskt läkemedel samt lokalanestesi ökade också mer i vikt fem dagar efter brännmärkningen (p=0,0092), i jämförelse med de andra grupperna. Kompletterande försök med en större försöksgrupp och under en längre tid skulle göra resultaten mer tillförlitliga. Om möjlighet fanns för en ytterligare kontrollgrupp som inte blev brännmärkta utan bara fick genomgå hanteringen så skulle det kunna ge viktig information. Observationer av denna grupp av djur skulle kunna belysa skillnaden mellan stress orsakad av smärta från brännmärkningen och den stress som kalvarna upplever bara genom att bli hanterade. Sammanfattningsvis tyder studiens resultat på en effekt av behandling med lokal anestesi och uppkomst av inflammation vid bränningstället vid brännmärkning av kalvar. Resultatet tyder även på en positiv effekt av anti-inflammatoriskt läkemedel för viktutvecklingen dagarna efter brännmärkningen. Vidare belyser studien svårigheterna när en experimentell studie genomförs på en kommersiell gård, samt att många faktorer inverkar på produktionsdjurs lidanden vid rutiningrepp, inte bara smärtan från själva ingreppet.

CONTENT

INTRODUCTION

Brazil is a country with a large and diverse agriculture sector, of which cattle ranching is the industry that occupies the largest land area. Brazil has the second largest number of cattle in the world (Schneider *et al.*, 2014). Private cattle ranching is one of the largest industries in Pantanal (Boulhosa and Azevedo, 2014), a vast wetland area located in the central-western parts of Brazil. In comparison with other large beef producing countries, Brazilian beef production has low costs, estimated to be 60% lower than in Australia and 50% lower than in the United States (Sterman Ferraza and de Felício, 2010).

As determined by Brazilian law (MAPA, 2016) all commercial cattle must be vaccinated against brucellosis and in association with this, marked by a hot-iron brand, representing the final digit of the year of vaccination. At many farms, also additional hot-iron branding placed elsewhere on the animal's body is carried out, for identification purposes, although not required by law.

The cattle breed Nelore (*Bos taurus ssp. indicus*), originating from India, is currently widely used across South America. The breed is well adapted to the climate and conditions of ranching in Pantanal, due to its ability to endure heat and poor-quality forage (Oklahoma State University Board of Regents, 2017). Nelore is the beef breed with the largest number of animals in Brazil currently (Sterman Ferraza and de Felício, 2010).

The impact of painful husbandry practices, such as branding and dehorning, on the welfare of farm animals and/or the production results has been debated and studied for a long time (McMeekan *et al.,* 1998; Schwartzkopf-Genswein *et al.*, 1997b; Webster *et al*., 2013). It is therefore important to examine the ways in which these procedures affect the animals and find ways to reduce pain during these procedures. Increased knowledge concerning these procedures might influence the way these procedures are performed, leading to improved animal welfare. Both at a small scale; individual farmers might change the way they treat their animals and at a larger scale; governments and policy makers might reconsider legislation, standards and guidelines and change these to further promote animal welfare.

The aim of this study was to examine the effects in relation to health status and growth rate that hot-iron branding can have on four-month-old Nelore heifers. A group of 32 heifers was divided into four different treatment groups: one group of heifers being traditionally branded (without any analgesia), the second group received a local anesthetic, the third group received an anti-inflammatory drug and the fourth group receive both a local anesthetic and an anti-inflammatory drug.

The hypothesis of this study was that the use of a local anesthetic, a non-steroidal anti-inflammatory drug (NSAID) or the combination of the two would improve the health status and weight gain of the calves. The drugs will decrease pain and decrease the expected stress effects, thereby hopefully minimizing the decrease in feed intake that painful procedures might cause. Lower stress levels might also decrease the risk of general infection, apart from the anti-inflammatory drug that has a direct effect on the risk of local inflammation/secondary infection at the branding site in the calves.

LITERATURE REVIEW

Scientific literature concerning hot-iron branding is scarce. A large number of studies have however been conducted regarding other painful procedures performed on calves and cattle, such as dehorning and different methods of castration (Earley and Crowe 2002; Faulkner and Weary, 2000; McMeekan *et al.,* 1998 and Petherick *et al.,* 2014). The results from these studies cannot be interpreted as equivalent of the results in this study or any study regarding hot-iron branding. However, there are a number of similarities and some comparisons can be made, keeping in mind, however, that these different procedures do not necessarily induce identical physiological reactions. The majority of studies regarding painful procedures have been carried out on *Bos taurus* and not on *Bos indicus*, which is the subspecies examined in this study.

What is pain?

According to the International Association for the Study of Pain, pain is described as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Merskey and Bogduk, 1994). Underwood described (2002) several ways of detecting pain in cattle; vocalizing (grunt, bellow), teeth grinding, reluctance to move, changing of facial expression, and decrease in production.

Molony and Kent (1997) stated that: ”animal pain is an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal’s physiology and behavior to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery; unnecessary pain occurs when the intensity or duration of the experience is inappropriate for the damage sustained or when the physiological and behavioral responses to it are unsuccessful at alleviating it.”

How is pain from burns perceived?

There is not much written on how cattle perceive pain from hot iron branding or burn injuries. There is, however, extensive research of human burn victims and their perception of pain. Burn injury is associated with severe pain and is described as one of the most excruciating pain sensations (Yuxiang *et al.,* 2012).

Burn injuries are described as having three main phases. First initial tissue damage causing cell death which is followed by inflammation of the tissue leading to a local oedema and the invasion of inflammatory cells. The last phase consists of re-epithelialisation of the damaged wound, which involves nerve healing, re-growth and sprouting (Laycock *et al.,* 2013).

Pain caused by burn injury is described as complex. Latarjet and Choinère (1995) define three types of pain associated with burn trauma. Nociception which is the immediate pain caused by the nociceptors located in the epidermis and the dermis are damaged. The inflammatory reaction that quickly ensues cause a hyperalgesia by sensitizing the nociceptors, causing a wind-up of pain. The third pain described is chronic neuropathic-like pain affecting healed burns. The etiology behind this is not fully known but it is thought to be linked to abnormalities in newly regenerated nerve endings, re-innervations of scars or more central mechanisms.

Pain management in farm animals

Walker *et al*. (2002) describe how the attitudes regarding pain management in agricultural animals have changed over the last two to three decades. At times the discussion within the veterinary community have been circling around apprehensions related to possible adverse effects related to the drugs used, but the general opinion seems to lean more in the direction of using pain management strategies amongst veterinarians working with farm animals (Walker *et al*., 2002)

It is, however, still common for veterinarians in many countries to perform procedures that are known to be painful without analgesia. In a study by Huxley and Whay (2006) questionnaires were sent to veterinarians in the UK and only a small proportion of the veterinarians used anti-inflammatory drugs as post-op analgesia during castration (4-6%) and disbudding (1-7%) of cattle. Though in some countries, Sweden for example, pain management for such procedures is mandatory (SFS 2012:701).

Assessing pain in humans and animals

Our understanding of pain in animals is often affected by research and empirical knowledge of human pain. Flecknell (2008) discussed how similar we can assume the human-animal experience of pain to be. It is safe to assume that all vertebrates have similar sensory mechanisms to recognize and respond to noxious stimuli. A procedure, such as branding for example, would therefore evoke a similar experience in animals as in humans. In contrast to this, it is likely that other instincts within the animal may cause the animal to mask the pain partly or completely, thus not showing the expected pain-related behavior. This is a great risk when assessing pain in animals. Consequently, it is important to develop robust pain scoring systems adapted to the species studied (Flecknell, 2008), especially for prey species who are unlikely to display pain or weakness (Underwood, 2002).

When assessing pain in animals, researchers have sometimes found it useful to use a pre-surgery baseline measuring for example heart rate and respiratory rate. It can, however, be difficult to differentiate what changes in behavior that are originating from the pain experienced by the animal from behaviors which are due to the fact that the animal is being restrained during the procedure or fatigue after surgery (Walker *et al.,* 2002).

Hot-iron branding

There has been a long tradition of hot-iron branding calves in all of South America, mostly on the upper hind limb. Recently, cheek branding has become more popular due to its visibility and it being easier to restrain the calves in order to brand them (Grobler, 2012). In a study conducted by Grobler (2012) calves were divided into four groups; calves that were branded on the cheek, hind leg or sham branded (control group 'branded' with a cold iron), on the cheek or hind leg. After branding, cortisol levels were measured in blood and feces. The study did not show any significant results when comparing the different groups. The calves that were branded on the cheek vocalized more, but this was the only significant result that was found in the study, no significant differences were found in cortisol levels (Grobler, 2012).

In a study conducted by Schwartzkopf-Genswein *et al*. (1997a), the effects of hot-iron branding and freeze branding were studied. Average body weight gain, antibiotic treatment and subsequent handling ease were measured every other day for 10 days after the branding. The results did not show any significant result comparing the two types of branding to the control group (sham branded) regarding antibiotic treatments or average body weight gain. The group of calves that were freeze branded did however need more time handling pressure than the control group or the control group on day 6 after branding. This, according to Schwartzkopf-Genswein *et al*. (1997a), might indicate that freeze branding may be associated with a more lingering pain than hot-iron branding.

Ley *et al*. (1992) conducted a study where the difference between hot-iron branding and freeze branding was examined. Twenty-seven crossbred calves (1/2 Simmental, 1/4 Hereford, 1/4 Brahman) were either freeze branded, hot-iron branded, or sham branded. The results showed that all calves had elevated cortisol levels from handling. All calves also had elevated heart rates, and it is discussed in the article that the stress of handling might mask signs of pain. Plasma epinephrine was higher for the hot-iron branded group than the other groups at 0.5 minutes into the procedure, as were levels of noradrenaline. During the work with the study it was also observed that the hot-iron branded calves seemed to show a quicker behavioral response to the branding, immediately lurching away from the iron and occasionally falling to their knees. The freeze-branded calves showed a similar reaction but approximately 8 seconds later. The group of sham branded calves did not show any behavioral reaction in particular (Ley *et al*., 1992).

Similar results in cortisol levels as the ones described by Ley *et al.* (1992) were found by Schwartzkopf-Genswein *et al*. (1997b) in a study comparing hot-iron branding to freeze branding. Thirty yearling heifers (450–500 kg) of mixed breed (Hereford, Charolaise, Angus and Shorthorn) where either hot-iron branded, freeze branded or sham branded. The results showed that both freeze branded and hot-iron branded heifers had higher levels of plasma cortisol 20 and 40 minutes after branding. The authors concluded, in agreement with the study conducted by Ley *et al.* (1992), that hot-iron branding causes a more pronounced cortisol response than freeze branding at 40 min (Schwartzkopf-Genswein *et al.*, 1997b).

Pain management efforts at branding

In a study by Tucker (2014b), the effects of a single injection of flunixin (an NSAID) given before hot-iron branding a group of Angus–Hereford weaned steers. The treatment with flunixin showed no difference regarding sensitivity of the area or healing of the wound but improved weight gain during the days after branding in all groups treated with flunixin.

Other methods except anti-inflammatory drugs and/or local anesthetics while performing hot-iron branding have also been discussed and investigated in various studies. In a study by Tucker *et al*. (2014), the effects of a cooling gel (active ingredient: tea tree oil) was tested on 48 Angus–Hereford weaned steers. The steers were divided into three groups; cooling gel was applied on the branding site once directly after the branding, gel applied twice directly after the branding and one day later and one group branded without cooling gel. The results showed that the cooling gel did lower the temperature at the branding site, especially directly after the branding. The group with gel applied twice had slower wound healing than the other groups. Only 43% of all the calves (regardless of group) had completely healed wounds 70 days after the branding. The sensitivity of the branding site was measured with an anesthesiometer and subsequently observing the animals' behavioral response. No difference could be seen between the groups regarding sensitivity. It should be noted that the area seemed to be painful throughout the 70-day study period. Weight gain was significantly lower than average weight gain for all groups one day after branding, there was however no significant difference in weight gain when comparing the different groups. The authors concluded that the cooling gel seemed to have only little effect on branding wounds, but that the effects of hot-iron branding seemed to be lasting for a longer period of time than generally perceived before (Tucker *et al*., 2014a).

Pain management in relation to other painful procedures in cattle

Several studies have been performed recently investigating various non-steroidal anti-inflammatory drugs (NSAIDs) and their effects on cattle during painful procedures. In a study by Petherick *et al.* (2014) a total of 32 *Bos indicus* bulls, 7-10 months old, were castrated and physiological and productivity related measurements were performed. The main aim of the study was to examine the difference between surgical- and tension band castration but the effects of a NSAID (ketoprofen) was also examined. The results of the study showed that the most prominent effect of ketoprofen was that the cortisol concentrations of the bulls surgically castrated were significantly reduced both 40 minutes and also 2 hours after the surgery. There was no significant result regarding ketoprofens' effect on the band castrated bulls (Petherick *et al*., 2014).

Webster *et al.* (2013) examined the effects of a local anesthetic and the NSAID flunixin; 30 two to three-month-old calves were castrated surgically. The group of calves were divided in five groups; sham castration, castration without any drugs, castration with lidocaine and castration with flunixin and castration with lidocaine and flunixin. Plasma cortisol levels, frequency of behavioral changes, body weight gain and feed intake were measured. Mean cortisol levels were significantly lower for the sham castrated group and the group given lidocaine and flunixin. The group treated with only flunixin had elevated cortisol levels but during a shorter period of time than the group castrated without any drugs. The two groups being given flunixin showed less crouching than the groups being given only lidocaine or no treatment. This study did not show any significant result regarding feed intake and weight gain. In conclusion, the outcome of the study indicated that a combination of flunixin and lidocaine reduce cortisol levels and behavioral changes. Treatment with flunixin alone also had a significant effect on cortisol and behavior (Webster *et al*., 2013).

In a study conducted by Earley and Crowe (2002), 40 Frisian bull calves were surgically castrated. The calves were divided into five groups given ketoprofen, lidocaine, ketoprofen and lidocaine, castration without any drugs and one control group (exposed to sham handling). After the castration the cortisol plasma levels, acute phase proteins, average feed intake and daily weight gain were measured for 72 hours. The results of the study showed that both ketoprofen and lidocaine reduced the cortisol peak after castration. Ketoprofen also suppressed the total cortisol response (area under cortisol curve) following castration, whereas lidocaine did not. Earley and Crowe conclude that the application of a local anesthetic only is not sufficient as pain relief and that hence e.g. ketoprofen or another analgesic drug should be added.

Similar results were also found in a study by McMeekan *et al.* (1998), where 100 Frisian calves were dehorned (scoop-dehorning) and treated with ketoprofen, local anesthetics or both. Plasma cortisol levels were measured after disbudding. The results showed that giving only a local analgesic reduced the initial peak of cortisol but that the levels increased again after the effect of the analgesic has abated (after approximately 2 hours in this case). Using only ketoprofen did not show a significant effect on the initial cortisol peak but kept the cortisol levels at pre-treatment levels after the first 1,3 hours after dehorning. McMeekan *et al.* (1998) therefore suggest that both a local anesthetic and an anti-inflammatory drug is to be used when performing dehorning, to reduce stress and pain effectively (McMeekan *et al.,* 1998).

The effects of ketoprofen were also tested in a study by Faulkner and Weary (2000), where it was found that the calves treated with ketoprofen prior to hot-iron dehorning showed significant less head shaking and ear flicking after the dehorning. The group of ketoprofen treated calves also tended to gain more weight during the 24 hours after the procedure compared to the control calves. In this study all of the animals were given a local anesthetic and the sedative xylazine to make the animals lie down prior to the procedure. The combination of xylazine and a local anesthetic resulted in the calves not having to be restrained in any way and they did not react at all to the application of the hot-iron (Faulkner and Weary, 2000).

In a study by Coetzee *et al*. (2015), the effect of meloxicam was examined when surgical castration was performed on British-Continental bulls and steers. Similar to other studies, no difference in feed intake or average daily weight gain could be found. Meloxicam did however reduce the risk of respiratory disease in calves, post-surgical castration (Coetzee *et al*., 2015).

Pharmaceutical applications

Local anesthetics

Local anesthetics work by blocking the initiation and propagation of action potentials by preventing the voltage-dependent increase in Na+ conductance in nerve fibers. The molecules physically plug the transmembrane pores by interacting with transmembrane amino acids. At lower concentrations, the drug lowers the rate of rise of the action potential. At higher concentrations, the action potential firing in the nerve fibers is prevented by local anesthetics. Local anesthetics are in general more efficient at blocking nerve fibers with a smaller diameter than nerves with a larger diameter. Consequently: nociceptive nerves which mitigate pain, (Aδ and C fibers) are blocked to a fuller extent than other sensory processes such as touch and proprioception. (Rang and Dale, 2012)

Main adverse effects of local anesthetics are associated with the drug reaching the vascular system. The adverse effects that are mostly discussed in literature are central nervous system effects such as agitation, confusion, tremor, convulsion and respiratory depression. Cardiovascular effects also occur, mainly myocardial depression and vasodilatation with can lead to fall in blood pressure. Occasional hypersensitivity reactions also occur. (Rang and Dale, 2012)

It has been commonly stated among practitioners that local anesthetics delay the healing of wounds. This has also been described in some literature (Marongiu, 2012) as an adverse effect of local anesthetics, together with irritation, distortion of the wound and swelling.

There are however few studies investigating the effects local anesthetics infiltrated in the skin has on the inflammatory response at the site of infiltration. This is due to the resolution characteristics of many commonly used anesthetics not being evaluates at the time of their classical development (Chiang *et al*., 2008).

Some studies have been conducted to investigate whether local anesthetics delay the healing process. To be noted about these studies on the effect on healing by local anesthetics is that they are all made studying surgical incisions and not the type of wounds caused by burns, which is the case in our experiment.

Carvalho *et al*. (2010) conducted a study where results showed that the use of bupivacaine in surgical wounds changed the composition of wound mediators. The levels of interleukin 10 were increased and the levels of substance P were lowered. This could result in a proinflammatory environment in tissue treated with bupivacaine (Carvalho *et al*., 2010).

In a study conducted by Drucker *et al*. (1998), it was investigated if lidocaine had any effects on wound healing. The in vivo study on Guinea pigs *(Cavia porcellus)* showed that the use of lidocaine in surgical wounds produced significant histopathologic changes of the tissues. The animals treated with lidocaine had less vascularisation and less collagen fibers than the control group. However, no differences in number of inflammatory cells could be seen (Drucker *et al*., 1998).

Other studies have shown that local anesthetics have anti-inflammatory properties; an in vitro study conducted by Sculley and Dunley (1980) showed that 2% lidocaine inhibited 28 different species of bacteria, five of these were gram-positive and 23 of the species were gram-negative (Sculley and Dunley, 1980).

Local anesthetics also cause pain when administered (skin infiltration). The cause of this is believed to be that local anesthetics have an acidic pH (5.0-7.0). The acidity is a side effect from giving the local anesthetics a longer shelf life and facilitate solubility (Burns *et al.*, 2006).

Non-Steroidal Anti-inflammatory drugs (NSAIDs)

The non-steroidal anti-inflammatory drug (NSAID) used in this experiment is meloxicam which is widely used on cattle all over the world. Anti-inflammatory drugs work by inhibiting the COX enzyme with leads to an inhibition of prostaglandins and tromboxanes. There are several types of COX enzymes. COX-1 enzymes regulate a number of physiological processes in the body, for example tissue homeostatic and inducing prostaglandin production involved in for example gastric cytoprotection, platelet aggregation and renal blood regulation. COX-2, on the other hand, is mainly involved in the prostaglandin mediators induced during inflammation (Bacchi *et al., 2012).* The main effects that are sought after when treating animals with anti-inflammatory drugs are:

-Anti-inflammatory. The reduction of prostaglandin E and prostacyclin reduced vasodilatation and edema.

-Analgesic effect. Less sensitization of nociceptive nerve endings to inflammatory mediators.

-Antipyretic effect. Prostaglandins are released in the central nervous system which elevates the hypothalamic set point for fever. This is prevented by the NSAID lowering the levels of COX-2 and prostaglandins.

Adverse effects associated with NSAIDs include: gastrointestinal effects such as nausea and acute and chronic gastric ulcers, reversible renal insufficiency and liver disorders. Most adverse effects are associated with long term use of the drug. (Rang and Dale, 2012)

AIM OF THE STUDY

The aim of this study was to examine the effects on health and productivity of NSAID (meloxicam) and local anesthetic (bupivacaine and lidocaine) when hot-iron branding calves. The parameters examined were general health, weight gain and inflammation at the site of branding.

MATERIAL AND METHODS

The farm, the animals and the procedures

Data collection was performed at a commercial farm located in Araguaíana-MT, Brazil. The main activity of the farm is breeding of pure and crossbred Nelore cattle (*Bos indicus*), with approximately 1300 breeding cows. All of the heifers on the farm are vaccinated against brucellosis, as determined by Normativ Instruction no. 19 of October 10, 2016 (MAPA, 2016). In association with heifers being vaccinated they are also mandatorily receiving a mark by a hot iron on the face, representing the final digit of the year of vaccination. In this study 32 four-month-old heifers (age min 103 d, max 163 d, mean age 145 d, standard deviation 19.8 d) were vaccinated and hence branded. All of the calves where their mothers first calves, i.e. the offspring of primiparous heifers.

On the day before the branding the methods were tested. This was done with the same groups of heifers that were to participate in the actual experiment. The heifers were divided in groups of eight or nine and a test drive was carried out. The heifers were kept in a small pen and led to the crush one by one. The heifers were kept restrained in the crush for two minutes before being released. The purpose of keeping the animals in the crush for two minutes was to film the faces´ of each animal, this material was later used for behavioral analysis. This data, however, is not included in this thesis.

Two heifers were excluded from the experiment, as they were considered outliers in relation to body size. The first one because it was a very large heifer and it was suspected that the animal was much older than the other heifers. The second heifer that was excluded was very small compared to the other heifers of the same age.

Treatment groups

The heifers were divided into four different groups with stratified randomization, using four different parameters: weight, daily growth, age and temperament. The temperament was assessed by measuring the time it took for the heifers to walk/trot across half of the pen (an area with a length of approximately 20 meters) after the heifers were subject to the restrain treatment in the crush. The time was measured with a stop watch and after the heifers had been restrained in the crush for one minute. The speed of flight was also used to assess the temperament and measured directly after the calves were released from the crush. The speed was measured using an electronic device composed of a pair of photoelectric cells and a processor programmed to register the time taken by each heifer to cover the distance of two meters. These two measurements were conducted when the calves’ baseline measures were taken, before any anesthesia or branding.

All of the heifers underwent a short assessment of health status before the marking procedure, as described below. The heifers were weighed once (with a Tru-Test SR3000 scale) prior to the branding, during the baseline measurement.

The weighing and health assessments were redone in the same manner five days after the branding.

On the day of the branding, the heifers were divided into groups of eight animals per group. The heifers were kept in a small pen before being led to the crush one by one where they were given the vaccine and anesthesia. The animals were kept in the crush for one minute for the purpose of filming the face of each calf. The calves were subsequently released and led back to a pen. When all of the heifers had been given the vaccine and anesthesia (with exception of the control group), they were again collected in the small pen and led one by one to the crush where the branding was done. It was ensured that there was a minimum of ten minutes after the administration of anesthesia and the branding, to allow for the local anesthetic to have an effect before the branding was conducted. After the branding the animals were kept in the crush for one minute for the purpose of filming the face of each heifer.

The first group (control group = C) were marked in a traditional way, without any procedure to relieve pain. The second group of heifers received a subcutaneous block in the area of the branding (local anesthetic = LA). The local anesthetic consisted of 5 milliliters of 2% lidocain (75%) + 0.5% bupivacaine (25%) and the subcutaneous injection was administered approximately 10-20 minutes prior to the branding. The local anesthetic was injected in an area on the left cheek between *N. facialis* and *N. auriculotemporalis*. The area was massaged after the injection to facilitate the infiltration of the anesthetics. The third group (NSAID) received a dose of the non-steroidal anti-inflammatory drug meloxicam 0.5 mg /kg intramuscular in the left gluteus muscle. The fourth group received both local anesthetics subcutaneous and meloxicam 0.5 mg /kg intramuscular in the left gluteus muscle (LA + NSAID). The calves that were not injected with local anesthetics were touched on the left cheek.

The heifers were branded on the left cheek and the hot iron was placed on the skin for approximately 1-2 seconds. The branding site was wiped with a cloth prior to branding and the area was not clipped.

The assessments

The assessment of the health of each heifer was restricted to maximum 1 minute per heifer and was conducted by the same person. The assessment was made without touching or interacting with the heifers. The evaluation was made standing approximately 1-2 meters above and in front of the animals, looking down on a group of 8-9 heifers in a small pen. The assessment was made using part of WQ protocols for dairy and beef cattle (Welfare Quality® Assessment protocol for cattle, 2009); the part related to health (1.3.1 Absence of disease and 1.3.2 Absence of disease).

The part of the protocol used for this study consists of 8 different parameters. The protocol is to be used on a herd basis, for this study the assessment was however made on an individual basis of each heifer. The parameters observed were:

-Lameness. Mainly observed when the animal was led to the containment trunk and when standing in the containment trunk. The animal was considered lame if it is showing one or several of the following indicators: When the animal was walking: irregular foot fall, reluctance to bear weight on a foot, uneven temporal rhythm between hoof beats or weight not borne for equal time on each of the four feet. Indicators used in standing animals: resting a foot (bearing less/no weight on one foot), frequent weight shifting between feet ("stepping"), repeated movements of the same foot or standing on the edge of a step

-Coughing. Described by the WQ protocol as a sudden and noisy expulsion of air from the lungs. Number of coughs per animal was counted.

-Nasal discharge. Described by the WQ protocol as clearly visible flow/discharge from the nostrils; it can be transparent to yellow/green and is often of thick consistency.

-Ocular discharge. Described by the WQ protocol as clearly visible flow/discharge (wet or dry) from the eye, at least 3 cm long.

-Hampered respiration. Described by the WQ protocol as deep and overtly difficult or laboured breathing. Expiration was visibly supported by the muscles of the trunk, often accompanied by a pronounced sound. Breathing rate may only have been slightly increased.

-Diarrhoea. Described by the WQ protocol as loose watery manure below the tail head on both sides of the tail, with the area affected at least the size of a hand.

-Bloated rumen. Described by the WQ protocol as a characteristic "bulge" between the hip bone and the ribs on the left side of the animal.

Apart from these parameters taken from the WQ protocol the site of the branding was also checked for signs of infection such as discharge, redness or abscesses. The left side of the heifers' faces were filmed for one minute each, five days after branding, with the heifers standing in the crush. The videos were watched and analyzed to see if any signs of infection could be seen. If signs of inflammatory reaction were observed at the site of the branding the heifers were treated with an antibacterial spray to shield the area from further complications.

Statistics

The variables related to weight, performance and health were to be analyzed in order to evaluate the effects of treatments (iron branding without and with the three possibilities of pain relief). The statistical test used was dependent on the normal distribution of the data.

The statistical analyses were made using Minitab Express (Minitab Express 1.3.0, 2014). To see if there was any difference between the different treatment groups regarding inflammation at the site of branding, a Chi-square test was applied. To see if there was any difference regarding weight gain between the groups, an ANOVA test was used.

RESULTS

Health parameters

The baseline ocular examination of the calves that was carried out the day before the branding showed that the groups of heifers were generally healthy. Three heifers showed signs of diarrhea (according to the WQ protocol). The health assessment was repeated five days after the branding. During this assessment only one of the heifers showed signs of diarrhea, and the group of heifers showed no other signs of illness. Due to lack of clinical findings the health assessment was not analyzed statistically.

Inflammation at the site of branding

A chi-square test was carried out on the four different treatment groups to see if there were any correlation of any of the treatment groups on the risk of developing an inflammatory reaction at the site of branding. A statistically significant correlation was found between the group of heifers given local anesthetics (16 heifers) and the presence of inflammation (P = 0.033) (Table 1:1). No effect was found between treatment with the anti-inflammatory drug and inflammation (P = 1.000). No effect was found between the control group of heifers that were not treated with anything and inflammation (p = 0.217). No effect regarding inflammation at the site of branding was found between the group treated with both local anesthetics and the anti-inflammatory drug. (Figure 1).

Table 1:1. *Chi square test concerning the animals given local anesthetics (LA) and the correlation regarding inflammation at the site of branding, p = 0.033*

	No inflammation	Inflammation	Total
Without LA	16	0	16
With LA	12	4	16
Total	28	4	32

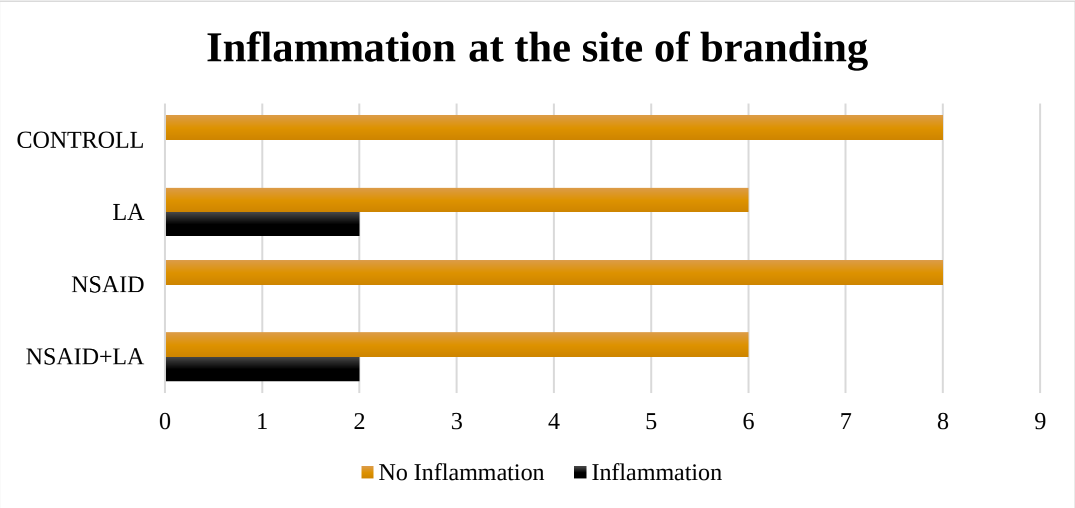

Figure 1. *Incidence of inflammation at the site of branding within the different groups. As can be seen, only heifers that had received local anesthetics developed an inflammatory reaction.*

Weight difference five days after branding

One of the calves in the group given only local anesthetics had to be excluded from the statistics regarding weight difference. This was due to the fact that there was a large difference between the two weights recorded from this calf five days post branding, and hence the data was considered unreliable.

A one-way ANOVA-test was carried out with the different treatments to see if there was any effect of the different treatments regarding the weight gain/ weight loss five days after the branding. A significant effect was found regarding the calves given NSAID ($P = 0.034$). When a one-way ANOVA was made testing the effect of giving both treatments (NSAID and LA) a significant effect was found ($P = 0.009$). No other significant effects could be found regarding the effect of treatment on weight gain five days after treatment. (Table 1:2).

Table 1:2. *Weight differences and standard error of the mean (SE) five days after the branding, for the four different treatments*

Groups	Mean Weight diff. (kg)	SE
NSAID+LA	2.563	0.438
NSAID	0.938	0.764
LA	-0.214	0.714
CONTROL	0.625	0.680

DISCUSSION

The results of our study indicate that the use of NSAID before branding result in the calves gaining more weight during the five days after branding. This effect is thought to be a result of a long-term pain analgesic drug decreasing the loss in feed intake during the days after the painful procedure. In the studies previously made regarding hot-iron branding and the use of anti-inflammatory drugs and/or local anesthetics in cattle the results have varied regarding the productivity (weight gain) of the calves. Some studies have not been able to find any difference in weight gain (Schwartzkopf-Genswein *et al*., 1997a, Tucker *et al*., 2014). Other studies have found effects related to the use of NSAIDs on weight gain after the procedure (Tucker, 2014b). Results indicating that the use of NSAIDs increase weight gain, or decreases weight loss, have also been reported in studies regarding other painful husbandry practices such as dehorning (Faulkner and Weary, 2000).

Our results also indicate that the group of animals given both local anesthetics and NSAIDs gained more weight than the other groups during the days immediately after the procedure. This implies that the use of both of the treatments has a positive effect on productivity. Contrary to this, the group of animals given local anesthetics only had the lowest mean of weight gain of all the groups. The experiment would have to be repeated with a larger group of animals to further investigate if there is a connection between local anesthetics and decreased weight gain after branding.

A statistically significant effect was found linking the use of local anesthetics and inflammation at the branding site. The question whether local anesthetics delay healing and/or increase the risk of inflammation has been discussed both in literature (Marongiu, 2012) and by practicing veterinarians. Despite the obvious risk of injecting some sort of pathogen when administering the anesthesia, some studies have demonstrated results indicating that the actual drug have components that delay the healing of wounds (Carvalho *et al*., 2010). Other studies have showed that local anesthetics has direct anti-inflammatory properties and has the potential to lower the number of bacteria (Sculley and Dunley, 1980).

Other studies have found that the use of local anesthetics changes the histological appearance of the tissues but have not been able to establish in what way this could affect the animal clinically (Drucker *et al*., 1998).

One could argue that there are other benefits to gain from the fact that the animals would experience less pain during the branding, benefits that would outweigh the fact that the incidence of inflammation at the site of branding could be more frequent. The increase of the calves' welfare in terms of less pain is one of the factors to consider. The administration of the local anesthetic is known to cause some pain and/or discomfort (Burns *et al.,* 2006). It is however safe to assume that the pain of being burned with a hot iron would cause a pain more severe, as pains associated with burn injuries in humans has been described as one of the most excruciating pain sensations (Yuxiang *et al.,* 2012).

Overall the calves in our study were healthy, which might have been a strong contributor to the lack of difference regarding the calves' health status before and five days after the branding. The stress reaction of the branding might not be severe enough to increase the cortisol levels and therefore making the health of the calves affected. This is supported by the study by Grobler (2012), who did not find any change of cortisol levels after branding.

Contrary to this, other studies (Schwartzkopf-Genswein *et al.,* 1997b; Ley *et al*., 1992), found an increase in plasma cortisol after hot iron branding.

Several studies examining the effects of surgical castration have not only found that the procedure led to increased plasma cortisol levels after the procedure, but these studies also found that the use of a combination of local anesthetics and an NSAID lowered the increase of cortisol after the surgery (Earley and Crowe, 2002; McMeekand *et al., 1998;* Webster *et al., 2013).*

However, none of these studies have shown an increase in diseases in animals being hot iron branded or subjected to other husbandry practices. Nonetheless, high cortisol levels are known to be related to an increases risk of disease/inflammation due to cortisol lowering the response of the immune system. Animals used in experimental studies are housed in a different and possibly better environment compared to the average animals at a commercial farm. For example, having better food, more space and better housing, or at least more standardized and controlled conditions, including better biosecurity. These animals might therefor naturally have a decreased risk of disease. A hypothesis is that animals at commercial farms may be subjected to aggravating circumstances, such as overcrowded housing (this was however not an issue at the farm of our study), which would further could burden the animal's immune system. Hence, the lowering of cortisol levels that the hot iron branding according to some studies cause, might have a larger impact on animals living on commercial farms.

The calves in our study were generally healthy. A painful and stressful procedure such as branding might have had a negative effect on the calves' general health if the conditions had been different, for example difficult weather (during the rainy season), bad supply or poor quality of forage or any other external circumstance that would lower the calves' immune response. The stress induced by the pain of branding may in that case decrease their immune response further and increase the risk of disease. Our results in this study however, do no indicate that hot-iron branding has any negative effect on the calves' general heath, regardless of pain management strategy.

Due to practical reasons at the farm the test of the methods that was carried the day before the branding had to be conducted with the calves that were to participate in the study. This was not optimal as the calves were exposed to stress one day prior to the actual experiment. It would have been better to use a different group of calves of the same age. Having a separate group of calves would make it possible to test the methods without affecting the calves later used in the study.

Methodological considerations

Other studies have measured the feed intakes of the animals (Webster *et al*., 2013) to gather more precise information. This was not possible in our study, as the calves were on pasture and intake could hence not be monitored, but could be something to consider in a future project. The calves in our experiment were only weighed five days after the procedure, more frequent measurements taken for a longer period of time of the calves' weights would likely provide useful information.

As in this experiment, many studies are conducted with a smaller group of calves. As several other authors have discussed (Schwartzkopf-Genswein *et al*., 1997a, Tucker *et al*., 2014) a larger group of animals might have increased the probability of a robust result. It would have been beneficial to the study if the calves could be monitored and their parameters (weight for example) could be measured for a longer period of time. The time restraints on the farm while the experiment was conducted were quite strict, both due to practical reasons and the fact that the animal welfare of the animals in the study was to be regarded at all times

Other studies concerning hot-iron branding have sometimes included a second control group of sham-branded cattle (Grobler, 2012, Schwartzkopf-Genswein *et al*., 1997b and Ley *et al*., 1992). The group of sham-branded cattle would represent a placebo group. This to be able to determine the difference between the stress induced by pain due to the branding and the stress of only being restrained and handled. This was however not possible in our study due to the fact that all calves had to be vaccinated and branded by law and no group of calves could be spared from branding. A possible solution to this might have been to have the sham branded group consisting of slightly younger calves. This would however not be optimal, as these calves might have different growth rates and possibly an immune response that differs from the branded calves. The welfare of the animals in an experiment is of course always to be considered and to involve a new group of calves raises the question whether this would be ethically reasonable. It would also only be possible to follow the sham-branded control group for a short period of time, as these calves would also be branded at four months of age. If the study is to be repeated on a larger scale a sham branded control group might however be something to consider.

Additional procedures to standardize the treatment of the calves during the experiment could be improved by administering a placebo to the groups that were not receiving medicine, as done for example by Webster *et al. (2013)* and Earley and Crowe (2002). In our study the animals that were not given a skin infiltration of local anesthetics were touched on the injection site, to standardize the stress of being handled amongst the four groups. This would, in a future study, benefit from injecting the calves with a placebo (for example a saline solution), both on the cheek where the local anaesthetic is administered and in the gluteus muscle where the NSAID is injected.

The question whether the largest impact on the animal is the actual painful procedure or the stress of being handled and restrained has been discussed in literature (Walker *et al*., 2002). In addition, the calves in this study were also exposed to the stress of being separated from their mothers. The mothers were kept in a pen close to the branding site. A factor affecting the calves' stress levels during and after the branding might be the proximity of the mothers to the calves, as the herd of vocalizing cows sometimes were further away and sometimes closer.

Other ways to distinguish between the stress caused by the pain of a procedure and the stress of being handled might be to incorporate some sort of behavioral measurement to assess the stress the cattle are being exposed to. For example, vocalizing (grunt, bellow), teeth grinding, reluctance to move, and changing of facial expression (Underwood, 2002), might give further insight and information which would make it easier to distinguish the cause of the stress the animals are experiencing. Alongside this study a behavioral study was also conducted with the same group of animals, analyzing body movement during the branding, pain face during and after the branding and flight speed after the branding. Combining the results from these two studies may give further understanding of the animals' experience of the hot-iron branding and what type of treatment would be optimal to achieve improved animal welfare.

There are however challenges assessing pain in cattle as it is natural for these animals not to show fear and pain, as they are flight animals (Flecknell, 2008).

An additional way of measuring the stress level of the animals would be to measure the concentration of cortisol in plasma, as have been in several studies (Grobler, 2012; Ley *et al.*, 1992 and Schwartzkopf-Genswein *et al.*, 1997b). There is, however, challenges and considerations to be taken when sampling blood from cattle and analyzing cortisol levels. The sampling of blood will likely add to the stress level of the animals which could make the cortisol levels rise. This is especially to be considered when handling cattle that have had very little handling prior to the study.

Though the crush used on the farm where our experiment was performed is relatively new (approximately 10 years) it is made for adult cattle and relatively noisy. There are more modern crushes on the market that are quieter and that can be adapted to the size of adult or young animals. A new, less painful and less noisy crush could be a way to lessen the stress of the calves when being restrained.

A way of decreasing the stress of having to be restrained in the crush would be if the calves were able to be sedated prior the branding. It was not possible to try this in our experiment due to practical reasons. It is in Sweden legia artis to use an alfa-2 agonist such as xylazine to sedate calves before dehorning. The sedative drug is administered intra muscular in the neck of the calves while a group of calves are in a small pen. Approximately 10 minutes after the sedative is administered the calves will lay down by themselves. This was for example used in a dehorning study by Faulkner and Weary (2000) where the sedative xylazine was used, adequate recumbency was achieved in the study and the calves did not have to be restrained prior to the local anesthesia or the painful procedure. The most common treatment when dehorning calves in Sweden is also to use a nerve block such as used in our experiment and in addition to this a dose of NSAID. The combination of the alfa- 2 agonist and the nerve block makes it possible to perform a painful procedure on the calves without having to restrain them at all. If sedation was used when branding calves, it would subsequently make it possible to brand the calves without restraining them in the crush. The intra muscular injection of the alfa-2 agonist is given first and is quickly administered, one person is holding the calf and one is administering the drug in the muscles of the neck of the calf. This injection is not painful and would, in comparison to the stress of being restrained in the crush, be considered as much less stressful. This might also lessen the negative annotations that the crush might have for the calves in their future lives. If painful procedures such as branding, and dehorning could be done in a pen with the help of sedation, the less painful procedures such as vaccination and deworming could be done in the crush without sedation. This may lessen the stress that being restrained in the crush poses on the calves and hence decrease the traumatic memories that the calves might associate with the crush.

This thesis is mostly aimed towards examining the effects that hot-iron branding has on the productivity of the calves, such as general health and weight gain. These parameters are perhaps the ones mostly considered as economically important for the farmers. Factors such as animal behavior may appear to constitute less of an economic incentive to try to create an improvement within animal welfare. At a commercial farm it is, however, important that the animals are as calm and easy to manage as possible, as a lot of the cowboys' time is spent handling animals. Analyzing and trying to minimize the trauma that these animals for example associate with being restrained in general and being held in the crush at the farm in particular could decrease the workload of the employees substantially, in addition to animal welfare being a goal in itself.

CONCLUSIONS

- The results of this study indicate a positive effect of the use of NSAID as a treatment before hot-iron branding regarding weight gain five days after the branding. The pain-relieving effect of the drug may decrease the loss in feed intake after a painful procedure such as hot-iron branding.
- The results of this study also indicate that the use of local anesthetics seem to have a negative impact on the healing of the wound caused by hot-iron branding. Previous literature has reported conflicting results on the possible negative inflammatory effects of local anesthetics.
- The effect of the branding on the heifers' general health was also recorded. The results of this study do not indicate that the branding made the heifers more prone to illness.

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