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Evaluating the efficacy of a topical anaesthetic formulation and ketoprofen, alone and in combination, on the pain sensitivity of dehorning wounds in Holstein-Friesian calves

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Abstract. The aim of this study was to investigate the effect of a topically applied local anaesthetic and the non-steroidal anti-inflammatory drug ketoprofen, alone and in combination, on the pain sensitivity response of calves to dehorning (mean age 2.2 months). Calves were randomly allocated and blocked by age to one of four groups. Groups were: scoop dehorning (D, n = 8), scoop dehorning + i.m. administration of 3 mg/kg ketoprofen (DK, n = 8), scoop dehorning + application of topical anaesthetic (DTA, n = 7) and scoop dehorning + application of topical anaesthetic and i.m. administration of ketoprofen (DKTA, n = 7). A pressure algometer was used to determine the mechanical nociceptive threshold (MNT), being the pressure (kg/f) at which calves withdrew from the stimulus. Measurements were taken before dehorning and at 1 min, 1, 2, 5 and 24 h post-dehorning at both the cut skin edge of the wound and the peri-wound area. The effect of treatment changed over time ($P < 0.001$). MNT was highest before treatment (MNT = 5.03 kg/f) and tended to decrease over time (MNT = 1.16 kg/f 24 h post-treatment). Overall, D calves exhibited the lowest MNT with an average of 1.77 kg/f. DTA calves had the highest MNT (3.89 kg/f), followed closely by DKTA calves (3.24 kg/f). DK calves exhibited an intermediate MNT of 2.61 kg/f. MNT of the cut skin edge was generally lower than that of the peri-wound area (2.01 vs 3.81 kg/f, respectively, $P = 0.02$).The topical anaesthetic formulation significantly reduced the pain sensitivity of dehorning wounds. There was no observed enhanced analgesic effect with addition of ketoprofen. The cut skin edge was more sensitive to pressure than the peri-wound area.

Additional keywords: calf, non-steroidal anti-inflammatory drug, topical anaesthesia, welfare.

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Introduction

Dehorning is a common procedure in the dairy and beef cattle industries and is well documented as being painful (Fisher *et al.* 2008). It is an important management tool for production and safety reasons. Apart from increased probability of injury and difficulty in handling, horns may have additional economic impacts through bruising and hide damage to other cattle, and increased requirements for space during feeding and transport (Vowles 1976; Marshall 1977; Stookey and Goonewardene 1996; Stafford and Mellor 2005). Dehorning cattle reduces bruise trim by ~50% thus helping to reduce economic losses (Prayaga 2007).

Despite the justifications for dehorning, there is growing public concern for production animal welfare, including the welfare of animals undergoing painful husbandry procedures (Fisher *et al.* 2008). This requires evaluation of alternative options to dehorning or modification of the dehorning procedure to reduce or eliminate pain. Although the long-term solution to dehorning is the breeding of polled cattle, this is currently not a feasible or practical solution for many dairy and beef producers. In the interim, pain management is one realistic alternative to allay welfare concerns at dehorning (Fisher *et al.* 2008). Although dehorning is traditionally performed without pain relief in many countries (Huxley and Whay 2006; Fisher *et al.* 2008; Paull *et al.* 2009), changing legislation especially in the EU recommends pain relief be used for calves over 4 weeks of age (Council of Europe 1988). Research has been conducted on the application of anaesthesia and analgesia for the procedure, however the cost-effectiveness and practicality of this approach for routine on-farm use is questionable (Petherick 2005). Studies on the attitudes of producers and practitioners to pain alleviation when dehorning, have shown that the main reasons for lack of use of anaesthesia and analgesia were time constraints, cost, and lack of information or skill (Huxley and Whay 2006; Gottardo *et al.* 2011; Guatteo *et al.* 2012).

Regardless of existing constraints and limitations, there is evidence that livestock industries are responding to welfare concerns, with consideration of pain relief for calves undergoing dehorning. Legislation requiring the administration of local anaesthesia before castration and dehorning has been enacted by several European countries (Graf and Senn 1999; Rollin 2004). The Farm Animal Welfare Council in the UK recommends that analgesics and local anaesthesia be used when dehorning calves, and that the procedure be performed before 2 months of age (Anonymous 1997). 'The Australian Model Code of Practice for the Welfare of Animals: Cattle' encourages dehorning as young as possible and recommends the use of a local anaesthetic when dehorning calves over 6 months of age (Anonymous 2004*a*). The Australian Veterinary Association also states that cattle should be dehorned as young as possible and that analgesia should be used where appropriate (Anonymous 2004*b*).

Issues of impracticality and cost of pain relief for dehorning can be addressed through the introduction of a topical anaesthetic that can be easily applied on-farm by producers. Recently, a topical anaesthetic wound dressing was shown to significantly reduce short-term pain sensitivity in scoop dehorned dairy calves (Espinoza *et al*. 2013). This multipurpose formulation contained lignocaine and bupivacaine for local anaesthesia, cetrimide for antisepsis, and aluminium chlorohydrate for astringency of the wound. These ingredients were carried in a viscous gel base to improve adhesion to the wound and surrounding tissue. Lignocaine, when administered as a cornual nerve block or a ring block, provides rapid onset of anaesthesia at 2–5 min after application, and the anaesthetic effects are documented to last up to 3 h (McMeekan *et al*. 1999; Faulkner and Weary 2000; Stafford and Mellor 2005; Stewart *et al*. 2009; Coetzee 2011). Bupivacaine is a longer acting local anaesthetic that begins to take effect 20–30 min after administration, with anaesthesia lasting up to 8 h (Faulkner and Weary 2000; Stafford and Mellor 2005; Coetzee 2011). Although the use of injected local anaesthetic agents has been well documented, their topical application requires further examination.

Non-steroidal anti-inflammatory drugs (NSAID) have analgesic effects that extend into the post-operative period by inhibiting cyclooxygenase and prostaglandin production and reducing nociceptor sensitisation (Coetzee 2011). Subcutaneous infiltration with a local anaesthetic, in combination with the NSAID ketoprofen, has been shown to reduce the cortisol response, pain-related behaviour, and heart rate in calves undergoing dehorning to a greater degree than local anaesthesia alone (Faulkner and Weary 2000; Stafford and Mellor 2005; Coetzee 2011). Thus, if used in combination with a topical anaesthetic, the inclusion of an NSAID may provide enhanced relief of the pain associated with dehorning.

Algometry is a form of quantitative sensory testing that provides objective, repeatable results (Duarte *et al*. 2005) when measuring sensation, pain detection and pain tolerance (Clark *et al*. 2011). An algometer applies increasing pressure onto a target site and measures the pressure, usually in kilograms or pounds of force, at which the animal withdraws from the stimulus (Clark *et al*. 2011). The maximum pressure applied is interpreted as the pain threshold response (or mechanical nociceptive threshold; MNT) of the animal (Fitzpatrick *et al*. 2013). Algometry has been used to successfully provide an indication of pain in a variety of conditions in cattle including lameness, digital dermatitis, ocular pain in calves affected with bovine keratoconjunctivitis, and mastitis (Dyer *et al*. 2007; Cutler *et al*. 2013; Fitzpatrick *et al*. 2013; Dewell *et al*. 2014). Algometry has also been previously validated as a tool to quantify pain sensitivity following dehorning (Heinrich *et al*. 2010; Tapper 2011). Calves undergoing cautery disbudding were found to have lower MNT than calves that had not been disbudded, suggesting that the procedure causes a pain response (Heinrich *et al*. 2010). It was also found that calves that were dehorned following administration of meloxicam, had higher MNT than calves that were dehorned without analgesia, suggesting that meloxicam helped to relieve the pain response (Heinrich *et al*. 2010).

This experiment aimed to investigate the duration and effect of a topical anaesthetic and the NSAID ketoprofen, alone and in combination, on the pain sensitivity of calves undergoing scoop dehorning. It was hypothesised that calves receiving the topical anaesthetic and ketoprofen would exhibit lower pain sensitivity than calves dehorned alone, and that their combined use would result in an additive effect on pain sensitivity.

Materials and methods

Animals and housing

Thirty Holstein-Friesian heifer calves (51–86 days old) were sourced from 'Corstophine' Dairy Unit at The University of Sydney (New South Wales, Australia) in May 2013. The calves had been raised under commercial dairy operational conditions. The experimental protocol was conducted under institutional animal ethics committee approval (Approval No. 5832).

Calf weights were estimated using a Holstein-Friesian dairy calf weight estimation tape (The Coburn Co., Inc., Whitewater, WI, USA) with a range of 65.5–122.3 kg. Horn bud diameter (cm) for each calf was measured using a standard metric ruler. Diameter was set as the distance across the base of the horn where it attached to the skull. Calves were group-housed in 50 × 20-m paddocks from 2 days of age and for the duration of the experimental period. The calves were fed a milk ration at ~10% of their bodyweight twice daily at 0730 hours and 1530 hours via an artificial teat and had *ad libitum* access to water and kikuyu-based pasture.

Experimental design and treatments

On the day of the experiment, calves were randomly allocated to one of four treatment groups: (1) scoop dehorning (D, $n = 8$), (2) scoop dehorning with pre-operative administration of ketoprofen (3 mg/kg) (Key Injection, Parnell Technologies NZ Pty Ltd, Auckland) (DK, $n = 8$), (3) scoop dehorning with post-operative application of topical anaesthetic (TA) (Technical Consultancy Services Pty Ltd, Rockdale, NSW, Australia) (DTA, $n = 7$) and (4) scoop dehorning with pre-operative administration of ketoprofen and post-operative application of TA (DKTA, $n = 7$). The experiment was conducted over 2 days, with 16 calves being treated on the first day (D and DK treatment groups) and 14 calves being treated on the second day (DTA and DKTA treatment groups). Calves were quietly moved from their housing paddocks to a holding pen. Ketoprofen was administered intramuscularly in the neck 20 min before dehorning at a dosage of 3 mg/kg. All calves that did not receive ketoprofen (D and DTA calves) were administered a placebo injection of buffered saline intramuscularly in the neck equivalent to the volume it would have received if given ketoprofen. This was conducted to eliminate any possible effect the injection may have on the calves' pain sensitivity. Individual calves were then moved through the race and restrained in a standing upright position in a calf cradle (Arrow Farmquip, Tamworth, NSW, Australia) for treatment and data collection. Scoop dehorning was performed by a single experienced technician. The dehorning device was positioned over the horn bud and the handles pulled apart to excise the horn bud and surrounding tissue. TA was administered immediately after dehorning with a 10-mL syringe and a soft silicone brush. Approximately 7 mL of TA was applied per wound (~14 mL per calf), or enough to completely cover the dehorned wound and the peri-wound area. The peri-wound area covered by TA was ~1 cm wide for the total circumference of the dehorned wound. The brush was cleaned between calves in a bucket of 100 mL/L chlorhexidine solution (Hibitane, Coopers Animal Health, Sydney, NSW, Australia). Following treatment and data collection, calves were quietly returned to their paddocks until subsequent data collection was required, where they were quietly moved back to the holding pen and cradle.

Assessment of pressure/pain threshold

Pressure algometry was used to measure changes in pain sensitivity over time, with the amount of force required to elicit a head withdrawal response denoted as the MNT (in kg/f). This value can also be interpreted as the minimum pressure required to induce a pain response. For this experiment, a pain response was defined as a full head withdrawal reflex away from the mechanical stimulus. Testing was performed using a calibrated hand-held pressure algometer (Wagner Pain Test FPIX Digital Algometer, Wagner Instruments, Riverside, CT, USA) with a maximum threshold of 10 kg force (to 50 g accuracy). Measurements were taken after each calf had settled in the calf cradle and there was minimal noise or external activity to distract the animal. The algometer, equipped with a round rubber tip ~1 cm in diameter, was placed perpendicular to the target site for each measurement. Increasing pressure was slowly applied until the calf withdrew its head, and the maximum pressure applied was recorded as the MNT (kg/f). Calves that did not respond to pressure testing had its MNT recorded as 10 kg/f (maximum threshold of the algometer). The algometer was reset to zero after each pressure sensitivity test.

Pressure sensitivity was measured immediately before dehorning, and 1 min, 1 and, 2, 5 and 24 h post-dehorning. The algometer was operated throughout the experiment by a single and trained technician. Pressure measurements were taken from eight sites on each calf in total: four sites located on the cut skin edge of the wound and four sites located on the surrounding tissue (peri-wound area ~1 cm from the cut skin edge) (Fig. 1). The cut skin edge sites were chosen as it has previously been shown that the sensitivity of cut skin edges in mulesing wounds are more sensitive to pain due to damaged nerve endings (Lomax *et al.* 2008). The peri-wound sites were chosen to investigate secondary hyperalgesia (exaggerated pain response in tissue surrounding the wound). The visibility of the TA on the wound surface meant that the technician was able to differentiate between calves that had been treated with TA and those that had not.

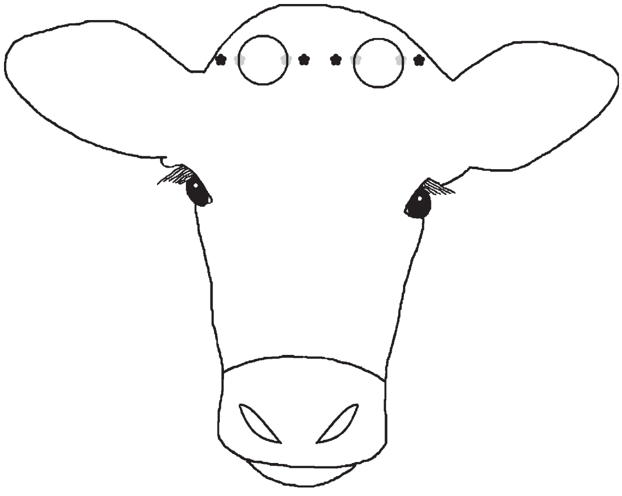

Fig. 1. Sites subjected to pressure sensitivity testing (peri-wound sites: black stars; cut skin edge sites: grey stars).

Statistical analyses

Data was normalised by log-e transformation. Pressure readings were analysed by conducting residual maximum likelihood using the statistical software package, GENSTAT (VSN International Ltd, Hemel Hempstead, UK). The fixed effects of the model were treatment (D, DK, DTA, DKTA), time (pre-treatment, 1 min, 1, 2, 5, and 24 h post-treatment), and their interaction, area tested (peri-wound or cut skin edge), calf weight and age, and horn bud diameter. The random effect of the model was calf. For all statistical calculations, P-values ≤ 0.05 were considered statistically significant.

Results

Overall

There was a significant interactive effect of time and treatment ($P < 0.001$), and area tested and treatment ($P = 0.02$) on MNT. There was no significant effect of calf age, weight or horn bud diameter on MNT.

Time and treatment interactive effect

There was a significant effect of time and treatment, and a significant time and treatment interaction ($P < 0.001$). All calves had an MNT between 4.44 and 6.12 kg/f before treatment (mean 5.03 kg/f) (Fig. 2). Thereafter MNT decreased over time reaching the lowest mean MNT at 24 h post-treatment. Overall, D calves exhibited the lowest MNT with an average of 1.77 kg/f. DK calves had a mean MNT of 2.61 kg/f. DTA calves had the highest mean MNT (3.89 kg/f), followed closely by DKTA calves (3.24 kg/f).

D versus DK At 1 min post-dehorning, the mean MNT of DK calves was greater than that of D calves (5.22 and 3.65 kg/f, respectively). At 1 and 2 h post-treatment, DK calves had a significantly greater mean MNT than D calves ($P < 0.05$). Mean MNT of these calves were similar 5 and 24 h post-treatment.

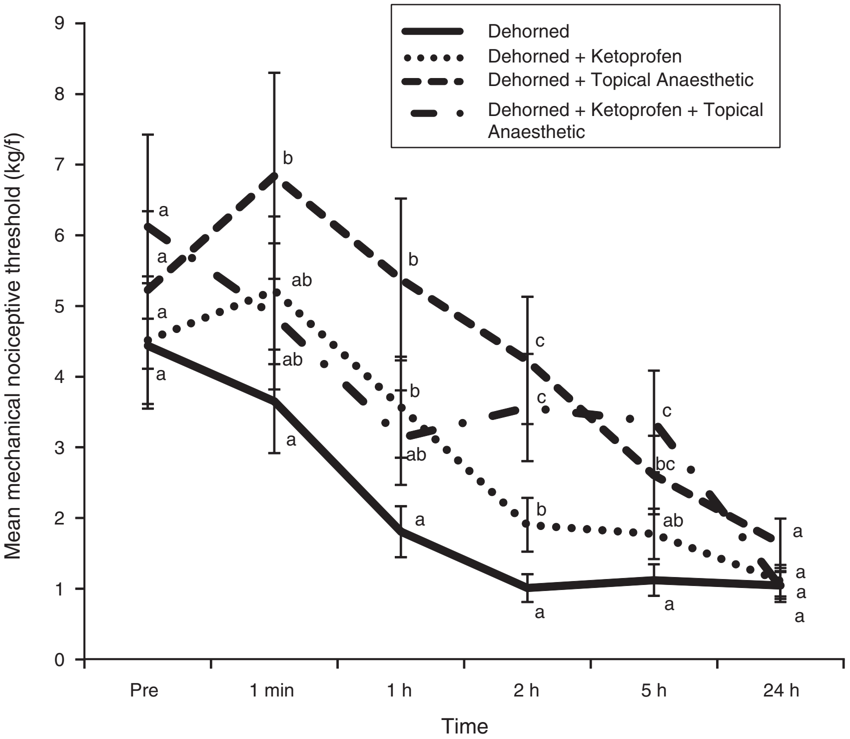

Fig. 2. Predicted mean mechanical nociceptive thresholds following algometer mechanical stimulation on various sites over time on the peri-wound and cut skin edge of dehorned calves subjected to the following treatments: dehorned alone (*n* = 8), dehorned with ketoprofen (*n* = 8) dehorned and treated with topical anaesthesia (*n* = 7), and dehorned with ketoprofen and treated with topical anaesthesia (*n* = 7). Calves not sharing the same superscript within each time point are considered statistically significant ($P < 0.05$).

D versus DTA The mean MNT of DTA calves was significantly higher than D calves at all time points post-treatment except 24 h ($P < 0.05$).

D versus DKTA The mean MNT of DKTA calves was greater than that of D calves at all time points, with significant differences 2 and 5 h post-treatment ($P < 0.05$).

DK versus DTA MNT was numerically higher in DTA than DK calves at most time points, with a significant difference detected 2 h post-treatment ($P < 0.05$).

DK versus DKTA MNT of DK and DKTA calves were similar 1 min, 1 and 24 h post-treatment. DKTA calves had significantly greater MNT than DK calves 2 and 5 h post-treatment ($P < 0.05$).

DTA versus DKTA MNT was numerically higher in DTA than DKTA calves 1 min, 1, 2 and 24 h post-treatment. MNT was numerically higher in DKTA than DTA calves 5 h post-treatment. No significant differences were detected between groups at any time point.

Area and treatment interactive effect

Mechanical stimulation of the cut skin edge area resulted in lower mean MNT than stimulation of the peri-wound tissue (2.01 vs 3.81 kg/f, respectively) (Fig. 3). Differences varied depending on treatment with the greatest difference seen in DTA calves (2.09 kg/f) and similar differences in D, DK and DKTA calves (1.63–1.7 kg/f; $P = 0.02$).

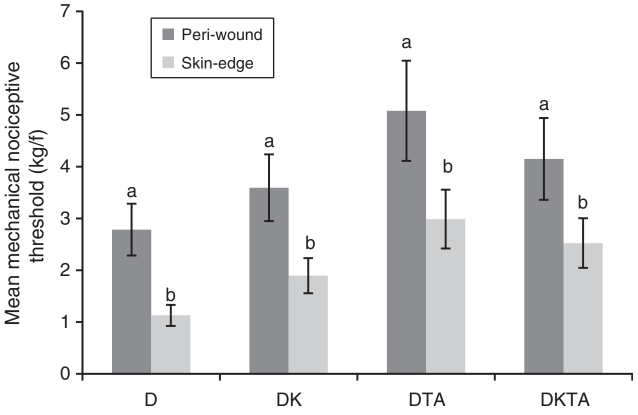

Fig. 3. Predicted mean mechanical nociceptive thresholds following algometer mechanical stimulation on the peri-wound and cut skin edge of dehorned calves subjected to the following treatments: dehorned alone (D) (*n* = 8), dehorned with ketoprofen (DK) (*n* = 8), dehorned and treated with topical anaesthesia (DTA) (*n* = 7), and dehorned with ketoprofen and treated with topical anaesthesia (DKTA) (*n* = 7). Calves not sharing the same superscript within each treatment are considered statistically significant ($P < 0.05$).

Discussion

This study assessed the efficacy of a topical anaesthetic formulation in combination with the NSAID ketoprofen, for the pain amelioration of scoop dehorning wounds in calves. As hypothesised, calves treated with TA were significantly less pressure-sensitive than calves dehorned without TA (except at 24 h post-treatment). Treatment with ketoprofen reduced pressure sensitivity with significant reductions observed 1 and 2 h post-treatment. The combined use of TA and ketoprofen resulted in some reduced pressure sensitivity compared with calves dehorned without analgesia, however no additive effect was observed with DKTA calves displaying similar sensitivity to DTA calves.

Previous research has shown that TA significantly reduces pain sensitivity for at least 1.5 h post-dehorning (Espinoza *et al.* 2013). However, as the pain of dehorning appears to be most severe during the 7–9 h post-procedural period, and may still be evident 1–2 days later, addressing chronic pain is considered important (Sylvester *et al.* 1998, 2004*a*; Stafford and Mellor 2005). Therefore, a combination of drugs, as used in the present study, may provide a multimodal approach to analgesia, which has been demonstrated to be more efficacious (Coetzee 2011).

The post-operative application of TA on dehorning wounds resulted in significantly greater mean MNT than calves dehorned without TA. This reduced pain sensitivity in DTA and DKTA calves was observed for up to 5 h post-treatment, exceeding the duration of efficacy of 1.5 h detected previously (Espinoza *et al.* 2013). This may be due to the increased concentration of bupivacaine in the current formulation (1% vs 0.5% in Espinoza *et al.* 2013). The quantity of TA may also be important for efficacy, with a larger volume used here than in previous work (7 mL vs 4 mL per dehorning wound). An important consideration however is the different modes of pain assessment used to gauge efficacy in the two experiments. The von Frey monofilaments used in Espinoza *et al.* (2013) were designed to bend at an even, repeatable force (10 and 300 g/f). Thus the same force was exerted on the wound and surrounding skin of all calves in order to invoke a pain response. This response to stimulation was then visually scored using a numerical rating scale ranging from 0 to 3 (no, minor, moderate or severe response). The present study utilised algometry whereby tolerance (up to 10 kg/f) to increasing mechanical stimulation was measured and a quantitative outcome obtained. Therefore, the type of pain response being measured is different between the two trials, which does not allow for direct comparison.

The administration of ketoprofen provided significant short-term reduction in the pain sensitivity of dehorning wounds when compared with D calves (up to 2 h). However, no reduction was evident 5 and 24 h post-dehorning. The onset of ketoprofen has been documented at 2 h post-dehorning (McMeekan *et al.* 1998*a*; Stafford and Mellor 2011) whereas other sources suggest a half-life in most animals of less than 2 h (Papich 2010). Hence the pharmacokinetics of ketoprofen are unclear. DTA and DKTA calves exhibited similar mean MNT at all time points following dehorning, whereas in other studies, the combined use of ketoprofen and lignocaine has been shown to be effective at reducing the pain response to dehorning (McMeekan *et al.* 1998*a*; Faulkner and Weary 2000; Sutherland *et al.* 2002). Route of administration could account for these results. Although the dose is the same in the present study as in the literature (3 mg/kg), ketoprofen was administered intramuscularly into the neck rather than intravenously into the calf jugular vein (McMeekan *et al.* 1998*a*; Sutherland *et al.* 2002), or orally (Faulkner and Weary 2000). In addition, local anaesthesia was administered topically rather than via subcutaneous infiltration, allowing for slower absorption and concentration of the actives at the injury site.

The effect of an NSAID on the pain sensitivity of disbudding wounds has been previously investigated. Calves that were cautery disbudded following the administration of meloxicam and lignocaine (cornual nerve block), displayed lower pain sensitivity (as determined through algomtery) 4 h after disbudding than calves given lignocaine alone (Heinrich *et al.* 2010). This indicates the effectiveness of an NSAID as an ameliorant for acute dehorning pain. Results from the present study also found significantly lower pain sensitivity in ketoprofen-treated calves 1 and 2 h post-dehorning compared with calves not given ketoprofen. Another study, which investigated the effect of meloxicam on the pain sensitivity of disbudding wounds, failed to detect any analgesic effect but attributed this finding to the probable inability of von Frey monofilaments in exerting sufficient pressure to induce a response (Mintline *et al.* 2013). Heinrich *et al.* (2010) and Mintline *et al.* (2013) studied meloxicam, believed to be a preferential cyclooxygenase-2 isoform inhibitor with a half-life between 20 and 43 h. Ketoprofen is a nonspecific cyclooxygenase inhibitor with a half-life in most animals of less than 2 h but duration of action up to 24 h (Papich 2010). The much shorter half-life of ketoprofen compared with meloxicam may explain the seemingly diminishing effect from 1 to 2 h in the DK group.

Method of dehorning is an important consideration given that cautery (used in Heinrich *et al.* 2010; and Mintline *et al.* 2013) and amputation dehorning (used in the present study) elicit very different wounds and subsequent pain responses. Cautery disbudding elicits first, second and third degree burns resulting in the damage and destruction of nociceptors leading to altered or loss of sensation (Petrie *et al.* 1996). Thus cautery disbudding has been suggested as less painful than amputation dehorning, which results in a surgical wound resulting in bleeding, and exposed and severed nociceptors.

The duration of nerve blockade provided by local anaesthetics has been suggested as having an impact on the efficacy of NSAID for the amelioration of dehorning pain (Stafford and Mellor 2005). Ketoprofen in combination with lignocaine administered around the cornual nerve has been shown to virtually eliminate the cortisol response to dehorning, whereas the use of bupivacaine appears to produce a delayed cortisol response once the bupivacaine wears off (McMeekan *et al.* 1998*a*). While in this circumstance it is not clear why shorter-acting local anaesthesia is more effective than longer-acting local anaesthesia, this occurrence may explain why the ketoprofen and TA combination did not appear to have an enhanced analgesic effect.

Important information is presented in the present study with respect to the onset of topical anaesthesia for dehorning wounds. Evidence of local anaesthesia may be seen 1 min post-dehorning and treatment with TA. Some indication of local anaesthesia was seen in previous dehorning work at 1 min, though it was not statistically significant when compared with control animals and could be due to the different tool used for assessment: von Frey monofilaments (Espinoza *et al.* 2013). Previous research on TA for castration and mulesing in lambs reported evidence of anaesthesia between 1 and 3 min post-treatment, respectively (Lomax *et al*. 2008, 2010). TA enables a faster onset of local anaesthesia than injectable administration, (which is 3–5 min for a cornual nerve block in calves) as agents are applied directly to open wounds and mucosal surfaces allow rapid absorption and action through exposed nerve endings and blood vessels.

The cut skin edge of the dehorning wound was found to be more sensitive than the peri-wound area, regardless of treatment. This supports previous findings of increased sensitivity of the cut skin edge of mulesing wounds in lambs (Lomax *et al*. 2008). This sensitivity is likely to be caused by direct injury to nociceptors (Meyer *et al*. 2005). At the site of injury (the dehorning wound), sensitisation of exposed nerve endings leads to an enhanced response from these nociceptors. Production of inflammatory mediators and upregulation of proinflammatory enzymes, along with changes in tissue pH and electrolyte composition (Tapper 2011) can explain the significant decrease in mean MNT over time observed in the present study. There was a significant decrease in mean MNT over time indicating the effect of inflammatory mediators on exposed nerve endings in addition to uninjured tissue (Meyer *et al*. 2005).

The TA formulation used previously and in this study, was modified from the original formulation designed for the mulesing of sheep in Australia (Tri-Solfen, Bayer Animal Health, Pymble, NSW, Australia). Scoop dehorning wounds are usually significantly smaller than mulesing wounds although there is often increased haemorrhage, particularly if arterial bleeding occurs (Espinoza *et al*. 2013). Aluminium chlorohydrate was included as a vasoconstrictive agent to promote haemostasis and slow the rate of systemic absorption of anaesthetic actives, which aids in extending the duration of local anaesthesia (Lomax *et al*. 2013).

There are several advantages to the post-operative application of local anaesthetics. In animal production, routine husbandry procedures need to be performed quickly and efficiently due to time and labour constraints. TA can be applied easily, has a rapid onset, and there is no need for double-handling of animals. Post-operative application of TA allows direct contact of the anaesthetic agents, lignocaine and bupivicaine, with damaged tissue and nerve endings. This direct contact between the source of pain and the pain relieving actives improve efficacy and speed of pain alleviation. Additionally, the haemostatic and antiseptic agents are applied directly to the wound allowing pain, blood loss and infection to be treated simultaneously. Post-operative application of TA is therefore an effective, practical and cost-effective option for farmers, which minimises the amount of handling required (Lomax *et al*. 2010).

The high cost and impracticality of injectable pain relief methods have prevented the widespread uptake of pain relief by the majority of cattle producers. Although a significant amount of research has focussed on the use of injected local anaesthesia for ameliorating dehorning pain (Morisse *et al*. 1995; McMeekan *et al*. 1998*a*, 1999; Graf and Senn 1999; Sutherland *et al*. 2002; Stafford and Mellor 2005; Doherty *et al*. 2007; Stewart *et al*. 2009), this study is one of the first to investigate the use of TA. TA has a potential welfare benefit for young calves undergoing scoop dehorning, as well as a potential benefit for beef and dairy industries to address welfare concerns with a relatively practical approach.

All dehorned calves demonstrated increased sensitivity over time. A limitation of this study is the inability to compare with non-dehorned animals as no sham (positive control) animals were included in the study. Rather, a reduction in pain from the dehorned animals (negative control) with a known painful wound can be performed. It is likely that the decrease in MNT over time observed in all animals is due to tissue damage and the inflammatory process, and not an artefact of repeated testing. The pain following dehorning has been well documented (Petrie *et al*. 1996; McMeekan *et al*. 1997, 1998*b*; Sylvester *et al*. 2004*b*). Sham dehorned calves subjected to mechanical stimulation with von Frey monofilaments did not display increasing sensitivity but an unchanging response up to 24 and 75 h post-sham dehorning (Espinoza *et al*. 2013; Mintline *et al*. 2013). Whereas scoop dehorned calves showed an increased sensitivity to von Frey stimulation up to 24 h post-dehorning (Espinoza *et al*. 2013). Sham dehorned calves subjected to pressure algometry showed a reduction in MNT over time (Tapper 2011) and this may be due to the return of sensation, or consequence, from the administration of two lignocaine cornual nerve blocks given to all animals throughout the experimental period. Repeated mechanical stimulation in sheep has produced mixed results. Healthy sheep with an algometer strapped to their hind leg did not become sensitised during the testing period (Nolan *et al*. 1987*a*, 1987*b*, 1987*c*; Welsh and Nolan 1995). Another study using a handheld algometer to determine the MNT of the forelimb of sheep observed a decrease in MNT over 3 days (Stubsjoen *et al*. 2010). The number of chosen time points may be an important consideration as each sheep was tested 30 times over a 30-min period. The inclusion of this group in future work would be beneficial to compare sensitivity of intact and dehorned calves.

Pressure algometry is a novel indicator of the pain induced by dehorning despite its widespread use for the quantification of pain sensitivity for other bovine diseases (Dyer *et al*. 2007; Cutler *et al*. 2013; Fitzpatrick *et al*. 2013; Dewell *et al*. 2014). Algometry is useful for the assessment of localised wound pain and anaesthesia but does not quantify systemic analgesia. Algometry can be invasive as mechanical pressure is being used to measure hyperalgesia. The re-handling and restraint of animals, which is required for pressure testing, can also cause stress and potentially disturb the wound.

Algometry was chosen as the sole pain assessment tool in this study for several reasons. Algometry is a form of mechanical sensory testing, which induces a pain response in order to establish maximum nociceptive threshold. This induction of pain would likely induce a cortisol response, thus interfering with the measurement of cortisol. In addition, animals should be minimally handled for assessment of cortisol so that handling and restraint do not induce stress and a rise in cortisol (Hemsworth *et al*. 2011). Behaviour assessment could not be combined with mechanical sensory testing as repeated-measures on each animal was being performed to document changing sensitivity over time. This required animals to be re-handled and restrained at regular intervals, which would disturb and confound any behaviour observations significantly.

To obtain quality data it is vital that behaviour observations be unobtrusive (Ploger and Yasukawa 2003), performed alone, and with minimal to no interference or handling for the observation period. Evaluation of TA and ketoprofen for dehorning pain could be performed using physiological and behavioural indicators but in isolation and not in conjunction with pressure algometry.

Other limitations to the present study would require further examination. In future trials, the inclusion of a placebo gel would allow for effective blinding to treatment, in addition to evaluating a wound barrier effect that has previously been observed in sheep (Lomax *et al*. 2008). Although the experiment was conducted in an identical manner on both days of the study, future experiments should represent all treatments groups on each day of the study. This would eliminate any potential confounding by ‘day of experiment’.

Through the use of pressure algometry, it can be concluded that TA is able to provide pain relief in under 3-month-old dairy calves for up to 5 h post-scoop dehorning. This was evident through reduced pressure sensitivity at both the cut skin edge of the wound and the peri-wound area. A combined effect of TA and ketoprofen was not detected with the methods used and should be further investigated. The low MNT observed 24 h after dehorning suggests that dehorning causes prolonged pain. Extending the observation period beyond 24 h will help understand the generation and extent of pain following dehorning. TA offers a practical and effective option for improving the welfare of young calves undergoing scoop dehorning.

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