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Pain management during adversive procedures in extensively raised beef cattle

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Executive Summary

Routine husbandry procedures including castration, dehorning, branding, ear notching, ear tagging and spaying are commonly performed on beef cattle and usually without any form of pain relief. Extensive literature exists demonstrating the pain and distress resulting from these procedures. Some alternatives to these painful practices are being developed, with variable success in their uptake, including immunocastration and more widespread use of polled *Bos indicus* cattle. However, the current practices do need to continue for an extended interim period for numerous reasons, including ease of management, improved safety and enhanced productivity. Importantly, consumer demand for improved animal welfare is driving increasing corporate responsibility for stakeholders throughout the beef industry to provide effective analgesia for these husbandry interventions, preferably at a reasonable cost that can encourage producer uptake.

Recently, the practical constraints associated with provision of conventional forms of anaesthesia and analgesia in beef cattle, have been addressed through the development and registration of 'farmer applied' pain relief products. A topical anaesthetic (TA) gel (Tri-Solfen®, Bayer Animal Health Australia) designed to be applied to open wounds, and a buccal meloxicam (BM) gel (Ilium® Buccalgesic OTM, Troy Laboratories) designed for oral trans-mucosal absorption, have been developed for post-operative anaesthesia and analgesia of lambs and calves undergoing surgical husbandry procedures. A meloxicam injection (MI) is also available for use in cattle. Other forms of anaesthesia, including cryoanaesthesia have also been investigated.

This project aimed to assess the efficacy of cryoanaesthesia, TA, BM and MI alone and in various combinations, for the relief of post-operative pain caused by routine husbandry procedures in beef cattle. In addition, the effects of provision of analgesia on production parameters, including weight gain and mortality, were noted.

Cryoanaesthesia provided a significant reduction in pain associated with ear tagging and ear notching in weaner calves. A three second spray of vapocoolant was found to provide sufficient anaesthesia for short periods of time and was applied immediately prior to ear tagging and notching. A topical vapocoolant spray, applied to the scrotum and spermatic cords, and lignocaine injected into the scrotum and spermatic cords, were used to address intra-operative pain associated with castration. The results suggested that both the vapocoolant spray and lignocaine were inadequate pain relief interventions during surgical castration of unweaned beef calves.

Post-operative pain relief for castration was initially investigated through examination of the effect of TA on plasma cortisol. There was a trend for TA to reduce plasma cortisol, although no significant treatment effect was shown. The effect of TA and BM alone and in combination, on production, behaviour and wound inflammation of unweaned beef calves following surgical castration, was investigated. TA and BM, alone and in combination all reduced pain, as demonstrated through a reduction in some pain-related behaviours. BM reduced inflammation, as demonstrated through reduced maximum scrotal temperature over time.

Pain relief for dehorning was examined, initially by using modified formulations of TA to try and improve adhesion to the wound. This study used mechanical stimulation of wounds to

assess analgesia and results indicated that all TA formulations were comparable to a cornual nerve block of lignocaine in their ability to anaesthetise dehorning wounds post-operatively.

Behaviour and wound inflammation following amputation dehorning of unweaned beef calves was assessed both without treatment and with TA or BM. There were no clear effects of TA or BM on pain and inflammation in this study, suggesting further trials and assessments for this procedure are required.

The effects of TA and BM, alone and in combination, were examined on production and behaviour of weaned beef calves following concurrent castration and dehorning. A combination of TA and BM resulted in a reduction in weight loss associated with castration and dehorning and increased lying activity, thought to be due to a reduction in pain-related restlessness.

Finally, the effects of TA and MI were investigated for use during spaying of beef cattle in northern Australia. In this investigation a Willis spay tool was modified to enable delivery of TA to the internal sites of vaginal wall penetration and ovarian resection at the time of the procedure. Behavioural responses following treatment are currently being analysed to provide information on the analgesic efficacy of TA and MI.

The results of this project suggest that cryoanaesthesia is a suitable form of analgesia for ear tagging and ear notching but does not provide significant benefit for any other major procedures. TA and BM both provide some amelioration of pain caused by castration and dehorning in calves, with indications of increased efficacy when a combination of TA and BM are used. The results suggest that TA and BM do not completely abolish pain following castration and dehorning of calves and therefore further improvements to analgesic therapies for these procedures should be investigated.

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1 Background

1.1 Introduction

Ear tagging, ear notching, branding, dehorning, castration and spaying are routine husbandry procedures performed on cattle in extensive beef production systems in Australia. These procedures are necessary to improve animal welfare, management and productivity and are typically conducted without any form of anaesthesia or analgesia. Until recently, most analgesic products registered for use in cattle required injection and veterinary prescription, making them impractical for use during routine husbandry operations on commercial beef farms. However, consumer concerns for improved animal welfare are increasing and the livestock industries are aware of their need to respond with practical options for providing pain relief to cattle undergoing husbandry procedures.

1.2 Animal welfare

Public concern for the wellbeing of animals has continued to increase over recent decades, with animals now widely recognised as sentient beings that have the ability to experience pain and distress (Cornish *et al.* 2016). In Australia, animal welfare is increasingly important to the public, as reflected through a willingness for people to become actively involved in animal welfare issues and evidence that consumers will adjust their purchasing of livestock products when improved welfare perceptions are promoted. Public concern for animal welfare is increasingly affecting the economics and sustainability of livestock production industries as it directly influences actions from retailers, regulators and legislators (Coleman 2008). However, there is limited published information on the attitudes of Australians towards painful husbandry procedures performed on livestock, although it is believed the Australian public considers animals should not have to experience unnecessary pain (Coleman 2008). Pressure to provide pain relief to livestock undergoing routine husbandry procedures is very likely to increase as the public becomes more aware of the pain and distress caused by such procedures (Mellor *et al.* 2008).

The World Organisation for Animal Health (OIE) recommends that surgical husbandry procedures performed on beef cattle should be conducted at as early an age as possible and in a way that minimises pain and stress to the animal (OIE 2015). Similarly, the objective of the Australian Animal Welfare Standards and Guidelines (S&G's) for cattle, derived from the Australian Animal Welfare Strategy (AAWS), is for painful husbandry procedures to be conducted appropriately and in a manner that minimises the risk to the welfare of cattle, including pain and distress (AHA 2014). The Standards state that pain relief must be used when castrating and dehorning cattle older than 6 to 12 months, depending on when they are first brought into the yards, and when performing flank laparotomy for spaying or webbing of cattle. Further, the Guidelines state that pain relief should be used for all surgical procedures (AHA 2014).

1.3 Pain

1.3.1 Pain in cattle undergoing husbandry procedures

Pain associated with routine husbandry procedures in beef cattle has been well documented in the literature, including papers on pain during: dehorning (Stafford and Mellor 2005a; Kupczynski *et al.* 2014); castration (Stafford and Mellor 2005b; Coetzee 2013); ear tagging

(Stewart *et al.* 2013); ear notching (Friend *et al.* 1994); branding (Lay *et al.* 1992a; Lay *et al.* 1992b; Lay *et al.* 1992c; Schwartzkopf-Genswein and Stookey 1997; Schwartzkopf-Genswein *et al.* 1997a; Schwartzkopf-Genswein *et al.* 1997b; Tucker *et al.* 2014a; Tucker *et al.* 2014b); and spaying (McCosker *et al.* 2010; Petherick *et al.* 2011; Petherick *et al.* 2013). Despite multiple husbandry procedures often being performed at the same time, there is limited literature on the pain and distress of concurrently performed procedures (Baldridge *et al.* 2011; Mosher *et al.* 2013).

1.3.2 Pain relief in cattle undergoing husbandry procedures

The efficacy of various analgesic products for cattle undergoing dehorning and castration is well documented in the literature (Stafford and Mellor 2005a; Coetzee 2013; Stock *et al.* 2013). Most research has focused on local anaesthetics (LA) and non-steroidal anti-inflammatory drugs (NSAIDs) (Stafford and Mellor 2005a; Coetzee 2011; Stock *et al.* 2013), with a combination of an LA and an NSAID considered best practice (Mellor and Stafford 1999; Hudson *et al.* 2008). Less research has focused on α-2 agonists and opioids as their sedative effects, variable efficacy and costs, make them potentially unsafe and less appropriate for use by producers on-farm (Anderson and Muir 2005; Hudson *et al.* 2008). Few studies have examined the efficacy of analgesics for ear tagging and notching, branding (Tucker *et al.* 2014a; Tucker *et al.* 2014b) and spaying. Until recently, most LA and NSAID products required subcutaneous (SC), intravenous (IV) or intramuscular (IM) administration and most research has focused on pre-operative administration of LA and NSAID injections (Stafford and Mellor 2005a; Coetzee 2011; Stock *et al.* 2013). There has been some research conducted using oral NSAIDs for the relief of pain caused by castration and dehorning in calves (Coetzee *et al.* 2012a; Allen *et al.* 2013; Glynn *et al.* 2013; Brown *et al.* 2015), with advantages of oral NSAIDs compared to injectable NSAIDs including longer duration of action, ability to be delivered via feed, reduced risk of injection site reactions or needle related injuries (Olson *et al.* 2016), and reduced operator skill required (Small *et al.* 2014)). The uptake and sustained use of analgesia in production animals depends on the cost, ease of administration and time to effect of the available products (Mellor and Stafford 1999). In addition, requirements for veterinarians or skilled personnel under veterinary supervision, plus the time required for registration of pharmaceutical products, both affect the uptake of pain relief treatments on-farm (Mellor *et al.* 2008).

1.4 Australian beef production

1.4.1 Context

The Australian beef industry contributes approximately 23% of the total gross value of farm production and 22% of the total value of farm export income in Australia (figures from 2015-2016) (ABARES, 2017) . Beef production is the most prevalent agricultural activity in Australia, with 57% of farms and 75% of total agricultural land carrying beef cattle (ABARES, 2017).

The research in this report is particularly relevant for the northern component of Australia's beef industry. Northern Australia contains extensive tropical and sub-tropical areas where numerous environmental stressors exist that affect the management of beef cattle. Beef production in northern Australia is mostly conducted on unimproved pastures in the dry tropics, with highly seasonal and variable rainfall, poor soil fertility, woody vegetation and

fluctuating quantity and quality of forage. *Bos indicus* and *Bos indicus* x *Bos taurus* are now the dominant breeds of cattle in northern Australia as they are better adapted to cope with these environmental stressors (Bortolussi *et al.* 2005). Beef cattle properties and herds in northern Australia do vary, although most are large with cattle managed in large numbers at low stocking rates under extensive management systems (McLean *et al.* 2014). On most properties, management is relatively low-input, mating is typically uncontrolled, and the cattle are usually only handled once or twice a year when they are mustered for calf weaning, usually in the periods April to July and August to September (Petherick 2005). The age range of calves at weaning can be wide (between 3.5 to 10 months of age) due to uncontrolled mating and extended calving periods and intervalving intervals. Occasionally, calves may be missed during the annual muster, resulting in calves that can be significantly older than the herd average at the next round of weaning (Prayaga 2007).

1.4.2 Concerns regarding painful procedures

Surgically performed procedures have welfare implications that can be exacerbated by the nature of some Australian beef production systems, especially those located in northern Australia. As most cattle in northern Australia are *Bos indicus* or *Bos indicus* x *Bos taurus* genotypes, dehorning becomes necessary due to a lack of polled animals and a complex mode of inheritance of the poll gene in these breeds (Prayaga 2007). Performing mustering and 'marking' procedures on older calves that are unaccustomed to handling by humans also causes considerable stress (Petherick 2005), resulting in larger wounds, increased blood loss, longer healing periods, and potentially both higher mortalities and levels of pain (Stafford and Mellor 2015). Spaying of beef cattle is also necessary on some properties in northern Australia as it is difficult to prevent bulls from accessing females in such extensive environments (Petherick 2005).

1.4.3 Constraints of analgesia use

Widespread uptake of analgesia use during routine husbandry procedures may prove difficult, especially in extensive production systems as the additional time and skills required for administration of injectable anaesthetics and analgesics to large numbers of cattle is a potential hindrance to producer adoption of such products (Petherick 2005). As most northern Australian cattle are unaccustomed to human handling, longer restraint times or doubling handling could pose additional animal welfare issues from the stresses caused by these activities (Petherick 2005).

1.5 Practical options for pain relief

The necessity for a practical method of delivery of analgesia in livestock production systems has been recognised for over a decade in Australia. Research into practical methods of providing analgesia led to the registration of a wound treatment formulaton containing topical anaesthetic (TA) (Tri-Solfen®, Bayer Animal Health, Pymble, NSW, Australia) initially for use during mulesing of lambs, but then later registered for use for tail docking of lambs and surgical castration of both lambs and calves. Subsequently, a buccal meloxicam (BM) formulation was developed (Ilium Buccalgesic OTM®, Troy Laboratories, Glendenning, NSW, Australia) for use during tail docking of lambs and surgical castration of both lambs and calves. Tri-Solfen® contains lignocaine (40.6 g/L), bupivacaine (4.2 g/L), cetrimide (5 g/L) and adrenaline (24.8 mg/L) in a gel base and is applied to wound surfaces during or

immediately following painful procedures, using a spray applicator, where it is absorbed at the site of injury for provision of local anaesthesia and enhanced wound healing. BM contains meloxicam (10 mg/mL) in a gel base and is administered into the buccal cavity immediately prior to the procedures using a gun applicator where it is absorbed through the oral mucosa and provides anti-inflammatory and analgesic effects. Administration of TA and BM adds a small amount of time during processing of each animal, although results in an easier, faster and safer drug administration process compared to pre-surgical injections of local anaesthetic and/or NSAIDs. In addition, BM may offer some advantages over oral NSAIDs with the bioavailability of the drug improved due to avoidance of degradation in the gastrointestinal tract and hepatic first-pass metabolism (Habib *et al.* 2011).

This research project builds on previous studies on the use of TA (Lomax *et al.* 2008; Lomax *et al.* 2010; Lomax *et al.* 2013) and BM (Small *et al.* 2014) in lambs for various husbandry procedures and the use of TA for surgical castration in calves (Lomax and Windsor 2014). It builds on previous studies on the use of modified formulations of TA for dehorning of calves (Espinoza *et al.* 2013; Espinoza *et al.* 2015). Modified formulations of TA were developed and tested as it was recognised that the excessive haemorrhage that often occurs following dehorning, may inhibit sufficient absorption of anaesthetic agents at the wound site. These previous studies in calves showed a reduction in post-operative wound sensitivity for at least 24 h and pain-related behaviours for at least 4 h following surgical castration when TA was used (Lomax and Windsor 2014) and a reduction in wound sensitivity up to 1.5 h (Espinoza *et al.* 2013) and 5 h (Espinoza *et al.* 2015) following dehorning when modified formulations of TA were used.

As TA inhibits nociception following application of TA to exposed nociceptors and BM inhibits sensitisation of wounds by amelioration of COX pathways, neither drugs address the intra-operative pain of procedures. Cryo-anaesthesia may be a practical option for relieving intra-operative pain of due to minor surgical procedures if shown to be effective. Cryo-anaesthesia provides temporary local anaesthesia by disruption of local sensory nerve function through reduced tissue temperature (Griffith *et al.*, 2016). Topical vapocoolants can be applied to the skin surface where they rapidly evaporate causing a reduction in tissue temperature and have previously been shown to reduce pain caused by initial intradermal anaesthetic injection (Collado-Mesa et al., 2015), venipuncture (Mace, 2016), vaccinations (Mawhorter et al., 2004), cosmetic botulinum injections (Weiss and Lavin, 2009) and intravenous cannulation (Griffith et al., 2016) in humans, and arthrocentesis in horses (Fjordbakk and Haga, 2011).

1.6 Summary and overarching aims

Minimising pain and suffering in animals is an important for improving livestock welfare and there is a growing expectation by consumers for provision of analgesic treatments when farmed animals experience painful routine husbandry procedures. Improvements to current routine husbandry procedures performed on Australian beef cattle are required to ensure sustainability of the beef industry. Most research to date has focused on SC, IV, or IM administration of pharmaceuticals, although practical constraints have limited their widespread adoption by producers. This has been a particular issue for large-scale, extensively managed cattle operations. The availability of producer-applied products including TA, BM and CRYO may improve producer uptake of analgesia in cattle. Further, if improvements to production can be demonstrated in the use of these products, this may

offset the extra costs associated with their use.The aim of this project was to examine the efficacy and practicality of producer-applied analgesic therapies when administered to beef cattle on commercial farms during routine surgical husbandry procedures.

2 Project Objectives

2.1 Objective 1

Examine the efficacy of topical anaesthetic, cryoanaesthetic and non-steroidal anti-inflammatory drugs alone and in various combinations, during single routine surgical procedures including dehorning, castration, ear notching, branding and spaying, using behavioural scores, pressure algometry, thermography and physiological parameters including cortisol to enable quantification of pain responses

2.2 Objective 2

Examine the efficacy of topical anaesthetic, cryoanaesthetic and non-steroidal anti-inflammatory drugs alone and in various combinations for multiple combinations of routine husbandry practices as practised routinely on northern beef properties, as directed by results from Objective 1.

2.3 Objective 3

Monitor the effect of analgesia provided during aversive procedures on production parameters including weight gain, morbidity and mortality.

3 Methodology

3.1 General methodology

3.1.1 Animal ethics

All experimental protocol was approved by the Animal Ethics Committee of the University of Sydney (approval number 5832). All animals were sourced from commercial cattle herds in the southern highlands and southern tablelands of NSW, western Qld and far northern WA, Australia. Surgical husbandry procedures performed on the animals were conducted as per normal farm management practice on the properties where the studies were conducted.

3.1.2 Husbandry procedures

3.1.2.1 Ear tagging and notching

Ear tagging was conducted using a flexible tag applicator (Allflex Australia Pty Ltd, Landmark, Goulburn, NSW, Australia). National Livestock Identification System (NLIS) tags were placed in the right ears and individual animal identification tags were placed in the left ears of all animals. Tag attachments were placed in the middle of the ears between the two main cartilage ridges. Ear notching was conducted by removing a section of the edge of the ear using the property's ear notching device. The tag applicator and the ear notching device were chemically sterilised before each use.

3.1.2.2 Surgical castration

Surgical castration techniques varied between studies conducted on different properties due to operator preference. For the studies conducted at the University of Sydney owned property 'Arthursleigh' in the southern tablelands of NSW (outlined below in sections 3.3, 3.4 and 3.5), castration was initially performed by transversely excising the distal third of the scrotal skin with a knife. Each testis was manually exteriorised by applying downward pressure and then pulling from the tunica vaginalis. The spermatic cord was then severed approximately 12 cm proximal to the head of the epididymis using a scraping motion. For the study conducted at the property in western Qld (outlined below in section 3.8), castration was performed by pushing the testicles to the distal end of the scrotum and incising the scrotum and tunica dartos with a scalpel blade, from the base dorsally on each lateral aspect of the scrotum. Each testis was manually exteriorised through the openings by applying downward pressure to expose the spermatic cords and these were severed approximately 10 cm proximal to the head of the epididymis using the scalpel blade. The knife and scalpel were chemically disinfected before and between each use.

3.1.2.3 Amputation dehorning

Amputation dehorning techniques varied between studies due to horn size variation between animals of different ages. For the study conducted at both the properties in the southern highlands and the southern tablelands of NSW (outlined below in section 3.6), dehorning was performed using a medium size scoop dehorning device (Barnes Dehorner; Bainbridge Pty Ltd, Murarrie, Qld, Australia). For the study conducted at the property in the southern tablelands of NSW (outlined below in section 3.7) and for the study conducted in western Qld (outlined below in section 3.8), dehorning was performed using a yearling cup dehorner (Dominion Yearling Cup; Bainbridge Pty Ltd, Murarrie, Qld, Australia). Dehorning was performed by placing the dehorning device over and to the base of the horn, then applying downward pressure and either opening (Barnes Dehorner) or closing (Dominion Yearling Cup) the handles to excise the horn and surrounding skin. The dehorning tools were chemically disinfected before and between each use.

3.1.2.4 Spaying via the Willis dropped ovary technique (WDOT)

Spaying was conducted using a large ovariotome (Willis Spay Tool; Bainbridge Pty Ltd, Murarrie, Qld, Australia). Firstly, the vulva was wiped clean, then the ovariotome, modified from its original design to allow delivery of topical anaesthetic (TA) (by addition of a narrow tube attached to the arm of the tool ebabling TA to be delivered to the head of the tool), was introduced into the vagina and inserted through the vaginal fornix into the caudal abdominal cavity. Each ovary was then manipulated by transrectal palpation, into the cutting slot of the ovariotome, then bluntly severed. The ovariotome was chemically disinfected before and between each use.

3.1.3 Sham husbandry procedures

Sham castration was performed by physically manipulating the scrotum without surgery. Sham dehorning was performed by placing the dehorner over the horn bud and applying light pressure to the surrounding skin, without penetrating and excising any tissue.

3.1.4 Pain relief treatments

3.1.4.1 Cryoanaesthetic

The cryoanaesthetic (CRYO) used in the study on ear tagging and ear notching (outlined below in section 3.2) and in the study on surgical castration (outlined below in section 3.3) was a topical vapocoolant spray (Animal Ethics Pty Ltd, Vic, Australia) comprising a hydrocarbon propellant in an aerosol canister. For ear tagging and ear notching, vapocoolant spray was applied to both sides of the ear at the relevant site of ear tagging or ear notching from a distance of 10 cm and for a duration of 3 seconds (s). This application method was based on the results of prior temperature validation studies (outlined below in sections 3.2.2.1 and 4.1.1). The ear was resprayed at each site prior to each procedure, i.e. three sprays per animal (notch, tag, tag). For surgical castration, vapocoolant spray was applied to the distal scrotum immediately prior to incision and then to each exposed spermatic cord following extrusion, prior to excision, from a distance of 10 cm for a duration of 3 s. This application method was based on the results of the study on ear tagging and ear notching (outlined below in sections 3.2.2.2 and 4.1.2) .

3.1.4.2 Local anaesthetic injection

The local anaesthetic (LA) injections used in the study on castration (outlined below in section 3.3) and the study on dehorning (outlined below in section 3.6) were lignocaine HCl (20 mg/mL) (Ilium Lignocaine 20 Local Anaeshetic Injection®; Troy Laboratories, Glendenning, NSW, Australia). For castration, 3 mL of LA were injected into each scrotal chamber and a further 3 mL into the spermatic cord, using a 10 mL syringe and an 18 G needle, 5 min prior to castration. For dehorning, 5 mL of LA was injected into the tissue in the vicinity of the cornual nerve of each horn using an 18 G needle inserted to a depth of 1 cm immediately behind the temporal ridge at a point midway between the lateral canthus of the eye and the base of the horn.

3.1.4.3 Topical anaesthetic

The topical anaesthetic (TA) used in the studies on castration (outlined below in sections 3.4 and 3.5), dehorning (outlined below in sections 3.6 and 3.7), concurrent castration and dehorning (outlined below in section 3.8) and spaying (outlined below in section 3.9) was a wound treatment with local anaesthetic formulation (Tri-Solfen®; Bayer Animal Health, Pymble, NSW, Australia) topically applied to wounds or tissue during or immediately following the procedures. Tri-Solfen® consists of lignocaine (40.6 g/L), bupivacaine (4.2 g/L), adrenaline (24.8 mg/L) and cetrimide (5 g/L) in a gel base. There were two novel TA formulations (Bayer Animal Health, Pymble, NSW, Australia) that were also used in the first study on dehorning (outlined in section 3.6). Both of these formulations contained 20% w/v lignocaine and 4% w/v bupivacaine. These novel formulations were specifically designed for application to amputation dehorning wounds where haemorrhage may affect absorption of anaesthetic agents. Higher concentrations of lignocaine and bupivacaine were included in these novel formulations with the intention of increasing the amount of active ingredients coming into contact with the tissue surface immediately upon application. The first formulation used an inert powder base as a carrier in an attempt to improve adherence to the wound. The second formulation used an ethanol/water base as a carrier designed to evaporate following application.

For dehorning, all formulations of TA were applied to wounds immediately post procedure, covering the entire wound and immediate surrounding skin. The powder was applied using a measuring spoon and the ethanol liquid and Tri-Solfen® were applied using a household

spray bottle. For each dehorning wound, 5 to 10 g of powder or 4 mL of the ethanol liquid or Tri-Solfen® was applied.

For castration, before removal of each testis the exposed testicular tissue was coated with TA by inserting the nozzle of a spray applicator along the spermatic cord inside the tunica vaginalis, into the vicinity of the exteral ring of the inguinal canal and applying 2 to 3 mL of TA. Another 2 to 3 mL of TA was also applied to the cut skin edge of the scrotum.

For spaying, the ovaritome was modified with the addition of a hollow steel tube (3 mm outside diameter) running alongside the rod. The tube had an opening at both ends (near the handle and at the cutting slot) to allow delivery of TA. Topical anaesthetic was applied, using a 15 mL syringe, by squirting the product into the opening near the handle of the ovariotome. 2 mL of TA was applied to the vaginal fornix prior to penetration and a further 2 mL was applied to each ovarian pedicle whilst in the cutting slot of the ovariotome prior to excision.

3.1.4.4 Buccal non-steroidal anti-inflammatory drug

Buccal meloxicam (10 mg/mL) (BM) in a gel base (Ilium Buccalgesic OTM®; Troy Laboratories, Glendenning, NSW, Australia) was the non-steroidal anti-inflammatory drug (NSAID) product used in the studies on castration (outlined below in section 3.5), dehorning (outlined below in section 3.7) and concurrent castration and dehorning (outlined below in section 3.8). For all studies, the BM was administered (0.5 mg/kg body weight) into the oral cavity adjacent to the upper molar teeth, using a hooked nozzle, for absorbtion through the buccal mucosa.

3.1.4.5 Subcutaenous non-steroidal anti-inflammatory drug

Subcutaneous meloxicam (20 mg/mL) (SCMEL) (Metacam® 20 mg/mL Solution for Injection; Boehringer Ingelheim Pty Ltd, North Ryde, NSW, Australia) was the NSAID product used in the study on spaying (outlined below in section 3.9). For this study, SCMEL was administered (0.5 mg / kg body weight) under the skin of the rump.

3.2 Effect of topical vapocoolant spray on perioperative pain response of unweaned calves to ear tagging and ear notching

3.2.1 Animals and location

Twenty unweaned, Angus calves (10 males, 10 females), aged 3 to 4 months, were randomly selected from a commercial beef herd at the University of Sydney owned property ‘Arthursleigh’ in the southern tablelands of NSW, Australia.

3.2.2 Experimental design and treatments

3.2.2.1 Temperature validation studies

Temperature validation studies were conducted on dead and live tissue to determine the ability of the vapocoolant spray to reduce tissue temperature to below 10 °C and to measure the duration of the cooling..The head of a single Holstein Friesian calf that had died at birth was sourced from the University of Sydney owned property ‘Corstorphine’ near Camden, NSW. Two K-type temperature thermocouples attached to a dual-channel thermometer data logger (Yu Ching Technology Co. Ltd, Taipei city, Taipei, Taiwan) were validated against a mercury thermometer using a water bath. The water bath was heated to 50 ± 10 °C.

To assess temperature conduction of the tissue in response to the vapocoolant spray, the K-type thermocouples were: (1) sutured onto the surface of the ear to assess cooling of skin surface; and (2) inserted (using a 16 gauge needle) beneath the epidermis on each side of the ear to assess penetration through the skin and cartilage. The calf's head was then placed into a plastic bag (with thermocouple CDections to the data logger remaining outside the bag), vacuum-sealed, and tied and secured with string. The bag was then lowered into the water bath until the ear pinnae reached a temperature of approximately 30 °C (the estimated ear pinnae temperature of a live calf) as indicated by the data logger. The head was then removed from the water bath and bag, and a vapocoolant spray was applied from a distance of 10 cm (Fjordbakk and Haga 2011) to each thermocouple site with four spray durations: 1, 2, 3 and 5 seconds. The head was re-warmed in the water bath between spray applications.

Following successful temperature studies in dead tissue, live tissue testing was conducted, with consideration of the effect of tissue perfusion on efficacy of the vapocoolant spray. A 4-month-old Angus calf was restrained in a 'swing-away' calf cradle (Arrow Farmquip, Tamworth, NSW, Australia) in right lateral recumbency. An area of 5 cm^2 on both sides of the ear was infiltrated with 3 mL lidocaine hydrochloride (Ilium Lignocaine 20 Local Anaeshetic Injection ®; Troy Laboratories, Glendenning, NSW, Australia) to anaesthetize the tissue. A 16 gauge needle was then used as a catheter to insert the thermocouple into the subdermal tissue. The vapocoolant spray was applied from a distance of 10 cm directly onto the area of skin where the thermocouple was inserted. Following consideration of the results obtained from the dead tissue study, spray duration times of 1, 2 and 3 seconds were assessed. The ear temperature was allowed to return to normal (~30–35 °C) between spray applications. Following the experiment, the thermocouples were removed, the ear cleaned with antiseptic (Hibitane; Coopers Animal Health, Macquarie Park, NSW, Australia) and the calf returned to its mother.

3.2.2.2 Behavioural study

On the day of the trial, calves were separated from their mothers into male and female groups and allowed to settle for 1 hour. The calves were then moved calmly through the race towards the calf cradle for restraint. The race was curved with high, solid walls to reduce the impact of external stressors. Cattle prodders, dogs and aggressive stockmanship were avoided to minimise stress.

Calves were restrained in right lateral recumbency in the calf cradle and blindfolded with a blacked-out pony fly mask (Roma® 'Buzz Away' fly mask; WeatherBeeta, Melbourne, Vic, Australia) to minimise the reaction to the stockman's approach. Calves were randomly allocated to one of two treatments: application of the vapocoolant spray (VS; the test group) or an aerosol water spray (WS; the negative control group) (Thermal Water Spray; Avène, North Sydney, NSW, Australia) to the ear tagging and ear notching sites prior to the procedure. Three procedures were conducted on each animal: (1) ear tagging with property's National Livestock Identification System (NLIS) tag in the right ear; (2) ear tagging with the animal identification tag in the left ear; and (3) ear notching (right ear for females, left ear for males). Therefore, each animal received two tags (one in each ear) and one ear notch, although the order of procedures was randomised for each animal. Treatment did not commence until the calf settled (not moving or vocalising). Application of the water spray

was conducted as per the application of the vapocoolant spray (outlined above in section 3.1.3.1).

3.2.3 Measurements

3.2.3.1 Ear temperature (Temperature validation studies)

For both dead and live tissue, the data logger recorded the change in temperature at 1 second intervals over a period of 1 minute after each vapocoolant spray application. Data were uploaded to Microsoft Office Excel via the Temp Monitor S2 Software (Yu Ching Technology Co. Ltd, Taipei city, Taipei, Taiwan) for analysis.

3.2.3.2 Behavioural scoring (Behavioural study)

A video camera was mounted on a tripod facing the experimental area to record the animals' responses for behavioural scoring. The video was recorded for the duration of the procedure only (~1–2 minutes per calf), and it was stopped once the calf was released. Videos were edited prior to scoring to remove the treatment from the videos so that only the procedure and animal response were visible to the observer. The tag number was visible, allowing each individual animal to be identified. Following the trial, behaviour was scored by an experienced observer. The videos were played in a random sequence. Pain response scores for each separate procedure in each calf were assigned using a categorical numerical rating scale (NRS) [with 0 = zero movement; 1 = mild movement (mild head and/or body movement, including ear and/or tail flick, wince or nasal flare); 2 = moderate movement (moderate head and/or body movement, including head shake, twisting, mild kicking and mild vocalisation); and 3 = severe movement (severe head and/or body movement including kicking, full head movement from cradle and severe escape response, bellowing)]. The scale was adapted from previous studies (Meyer *et al.* 2007; Lomax *et al.* 2008; Fjordbakk and Haga 2011; Lomax and Windsor 2014).This resulted in a total of 40 tag response scores and 20 notch response scores (Table 3.2.1).

Table 3.2.1 Number of scores obtained from 20 calves, subdivided into categories used for analysis (sex, procedure, side and treatment)

Variable	Category	Frequency	Percentage
Treatment	Control	30	50
	Vapocoolant spray	30	50
Sex	Female	30	50
	Male	30	50
Procedure	Notch	20	33
	Tag	40	67
Side	Left	20	50
	Right	20	50

3.2.3.3 Postoperative inspection for tissue damage (Behavioural study)

All animals were re-mustered 1 week after the procedures, with each calf restrained in a head bail and their ears examined by a registered veterinarian for any signs of tissue damage. In particular, tissues were assessed for clinical signs of frostbite, including oedema or inflammation, demarcated limits, discoloration of the skin (although this is difficult to see in black Angus calves), and necrosis or sloughing of the skin (Cruz and Naylor 1993).

3.2.4 Statistical analysis

Descriptive analyses of data were conducted using Genstat® statistical software (15th edition; VSN International Ltd, Hemel Hempstead, Hertfordshire, UK). Data were analysed by ordinal logistic regression (OLR) using SAS statistical software (release 9.2; SAS Institute Inc., Cary, NC, USA). The fixed effects considered for the final model were treatment, sex, procedure and side. Random effects (animal identification) were included to account for the multiple measurements taken on each animal (one ear notch, two ear tags). A univariate OLR analysis was conducted to evaluate the association of each individual variable with the ordinal outcome. Variables with $p < 0.25$ were included in multivariate analyses. This p value was selected as a cut-off point so as not to exclude any scientifically relevant variables, while avoiding inclusion of any unnecessary variables (Mickey and Greenland 1989; Bursac *et al.* 2008). Multivariate models were built using a stepwise approach. First order interactions were tested between all significant variables and retained if significant; $p \leq 0.05$ was considered significant for statistical associations. Cumulative probabilities were obtained from the final model and plotted using Microsoft Office Excel for presentation of results.

3.3 Effect of lignocaine and a topical vapocoolant spray on the pain response to surgical castration in beef calves

3.3.1 Animals and location

Forty unweaned, Angus bull calves, aged 2 to 4 months and weighing 103.5 ± 30.2 kg, were randomly selected for the trial from a commercial herd at the University of Sydney owned property, ‘Arthursleigh’ in the southern tablelands of NSW, Australia. The calves were routinely ear tagged and ear notched one week prior to the trial.

3.3.2 Experimental design and treatments

On the day of the trial, calves were separated from their mothers and held for 1 h in a yard adjacent to the cattle race. The calves were quietly moved through the race towards a calf cradle where they were weighed using cattle scales (Weigh scale and data recorder W810; Gallagher Group Ltd, Hamilton, Waikato, New Zealand) within the race and then restrained in right lateral recumbency in a ‘swing-away’ calf cradle (Arrow Farmquip, Tamworth, NSW, Australia) for treatment and data collection.

The calves were randomly allocated to one of four treatments: (1) sham castration (SHAM, n = 10); (2) surgical castration (CAST, n = 10); (3) surgical castration following pre-operative injections of local anaesthetic lignocaine (Ilium Lignocaine 20®, Troy Laboratories, Glendenning, NSW, Australia) (LIG, n = 10); and (4) surgical castration following pre-operative application of topical vapocoolant spray (Animal Ethics Pty Ltd, Vic, Australia)

(VAPO, n = 10). The castration procedure took approximately 30 to 40 s. For SHAM calves, testes were physically manipulated for 30 s with no surgical intervention.

3.3.3 Measurements

3.3.3.1 Behavioural scores

A video camera mounted on a tripod was used to film the behavioural responses of each calf to the procedures. Ear tag numbers were visible in the videos, allowing for individual animal identification. Each video was later scored individually by two trained observers. There were two scores that differed between observers. These scores were reassessed following discussion, resulting in agreement between both observers for all scores. Behavioural responses were scored on a numerical rating scale of 0 to 3, taken from the previous study on the behavioural response to ear tagging and ear notching (outlined above in section 3.2) and were as follows: 0 = no movement; 1 = mild movement (mild head and/or body movement, including ear and/or tail flick, wince or nasal flare); 2 = moderate movement (moderate head and/or body movement, including head shake, twisting, mild kicking and mild vocalisation); and 3 = severe movement (severe head and/or body movement including kicking, full head movement from cradle and severe escape response, bellowing). An individual score was assigned to each consecutive stage of the castration procedure: (1) excision of scrotum; (2) extrusion of right spermatic cord; (3) severing of right spermatic cord; (4) extrusion of left spermatic cord; and (5) severing of left spermatic cord. This resulted in a total of five scores for each calf. For SHAM calves, scores for each of these stages were assigned throughout the sham castration procedure at an estimated time-point at which they were likely to occur, based on the average timing of the stages in the video recordings of the castration procedure.

3.3.3.2 Ocular temperature

Infrared photographs of the left eye were captured from calves using a handheld infrared camera (FLIR®E50; FLIR Systems, Inc., Mulgrave, Vic, Australia), with a thermal range of -20°C to 120°C and a sensitivity of 0.045°C. Infrared photographs were taken at three time-points whilst calves were restrained in lateral recumbency in the calf cradle; immediately following restraint, immediately following administration of lignocaine or vapocoolant spray (or 1 min after the first photograph for SHAM and CAST calves) and immediately following sham castration or castration. There was approximately one min between each time-point. A 10 x 10 cm cardboard frame was used to standardise the image area by holding it over the eye with the eye in the centre. The camera frame was then aligned with the cardboard frame for each photograph. This ensured the camera lens was at a consistent distance of 0.5 m from the eye. This distance, along with an emissivity value of 0.95 were entered into the infrared camera for calibration. Ambient temperature and humidity was monitored and entered into the infrared camera for calibration every 30 min during the data collection period. Images were analysed for maximum temperature using a thermal imaging software program (FLIR® Tools Software; FLIR Systems, Inc., Mulgrave, Vic, Australia). This software allowed for analysis of a specific area using a geometric figure drawn on the photograph. A circle figure was drawn around the eye in each photograph and the maximum temperature within this area was calculated.

3.3.4 Statistical analysis

Behavioural score data were subjected to ordinal logistic regression (OLR) in ASReml® 3 statistical software (VSN International, Hemel Hempstead, Hertfordshire, UK). The fixed effects of this model were treatment x stage of procedure and body weight. Maximum ocular temperature data was subjected to restricted maximum likelihood (REML) for repeated measures using the mixed models procedure of Genstat® statistical software (17th edition; VSN International Ltd, Hemel Hempstead, Hertfordshire, UK). Correlations were utilised to identify any collinear effects of which only one was selected for the model. Data on ambient temperature and ambient humidity were subjected to a Spearman's rank correlation using the nonparametric correlations procedure of Genstat®. A strong negative correlation (R = -0.92) was identified, therefore only ambient temperature was included in the model. The fixed effects of this model were treatment x time-point, body weight and ambient temperature. The random effect for both models was calf ID. Insignificant terms were dropped from the models using a backwards elimination approach. Significant variates in the fixed model for maximum ocular temperature were subjected to a Spearman's rank correlation with maximum wound temperature using the nonparametric correlations procedure of Genstat®. Data from the OLR analyses are presented as cumulative odds ratios with the statistical probabilities of calves displaying behavioural response scores of Y = 0, 1, 2 and 3. Data from the REML analyses are presented as predicted means (± standard error of the mean). For all statistical calculations, P values ≤ 0.05 were considered statistically significant and P values ≤ 0.1 were considered statistical tendencies. Where significant effects were found, post-hoc pair-wise comparisons using least significant differences at a level of $P \leq 0.05$ were conducted to analyse differences between groups. For ordinal data, individual odds ratios were generated for significant factors in R 3.4.3 statistical software (R Core Team 2017).

3.4 Effect of a topical anaesthetic formulation on the cortisol response to surgical castration of unweaned beef calves

3.4.1 Animals and location

Twenty-four unweaned, Angus bull calves, aged 3 months and weighing 77 to 102 kg, were randomly selected from a commercial beef herd at the University of Sydney owned property 'Arthursleigh'in the southern tablelands of NSW.

3.4.2 Experimental design and treatments

Calves were held with their mothers for 5 days before the experiment in a 4 ha paddock, adjacent to the cattle handling facilities. During this time, cows and calves had *ad libitum* access to water and pasture. Cows and calves were supplemented with lucerne hay daily due to low pasture levels in the holding paddock and to encourage a positive association with the experimental environment. Calves were ear tagged 2 days before experimentation and weighed using cattle scales (Weigh scale and data recorder W810; Gallagher Group Ltd, Hamilton, Waikato, New Zealand). Before ear-tagging, calves had not been separated from their mothers and had minimal exposure to humans. Calves were habituated to movement through handling facilities twice daily (at 0930 and 1600 h) for 4 days before experimentation. Cows and calves were mustered into a holding yard and quietly moved through the race with their mothers; 1 day before experimentation, calves were restrained in the cattle crush

('Ultimate' Crush; RPM Rural Products, Gatton, Qld, Australia) for 2 min before exiting the race. Restraint involved manually catching the calf in the head bale in a standing position, and applying the squeeze on the chute to reduce movement. This emulated how the calves would be handled during the trial for treatment and blood collection. Cows and calves were released into the 4 ha paddock between habituation periods.

Calves were randomly allocated to one of three treatment groups: (1) sham castration/control (CON, n = 8); (2) surgical castration (C, n = 8); and (3) surgical castration with post-operative application of topical anaesthetic (Tri-Solfen®; Bayer Animal Health, Pymble, NSW, Australia) (CTA, n = 8). The experiment was conducted over 2 days, with 4 calves from each treatment group treated on each day. On each day, cows and calves were moved from the paddock into the holding yard adjacent to the cattle race. Calves were separated from their mothers into a separate holding pen, and the cows were released back into the paddock. Calves were moved through the race, restrained in the head bale and released after treatment and blood sampling for every time-point. Calves generally moved through the race well. If required, calves were gently touched on the back to encourage movement. Incorporated within the race were manual slide gates, which were used to separate calves. This avoided over-filling of races and facilitated with ordering of animals. The random order of treatments was predetermined before calves entering the race using the animals' identification numbers.

All calves were treated within a 0.5 h time period, between 1000 and 1030 h on each of the 2 experimental days. For castration, the side gate of the crush was opened after the calves were restrained in the head bale. A single, experienced operator manually restrained the calves in a standing position while performing the procedure. Calves were castrated standing up, instead of employing the use of a calf cradle, to eliminate any potential stress associated with lateral recumbency (Tagawa *et al.* 1994; Pesenhofer *et al.* 2006).

3.4.3 Measurements

3.4.3.1 Cortisol

Calves were numbered (1 to 24) on each flank with white road marking spray paint at the first blood sample collection to facilitate ordering of the calves for each sampling time-point. Calves were always sampled in the same order. Blood samples (~4 to 9 ml) were collected into 9 mL EDTA vacutainers (Vacuette®, Interpath Services Pty Ltd, West Heidelberg, Vic, Australia) via jugular venipuncture within 2 min of securing the calves in the head bale and manually restraining their heads. Samples for baseline cortisol were drawn 0.5 h and immediately (0 h) before treatment. Thereafter, samples were drawn at 0.5, 1, 1.5, 2, 4 and 6 h post-treatment. The first and last blood samples were collected at 0930 and 1600 h, respectively, on each day. Calves were kept as a group in the holding yard adjacent to the cattle race between sampling time-points where they had *ad libitum* access to water and lucerne hay and where they could see and hear their mothers through a fence. Blood samples were placed on ice immediately after collection and stored until centrifugation. Blood samples were centrifuged within 4 h of collection at 3000 r.p.m. for 15 min. The plasma component of the samples was separated using 1 mL sterile pipettes and stored in 2 mL collection vials at −20 °C. Plasma cortisol concentrations were determined using a commercially available radio-immunoassay kit (Coat-A-Count Cortisol RIA; Siemens Pty Ltd, Los Angeles, CA, USA). The inter-assay and intra-assay coefficients of variation were 5.05% and 5.15%, respectively.

3.4.4 Statistical analysis

GenStat® statistical software (VSN International Ltd, Hemel Hempstead, Hertfordshire, UK) was used to conduct all statistical analyses and generate least significant difference (LSD) values. Data on cortisol concentrations were subjected to residual maximum likelihood (REML) for repeated measures. The fixed effects of the model were treatment, time, day, and body weight. The random effect of the model was calf. The integrated cortisol response, or area under the curve (AUC−0.5 to 6), was calculated for each calf and then analysed using a one-way analysis of variance (ANOVA). The suitability of the AUC data for parametric ANOVA was tested using a probability plot of the residuals to determine the normality of the data, and a plot of residuals against fitted values to determine the homogeneity of variance. For all statistical calculations, $P \leq 0.05$ was considered statistically significant and $P \leq 0.05 \leq 0.1$ were considered statistical tendencies. Differences between means were considered statistically significant if they were greater than the generated LSDs.

3.5 Effects of topical anaesthesia and buccal meloxicam on average daily gain, behaviour and inflammation of unweaned beef calves following surgical castration

3.5.1 Animals and location

A total of 50 unweaned Angus bull calves, aged 2 to 4 months and weighing 107.68 ± 26.30 kg, were sourced from a commercial herd on the University of Sydney owned property, ‘Arthursleigh’ in the southern tablelands of NSW, Australia. Calves had previously been ear tagged and ear notched one week prior to the trial. Before and during the experimental period, calves and their mothers were held in a 10 ha paddock adjacent to the cattle handling facilities and had *ad libitum* access to water and pasture. On each day of the trial, calves were drafted from their mothers into the cattle yards, and held in groups of 25 in two holding pens that led into the cattle race. Calves were released back into the paddock with their mothers upon conclusion of data collection each trial day.

3.5.2 Experimental design and treatments

The calves were randomly allocated to one of five treatment groups in the order that they came first came through the race by use of computer generated random numbers using Microsoft Office Excel: (1) sham castration (SHAM, n = 10); (2) surgical castration (C, n = 10); (3) surgical castration with pre-operative administration of buccal meloxicam (Ilium® Buccalgesic OTM; Troy Laboratories, Glendenning, NSW, Australia) (CBM, n = 10); (4) surgical castration with post-operative application of topical anaesthetic (Tri-Solfen®; Bayer Animal Health, Pymble, NSW, Australia) (CTA, n = 10); and (5) surgical castration with pre-operative administration of buccal meloxicam and post-operative application of topical anaesthetic (CBMTA, n = 10).

The trial was conducted over 7 days, with all calves treated on day 0 and observations recorded on days 0, 1, 2 and 6 relative to treatment. On day 0, calves were drafted through the race twice. The initial draft included collection of body weight data and administration of BM to CBM and CBMTA calves. This enabled administration of BM 25 min prior to castration as per label instructions. Calves were also spray painted with an identification number (1 to 50) on both sides and the back of the body at this point. On the second draft, calves were sham castrated or castrated. CTA and CBMTA calves were also treated with TA at this point.

On other days, calves were drafted through the race once for data collection. To facilitate animal handling, the calves were restrained in lateral recumbency in a ‘swing-away’ calf cradle (Arrow Farmquip, Tamworth, NSW, Australia) for procedure and treatment on day 0, and for data collection on days 1, 2 and 6.

3.5.3 Measurements

3.5.3.1 Average Daily Gain

Calves were weighed in a cattle crush using cattle scales (weigh scales and a data recorder W810; Gallagher Group Ltd, Hamilton, Waikato, New Zealand), prior to restraint in the calf cradle. Average daily gain was calculated for each calf using the difference from the pre-treatment weight collected on day 0 and dividing by the number of days since day 0.

3.5.3.2 Behaviour

Each calf was released into a yard (10 m x 25 m) adjacent to the cattle handling facilities immediately following treatment on day 0 for 5 h and provided ad libitum access to water and lucerne hay. Six video cameras (HD 1080p Sports Action Cam; Sony Australia Ltd, North Sydney, NSW, Australia), were attached at various points around the yard to record the calves from numerous angles. The videos were later analysed using continuous sampling of the frequency or duration of specified behaviours displayed by each calf within a 5-min focal period. This was repeated every hour for 5 h following treatment, resulting in a total of 25 min of observation for each calf. The frequency or duration of specific behaviours was recorded by two trained observers blinded to treatment, using an observational data software package (The Observer® XT 12; Noldus Information Technology, Wageningen, The Netherlands), with an ethogram designed using this software. Each observer recorded the behaviour of 5 calves from each treatment group, to minimise any potential effect of observer bias. The ethogram was derived from previous published studies on surgical castration (Ting et al., 2003, Petherick et al., 2015). Behaviours were categorised as states or points (Table 3.5.1); behavioural states were recorded as the total duration (s) and point behaviours were recorded as the total frequency.

Table 3.5.1 Ethogram for continuous observations conducted on each calf during 5-min focal periods every hour for 5 h following treatment

Behaviour	Description
States[1]	
Walk	Walking forwards or backwards in any style at any pace (the sum of 'walk relaxed', 'walk with a stiff gait', and 'walk with a limp')
Walk relaxed	Walking with muscles relaxed
Walk with a stiff gait	Walking slowly with muscles stiff
Walk with a limp	Walking slowly with a limp
Stand	Standing in any style (the sum of 'stand relaxed' and 'stand statue')
Stand relaxed	Standing passively or actively with head held relaxed and muscles relaxed
Stand statue	Standing stationary with muscles stiff and head held below brisket
Lie	Lying down completely on the ground in any style (the sum of 'lie normal' and 'lie abnormal')
Lie normal	Lying in a normal posture (ventral position and no extension of limbs)
Lie abnormal	Lying in an abnormal posture (lateral recumbency, one or both hind limbs extended > 90°, both forelimbs extended)
Arch back	Curving of the spine
Scratch	Raising a hind leg and scratching part of the body or scratching body against the yard fence
Lick	Turning head back and licking body with lips or tongue, or both
Eat	Ingesting lucerne hay
Drink	Ingesting water
Points[2]	
Lick wound	Licking of scrotal area whilst lifting a hind limb
Stamp	Lifting front or hind foot and forcefully placing it on the ground
Kick	Kicking backward or towards the belly with a hind limb
Ease quarters	Shifting body weight from one side of body to the other whilst standing
Flick tail	Sideways movement of the tail from vertical to return to vertical
Flick ear	Quick movement of one or both ears

[1] States are behaviours with measurable duration and are quantified by duration of time (s).
[2] Points are behaviours without measurable duration and are quantified by frequency.

3.5.3.3 Scrotal diameter

Scrotal diameters (mm) of all castrated calves were measured on days 1, 2 and 6 of the trial using digital calipers (Budget 150 mm digital vernier calipers, Jaycar Electronics, Rydalmere, NSW, Australia) to evaluate oedema as an indicator of inflammation. The tips of the calipers were adjusted to measure the lateral distance between the two points of raised tissue furthest from the midline of the scrotum.

3.5.3.4 Maximum scrotal temperature

To measure scrotal surface temperature, infrared photographs of the scrotal area were captured from all castrated calves on days 1, 2 and 6 of the trial using a handheld infrared camera (FLIR®E50; FLIR Systems, Inc., Mulgrave, Vic, Australia), with a thermal range of -20°C to 120°C and a sensitivity of 0.045°C. A 10 cm x 10 cm cardboard frame was used to standardise the image area for each photograph. The camera frame was aligned with the cardboard frame and held above the scrotal area with the scrotum in the center for each photograph. This ensured the camera lens was at a consistent distance of 0.5 m from the scrotal area for each image. This distance, along with an emissivity value of 0.95 were entered into the infrared camera for calibration. The camera lens was focused appropriately for each photograph. The quality of each photograph was checked by the operator on the screen of the camera immediately after it was taken, allowing for additional photographs to be taken if necessary. Ambient temperature and humidity were monitored and recorded at the time each photograph was captured and were entered into the infrared camera for calibration every 30 min during the data collection period. Images were analysed for maximum temperature using a thermal imaging software program (FLIR® Tools Software; FLIR Systems, Inc., Mulgrave, Vic, Australia). This software allowed for analysis of a specific area using a geometric figure drawn on the photograph. A square was drawn immediately inside the cardboard frame in each photograph and the maximum temperature within this area was calculated.

3.5.3.5 Wound morphology score

Digital photographs of the scrotal area were taken from all castrated calves on days 1, 2 and 6. These photographs were later scored for visible evidence of inflammation and healing using a customised numerical rating scale of 1 to 5 described in Fig. 3.5.2.

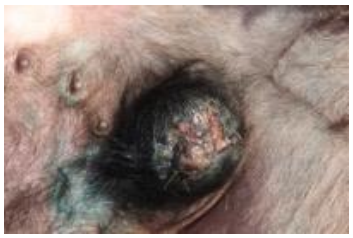
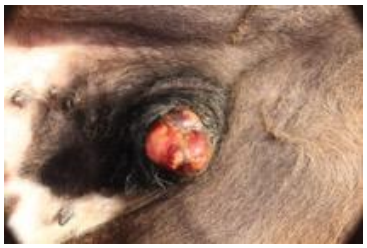
Score	Example	Wound description
1		Focal mild scrotal wound dermatitis with complete closure of the incision and absence of exudate and exposed underlying tissue
2		Focal mild scrotal wound dermatitis with incomplete closure of the incision and absence of exudate and exposed underlying tissue
3		Focal moderate scrotal wound dermatitis with incomplete closure of the incision, presence of some exudate, but absence of exposed underlying tissue
4		Focal to locally extensive moderate scrotal wound dermatitis with incomplete closure of the incision, presence of exudate, and limited extrusion of underlying tissue
5		Locally extensive moderate to severe scrotal wound dermatitis with incomplete closure of the incision, presence of exudate, and extensive exposure of underlying tissue

Figure 3.5.1 Customised numerical rating scale used to score calf castration wound morphology.

3.5.4 Statistical analysis

Data on ADG, each behavioural state (Table 3.5.1), scrotal diameter and maximum scrotal temperature were subjected to restricted maximum likelihood (REML) for repeated measures using the linear mixed models procedure in Genstat® statistical software (17th Edition; VSN International Ltd, Hemel Hempstead, Hertfordshire, UK). Data on each point behaviour (Table 3.5.1) was subjected to REML using the generalised linear mixed models (GLMM)

procedure with a poisson distribution. For ADG, the fixed effects of the model were treatment x day, treatment and day. For each behaviour (Table 3.5.1), the fixed effects of the model were treatment x time-point, treatment, time-point and body weight (day 0). Data on ambient temperature and ambient humidity was subjected to a nonparametric Spearman's rank correlation. A strong negative correlation (R = -0.84) was identified, therefore only ambient temperature was included in the model for scrotal diameter and maximum scrotal temperature. For scrotal diameter and maximum scrotal temperature, the fixed effects of the model were treatment x day, treatment, day, body weight (day 0) and ambient temperature. The reason body weight (day 0) and ambient temperature were included in the models as fixed effects, was because the assumption was made that there may be a correlation between these variates and the independent variables measured. The random effect of all models was calf ID. Due to the significance of body weight in the models for scrotal diameter and maximum scrotal temperature, data on body weight and scrotal diameter or maximum scrotal temperature were subjected to a nonparametric Spearman's rank correlation. Wound appearance scores were subjected to ordinal logistic regression (OLR) in ASReml® 3 statistical software (VSN International, Hemel Hempstead, Hertfordshire, UK). The fixed effects of the model were treatment x day, treatment, day and body weight (day 0) and the random effect of the model was calf ID. Insignificant fixed effects were dropped from all models in Genstat® and ASReml® using a backwards elimination approach. Data from the REML analyses are presented as predicted means ± s.e.m. Data from the OLR analysis are presented as cumulative odds ratios with the statistical probabilities of wounds having inflammation scores of Y = 1, 2, 3, 4 and 5. For all statistical calculations, P values ≤ 0.05 were considered statistically significant and P values > 0.05 and ≤ 0.06 were considered trends. Where significant effects were found, post-hoc pair-wise comparisons using least significant differences (LSDs) were conducted to analyse differences between groups.

3.6 Effect of topically applied anaesthetic formulation on the sensitivity of scoop dehorning wounds in calves

3.6.1 Animals and location

The study involved two trials on separate groups of calves. In trial 1, 21 female, horned, Holstein-Friesian calves, aged 8 to 24 weeks, were sourced from a commercial dairy herd at a property in the southern highlands of NSW, Australia. In trial 2, 18 castrated male and 18 female, unweaned, horned, Hereford calves, aged 16 to 20 weeks, were sourced from a commercial beef herd at a property in the southern tablelands of NSW, Australia.

In trial 1, calves were moved from group pens into a holding pen adjacent to the cattle handling facilities prior to experimentation, where they remained for the duration of the trial. The calves in this trial had been separated from their mothers at birth and hand reared as per routine dairy farm practice.

In trial 2, calves were moved from the paddock into a holding pen adjacent to the cattle handling facilities 2 days before the trial, where they were habituated to movement through handling facilities twice daily for 2 days before experimentation. Other than during time when moved through the handling facilities, the calves had access to their dams.

3.6.2 Experimental design and treatments

3.6.2.1 Trial 1

The trial was conducted over 1 day. On the day of the trial, calves were moved one at a time through the race and restrained in a head bale (Australian Stockyard Co, Goulburn, NSW, Australia) for treatment and data collection. Calves were blocked by age and randomly allocated to one of two treatments by use of random numbers generated in Microsoft Office Excel: (1) scoop dehorning with post-operative application of a novel topical anaesthetic powder (Bayer Animal Health, Pymble, NSW, Australia) (DTAP, n = 10); and (2) scoop dehorning with post-operative application of a novel topical anaesthetic ethanol liquid (Bayer Animal Health, Pymble NSW Australia) (DTAE, n = 11). The procedure of dehorning and applying the ethanol spray or the powder took approximately 15 s or 30 s per animal, for each of these products respectively. Data was collected immediately prior (0 h) to treatment, then 1 min, 90 min and 180 min post treatment. Between data collections, calves were released into a holding yard.

3.6.2.2 Trial 2

The trial was conducted over 2 days. On each day of the trial, 18 calves were moved one at a time through the race and restrained in a head bale (Australian Stockyard Co, Goulburn NSW Australia) for treatment and data collection. Calves were blocked by age, sex and day of trial and randomly allocated to one of four treatments by use of random numbers generated in Microsoft Office Excel: (1) scoop dehorning with a pre-operative cornual nerve block of lignocaine (Ilium® Lignocaine 20 Local Anaeshetic Injection; Troy Laboratories, Glendenning, NSW, Australia) (DCB, n = 9); (2) scoop dehorning with post-operative application of the ethanol liquid from Trial 1 (Bayer Animal Health, Pymble, NSW, Australia) (DTAE, n = 9); (3) scoop dehorning with post-operative application of a topical anaesthetic gel (Tri-Solfen®; Bayer Animal Health, Pymble, NSW, Australia) (DTAG, n = 9) and (4) sham dehorning (CON, n = 9). Calves in the DCB group were administered a cornual nerve block 15 min prior to dehorning and anaesthesia of the horn area was confirmed by the pinprick test with an 18 G needle immediately prior to dehorning. The procedure of dehorning and applying the ethanol liquid or the gel took approximately 15 s per animal. Data was collected immediately prior (0 h) to treatment, then 1 h, 2 h, 4 h and 6 h post treatment. Between data collections, calves were released into a holding yard.

3.6.3 Measurements

3.6.3.1 Wound sensitivity

Nociceptive and anti-nociceptive responses were noted in both trials. Mechanical stimulation of the horn or wound was performed using von Frey monofilaments (Touch-Test1 Sensory Evaluators; North Coast Medical and Rehabilitation Products, Morgan Hill, CA, USA). This was performed at two sites on the immediate edge of the horn base or wound (area 1) and two sites on the skin surrounding the horn or wound (area 2). Area 2 sites were 2 cm from the edge of the horn base or wound. Von Frey monofilaments are calibrated to bend at a pre-determined pressure. A 75 g/f (light touch) and 300 g/f (pain) monofilament were used to determine allodynia and hyperalgesia, respectively. Side (left or right horn) and site were randomised for each repeated measure. Calves were blindfolded during measurement to eliminate visual stimuli and reduce stress and consequent struggling behaviours. Sensitivity was assessed by scoring the behavioural responses of the calves to mechanical stimulation

on a numerical rating scale of 0 to 3 adapted from (Espinoza *et al.* 2013) whereby: 0 = no response; 1 = mild response including minor withdrawal reflex such as a slight head movement or an ear flick; 2 = moderate response including partial withdrawal reflex such as partial head rotation; and 3 = severe response including full withdrawal reflex such as full head jerk or rotation.

3.6.4 Statistical analysis

All data was analysed using ordinal logistic regression (OLR) in ASReml® 3 statistical software (VSN International Ltd, Hemel Hempstead, Hertfordshire, UK). For trials 1 and 2, the fixed effects of the OLR model were treatment x time, area and von Frey. In trial 1, calf ID was included as a random effect. In trial 2, calf ID, day of trial, age and sex were included as random effects. Data is presented as cumulative odds ratios with the statistical probabilities of calves in each treatment group displaying response score Y = 0, 1, 2 and 3. For all statistical calculations, P values ≤ 0.05 were considered statistically significant.

3.7 Evaluating treatments with topical anaesthesia and buccal meloxicam on pain and inflammation caused by amputation dehorning of calves

3.7.1 Animals and location

Twenty-one castrated male and 29 female, unweaned, Hereford calves, aged 6 to 8 months and weighing 235.67 ± 44.83 kg, were sourced from a commercial beef property in the southern tablelands of NSW, Australia. Steers had been castrated at 3 to 4 months of age. All castration wounds were fully healed by the time this study commenced. Before and during the experiment, calves and their mothers were held in a paddock adjacent to the cattle handling facilities. Both cows and calves had *ad libitum* access to water and pasture.

3.7.2 Experimental design and treatments

Calves were blocked by sex and randomly allocated to one of five treatment groups by use of random numbers generated in Microsoft Office Excel: (1) sham dehorned / control (CON, n = 14); (2) dehorned (D) (D, n = 12); (3) dehorned with pre-operative administration of buccal meloxicam (Ilium® Buccalgesic OTM; Troy Laboratories, Glendenning, NSW, Australia) (DBM, n = 12); and (4) dehorned with post-operative application of topical anaesthetic (Tri-Solfen®, Bayer Animal Health Australia, Pymble, NSW, Australia) (DTA, n = 12). Upon inspection at the point of randomisation and treatment, 7 calves were identified as being polled. These calves were allocated to the control treatment group that did not require dehorning.

The trial was conducted across 8 days in summer, with observations conducted on days 0, 1, 3 and 7 following treatment. Calf handling required that the calves were drafted from their mothers into one of three smaller holding yards adjacent to the cattle race, then drafted through the race and restrained in a head bale (Australian Stockyard Co, Goulburn, NSW, Australia) for treatment on day 0 and for data collection on days 1, 3 and 7. The calves were then released into the paddock with their mothers following data collections. On day 0, the calves were processed through the race twice. At the initial draft, the calves were ear tagged, weighed and spray painted with an identification number (1 to 50) on both sides and the back of the body, with BM administered to DBM calves 25 min prior to dehorning. At the second draft, calves were dehorned or sham dehorned and DTA calves were treated with

TA. On days 1, 3 and 7 of the trial, the calves were processed through the race once for data collection. All wounds were sprayed with spinosad (Extinosad Aerosol for Wounds; Elanco Animal Health, West Ryde, NSW, Australia) for fly control following data collection.

3.7.3 Measurements

3.7.3.1 Behaviour

On day 0, the calves were observed immediately following treatment for 3 h of behavioural observations. This was conducted in two round yards (80 m^2 each) adjacent to the cattle handling facilities with 3 video cameras (HD 1080p Sports Action Cam, Sony Australia Ltd, North Sydney, NSW, Australia), attached at various points along the fence of each yard to capture videos of the cattle from all angles of the yards. The videos continuously recorded the frequency or duration of certain specified behaviours displayed by each calf. For analysis, 5-minute focal periods were examined every hour for 3 h following treatment. The frequency or duration of behaviours were recorded using an observational data software package (The Observer® XT 12 ; Noldus Information Technology, International). An ethogram was designed using this software whereby behaviours were categorised as states or points (Table 3.7.1). The ethogram was derived from previously published studies on dehorning (McMeekan *et al.* 1999; Sylvester *et al.* 2004). State behaviours were quantified by duration (s) and point behaviours were quantified by frequency.

Table 3.7.1 Ethogram developed for behavioural observations conducted on calves following treatment

Behaviour	**Description**
States[1]	
Walk	Walking forwards or backwards in any style at any pace.
Stand	Standing in any style.
Lie	Lying down completely on the ground in any style.
Head down	Holding head below brisket.
Scratch	Raising a hind leg and scratching part of the body or scratching body against the yard fence.
Lick	Turning head back and licking body with lips or tongue, or both.
Points[2]	
Head shake	Rapid shaking of the head around a rostral to caudal axis.
Head turn	Rapid turning of the head to either side of the body.
Head paw	Lifting of hind leg and contacting with the head.
Head rub	Rubbing head against another calf or the yard fence.
Ear flick	Rapid movement of one or both ears.

[1] States are behaviours with measurable duration and are quantified by duration of time (s).
[2] Points are behaviours without measurable duration and are quantified by frequency.

3.7.3.2 Maximum wound temperature

Infrared photographs of both the left and right wounds were captured from all dehorned calves on days 1, 3 and 7 of the trial using a handheld infrared camera (FLIR®E50; FLIR Systems, Inc., Mulgrave, Vic, Australia), with a thermal range of -20°C to 120°C and a sensitivity of 0.045°C. A 10 cm x 10 cm cardboard frame was used to standardise the image area for each photograph. The camera frame was aligned with the cardboard frame held over the wound for each photograph, ensuring the camera lens was at a consistent distance of 0.5 m from the wound for each photograph. This distance, along with an emissivity value of 0.95 were entered into the infrared camera for calibration. Ambient temperature and humidity were monitored and recorded at the time each photograph was captured and were entered into the infrared camera for calibration every 30 min during the data collection period. Images were analysed for maximum temperature using a thermal imaging software program (FLIR® Tools Software ; FLIR Systems, Inc., Mulgrave, Vic, Australia). This software allowed for analysis of a specific area using a geometric figure drawn on the photograph. A circle was drawn around the wound within the cardboard frame in each photograph and the maximum temperature within this area was calculated.

3.7.3.3 Wound morphology

Digital photographs of the wound were taken from all dehorned calves on days 1, 3 and 7. These photographs were later scored for visible evidence of inflammation and healing using a customised numerical rating scale of 1 to 3 (Figure 3.7.1).

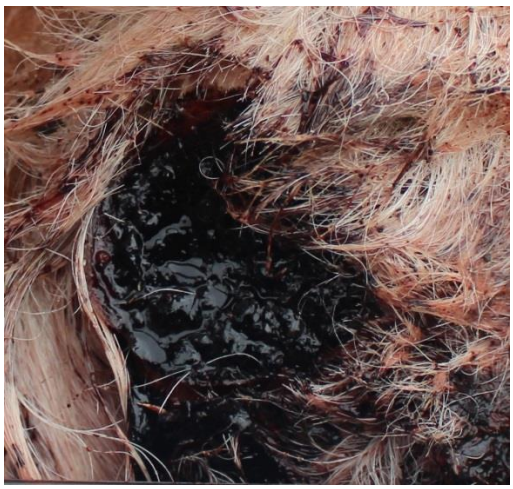
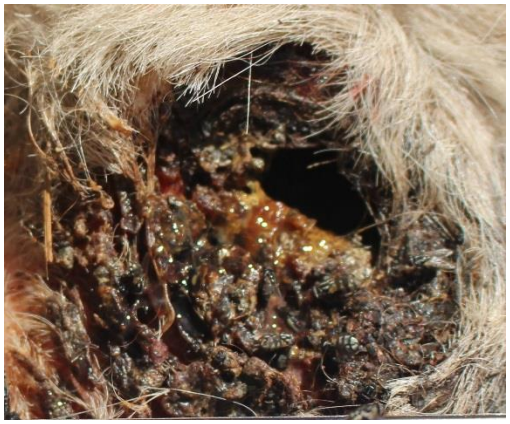
Score	Example	Wound description
1		Focal non-suppurative lesion characterised by a dried thickened fibrinous exudate and adequate closure of the wound surface
2		Focal mild suppurative lesion characterised by a mildly moist thickened serous exudate, and mildly inadequate closure of the wound surface
3		Focal moderate to severe suppurative lesion characterised by a moist thickened suppurative exudate and inadequate closure of the wound surface

Figure 3.7.1 Customised numerical rating scale used to score wound appearance

3.7.4 Statistical analysis

Data on each state behaviour and maximum wound temperature were subjected to restricted maximum likelihood (REML) for repeated measures using the linear mixed models procedure of Genstat® statistical software (17th Edition; VSN International Ltd, Hemel

Hempstead, Hertfordshire, UK). Data on each point behaviour were subjected to REML for repeated measures using the generalised linear mixed models (GLMM) procedure of Genstat® with a poisson distribution. The combined frequency of all point behaviours was also analysed this way. Wound appearance scores were subjected to ordinal logistic regression (OLR) in ASReml® 3 statistical software (VSN International, Hemel Hempstead, Hertfordshire, UK). For each behaviour and combined point behaviours (Table 3.7.1), the fixed effects of the model were treatment x time-point. For maximum wound temperature, the fixed effects of the model were treatment x day + ambient temperature. Data on ambient temperature and ambient humidity were subjected to a Spearman's rank correlation using the nonparametric correlations procedure of Genstat®. A strong negative correlation (R = -0.89) was identified, therefore only ambient temperature was included in the model. Data on ambient temperature and maximum wound temperature was subjected to a Spearman's rank correlation using the nonparametric correlations procedure of Genstat®. For wound appearance, the fixed effects of the model were treatment x day. The random effect for all models was calf ID. Data from the REML analyses is presented as predicted means. Data from the OLR analysis is presented as cumulative odds ratios with the statistical probabilities of wounds displaying scores of Y = 1, 2 and 3. For all statistical calculations, P values ≤ 0.05 were considered statistically significant.

3.8 Effects of topical anaesthetic and buccal meloxicam treatments on concurrent castration and dehorning of beef calves

3.8.1 Animals and location

A total of 307 *Bos indicus* or *Bos indicus* crossbred weaner bull calves, aged approximately 6 to 8 months, were sourced from a commercial beef herd on a property in western Qld, Australia, to be used in two experiments. One week prior to commencement of experiment 1, all calves were mustered, separated from their mothers and held in a set of 'weaning yards' with *ad libitum* access to water and lucerne hay, as is commonly practiced on northern Australian beef herds.

3.8.2 Experimental design and treatments

For both experiments, calves were randomly allocated to one of five treatments in the order that they were processed by use of random numbers generated in Microsoft Office Excel: (1) no castration or dehorning (CON); (2) castration and dehorning (CD); (3) castration and dehorning with pre-operative buccal meloxicam (CDBM); (4) castration and dehorning with intra-operative topical anaesthetic (CDTA); and (5) castration and dehorning with pre-operative buccal meloxicam and intra-operative topical anaesthetic (CDBMTA). There were 50 calves per treatment group for experiment 1. A subset of these calves (20 per treatment group) was fitted with global positioning system (GPS) units and a further subset of these calves (10 per treatment group) was fitted with accelerometers. In experiment 2, there were 12 calves in the CON and CDBMTA treatment groups and 11 calves in the CD, CDBM and CDTA treatment groups.

Experiment 1 was performed over 7 days, from the day of treatment (day 0) to 6 days post-treatment (day 6). On day 0, calves were processed through a race where they were weighed using cattle scales (Livestock Manager TSi 2; Gallagher Group Ltd., Hamilton, New

Zealand) within the cattle crush (Ultimate Crush; RPM Australia-Pacific Pty Ltd., Gatton, Qld, Australia). They were restrained in a head bale for ear tagging and ear notching. CDBM and CDBMTA calves were treated with buccal meloxicam (BM) at this point. Calves were then moved through a separate race to a weaner cradle (Morrissey & Co. Calves Handling Equipment, Jandowae, Qld, Australia) where they were restrained in left lateral recumbency for treatment and attachment of GPS and accelerometer units.

Commercially produced GPS units (17 × 25 × 5 mm) (CatLog™, Catnip Technologies Ltd., Anderson, CA, USA), designed for use on domestic cats and their attached battery packs (17 × 20 × 49 mm), were placed in acrylonitrile butadiene styrene plastic Jiffy enclosure boxes (130 × 68 × 44 mm), (Jaycar Electronics, Rydalmere, NSW, Australia) and secured in place with Styrofoam. The boxes were then enclosed with their supplied lids and fixing screws and attached to luggage straps (25 mm × 2 m) (Gripwell Australia Pty Ltd, Chatswood, NSW, Australia), using zip ties. On day 0, a single luggage strap was secured around the neck of each animal with the plastic box positioned on the upper right side of the neck to ensure the GPS antenna was unobstructed from satellite signals. In addition, the plastic boxes were fixed in place on the neck of the animal with fast grip contact adhesive (Parfix®, DeluxGroup Ltd, Clayton, Vic, Australia). On day 6, the luggage straps and boxes were quickly and carefully removed from the cattle by cutting the straps using a knife. The GPS units were used as they are lightweight (22 g) and low-cost and therefore practical and cost-effective for tracking greater numbers of individual animals (Forin-Wiart *et al.* 2015).

Accelerometers (HOBO Pendant® G Acceleration Data Loggers; Onset Computer Corporation, Bourne, MA, USA), were inserted into pieces of foam sponge and secured on each calf to the lateral aspect of the right hind leg proximal to the fetlock using bandaging tape (VetRap™ Bandaging Tape; 3M, North Ryde, NSW, Australia) and cloth tape (50 mm × 15 m) (Norton Bear 50 mm × 15 m Silver Cloth Tape; Saint Gobain, Somerton, Vic, Australia). The units were positioned such that the x-axis was perpendicular to the ground and pointing ventrally, the y-axis was parallel to the ground and pointing cranially and the z-axis was parallel to the ground and pointing toward the midplane.

All calves except CON calves were castrated and dehorned and CDTA and CDBMTA calves were treated with topical anaesthetic (TA) whilst still restrained in the cradle. Calves were released into another holding yard (300 m^2) where they remained until the last animal was processed.

The marking process commenced at 07:30:00 am and was concluded by 05:00:00 pm hours. When all calves had been processed, they were moved into a laneway (700 m^2) where they remained until 06:00:00 am the following day when they were moved to a large paddock (619 ha) for a further 6 days. During this time, calves had *ad libitum* access to pasture and water. On day 6 at 06:00:00 am, calves were mustered back into the holding yards adjacent to the handling facilities and processed through the first race. Whilst in the race, GPS and accelerometer units were removed, then the calves were weighed in the cattle crush and released.

Experiment 2 was conducted over 3 days (days A, B and C), with 17 calves (3-4 per treatment group) treated on day A and 20 calves (4 per treatment group) treated on days B and C. Each day, calves were processed as per experiment 1. Calves were individually numbered on both sides and the back of the body with spray while in the race. Following

treatment, calves were released into a holding yard (104 m^2) for behavioural recording, as described below. This process commenced at 07:30:00 am and concluded by 08:30:00 am.

3.8.3 Measurements

3.8.3.1 Weight gain (experiment 1)

Weight gain was calculated for each calf using the difference of the pre-treatment weight collected on day 0 and the post-treatment weight collected on day 6.

3.8.3.2 Paddock utilisation (experiment 1)

GPS units were programmed using CatLog™ software (Catnip Technologies Ltd, Anderson, CA, US) to record a positional fix every 10 s using the Navstar global positioning system from 10:00:00 am on day 0 for the entire experimental period (7 days). Location information was downloaded using the CatLog™ software and exported into Microsoft Excel 2007. Only positional fixes recorded whilst all animals were in the paddock were included. Hence, all positional fixes before 08:00:00 am on day 1 and after 11:59:59 pm on day 5 were disregarded. Positional fixes that were located outside the paddock boundary, which included a 40 m buffer to accommodate for possible large location errors associated with down antennas, short-fix intervals and sky obstructions (Forin-Wiart *et al.* 2015), were removed. In addition, location fixes that were greater than 1 h apart or with a speed greater than 3.66 m / s (Heglund and Taylor 1988) were removed. Paddock utilisation to determine 95% Minimum Convex Polygon (MCP) on a daily basis per animal was calculated in R 3.3.3 (R Core Team 2017) using the 'adehabitatHR' package (Calenge 2006).

3.8.3.3 Lying activity (experiment 1)

The accelerometer loggers were pre-programmed using HOBOware software (Onset Computer Corporation, Bourne, MA USA) to record the g-force on the x-, y- and z-axes every 10 s from 10:00:00 am on day 0. The loggers recorded until the memory was filled at 22:13:00 hours on day 2. Following removal of the loggers, the data was downloaded using the Onset HOBOware software which converted the g-force readings into degrees of tilt. The data was then exported into Microsoft Excel 2007 and the degree of tilt on the x-axis was used to determine whether or not the calves were in a lying position at each 10-second reading. All data points prior to 12:00:00 pm on day 0 were removed as the last accelerometer unit was attached at 11:45:00 am. Tilt values > 120° were interpreted as standing and tilt values ≤ 120° were interpreted as lying. These thresholds were based on values used in previous studies on dairy cows (Ito *et al.* 2009; Mattachini *et al.* 2013) and adjusted according to the orientation of the loggers on the legs of the animals.

3.8.3.4 Individual behaviours (experiment 2)

Calves remained in the holding yard for 6 h following treatment. During this time, calves were provided *ad libitum* access to water and lucerne hay. Six video cameras (HD 1080p Sports Action Cam; Sony Australia Ltd, North Sydney, NSW, Australia) were attached at various points along the fence of the yard to capture video footage of the calves. Cameras were placed strategically to capture footage from all angles of the yard. This footage was later used to continuously record the frequency or duration of certain specified behaviours displayed by each animal in 5-minute focal samples at 6 time-points (40, 80, 120, 180, 240 and 360 min following treatment). The frequency and duration of behaviours were recorded by a single, trained observer using an observational data software package (The Observer®

XT 12; Noldus Information Technology, Wageningen, The Netherlands). The observer was blinded to treatment, although it was clear which calves were CON calves due to the presence of intact horns. An ethogram was designed using The Observer® XT software whereby behaviours were categorised as states or points (Table 3.8.1). State behaviours were quantified by duration (s) and point behaviours were quantified by frequency. The ethogram was derived from previous published studies on surgical castration and amputation dehorning (McMeekan *et al.* 1999; Ting *et al.* 2003b; Sylvester *et al.* 2004; Petherick *et al.* 2015).

Table 3.8.1. Ethogram developed for behavioural observations conducted on calves following treatment

Behaviour	Description
	States [1]
Walk	Walking forwards or backwards in any style at any pace.
Stand	Standing in any style.
Lie	Lying down completely on the ground in any style.
Head down	Holding head below brisket.
Eat	Ingesting lucerne hay.
Drink	Ingesting water.
	Points [2]
Head shake	Rapid shaking of the head around a rostral to caudal axis.
Head turn	Rapid turning of the head to either side of the body.
Head paw	Lifting of hind leg and contacting the head.
Kick	Kicking backward or towards the belly with a hind limb.
Stamp	Lifting front or hind foot and forcefully placing it on the ground.
Ear flick	Rapid movement of one or both ears.
Tail flick	Sideways movement of the tail from vertical to return to vertical.

1 States are behaviours with measurable duration and are quantified by duration of time (s). 2 Points are behaviours without measurable duration and are quantified by frequency.

3.8.4 Statistical analysis

All data was subjected to restricted maximum likelihood (REML) using Genstat® statistical software (17th Edition; VSN International Ltd, Hemel Hempstead, Hertfordshire, UK). For weight gain, outliers within treatment groups were identified using the boxplot procedure of Genstat®. A linear mixed models procedure was used to analyse data on weight gain, paddock utilisation and observed state behaviours. A generalised linear mixed models (GLMM) procedure with a binomial distribution was used to analyse data on lying activity generated from accelerometer readings. A macro was used in Microsoft Office Excel to calculate the frequency of lying bouts and average duration of lying bouts. A GLMM procedure with a poisson distribution was used to analyse data on frequency of lying bouts and a linear mixed models procedure was used to analyse data on average duration of lying bouts. A generalised linear mixed models (GLMM) procedure with a poisson distribution was used to analyse data on observed point behaviours. For weight gain (experiment 1), the fixed effect of the model was treatment. For paddock utilisation (experiment 1) the fixed effects of the model were treatment x day. For total lying activity, frequency of lying bouts

and average duration of lying bouts (experiment 1), the fixed effects of the model were treatment x day + body weight. For each observed behaviour (Table 3.8.1) (experiment 2), the fixed effects of the model were treatment x time-point + day + body weight. The random effect for all models was calf ID. Insignificant terms were dropped from the models using a backwards elimination approach. Data on weight gain and observed behaviours is presented as predicted means. Data on lying activity is presented as the proportion of time calves spent lying. For all statistical calculations, *P* values ≤ 0.05 were considered statistically significant.

3.9 Investigating analgesia for control of pain and haemorrhage following spaying via the willis dropped ovary technique in cattle

3.9.1 Animals and location

Seventy-five heifers and 17 cows were sourced from a commercial beef herd in far northern WA, Australia. All animals were *Bos Indicus* or *Bos Indicus* crossbreeds. The heifers and cows weighed 260.75 ± 45.32 kg and 343.82 ± 40.13 kg, respectively.

3.9.2 Experimental design and treatments

Animals were randomly allocated to one of five treatments by use of random numbers generated in Microsoft Office Excel: (1) Transrectal palpation only / control (CON, n = 15 heifers and 4 cows); (2) Spaying (S, n = 15 heifers and 4 cows); (3) Spaying with peri-operative delivery of topical anaesthetic (Tri-Solfen®, Bayer Animal Health, Pymble, NSW, Australia) (STA, n = 15 heifers and 3 cows); (4) Spaying with peri-operative delivery of subcutaneous meloxicam (Metacam® 20 mg/mL Solution for Injection; Boehringer Ingelheim Pty Ltd, North Ryde, NSW, Australia) (SM, n = 15 heifers and 3 cows); and (5) Spaying with peri-operative delivery of topical anaesthetic and subcutaneous meloxicam (STAM, n = 15 heifers and 3 cows).

The trial was performed over 3 days, with 42 heifers and 15 cows treated on the first day and 33 heifers and 2 cows treated on the second day. On the first day, animals were processed through a race where they were weighed using cattle scales (model XR5000; Tru-Test™, Eight Mile Plains, Qld, Australia) within the cattle crush, (Immobilizer Pro-Chute; Leight's Country Industries Australia, Goombungee, Qld, Australia). Animals were then restrained in a head bale where they were ear tagged, ear punched and numbered consecutively on both sides and the back of the body with spray paint. Blood samples were then collected from the tail vein for later analysis of packed cell volume (PCV) and total plasma protein (TPP), as described below. Animals were then treated and released into one of three holding yards (each 20 m x 25 m) for behavioural recording, as described below. On the second day, at approximately 24 h following treatment, animals were again processed through the race, weighed within the cattle crush and restrained in the head bail for collection of a subsequent blood sample from the tail vein. These animals were then released into a different set of holding yards where they remained for 4 days with *ad libitum* access to water and lucerne hay. This entire process was then immediately repeated for the second group of heifers and cows.

3.9.3 Measurements

3.9.3.1 Change in body weight

Change in body weight was calculated for each animal using the difference of the pre-treatment weight collected immediately prior to treatment and the post-treatment weight collected 24 h following treatment.

3.9.3.2 Mortality

Animals were observed every 2 h up to 8 h following treatment, then every 24 h up to 4 days following treatment and any incidences of mortality were recorded. Post-mortems were conducted in the case of mortality.

3.9.3.3 Change in packed cell volume

Packed cell volume was measured manually. A small volume of blood was drawn from each sample into capillary tubes which were then sealed with plasticine and spun in a microhematocrit centrifuge for 5 minutes. The percentage volume of the whole blood sample occupied by red blood cells was then read using a microhematocrit card. Change in PCV was calculated for each calf using the difference of the pre-treatment PCV determined from the blood sample collected immediately prior to treatment and the post-treatment PCV determined from the blood sample collected 24 h following treatment.

3.9.3.4 Change in total plasma protein

Total plasma protein was measured using a refractometer. Each capillary tube was broken following determination of PCV and the plasma placed directly onto the refractometer. The TPP was then read directly from the refractometer scale by pointing it towards the light. Change in TPP was calculated for each calf using the difference of the pre-treatment TPP determined from the blood sample collected immediately prior to treatment and the post-treatment TPP determined from the blood sample collected 24 h following treatment.

3.9.3.5 Behaviour

Animals remained in the holding yards for 24 h following treatment. On the first day of the trial, there were 19 animals (14 heifers and 5 cows) in each of three yards. On the second day of the trial, only two yards were utilised, one containing 14 heifers and 2 cows and the other containing 19 heifers. During this time, animals were provided *ad libitum* access to water and lucerne hay. Three to four video cameras (HD 1080p Sports Action Cam; Sony Australia Ltd, North Sydney, Australia), were attached at various points along the fence of each yard to capture video footage of the animals. Cameras were placed strategically to capture footage from all angles of the yards. This footage is currently being used to instantaneously record the frequency of certain specified behaviours displayed by each animal every minute for 10 min of every hour for 6 h following treatment and again at 24 h following treatment. The frequency and duration of behaviours were recorded by a single, trained observer using an observational data software package (The Observer® XT 12; Noldus Information Technology, Wageningen, The Netherlands). The observer was blinded to treatment. An ethogram was designed using The Observer® XT software (Table 3.9.1). The ethogram was derived from previous published studies on WDOT spaying (McCosker *et al.* 2010; Petherick *et al.* 2013).

Table 3.9.1. Ethogram developed for behavioural observations conducted on cows and heifers following treatment

Behaviour	Description
Walk normal	Walking with muscles relaxed
Walk abnormal	Walking slowly with muscles stiff
Stand normal	Standing passively or actively with head held relaxed and muscles relaxed
Stand abnormal	Standing stationary with muscles stiff and head held below brisket
Lie normal	Lying in a normal posture (ventral position and no extension of limbs)
Lie abnormal	Lying in an abnormal posture (lateral recumbency, one or both hind limbs extended > 90°, both forelimbs extended)
Head down	Holding head below brisket
Head tuck	Tucking head to the side of the body
Repetitive head movement	Repeatedly or excessively moving the head by shaking or turning it to the side of the body
Back arch	Curving of the spine
Tail elevate	Holding tail stiffly in an upright position
Repetitive tail movement	Repeatedly or excessively flicking the tail
Lick self	Licking own body with lips, or tongue, or both
Lick other	Licking the body of another animal with lips, or tongue, or both
Eat	Ingesting Lucerne hay
Drink	Ingesting water
Ruminate	Chewing and regurgitating regularly

3.9.4 Statistical analysis

All data collected thus far was subjected to restricted maximum likelihood (REML) using Genstat® statistical software (17th Edition; VSN International Ltd, Hemel Hempstead, Hertfordshire, UK). A linear mixed models procedure was used to analyse data on change in body weight, change in PCV and change in TPP. For change in body weight, change in PCV and change in TPP, the fixed effects of the model were treatment + stage of maturity and the random effect of the model was animal ID. For change in PCV and change in TPP, outliers were identified using the boxplot procedure of Genstat®. Any animals showing a negative change in PCV or TPP outlying the normal range of the data were recorded.

4 Results

4.1 Effect of topical vapocoolant spray on perioperative pain response of unweaned calves to ear tagging and ear notching

4.1.1 Temperature validation studies

The thermocouples and data logger were found to be accurate to 0.2 °C. In dead tissue, a 1 second spray from 10 cm (on the same side of the ear as the thermocouple) was adequate to reduce the tissue temperature to below 10 °C (Fig. 4.1.1) for 10 seconds. In live tissue, it

was determined that a 3 second spray (on the same side of the ear as the thermocouple) reduced tissue temperature to below 10 °C from 4 seconds after spraying and it remained below 10 °C for 16 seconds (Fig. 4.1.2), which would allow sufficient time to conduct each procedure.

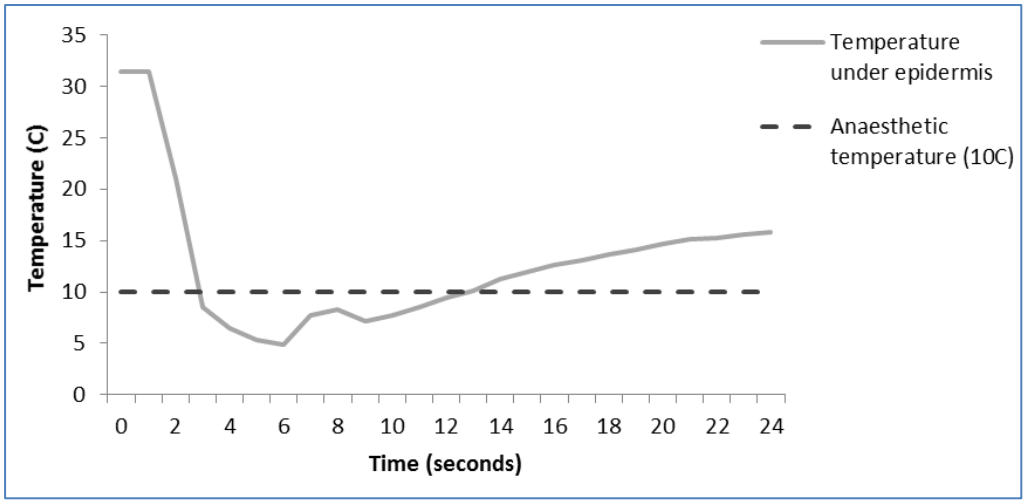


Figure 4.1.1 Tissue temperature beneath the epidermis of the back of the ear following a 1 second application of the vapocoolant spray from 10 cm to dead tissue. The area beneath the dotted line represents the period of time during which the tissue was at an adequate temperature to inhibit nociception (< 10 °C).

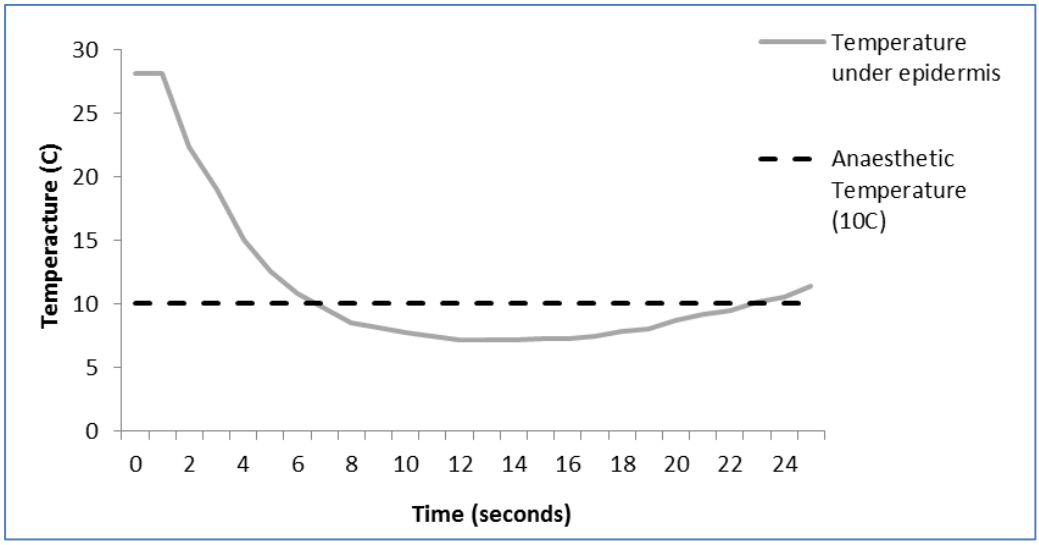


Figure 4.1.2 Tissue temperature beneath the epidermis of the ear following a 3 second application of the vapocoolant spray from 10 cm to live tissue. The area beneath the dotted line represents the period of time during which the tissue was at an adequate temperature to inhibit nociception (<10 °C).

4.1.2 Behavioural study

4.1.2.1 Descriptive and univariate analyses

Results of the descriptive and univariate analyses are presented in Tables 4.1.1 and 4.1.2, respectively. There was no difference in the response scores between the VS and WS groups (p = 0.051) (Table 4.1.3). There was no significant difference in pain response scores between males and females (p = 0.548) or side of procedure (p = 0.407) (Table 4.1.2). Ear notching resulted in statistically significantly greater pain response scores than did ear tagging (OR = 19.2, 95% CI: 5.34–68.99, p < 0.001) (Table 4.1.2). Earnotching resultedinthehighest response score (three) in 55% of calves, compared with only 5% of calves in response to ear tagging (Table 4.1.2).

Table 4.1.1 Contingency table for all categorical explanatory variables

Explanatory variable	**Category**	**Pain response score**			
		0	**1**	**2**	**3**
Treatment	WS	8 (27%)	6 (20%)	9 (30%)	7 (23.3%)
	VS	18 (60%)	2 (7%)	4 (13%)	6 (20%)
Sex	Female	15 (50%)	3 (10%)	5 (17%)	7 (23%)
	Male	11 (37%)	5 (17%)	8 (27%)	6 (20%)
Procedure	Notch	1 (5%)	3 (15%)	5 (25%)	11 (55%)
	Tag	25 (63%)	5 (13%)	8 (20%)	2 (5%)
Side	Left	11 (55%)	4 (20%)	3 (15%)	2 (10%)
	Right	14 (70%)	1 (5%)	5 (25%)	0 (0%)

WS, water spray; VS, vapocoolant spray.

Table 4.1.2 Univariate ordinal logistic regression results for the association of explanatory variables with the response variable

Explanatory variable	**Category**	***b***	**SE (*b*)**	**OR**	**95% CI**	***p*-value**
Treatment	VS			1.0		
						0.051
	WS	0.95	0.49	2.59	0.97–6.95	
Sex	Male			1.0		
						0.548
	Female	–0.28	0.47	0.75	0.29–1.96	
Procedure	Tag			1.0		
						< 0.001
	Notch	2.96	0.63	19.21	5.34–68.99	
Side	Right			1.0		
						0.407
	Left	0.54	0.65	1.71	0.44–6.68	

b, regression coefficient; OR, odds ratio; CI, confidence interval; WS, water spray; VS, vapocoolant spray.

Table 4.1.3 Multivariate ordinal logistic regression results for the association of explanatory variables with the response variable

Explanatory variable	**Category**	***b***	**SE (*b*)**	**OR**	**95% CI**	***P*-value**
Treatment	VS			1.0		
						0.011
	WS	1.41	0.55	4.08	1.34–12.42	
Procedure	Tag			1.0		
						< 0.001
	Notch	3.14	0.65	23.19	6.18–87.05	

b, regression coefficient; OR, odds ratio; CI, confidence interval; WS, water spray; VS, vapocoolant spray.

4.1.2.2 Multivariate analysis

Results of the multivariate analysis are presented in Table 4.1.3 and Fig. 4.1.3. When adjusted for procedure, there was a significant effect of treatment on response score, with WS calves having greater pain response scores to each procedure than VS calves (OR = 4.08, 95% CI: 1.34–12.42, p = 0.011; Table 4.1.3). WS calves were more likely to show the highest pain response score (three) following ear notching (70%) and ear tagging (9.1%) than were VS calves (36% and 2.4% for ear notching and ear tagging, respectively) (Fig. 4.1.3). There was a significant effect of procedure on response score, with ear notching resulting in greater pain response scores than ear tagging (OR = 23.19, 95% CI: 6.18–87.05, p < 0.001; Table 4.1.3). Animals were more likely to be allocated a pain response score of 0 for ear tagging in both the WS (47%) and VS (79%) groups than for ear notching (3.7% and 1.4% for WS and VS calves, respectively) (Fig. 4.1.3).

4.1.2.3 Postoperative inspection for tissue damage

No obvious adverse reactions to the sprays were observed. Mild inflammation and tissue granulation around ear tagging and notching sites were noted, which were attributed to wound healing.

4.2 Effect of lignocaine and a topical vapocoolant spray on the pain response to surgical castration in beef calves

4.2.1 Behavioural scores

There was no significant effect of body weight ($P = 1$). There was a strong trend for an interaction between treatment and stage of procedure ($P = 0.051$). There was a trend for SHAM calves to have lower scores in response to stages 2, 3, 4 and 5 compared to all other calves. There was also a trend for LIG calves to have lower scores in response to stages 2 and 4 compared to CAST and VAPO calves (Fig. 4.2.1). There was a significant effect of treatment ($P < 0.001$), with SHAM calves having significantly lower scores than all other calves and LIG calves having significantly lower scores than VAPO calves. Probabilities of calves having the lowest score (zero) for treatments SHAM, CAST, LIG and VAPO were 0.87, 0.20, 0.34 and 0.12, respectively (Fig. 4.2.2). SHAM calves were 9.1 (95% CI: 3.3 – 25.1), 5.7 (95% CI: 2.1 – 15.1) and 17.3 (95% CI: 6 – 50) times more likely to have lower scores than CAST, LIG and VAPO calves, respectively (Fig. 4.2.2). LIG calves were 3.1 (95% CI: 1.3 – 7.3) times more likely to have lower scores than VAPO calves. There was a significant effect of stage of procedure ($P < 0.001$), with calves having significantly greater scores in response to stage 2 than to all other stages and calves having significantly greater scores in response to stage 4 than to stage 5. Probabilities of calves having the greatest score (three) in response to stages 1, 2, 3, 4 and 5 were 0.05, 0.22, 0.05, 0.09 and 0.04, respectively (Fig. 4.2.3). Calves were 5.0 (95% CI: 2 – 12.4), 4.3 (95% CI: 1.8 – 10.4), 2.2 (95% CI: 1 – 5.3) and 5.7 (95% CI: 2.3 – 13.6) times more likely to have greater scores in response to stage 2 than to stages 1, 3, 4 and 5, respectively. Calves were 2.5 (95% CI: 1.1 – 5.9) times more likely to have greater scores in response to stage 4 than to stage 5.

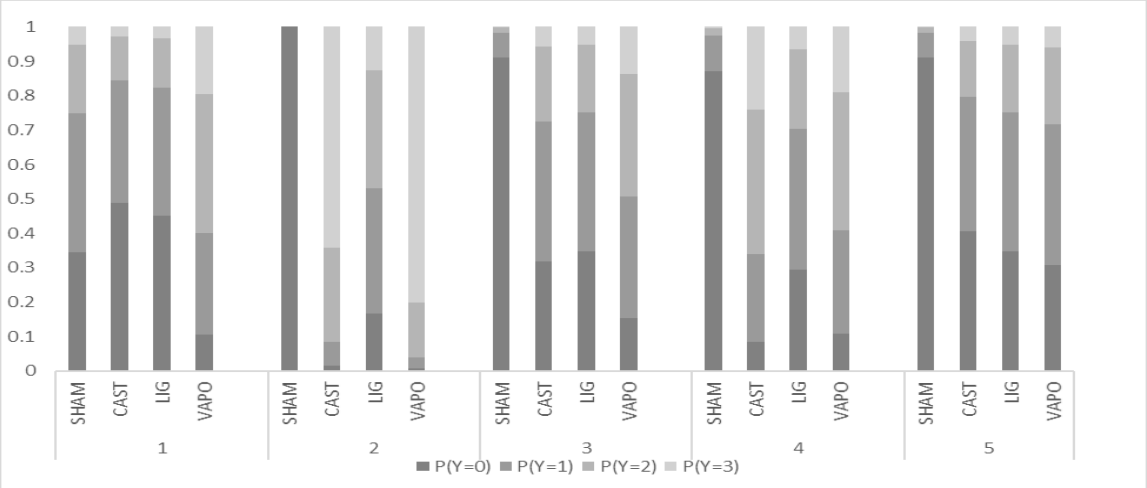


Figure 4.2.1 Probability of calves in each treatment group displaying behavioural response scores (Y; 0 = zero movement, 1 = mild movement, 2 = moderate movement, 3 = severe movement) to sham castration or castration at different stages of the procedure

Treatment: SHAM = sham castrated; CAST = castrated; LIG = castrated and treatment with local anaesthetic lignocaine; and VAPO = castrated and treatment with a vapocoolant spray.

Stage: 1 = excision of scrotum; 2 = extrusion of right testis and spermatic cord; 3 = excision of right spermatic cord; 4 = extrusion of left testis and spermatic cord; and 5 = excision of left spermatic cord.

A trend was found ($P = 0.051$).

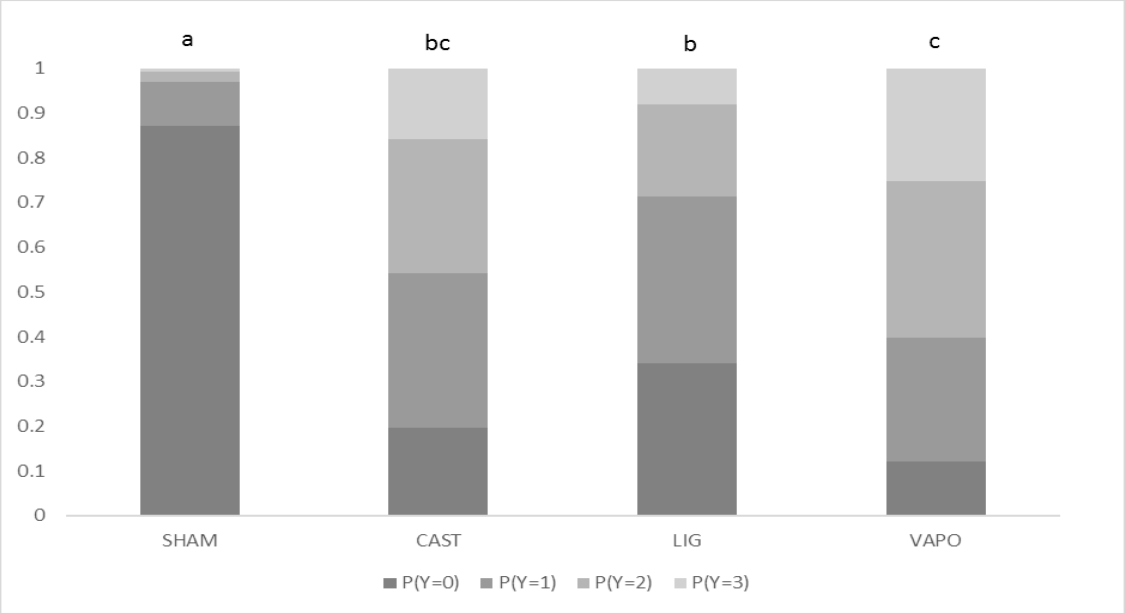


Figure 4.2.2 Probability of calves in each treatment group displaying behavioural response scores (Y; 0 = zero movement, 1 = mild movement, 2 = moderate movement, 3 = severe movement) to sham castration or castration

Treatment: SHAM = sham castrated; CAST = castrated; LIG = castrated and treatment with local anaesthetic lignocaine; and VAPO = castrated and treatment with a vapocoolant spray.

[a, b, c] Treatments with different superscripts differ significantly at $P \leq 0.05$.

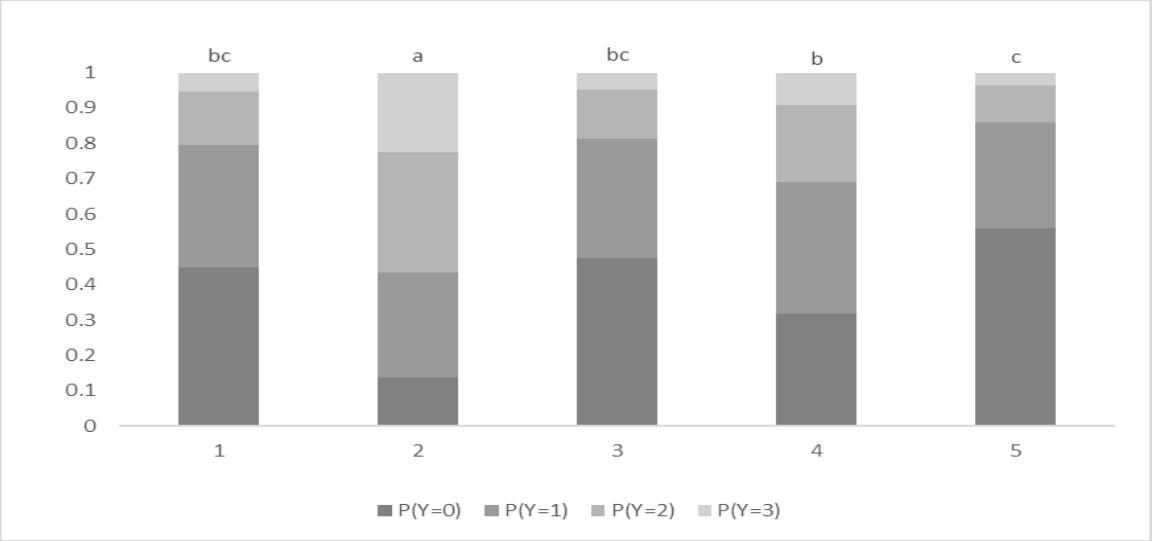


Figure 4.2.3 Probability of all calves displaying behavioural response scores (Y; 0 = zero movement, 1 = mild movement, 2 = moderate movement, 3 = severe movement) to sham castration or castration at different stages of the procedure

Stage: 1 = excision of scrotum; 2 = extrusion of right testis and spermatic cord; 3 = excision of right spermatic cord; 4 = extrusion of left testis and spermatic cord; and 5 = excision of left spermatic cord.

[a, b, c] Areas with different superscripts differ significantly at $P \leq 0.05$.

A significant effect was found ($P < 0.001$).

4.2.2 Ocular temperature

A strong negative correlation (R = -0.92) between ambient temperature and ambient humidity was identified, therefore only ambient temperature was included in the statistical model. There were no significant effects of treatment or body weight ($P = 0.739$ and $P = 0.479$, respectively). There were significant effects of time-point and ambient temperature ($P = 0.002$ and $P < 0.001$, respectively). Maximum ocular temperature was greater at time-point 3 (following sham castration or castration) (38.69 ± 0.09 °C) than at time-points 1 and 2 (38.44 ± 0.09 °C and 38.49 ± 0.09 °C, respectively). A low positive relationship between ambient temperature and maximum ocular temperature was identified (R = 0.30).

4.3 Effect of a topical anaesthetic formulation on the cortisol response to surgical castration of unweaned beef calves

There was no significant interaction between time and treatment (F = 0.99; d.f. = 14, 147; $P = 0.463$, Table 4.3.1). There was a significant effect of time on cortisol response across all treatment groups (F = 25.49; d.f. = 7, 161; $P < 0.001$, Table 2.2). Cortisol concentrations increased between −0.5 and 0.5 h relative to castration, and decreased between 1 and 6 h after castration. Lowest concentrations were at −0.5 h (29.96 nmol/l) and 6 h (23.39 nmol/l) and peak concentration at 0.5 h (77.98 nmol/l) was significantly higher than the cortisol response at 0 h (62.77 nmol/l). The cortisol response at 0 h was significantly higher than the cortisol response at −0.5 h. There was a statistical tendency for treatment to be significant (F = 2.95; d.f. = 2, 19; $P = 0.077$). CON, C and CTA calves had mean cortisol concentrations of 44.11 ± 10.05, 63.02 ± 11.5 and 59.03 ± 10.68 nmol/l, respectively. There was a significant

effect of treatment on integrated cortisol response (F = 3.78; d.f. = 2, 21; *P* = 0.04). The mean AUC for CON calves (253 ± 40.49 nmol/l per h) was significantly lower than the mean AUCs of C (394 ± 38.22 nmol/l per h) and CTA (372 ± 31.39 nmol/l per h) calves.

Table 4.3.1 Mean cortisol concentration (nmol/L ± s.e.m.) of calves in each treatment group over time

Time (h)	Cortisol concentration (nmol/L)					
	CON		C		CTA	
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.
-0.5	28.5	7.15	29.13	7.73	32.27	7.56
0	63.67	12.24	63.43	5.99	61.22	7.0
0.5	63.69	11.57	84.89	8.01	85.34	8.92
1	58	9.17	79.7	7.20	78.33	10.12
1.5	50.07	6.17	80.2	10.13	70.11	10.42
2	48.76	6.87	79.69	11.89	70.02	8.70
4	28.4	6.17	59.22	7.78	44.43	8.13
6	11.76	1.50	27.92	11.18	30.49	7.22

CON = sham castrated; C = Castrated; CTA = Castrated + topical anaesthetic
Descriptive statistics are based on predicted means (± s.e.m.).
No significant interaction was found (*P* = 0.463).

4.4 Effects of topical anaesthesia and buccal meloxicam on average daily gain, behaviour and inflammation of unweaned beef calves following surgical castration

4.4.1 Environment

Mean ambient temperature and humidity during data collection on days 1, 2 and 6 were 33.99ºC ± 0.28 (range 31.10 – 38.60ºC), 15.37% ± 0.16 (range 12.40 – 16.90%); 24.32ºC ± 0.30 (range 22.60 – 30.90ºC), 48.73% ± 1.05 (range 33.90 – 60.00%); and 38.76ºC ± 0.21 (range 36.80 – 41.70ºC), 18.66% ± 0.52 (range 13.60 – 27.00%); respectively.

4.4.2 Average Daily Gain

There was a significant effect of day (*P* < 0.001), with ADG lower on day 1 (-1.29 ± 0.56 kg) than on days 2 (0.75 ± 0.56 kg) and 6 (0.94 ± 0.56 kg). There was no significant effect of treatment (*P* = 0.7).

4.4.3 Behaviour

The behaviours, eating, walking with a limp, lying abnormally, back arching and kicking occurred too infrequently for statistical analysis. Behaviours influenced by time only are neither presented nor discussed.

There was a significant effect of treatment on the frequency of foot stamps (P = 0.005), with SHAM calves displaying less (0.19 ± 0.03), and C calves displaying more (1.11 ± 0.20) foot stamps than CBM, CTA and CBMTA calves (0.85 ± 0.15, 0.68 ± 0.12 and 0.56 ± 0.10, respectively). There was a trend for treatment to have a significant effect on duration of time spent walking with hypometria, observed as a 'stiff gait' (P = 0.06), with SHAM calves spending no time (0 ± 0.93 s), C calves spending the greatest duration of time (4.08 ± 1.00 s) and CBM, CTA and CBMTA calves spending an intermediate duration of time (0.99 ± 0.92 s, 1.18 ± 0.97 s and 1.85 ± 0.99 s, respectively) walking with a stiff gait. There was no significant effect of treatment on the frequency or duration of any other behaviour. There was no significant effect of BW on the frequency or duration of any of the behaviours.

4.4.4 Scrotal diameter

There was a significant effect of BW (P < 0.001), with a strong positive correlation (R = 0.73) between BW and scrotal diameter. There was no significant effect of treatment (P = 0.09), day (P = 1) or ambient temperature (P = 1) on the scrotal diameter.

4.4.5 Maximum scrotal temperature

There was a significant treatment x day interaction (P = 0.004) for maximum scrotal temperature. CBM and CBMTA calves had lower maximum scrotal temperatures on day 2 than C calves. CBMTA calves also had lower maximum scrotal temperatures on day 2 than CTA calves. Maximum scrotal temperatures of C and CTA calves was greater on day 6 than on days 1 and 2. Maximum scrotal temperatures of CBM and CBMTA calves were lower on day 2 than on days 1 and 6 (Table 4.4.1). There was a significant effect of BW (P < 0.001), with a weak negative correlation (R = -0.43) between BW and maximum wound temperature. There was no significant effect of ambient temperature (P = 0.8).

Table 4.4.1 Mean maximum scrotal temperature of castrated calves in each treatment group on days 1, 2 and 6 following treatment

Day	Mean maximum scrotal temperature (°C) ± s.e.m.			
	C	CBM	CTA	CBMTA
1	39.6^{Aa} ± 0.19	39.55^{Aa} ± 0.20	39.46^{Aa} ± 0.19	39.85^{Aa} ± 0.19
2	39.63^{Aa} ± 0.19	38.82^{Bb} ± 0.19	39.47^{Aa} ± 0.19	38.72^{Bb} ± 0.19
6	40.21^{Ba} ± 0.19	39.83^{Aa} ± 0.19	40.3^{Ba} ± 0.19	40.02^{Aa} ± 0.19

C = surgical castration; CBM = surgical castration with pre-operative buccal meloxicam; CTA = surgical castration with post-operative topical anaesthetic; CBMTA = surgical castration with pre-operative buccal meloxicam and post-operative topical anaesthetic

$^{A, B}$ Values within a column with different superscripts differ significantly at $P \leq 0.05$.

$^{a, b, c}$ Values within a row with different superscripts differ significantly at $P \leq 0.05$.

Descriptive statistics are based on predicted means (± s.e.m.).

A significant effect was found (P = 0.004).

4.4.6 Wound morphology score

There was a significant effect of day ($P < 0.001$), with wounds having lower scores on day 6 than on days 1 and 2 (Fig. 4.4.1). There was no significant effect of treatment ($P = 0.5$) or BW ($P = 0.5$).

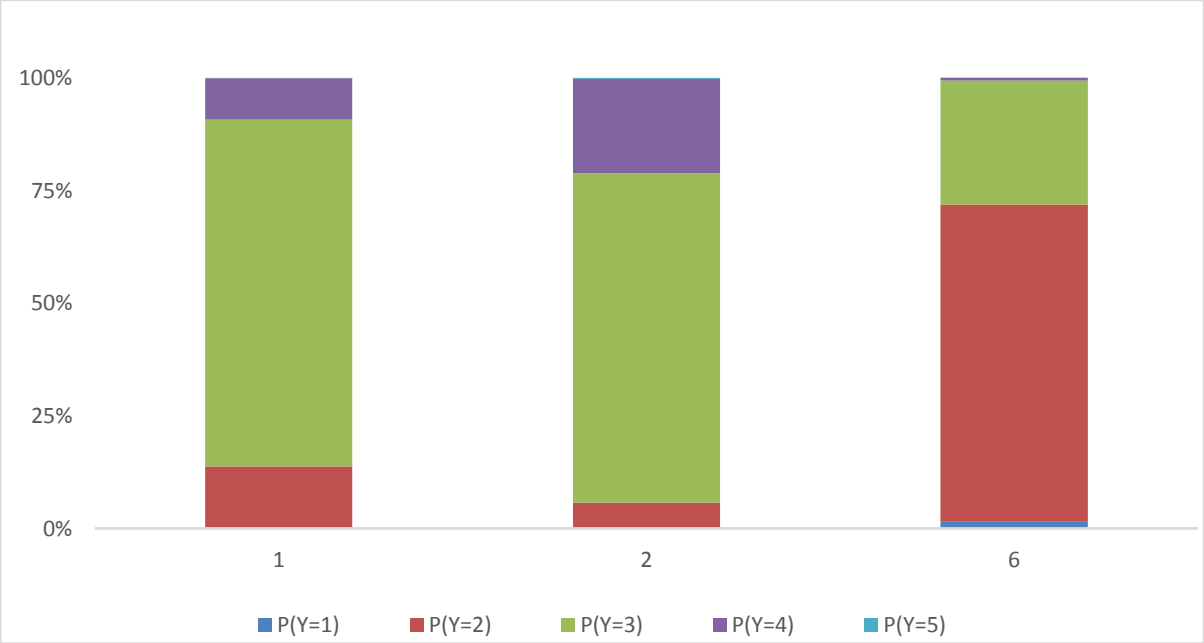


Figure 4.4.1 Probability of wounds of all castrated calves displaying inflammation scores (Y; 1, 2, 3, 4, 5) on days 1, 2 and 6 following treatment

[a, b] Days with different superscripts differ significantly at $P \leq 0.05$.

A significant effect was found ($P < 0.001$).

4.5 Effect of topically applied anaesthetic formulation on the sensitivity of scoop dehorning wounds in calves

4.5.1 Trial 1

There was a significant time x treatment interaction ($P < 0.001$) (Fig. 4.5.1). All calves had a greater probability of displaying a more severe response at 90 min than at 1 min. DTAP calves also had a greater probability of displaying a more severe response at 180 min than at 90 min. DTAP calves were more likely to display more severe responses than DTAE calves at 90 and 180 min.

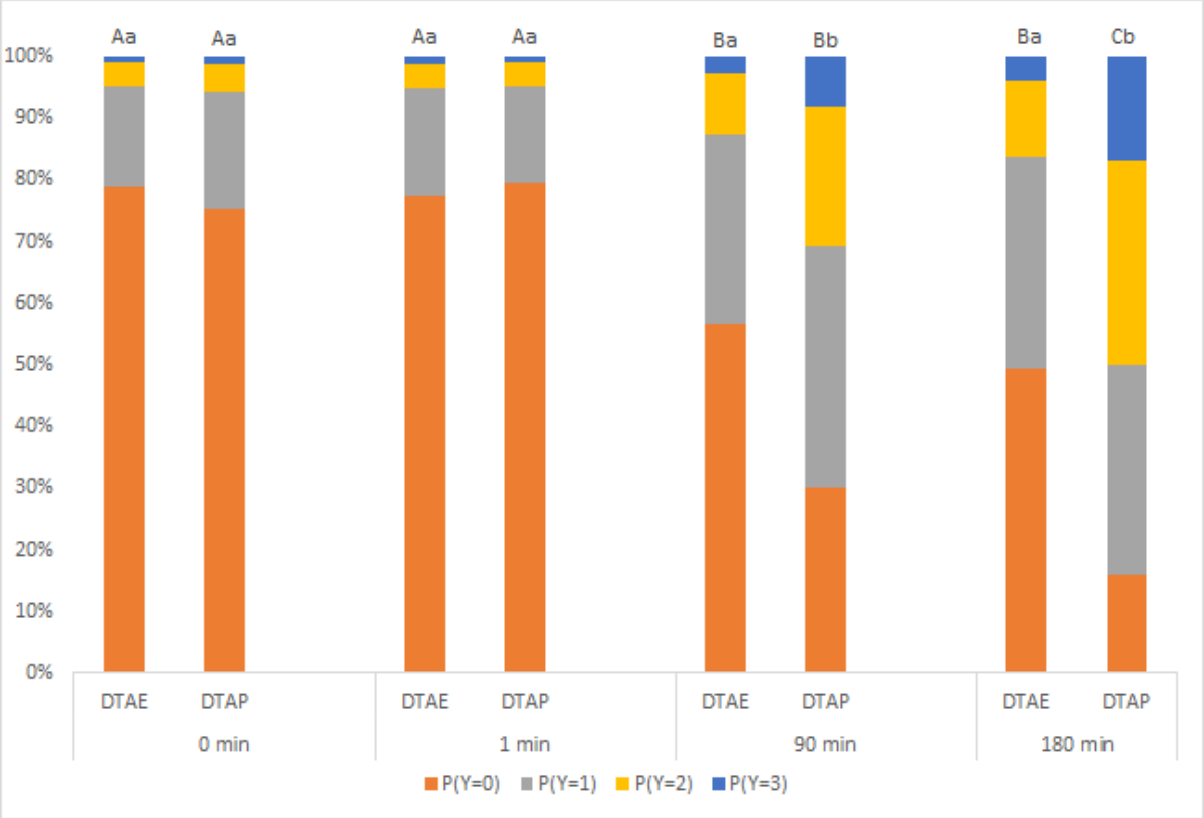


Figure 4.5.1 Probability of calves from Trial 1 in each treatment group displaying responses (Y; 0 = no response, 1 = mild, 2 = moderate, 3 = severe) at different time points

Results combine the effect of both von Frey monofilaments and all sites tested. (DTAE = scoop dehorned and treatment with topical anaesthetic ethanol spray; and DTAP = scoop dehorned and treatment with topical anaesthetic powder). a-b Within each time point, treatment groups not sharing a common letter are significantly different ($P < 0.05$). A-C Within each treatment, time points not sharing a common letter are significantly different ($P < 0.05$).

There was a significant effect of area ($P < 0.001$) (Fig. 4.5.2). Calves had a greater probability of displaying more severe responses for area 1 than for area 2.

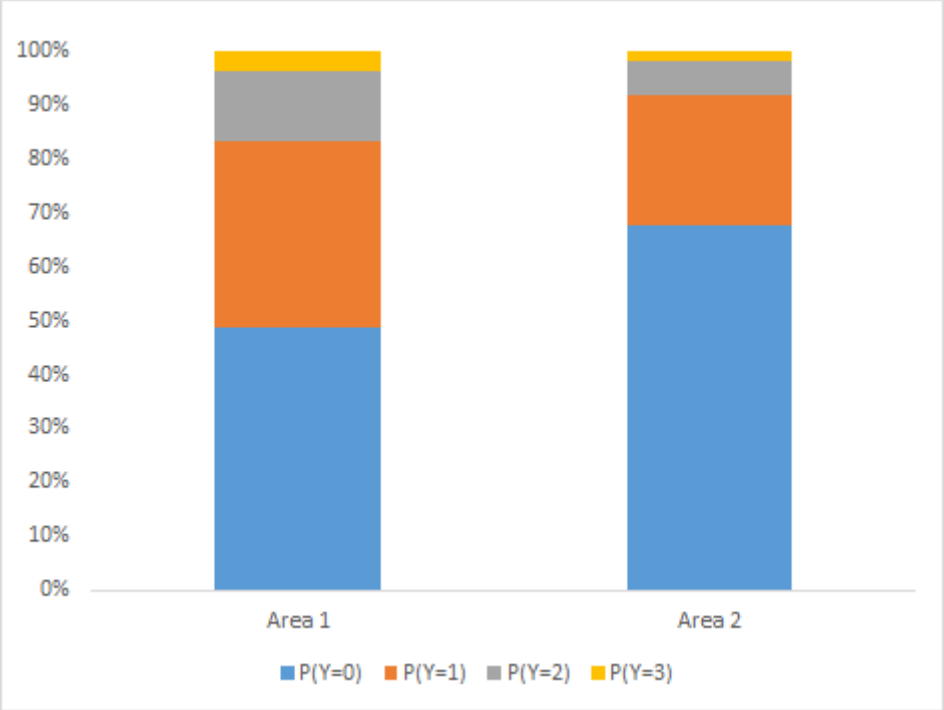


Figure 4.5.2 Probability of calves from trial 1 displaying responses (Y; 0 = no response, 1 = mild, 2 = moderate, 3 = severe) to stimulation of different areas
Results combine the effect of both von Frey monofilaments, both treatment groups and all time points. There was no significant effect of von Frey (*P* = 0.916).

Application of the ethanol liquid via a spray bottle was easier and quicker than application of the powder via a spoon, with less wastage of product. The powder was difficult to apply directly to the dehorned area and it was noted that wastage was an issue, particularly in windy conditions, raising health and safety concerns for the operator with potential inhalation of the powder during application.

4.5.2 Trial 2

There was a significant time x treatment interaction (P<0.001) (Fig. 4.5.3). DCB calves had an increasing probability of displaying more severe responses at each time-point from 1 h onwards. DTAG calves had an increasing probability of displaying more severe responses at each time-point from 0 to 2 h. DTAE calves had an increasing probability of displaying more severe responses at each time-point from 0 to 4 h. Prior to treatment (0 h), there were no differences between any treatment groups. At 1 h, DTAG calves were more likely to display a more severe response than CON calves. From 2 h onwards, all dehorned calves were more likely to display more severe responses than CON calves. There was a significant effect of area (*P* < 0.001) (Fig. 4.5.4). Calves had a greater probability of displaying more severe responses for area 1 than for area 2. There was a significant effect of von Frey (*P* = 0.007) (Fig. 4.5.5). Calves had a greater probability of displaying more severe responses to the 300 g/f von Frey than to the 75 g/f von Frey. Administration of the cornual nerve block took more time and requiredmore skill than application of the ethanol liquid and the gel via a spray bottle.

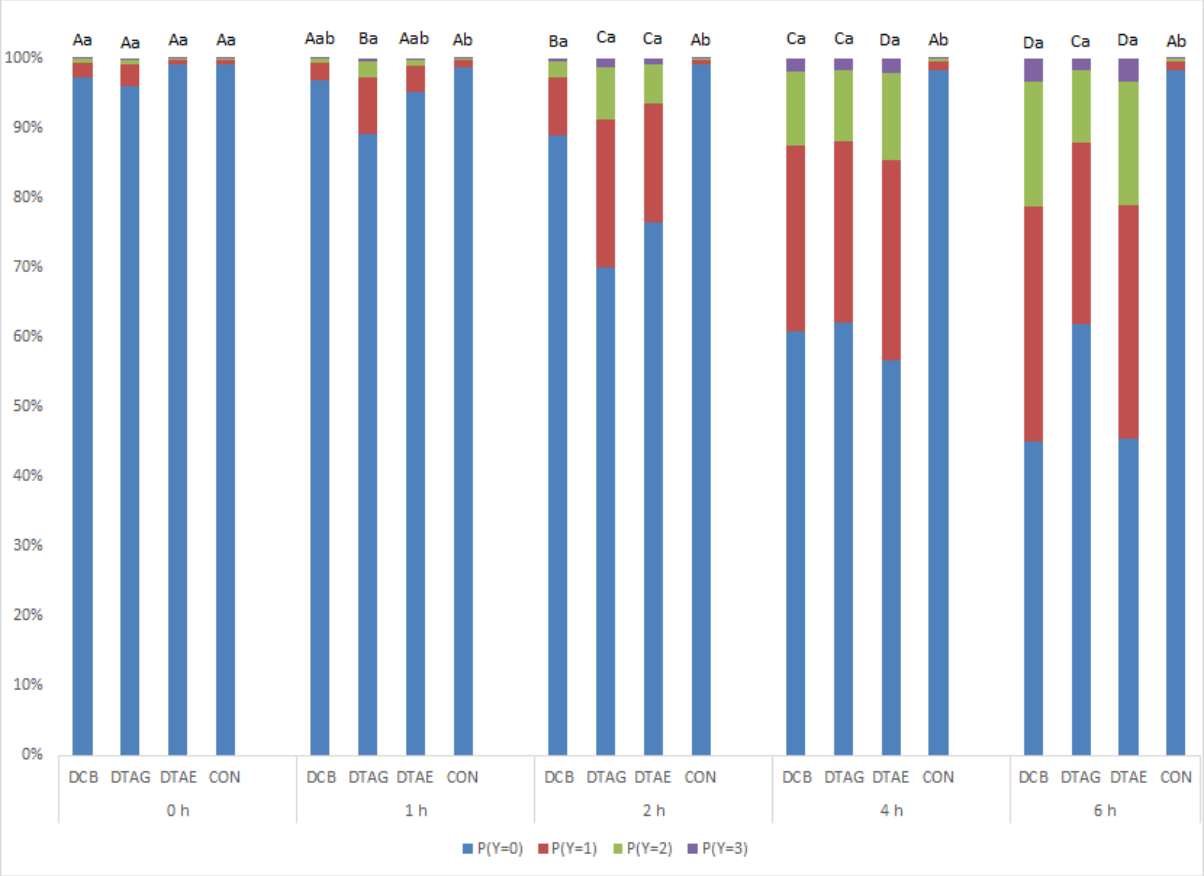


Figure 4.5.3 Probability of calves from trial 2 in each treatment group displaying responses (Y; 0 = no response, 1 = mild, 2 = moderate, 3 = severe) at different time points
Results combine the effect of both von Frey monofilaments and all sites tested. (DCB = scoop dehorned and treatment with cornual nerve block; DTAG = scoop dehorned and treatment with a topical anaesthetic gel; DTAE = scoop dehorned and treatment with a topical anesthetic ethanol spray; and CON = sham dehorned). a–c Within each time point, treatment groups not sharing a common letter are significantly different ($P < 0.05$). A-D Within each treatment, time points not sharing a common letter are significantly different ($P < 0.05$).

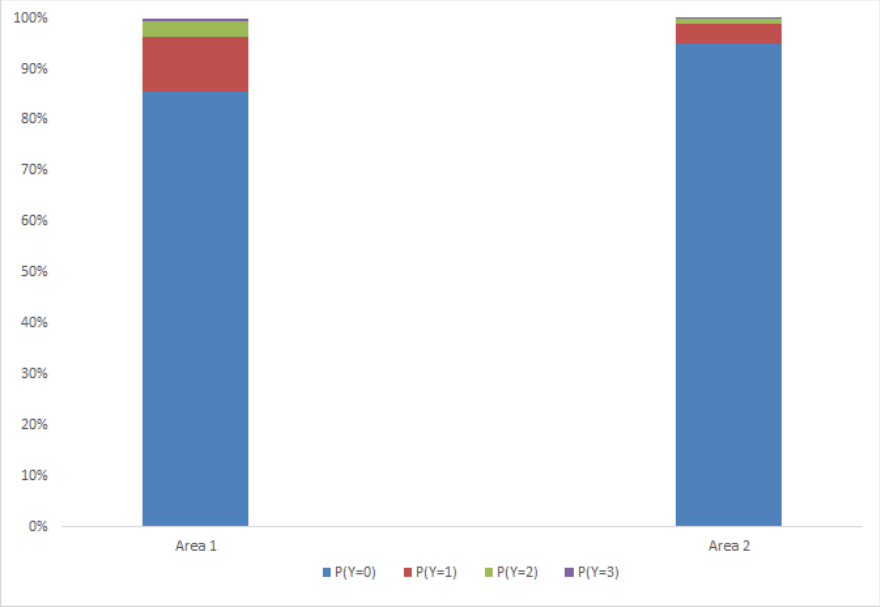


Figure 4.5.4 Probability of calves from Trial 2 displaying responses (Y; 0 = no response, 1 = mild, 2 = moderate, 3 = severe) to stimulation of different areas
Results combine the effect of both von Frey monofilaments, all treatment groups and all time points.

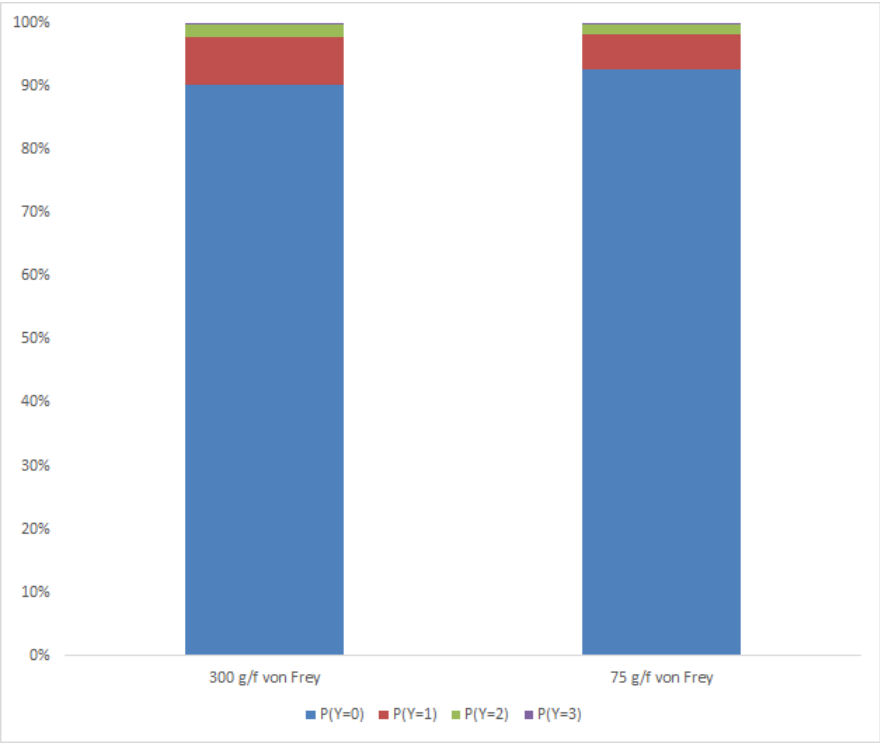


Figure 4.5.5 Probability of calves from Trial 2 displaying responses (Y; 0 = no response, 1 = mild, 2 = moderate, 3 = severe) to stimulation with different von Frey monofilaments
Results combine the effect of both Areas, all treatment groups and all time points.

Administration of the cornual nerve block took more time and required more skill than application of the ethanol liquid and the gel via a spray bottle.

4.6 Evaluating treatments with topical anaesthesia and buccal meloxicam on pain and inflammation caused by amputation dehorning of calves

4.6.1 Environment

Average ambient temperatures during the data collection period on days 1, 3 and 7 were 33.89°C, 30.30°C and 33.85°C, respectively. Average ambient humidities during the data collection period on days 1, 3 and 7 were 33.97%, 33.99% and 9.63%, respectively.

4.6.2 Behaviour

There were 16 missing focal periods due to calves being unidentified in the video footage. Of these missing samples, there were 2 from time-point 1 (2 x CON calves), 5 from time-point 2 (2 x CON, 2 x DBM and 1 x DTA calves) and 9 from time-point 3 (4 x CON, 2 x DBM and 3 x DTA calves). Behaviours influenced by time only are neither presented nor discussed.

There was a significant treatment x time interaction on the frequency of head shakes (P = 0.025) head turns (P = 0.036) and combined point behaviours (P = 0.037) (Table 4.6.1). CON calves displayed fewer head shakes than all dehorned calves at 2 and 3 h following treatment and CON and DTA calves displayed fewer head turns than DBM calves at 2 h following treatment. CON calves displayed fewer combined point behaviours than all dehorned calves at 2 h following treatment. There was no significant effect of treatment on any other behaviours (P > 0.05).

4.6.3 Maximum wound temperature

On day 1, infrared photographs from 15 calves (5 x D, 5 x DBM and 5 x DTA calves) were missing due to a temporary technical malfunction with the infrared camera. On day 3, infrared photographs from 1 D calf were missing as they could not be located.

There was a significant effect of day (P = 0.003), with greater maximum wound temperatures on days 3 and 7 compared to day 1 (Table 4.6.2). There was a significant effect of ambient temperature (P < 0.001). A moderate positive relationship between ambient temperature and maximum wound temperature was identified (R = 0.52). There was no significant effect of treatment (P = 0.797).

Table 4.6.1 Mean frequency of head shakes, head turns and combined point behaviours displayed by calves in each treatment group within a 5-minute focal sample at each time-point

Behaviour	*P* – value	Time-point (h)	Mean frequency (± s.e.m.)			
			CON	**D**	**DBM**	**DTA**
Head shakes	0.025	1	0.94^{Aa} ± 0.42	1.59^{Aa} 0.60	0.98^{Aa} ± 0.41	1.80^{Aa} ± 0.66
		2	0.13^{Ba} ± 0.13	1.72^{Ab} ± 0.64	2.24^{Ab} ± 0.82	2.13^{Ab} ± 0.76
		3	0.17^{Ba} ± 0.16	2.76^{Ab} ± 0.92	0.99^{Ab} ± 0.43	2.14^{Ab} ± 0.82
Head turns	0.036	1	2.33^{Aa} ± 0.51	4.17^{Aa} ± 0.67	4.17^{Aa} ± 0.67	4.25^{Aa} ± 0.68
		2	1.25^{Aa} ± 0.37	2.08^{Bab} ± 0.48	3.70^{Ab} ± 0.70	1.27^{Ba} ± 0.39
		3	2.10^{Aa} ± 0.52	1.58^{Ba} ± 0.42	1.60^{Ba} ± 0.46	2.22^{Aba} ± 0.57
Combined point behaviours	0.037	1	1.70^{Aa} ± 1.14	2.04^{Aa} ± 1.42	1.87^{Aba} ± 1.26	2.01^{Aa} ± 1.39
		2	0.88^{Ba} ± 0.69	1.58^{Ab} ± 1.05	2.08^{Ab} ± 1.58	1.68^{Ab} ± 1.15
		3	1.32^{Aba} ± 0.98	2.05^{Aa} ± 1.43	1.54^{Ba} ± 1.10	2.08^{Aa} ± 1.62

CON = Sham dehorning / control; D = amputation dehorning; DBM = amputation dehorning with pre-operative buccal meloxicam; DTA = amputation dehorning with post-operative topical anaesthetic.

$^{a, b}$ Values within a row with different superscripts differ significantly at $P \leq 0.05$.

$^{A, B}$ Values within a column with different superscripts differ significantly at $P \leq 0.05$.

Descriptive statistics are based on predicted means (± s.e.m.).

Table 4.6.2 Mean maximum wound temperature of all dehorned calves on days 1, 3 and 7 following treatment

Day	Mean maximum wound temperature (°C) ± s.e.m.
1	38.83[a] ± 0.42
2	40.43[b] ± 0.35
6	40.30[b] ± 0.40

[a, b] Values with different superscripts differ significantly at $P \leq 0.05$.

Descriptive statistics are based on predicted means (± s.e.m.). A significant effect was found (P = 0.003).

4.6.4 Wound morphology

Photographs from two calves (1 x D and 1 x DTA calf) on day 3 were excluded due to poor quality. On day 3, it was noted anecdotally that some of the open wounds were in the early stages of flystrike, as indicated by putrefactive odour and weeping of the wound. On day 7, the severity of flystrike had increased, as indicated by the presence of maggots in some open wound sinuses, serous exudate and the characteristic foul odour.

There was a significant treatment x day interaction (P = 0.03). All wound appearance scores decreased from day 1 to day 3. Wound morphology scores of DBM and DTA calves increased from day 3 to day 7 (Fig. 4.6.1).

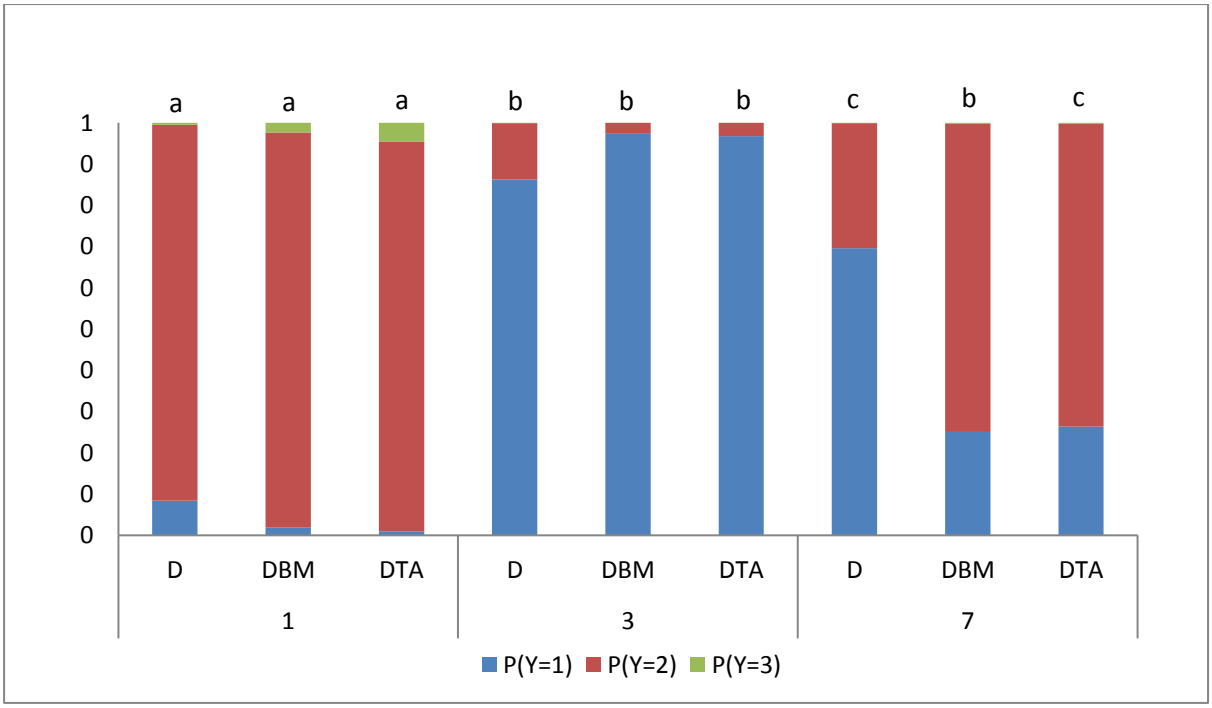


Figure 4.6.1 Probability of dehorning wounds from calves in each treatment group displaying appearance scores (Y; 1, 2, 3) on days 1, 3 and 7 following treatment

D = dehorned; DBM = dehorned with pre-operative buccal meloxicam; DTA = dehorned with post-operative topical anaesthetic.

[a, b] Days with different superscripts differ significantly at $P \leq 0.05$.

There were no significant differences between treatments within each time-point (P > 0.05).

A significant effect was found ($P < 0.03$).

4.7 Effects of topical anaesthetic and buccal meloxicam treatments on concurrent castration and dehorning of beef calves

4.7.1 Animals and environment

For experiment 1, calves weighed 198.77 ± 36.39 kg at the beginning of the trial. Daily maximum temperature throughout this experiment was 21.4°C, 21.1°C, 20.7°C, 23.8°C, 19.7°C, 19.9°C and 23.6°C for days 0, 1, 2, 3, 4, 5 and 6, respectively. Daily global solar exposure throughout this experiment was 13.5, 7.8, 15.0, 13.2, 14.9, 6.4 and 4.8 MJ/m^2 for days 0, 1, 2, 3, 4, 5 and 6, respectively.

For experiment 2, calves weighed 206.88 ± 40.23 kg. Days A, B and C of experiment 2 correspond with days 3, 4 and 5 of experiment 1.

4.7.2 Weight gain (experiment 1)

Ten data points were excluded, as 3 (1 x CD, 1 x CDTA and 1 x CDBMTA) were missing upon the second weighing and 7 (1 X CON, 2 x CD, 1 x CDBM and 3 x CDBMTA) were identified as outliers within their treatment groups using the boxplot procedure of Genstat®.

There was a significant effect of treatment on weight gain ($P < 0.001$). CON and CDBMTA calves had significantly greater weight gain values than CD calves. CON calves also had significantly greater weight gain values than CDBM and CDTA calves (Table 4.7.1).

Table 4.7.1. Mean weight gain of calves in each treatment group over 6 days.

Treatment	Mean Weight Gain (kg) ± s.e.m.
CON ($n = 50$)	−3.69 [a] ± 0.77
CD ($n = 50$)	−8.30 [c] ± 0.77
CDBM ($n = 50$)	−6.62 [bc] ± 0.76
CDTA ($n = 50$)	−6.59 [bc] ± 0.76
CDBMTA ($n = 50$)	−5.40 [ab] ± 0.79

CON = no castration and dehorning/positive control; CD = castration and dehorning/negative control; CDBM = castration and dehorning with pre-operative buccal meloxicam; CDTA = castration and dehorning with intra-operative topical anaesthetic; and CDBMTA = castration and dehorning with pre-operative buccal meloxicam and intra-operative topical anaesthetic. [a], [b], [c] Values with different superscripts differ significantly at $p \leq 0.05$. Descriptive statistics are based on predicted means (± s.e.m.). A significant effect was found ($p < 0.001$).

4.7.3 Paddock utilisation (experiment 2)

As part of the data 'cleaning' procedures, 8.4% of the total data points were removed; 16.5%, 4.1%, 4.6%, 11.9% and 3.7% of the data points were removed for treatment groups CON, CD, CDBM, CDTA and CDBMTA, respectively. There was no significant effect of treatment on paddock utilisation ($P = 0.167$). While there was a significant effect of day on paddock utilisation ($P < 0.001$), this is not presented nor discussed further due to acknowledged logging time and duration differences across days.

4.7.4 Lying activity (experiment 2)

There was no significant effect of body weight on total lying activity ($P = 0.724$). There was a significant interaction between treatment and day ($P < 0.001$) on total lying activity. CD calves spent the least proportion of time lying and CDBMTA calves spent the greatest proportion of time lying on all days. All other calves spent an intermediate proportion of time lying compared to CD and CDBMTA calves on all days. The proportion of time spent lying increased from day 0 to day 1 for all calves and again from day 1 to day 2 for all calves except CON calves (Table 4.7.2).

There was no significant effect of body weight on the frequency of lying bouts or the average duration of lying bouts ($P = 0.743$ and $P = 0.079$, respectively). There was no significant effect of treatment on the frequency of lying bouts or the average duration of lying bouts ($P = 0.225$ and $P = 0.141$, respectively). While there was a significant effect of day on the average frequency of lying bouts and the average duration of lying bouts ($P < 0.001$ and $P <$

0.001, respectively), this is not presented nor discussed further due to acknowledged logging time and duration differences across days.

Table 4.7.2. Proportion of time spent lying by calves in each treatment group on days 0, 1 and 2.

Day	Proportion of Time Spent Lying down (%)				
	CON	**CD**	**CDBM**	**CDTA**	**CDBMTA**
	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)
0	30.09 ^{Aab} ± 0.37	16.55 ^{Aa} ± 0.46	39.11 ^{Aab} ± 0.35	29.53 ^{Aab} ± 0.37	50.46 ^{Ab} ± 0.26
1	50.57 ^{Bab} ± 0.26	24.84 ^{Ba} ± 0.42	44.37 ^{Bab} ± 0.32	41.63 ^{Bab} ± 0.30	66.81 ^{Bb} ± 0.17
2	49.19 ^{Bab} ± 0.27	27.64 ^{Ca} ± 0.40	45.81 ^{Cab} ± 0.31	43.58 ^{Cab} ± 0.29	67.80 ^{Cb} ± 0.17

CON = no castration and dehorning/positive control; CD = castration and dehorning/negative control; CDBM = castration and dehorning with pre-operative buccal meloxicam; CDTA = castration and dehorning with intra-operative CDTA; and CDBMTA = castration and dehorning with pre-operative buccal meloxicam and intra-operative topical anaesthetic. ^{a}, ^{b} Values within a row with different superscripts differ significantly at $p \leq 0.05$. ^{A}, ^{B}, ^{C} Values within a column with different superscripts differ significantly at $p \leq 0.05$. Descriptive statistics are based on predicted means (±s.e.m.). A significant effect was found ($p < 0.001$).

4.7.5 Individual behaviour (experiment 2)

There were 6 missing focal samples due to calves being unidentified in the video footage. Of these missing samples, there was one from time point 1 (1 × CDBMTA calf), one from time point 2 (1 × CDBMTA calf) and 4 from time point 6 (1 × CON, 1 × CDBM and 2 × CDTA calves). Behaviours influenced by time only are neither presented nor discussed. As the behaviours ‘walk with a stiff gait,’ ‘walk with a limp,’ ‘stand statue’ and ‘lie abnormal’ occurred infrequently, it was decided to only analyse the behaviours ‘walk,’ ‘stand’ and ‘lie,’ instead of their modifiers (‘walk relaxed,’ ‘walk with a stiff gait,’ ‘walk with a limp,’ ‘stand relaxed,’ ‘stand statue,’ ‘lie normal’ and ‘lie abnormal’). The behaviours head pawing and kicking occurred too infrequently for statistical analysis.

There was a significant effect of treatment × time on the frequency of ear flicks ($p = 0.006$) displayed by the calves. The frequency of ear flicks was significantly greater in CDTA calves than in CON, CD and CDBMTA calves at 120 min and significantly greater in CDBM calves than in CDTA calves at 240 min (Table 4.7.3).

Table 4.7.3. Mean frequency of ear flicks, head turns and tail flicks displayed by calves in each treatment group within a 5-min focal sample at each time-point.

Behaviour	Effect and *p*-Value	Time (min)	CON	CD	CDBM	CDTA	CDBMTA
			(*n* = 12)	(*n* = 11)	(*n* = 11)	(*n* = 11)	(*n* = 12)
Ear flicks	Treatment × Time (*p* = 0.006)	40	0.53 ^{Aba} ± 0.31	1.84 ^{Aa} ± 0.71	0.66 ^{Aa} ± 0.35	1.59 ^{Ba} ± 0.61	0.50 ^{Aba} ± 0.30
		80	0.20 ^{Aa} ± 0.18	0.80 ^{Aa} ± 0.42	0.86 ^{Aba} ± 0.41	0.25 ^{Aa} ± 0.20	0.14 ^{Aa} ± 0.15
		120	0.27 ^{Aa} ± 0.21	0.56 ^{Aa} ± 0.34	0.72 ^{ABab} ± 0.37	3.24 ^{Bb} ± 1.05	0.48 ^{Aba} ± 0.29
		180	0.53 ^{Aba} ± 0.31	0.80 ^{Aa} ± 0.42	1.78 ^{Aba} ± 0.66	0.89 ^{Aba} ± 0.41	0.41 ^{Aba} ± 0.27
		240	0.47 ^{ABab} ± 0.28	1.36 ^{Aab} ± 0.58	2.57 ^{ABb} ± 0.87	0.38 ^{Aa} ± 0.25	0.55 ^{ABab} ± 1.22
		360	1.12 ^{Ba} ± 0.50	0.72 ^{Aa} ± 0.39	3.31 ^{Ba} ± 1.09	2.14 ^{Ba} ± 0.96	0.68 ^{Ba} ± 0.36
Head turns	Treatment (*p* = 0.049)		0.52 ^{a} ± 0.15	0.97 ^{ab} ± 0.24	1.04 ^{ab} ± 0.26	1.42 ^{b} ± 0.33	0.57 ^{a} ± 0.28
Tail flicks	Treatment (*p* = 0.04)		2.95 ^{a} ± 0.92	7.73 ^{c} ± 2.16	9.65 ^{c} ± 2.65	3.95 ^{ab} ± 1.21	6.13 ^{bc} ± 1.67

CON = no castration and dehorning/positive control; CD = castration and dehorning/negative control; CDBM = castration and dehorning with pre-operative buccal meloxicam; CDTA = castration and dehorning with intra-operative topical anaesthetic; and CDBMTA = castration and dehorning with pre-operative buccal meloxicam and intra-operative topical anaesthetic. ^{a}, ^{b}, ^{c} Values within a row with different superscripts differ significantly at $p \leq 0.05$. ^{A}, ^{B} Values within a column with different superscripts differ significantly at $p \leq 0.05$. Descriptive statistics are based on predicted means (± s.e.m.).

There was a significant effect of treatment on the frequency of head turns ($p = 0.049$) and tail flicks ($p = 0.04$) displayed by calves. CON calves displayed significantly less head turns than CDTA calves. CON and CDTA calves displayed significantly less tail flicks than CD and CDBM calves (Table 4.7.3). There was a significant effect of treatment on the duration of time calves spent walking ($p = 0.024$), eating ($p < 0.001$) and drinking ($p = 0.002$). The duration of time spent walking was significantly less in CON calves than in CD and CDBMTA calves and significantly greater in CDBMTA calves than in CDBM and CDTA calves. The duration of time spent eating was significantly greater in CON calves than in all other calves and significantly less in CDTA calves than in CDBMTA calves. The duration of time spent drinking was significantly greater in CON calves than in CDBMTA calves (Table 4.7.4). Treatment did not have a significant effect on the duration or frequency of any other behaviours.

Table 4.7.4. Mean duration of time (s) spent walking, eating and drinking by calves in each treatment group within a 5-min focal sample.

Behaviour	Effect and *p*-Value	CON (*n* = 12)	CD (*n* = 11)	CDBM (*n* = 11)	CDTA (*n* = 11)	CDBMTA (*n* = 12)
Walking	Treatment (p = 0.024)	23.82 ^{a} ± 6.62	47.09 ^{bc} ± 6.90	36.89 ^{ab} ± 6.92	32.78 ^{ab} ± 6.93	53.45 ^{c} ± 6.64
Eating	Treatment (p < 0.001)	127.64 ^{a} ± 14.00	33.01 ^{bc} ± 14.55	48.73 ^{bc} ±14.63	18.98 ^{c} ±14.71	67.88 ^{b} ± 14.08
Drinking	Treatment (p = 0.002)	9.43 ^{a} ± 1.86	5.30 ^{ab} ± 1.92	6.39 ^{ab} ± 1.95	2.65 ^{ab} ± 1.96	1.20 ^{b} ± 1.87

CON = no castration and dehorning/positive control; CD = castration and dehorning/negative control; CDBM = castration and dehorning with pre-operative buccal meloxicam; CDTA = castration and dehorning with intra-operative topical anaesthetic; and CDBMTA = castration and dehorning with pre-operative buccal meloxicam and intra-operative topical anaesthetic. ^{a}, ^{b}, ^{c} Values within a row with different superscripts differ significantly at $p \leq 0.05$. Descriptive statistics are based on predicted means (± s.e.m.).

There was a significant effect of day on the duration of time calves spent drinking (p < 0.001). Calves treated on day 1 spent a greater duration of time drinking compared to calves treated on days 2 or 3 (Table 4.7.5). There was a significant effect of day on the frequency of head shakes (p < 0.001), head turns (p < 0.001), ear flicks (p < 0.001), stamps (p = 0.022) and tail flicks (p < 0.001) displayed by calves. Calves treated on day 1 displayed more head shakes, head turns and ear flicks than those treated on days 2 and 3. Calves treated on days 1 and 2 exhibited more foot stamps on than those treated on day 3. The frequency of tail flicks decreased each day (Table 4.7.5). Day did not have a significant effect on the duration or frequency of any other behaviours.

Table 4.7.5. Mean duration of time (s) spent drinking and mean frequency of head shakes, head turns, stamps, ear flicks and tail flicks displayed by calves (n = 57) on each day within a 5-min focal sample.

Behaviour	*p*-Value	Outcome	Day A (*n* = 17)	Day B (*n* = 20)	Day C (*n* = 20)
Drinking	p < 0.001	Duration of time (s)	12.43 ^{a} ± 1.56	2.43 ^{b} ± 1.45	0.12 ^{b} ± 1.44
Head shakes	p < 0.001	Frequency	1.44 ^{a} ± 10.61	0.46 ^{b} ± 3.37	0.28 ^{b} ± 2.10
Head turns	p < 0.001	Frequency	1.62 ^{a} ± 0.30	0.60 ^{b} ± 0.13	0.61 ^{b} ± 0.13
Stamps	p = 0.022	Frequency	0.21 ^{a} ± 0.07	0.17 ^{a} ± 0.06	0.06 ^{b} ± 0.02
Ear flicks	p < 0.001	Frequency	1.57 ^{a} ± 0.34	0.52 ^{b} ± 0.13	0.51 ^{b} ± 0.13
Tail flicks	p < 0.001	Frequency	11.45 ^{a} ± 2.47	5.39 ^{b} ± 1.18	2.79 ^{c} ± 0.68

^{a}, ^{b} Values within a row with different superscripts differ significantly at $p \leq 0.05$. Descriptive statistics are based on predicted means (± s.e.m.). Body weight did not have a significant effect on the duration or frequency of any behaviours.

4.8 Investigating analgesia for control of pain and haemorrhage following spaying via the willis dropped ovary technique in cattle

4.8.1 Change in body weight

There was no significant effect of treatment or stage of maturity on change in body weight (*P* = 0.756 and *P* = 0.311, respectively).

4.8.2 Mortality

One S cow and one STA heifer died at approximately 4 and 44 h following treatment, respectively. The post mortem of the cow revealed that ovariotome penetration borrowed into cervical tissue before penetrating further along from the entry point than normal and there was evidence of haemorrhage from this point. Based on the reproductive anatomy of the cow, it appeared that parturition had occurred recently. The post mortem of the heifer reveleated that the point of ovariotome penetration skewed off to one side and the exit point was more lateral and closer to the broad ligament and potentially blood vessels than normal. There was also evidence that severe haemorrhage had occurred at the ovarian pedicle.

4.8.3 Change in packed cell volume

There was no significant effect of treatment or stage of maturity on change in PCV (*P* = 0.276 and *P* = 0.103, respectively). There were two animals that had decreased PCV outlying the normal range of the data. One of these animals was a STAMEL heifer and the other was a STA heifer. This STA heifer was the animal that died at approximately 44 h following treatment.

4.8.4 Change in total plasma protein

There was no significant effect of treatment on change in TPP (*P* = 0.884 and *P* = 0.104, respectively).

5 Discussion

5.1 Effect of a topical vapocoolant spray on perioperative pain response of unweaned calves to ear tagging and ear notching

This study contributes new information on the potential use of vapocoolant sprays to induce local anaesthesia of the skin of the ears of calves. Live tissue required vapocoolant spraying of longer duration to achieve adequate cooling than when spray was applied to dead tissue. Spray duration of 3 seconds was required in live tissue to reduce the ear temperature to anaesthetic levels of <10 °C, as compared with 1 second in dead tissue. This difference was most likely a result of reperfusion of live tissue with warming blood. Fjordbakk and Haga (2011) found that a 15 second vapocoolant spray duration was required for optimal reduction of pain associated with jugular vein cannulation in horses. Both species and the type of tissue targeted could result in differences in cooling rates, particularly as cooling can have differential effects on different types and lengths of nerve fibres (Douglas and Malcolm 1955). In addition, temperature reduction is less at greater tissue depths (Millis 2004). The depth of tissue of the neck and greater vascularization of that area would slow cooling rate as compared with the thinner skin over cartilaginous ear tissue. In the current study, both

sides of the ear were sprayed to allow maximum temperature penetration. The ability of the vapocoolant spray to induce cryoanaesthesia in other tissues, such as the scrotum and spermatic cord of calves for surgical castration, requires further investigation.

The validation study found that a 3 second spray reduced tissue temperature to below 10 °C for up to 16 seconds. In human skin, Farion *et al.* (2008) found that intravenous cannulation-associated pain was reduced for up to 60 seconds following a 10 second vapocoolant application. As ear tagging and ear notching are performed rapidly in bovines, it is expected that a 16 second interruption of nerve conduction would be sufficient for the duration of the procedure. In a production animal situation, where time, product use and practicality are important because of high stock numbers (Anil *et al.* 2005), a short spray duration (3 second) is likely to be beneficial in reducing local pain from production procedurs conducted on the skin of bovine ears. The time taken for the tissue to reach <10 °C is not expected to impact on the practicality of using vapocoolant ear skin sprays as it is unlikely that the procedure would be conducted within 4 seconds of spraying. Further work may be needed to examine this issue if current ear tagging procedures should alter. Future studies could focus on validation of the presence of tissue anaesthesia following spraying but prior to the tagging procedure. A skin prick test has been used for this previously (Fjordbakk and Haga 2011).

Tissue injury can occur when local cooling exceeds -20 °C (Evans *et al.* 1981). While the risk of such injury would probably increase with use of spraying of a longer duration, no evidence of skin lesions or frostbite was detected by o 8 weeks following spraying of skin for up to 15 seconds in horses (Fjordbakk and Haga (2011). Our observations that tissue temperatures did not reach -20 °C in the preliminary spray validation studies, and that no tissue damage was observed for at least a week after use of a spray duration of 3 seconds, suggest that vapocoolant spraying of bovine ear skin is very likely to be safe.

The vapocoolant spray reduced pain responses to both ear tagging and ear notching. That 60% of VS animals showed no response (Table 4.1.2) suggests there is adequate cooling with blocking of nociception and nerve conduction from induced anaesthesia in most animals. Lower pain response scores were found in the VS than in the WS groups overall. The rapid action and ease of application of the spray indicate the technique is practical and suggests that potentially, cryoanaesthesia is a practical alternative to other pain management options, including both local anaesthesia and non-steroidal anti-inflammatory drugs, which may require administration up to 90 minutes before the procedure (Earley and Crowe 2002; Stafford *et al.* 2002; Ting *et al.* 2003a; Meyer *et al.* 2007). Further, in comparison to both epidural anaesthesia and use of sedative analgesics such as xylazine, where there is risk of locomotor dysfunction that may result in pelvic limb ataxia and/or recumbency (Ting *et al.* 2003a; Currah *et al.* 2009), the brief, localized action of the vapocoolant spray allows farmers to release the calf immediately after the procedure. This is beneficial for both producers and animals, enabling more rapid delivery of procedures and reduced stress of calves due to reduction in duration of both restraint and separation from dams. Once producers observe and experience the ease of application of vapocoolant sprays and their efficacy in reducing pain of ear tagging, they may well be willing to adopt routine cryoanaesthesia.

It was noted that some VS calves exhibited greater pain scores than others in response to ear tagging and notching. It is unclear whether this was due to inadequate cooling or responses to external stressors, including presence of the human operator or sound of the tagging or notching devices. Although animals were allowed to settle and were handled in a calm manner, restraint and close proximity to humans can result in avoidance behaviours similar to those observed during pain (Mitchell *et al.* 2004). Calves were blindfolded, which has been found to reduce struggling and lower heart rate (Mitchell *et al.* 2004). However, we did not account for the sound of the procedures and proximity to the calf's head. In future studies, a positive sham-treated control group could be included to observe the effects of handling and external stressors on pain response scores.

An increase in heart rate following ear tagging has been reported, although no significant changes in behaviour were noted (Stewart *et al.* (2013) although their study did not report on perioperative pain behaviour. Our study noted that discomfort was displayed during the procedure, suggesting that pain associated with ear tagging is predominantly procedural.

Ear notching resulted in greater pain response scores in over half of the animals across both treatments. This was an expected outcome due to the amount of tissue damage caused by notching, and previous observations of prolonged wound healing in ear-notched calves (Petherick 2005). The behavioural responses observed in our study were similar to those reported previously (Friend *et al.* 1994), where evasive behaviour (head jerking) was observed during ear notching, but resolved within 30 seconds with minimal postoperative behavioural indicators of discomfort. With limited literature on studies of ear notching and ear tagging, there is a need for further studies documenting the pain and its amelioration of these important procedures.

There was a trend towards lower pain response scores for ear tagging on the right ear (NLIS tag) and in females (with ear notching on the right ear). When animals were restrained in lateral recumbency on their right side, movement to the right was restricted (Espinoza *et al.* 2013). Thus, a deviation between sides tagged and sex was expected, because of the method of restraint used in this study. The lack of statistical effect could reflect the general efficacy of the vapocoolant spray; although similar studies with larger sample sizes may be warranted, partuclarly as this would improve the confidence intervals obtained and validity of the results.

For efficacy trials, this study used a predetermined scale of expected behaviours, referred to by a trained, treatment-blinded observer to reduce the impact of assessor bias and strengthen assessment validity (Bateson 1991; Meyer *et al.* 2007; Millman 2013). Although many pain studies use physiological and biochemical indicators of pain (Coetzee 2011), these methods can be labour-intensive, expensive and invasive (Currah *et al.* 2009). Furthermore, pain indicators such as cortisol concentration and heart rate variability may be influenced by other factors, such as stress, fear, physical activity or sexual excitement (Anil *et al.* 2005; Currah *et al.* 2009). Observation of acute behavioural response to the procedure allowed us to document procedural pain, with minimal impact from external factors. Furthermore, videography allowed playback, size and speed alterations, and editing to remove view and sound of treatment for more accurate behavioural observations (Millman 2013). However, using a single observer, may have limited the objectivity of these observations and in future studies, it is recommended that multiple observers be used to score behaviours, and interobserver variation be calculated to further reduce bias.

In conclusion, this study provided new information on both the pain of ear tagging and ear notching, plus amelioration of pain responses by pre-procedural application of vapocoolant spray. These studies suggest that further investigation of the efficacy of cryoanaesthesia for other husbandry procedures in calves and for similar procedures in other farm species is warranted.

5.2 Effect of lignocaine and a topical vapocoolant spray on the pain response to surgical castration in beef calves

This study investigated the use of a topical vapocoolant spray to provide local anaesthesia during surgical castration of calves. The results suggested that due to its transient efficacy, cryoanaesthesia delivered in this manner is not an adequate form of pain relief for such an invasive procedure as surgical castration. The results of this study also added to concerns on the efficacy of intra-scrotal lignocaine when applied 5 min prior to the surgical castration of calves. This study also evaluated pain associated with different stages of the surgical castration operation in calves. Extrusion and extraction of the testes by pulling of the spermatic cords elicited the greatest pain response. It is noted that conclusions from this study are based on subjective behavioural scores only, as although ocular temperature was also measured, changes to this physiological variable were considered due to factors independent of pain.

Descriptive and numerical behavioural scoring systems have previously been used to assess the peri-operative pain of ear tagging and ear notching (Lomax *et al.* 2017), hot-iron disbudding (Stilwell *et al.* 2010) and surgical castration (Coetzee *et al.* 2014) in calves. In this study, behavioural scoring showed that SHAM calves reacted least to treatment, with a strong probability (0.87) of displaying no response (score zero). This differs from all castrated calves, with probabilities of displaying no response between 0.12 and 0.34 (Fig. 4.2.2). This was an expected finding which indicates that increasing behavioural scores correspond with increasing degrees of distress and pain. There was no difference between behavioural scores of CAST calves and either LIG or VAPO calves. However, there was a difference between those of LIG and VAPO calves, with LIG calves 3.1 times more likely to have lower scores than VAPO calves. Lignocaine may have induced a minimal degree of local anaesthesia, as the probability of calves displaying no response to castration tended to be stronger in treatment LIG (0.34) compared to treatment CAST (0.20). Conversely, the application of vapocoolant spray appeared to heighten the behavioural response to castration, with the probability of calves having score zero tending to be lower in treatment VAPO (0.12) than treatment CAST (0.20). A previous study investigating the efficacy of a vapocoolant spray for the relief of distress caused by pediatric immunisation found similar results. Children that received vapocoolant demonstrated stronger distress related behaviours towards immunisation compared to children that did not receive vapocoolant (Cohen *et al.* 2009). An explanation for findings such as these is that vapocoolant spray may have caused an irritating effect that offset any benefit of pain relief. Another explanation is that immediate prior application of vapocoolant spray may have drawn the attention of subjects to the procedure, heightening the distress response (Cohen *et al.* 2009).

Studies of the efficacy of lignocaine for pre and peri-operative pain relief of surgical castration in calves have produced a range of contrasting results. Studies that have measured heart rate, ocular temperature (Stewart *et al.* 2010) and cortisol (Fisher *et al.* 1996; Earley and Crowe 2002; Stafford *et al.* 2002; Stewart *et al.* 2010) have demonstrated

effective local anaesthesia induced by lignocaine applied during surgical castration of calves. However, other studies that considered cortisol may be an indicator of pain, have shown no peri-operative effect of lignocaine on the cortisol response to surgical castration in calves (Stafford *et al.* 2002; Mintline *et al.* 2013). These differences may be expected due to the numerous variables that exist between these studies, inlcuding the location and time of administration, the volume of lignocaine used, the type of surgical castration method (surgery-pull, surgery-cut, surgery with the Henderson castration tool), calf age and method of pain assessment. Further, cortisol responses are induced by several other factors independent of pain, including tissue damage, inflammation and haemorrhage (McCarthy *et al.* 2016). In our current study, lignocaine was administered 5 min prior to castration, considered a more realistic representation of use of this technique for routine husbandry procedures performed on cattle in a commercial setting. Previous studies have administered lignocaine at longer periods prior to castration, including 10 (Stewart *et al.* 2010), 15 (Fisher *et al.* 1996) or 20 (Earley and Crowe 2002; Stafford *et al.* 2002; Webster *et al.* 2013) mins. Longer periods prior to castration presumably aid local tissue perfusion with blockage of increasing numbers of nociceptors and it is possible that administration at 5 mins prior to surgery was insufficient to obtain optimal anaesthesia and may explain the minimal effect of lignocaine on the behavioural response of calves to surgical castration in the current study. However, additional time required for administration of pain relief is a major hindrance to its widespread adoption by commercial producers and is especially impractical for use in large, extensive beef cattle operations (Petherick 2005).

Vapocoolant spray was investigated in the current study as it offers a practical method of potentially providing local anaesthesia prior to painful procedures. Anaesthetic efficacy has been achieved using vapocoolant sprays prior to cosmetic botulinum injections (Weiss and Lavin 2009), intradermal anaesthetic injection (Collado-Mesa *et al.* 2015), venipuncture (Mace 2016) and vaccination in humans, arthrocentesis in horses (Fjordbakk and Haga 2011) and ear tagging and notching in calves (Lomax *et al.* 2017). In calves, a vapocoolant spray cooled ear tissue to < 10°C (temperature threshold required for anaesthesia) for 16 s when applied for 3 s and resulted in lower behavioural pain responses to ear tagging and ear notching (Lomax *et al.* 2017). In the current study, the same vapocoolant spray was also applied for 3 s to the scrotum and spermatic cords. Application of the spray did not lower the behavioural responses of calves to surgical castration. Similarly, other studies have found no effect of vapocoolant sprays on pain response to intravenous cannulation (Costello *et al.* 2006) and skin tests (Waibel and Katial 2005) in humans and jugular catheterisation in horses (Fjordbakk and Haga 2011). The effect of cooling can differ in relation to the type, length and depth of nerve fibres and the degree of tissue vascularisation (Lomax *et al.* 2017). The variation between studies could be attributed to the type of vapocoolant, duration of spray time and the type and location of tissue injury involved with each procedure. It is recognised that products with short application durations (≤ 3 s) are most practical for use during routine husbandry procedures performed in a commercial farm setting. For this reason and on the basis of the results from the previous study on ear tagging and ear notching (Lomax *et al.* 2017), a 3-second spray was investigated in the current study. Even if a longer spray would have induced anaesthesia, the pain associated with those stages of castration such as testicular extraction, where there is manual pressure and stretching applied to the spermatic cords, would likely still not be alleviated. Extraction of the testes invokes sensory responses along the length of the spermatic cords, into the inguinal canal and potentially visceral pain centres during externalisation of the tissues (Taylor and Weary

2000). As these stages of the procedure were identified as the most painful in the current trial and the effect of vapocoolant sprays is likely to be limited to the scrotal skin, cryoanaestehisa is considered ineffective for use during surgical castration of calves, pother than perhaps a reduction in the pain of the scrotal skin on insertion of the needle for injection of lignocaine, and/or the incision of the scrotum during surgical catsrtion.

This study provides information on the behavioural responses, as a measure of pain, associated with the different stages of castration in beef calves. Procedural sources of pain during surgical castration have been identified in piglets through analysis of vocal responses (Taylor and Weary 2000), with results similar to those of the current study (Fig. 4.2.3). In piglets, initial restraint, washing of the ano-genital area, incision of the scrotum, and extraction and incision of the spermatic cords were all compared. Extraction and incision of the spermatic cords evoked the greatest degree of vocalisation (Taylor and Weary 2000). Scrotal incision or excision, then testicular extraction and and severing of the spermatic cords, both affect different tissue types; cutaneous and visceral, respectively (Taylor and Weary 2000). Typically, visceral tissues are less sensitive to pain than non-visceral tissues (Baumans *et al.* 1994). Visceral pain is usually dull, diffuse and poorly localised compared to sharp, well localised somatic pain (Okafor *et al.* 2014). However, the testes are among the few viscera producing sharp, localised pain due to well innervated tissue (Taylor and Weary 2000) and the presence of true nociceptors (Baumans *et al.* 1994). Visceral pain can result from non-damaging stimuli such as distension or traction (Taylor and Weary 2000) and is often associated with exaggerated autonomic reflexes (Robinson and Gebhart 2008). Extraction of the spermatic cords likely invokes sensory stimuli within and along the length of the spermatic cords and extending into the inguinal canal and beyond, likely resulting in greater pain than both other stages of the castration procedure, particularly the rapid skin incision or excision and incision with severing of the spermatic cords (Taylor and Weary 2000). This may explain why the probability of calves displaying the most severe response (score three) was greatest for stage 2 (extraction with extrusion of the first spermatic cord) (0.22) and followed by stage 4 (extraction with extrusion of the second spermatic cord (0.09), in our study. There also appeared to be an effect of the order of procedural stages, with calves being 2.2 times more likely to have greater behavioural scores to extrusion of the first spermatic cord (stage 2) than to extrusion of the second spermatic cord (stage 4). Similarly, the final stage of the procedure resulted in the least severe behavioural response overall, with a low probability (0.04) of calves having a score three (Fig. 4.2.3). Behavioural responses of lame sows to thermal or pressure algometry of the rear legs have been shown to differ according to the leg (right or left) that was first tested. The right leg, tested first, was shown to tolerate less mechanical pressure or thermal stimulation than the left leg. This difference in nociceptive threshold was probably due to the sows being startled by the first manipulation (Pairis-Garcia *et al.* 2014). Similarly, this could well be the case in our study, with the pain associated with extraction of the first spermatic cord most likely startling the calves, resulting in a greater display of responses interpreted as representing pain..

There was a trend ($P = 0.051$) for an interaction between treatment and stage of procedure, with SHAM calves, as expected, tending to display lower behavioural responses to stages 2, 3, 4 and 5 compared to all castrated calves (Fig. 4.2.1). At stage 1 of both sham castration or castration, all calves tended to react similarly, suggesting that the initial handling of the testes invoking a response; this may have been either a startle or a distress response. This response may have diminished the expected response to the pain associated with excision

of the scrotum. Alternatively, the similarity between the responses from sham castrated calves and castrated calves at this stage could mean that the pain sensation associated with excision of the scrotum is of a milder intensity than has been assumed. LIG calves tended to display lower behavioural responses to extraction of the spermatic cords (stages 2 and 4) (Fig. 4.2.1), suggesting the insertion of lignocaine into the dorsal scrotum may have reduced the pain associated with this component of the castration procedure.

Measurement of ocular temperature has been used to evaluate stress and pain associated with disbudding (Stewart *et al.* 2008; Stewart *et al.* 2009; Stock *et al.* 2016) and castration (Stewart *et al.* 2010; Dockweiler *et al.* 2013) of calves. A decrease in ocular temperature following stress or pain is possibly due to vasoconstriction of capillary vessels caused by activation of the sympathetic nervous system. A subsequent increase in ocular temperature could be the result of increased dominance of the parasympathetic nervous system which results in vasodilation of blood vessels (Godyn *et al.* 2013). In our study, the increase in ocular temperature following castration or sham castration could not be attributed to the experience of pain, as there was no significant difference between SHAM and CAST calves for this measurment. These results contrast with those of some previous studies where differences in ocular temperature have been detected between control calves and calves undergoing disbudding (Stewart *et al.* 2008; Stewart *et al.* 2009) or castration (Stewart *et al.* 2010). However, as in our study, other previous research has found no difference in ocular temperature between control calves and calves undergoing disbudding (Stock *et al.* 2016) or castration (Dockweiler *et al.* 2013). Where no difference has been found between control and castrated calves, the methodology involved capturing a series of photographs before, during and after treatment (Dockweiler *et al.* 2013), similar to our study. However, differences have been detected when continuous recordings of ocular temperature were collected every 20 s for 10 min prior to treatment and 20 min post treatment (Stewart *et al.* 2010). Another difference in methodology refers to the location of where the maximum temperature was detected; either the whole eye (Dockweiler *et al.* 2013) or the medial posterior palpebral border of the lower eyelid (Stewart *et al.* 2010). Albeit a weak correlation, our study found a positive relationship between ambient temperature and maximum ocular temperature, despite calibration of the infrared camera for atmospheric conditions. The effect of ambient temperature on ocular temperature has not been examined in previous studies (Stewart *et al.* 2010; Dockweiler *et al.* 2013) and should be considered in future research. Habituation of calves to handling facilities and restraint was not conducted in our study. As it appears that stress rather than pain, may have had been dominant influence on ocular temperature, as has been previously suggested (Stock *et al.* 2016), habituation should be considered when conducting similar stideis in the future.

The results of this study showed that vapocoolant spray applied to the scrotum and each spermatic cord during surgical castration of beef calves did not reduce behavioural responses of the animals to the procedure. Lignocaine administered into the neck of the scrotum 5 min prior to castration also did not significantly reduce behavioural responses of animals to the procedure. The conocusion from these results is that adequate peri-operative anaesthesia for surgical castration of beef calves was not provided by either of the interventions tested, as assessed by scoring the behavioural response of the animals on a numerical rating scale. Future research should consider analgesic interventions that address the pain associated with extraction of the spermatic cords, as this stage of the surgical castration procedure appears to be the primary source of distress in beef calves. Due to

these results, , cryo-anaesthesia was not explored further as an option for intra-operative pain relief of amputation dehorning due to its lack of efficacy for surgical castration. Amputation dehorning is a significantly more invasive procedure than ear tagging and notching, potentially involving damage to bone and surrounding tissues. Hence, the assumption was made that using a topical vapocoolant spray in dehorning would only be relevant to transient anaesthesia of the skin and lack sufficient efficacy for significant alleviation of procedural pain associated with amputation dehorning.

5.3 Effect of a topical anaesthetic formulation on the cortisol response to surgical castration of unweaned beef calves

The results of this study did not support the hypothesis that provision of TA would reduce the post-operative cortisol response of calves following surgical castration. The main finding was that TA had no significant effect on cortisol concentrations of surgically castrated calves..

In this study we elected to use cortisol as a potential indirect measure of pain associated with castration; the cortisol response to castration of cattle, and the amelioration of this response by use of anaesthetics and analgesics, has been well documented (Coetzee 2011). However, it is still widely accepted that cortisol secretion occurs in response to a variety of stressors other than pain (Molony and Kent 1997). These stressors include weaning, social isolation, transport, social mixing, novelty, restraint and handling (Stilwell *et al.* 2010). In addition, there are numerous other variables, such as diurnal changes and individual variation, that influence cortisol concentration and have implication for the interpretation of experimental findings (Molony and Kent 1997). The results of our study highlight the responsiveness of cortisol secretion to factors other than pain. Whilst it is preferable to combine multiple physiological, neuroendocrine and behavioural measures to assits in managing the impact of non-painful factors on trial results, this usually requires using separate groups of animals for each measure. We were only able to utilise a single group of calves for this study so options for obtaining data in addition to cortisol concentrations were limited, partuclarly due to the constant interventions including movement and repeated handling of the calves for blood sampling. In addition, other measurements could have caused distress responses that may have affected our findings on cortisol. A previous study conducted by our research group, on the same property, provides information on the behavioural response and wound sensitivity of calves subjected to the same treatments as those in the current study (Lomax and Windsor 2014). That study found that calves treated with TA expressed significantly less pain-related behaviour than untreated calves and withstood greater pressure applied to the wound and surrounding skin as measured by an electric von Frey anaesthesiometer (0 to 1000 g; IITC Life Sciences, Woodland Hills, CA, USA). There were no significant treatment differences when wound sensitivity was measured with a von Frey monofilament (300 g; Bailey Instruments Ltd, Manchester, UK) (Lomax and Windsor 2014).

In our study, an increase in plasma cortisol from −0.5 to 0 h was apparent for all treatment groups (Tables 4.3.1 and 4.3.2). This likely reflects the stress of separation from mothers (Loberg *et al.* 2008), movement and handling through a race, and physical restraint in a head bale (Cooke *et al.* 2009) prior to treatment. Cortisol concentration further increased from 0 to 0.5 h to reach a peak, with the rise more apparent in C and CTA calves than CON calves (Table 4.3.1). In addition, the integrated cortisol response of CON calves was significantly lower than those of C and CTA calves. The significantly greater AUCs of C and

CTA calves, along with the tendency for these calves to display higher peak cortisol concentrations, is indicative of a response to castration which involves surgical tissue damage, stimulation of nociceptors (Earley and Crowe 2002) and localised haemorrhage (Gann and Egdahl 1965).

Our study was not the first to find a non-significant effect of locally administered anaesthetic on the cortisol response of castrated calves (Fisher *et al.* 1996; Webster *et al.* 2013). The effect of lidocaine HCl, a component of TA, has been widely investigated for its effects on the cortisol response to castration of calves (Coetzee 2011). One study found that 2% lidocaine HCl, injected into the testes and scrotum 15 min before castration, did not reduce the integrated cortisol response to surgical or burdizzo castration of Friesian calves (Fisher *et al.* 1996), despite significantly reducing cortisol concentrations from 0.25 to 1 h. Fisher *et al.* (1996) suggested that this was likely attributable to the short duration of action (~1 h) (Reichl and Quinton 1987) of lidocaine HCl. This suggestion does not explain the lack of difference between the integrated cortisol response of CTA and C calves in our study where the TA consists of the anaesthetic agent bupivacaine HCl in addition to lidocaine HCl. Bupivacaine is a long acting local anaesthetic with a duration of action of ~5 to 8 h (Coetzee 2011). In addition, the adrenaline and potentially the gel components of the TA formulation may have slowed the rate of systemic absorption and excretion of lidocaine and bupivacaine and as has been suggested, prolonging the duration of action of the anaesthetic actives (Lomax *et al.* 2013).

A study that measured cortisol concentrations of surgically castrated dairy calves found that 20 ml of 2% lidocaine HCl administered in a subcutaneous ring block at the neck of the scrotum, just above the testes, did not reduce the cortisol response to castration (Webster *et al.* 2013). It is likely that administration of lidocaine alone as a subcutaneous ring block was ineffective at mitigating the pain of castration, although it was suggested that the relatively high dose rate and the injection into the testes rather than the spermatic cords may have caused pain from tissue irritation or inflammation. It was also proposed that the twisting and severing of spermatic cords by the Henderson tool may have stimulated nociceptors proximal to the site of lidocaine injection (Webster *et al.* 2013). In our study, the castration procedure and the mode of anaesthetic application differs makedly to the study by Webster *et al.* (2013). The spermatic cords were severed using a knife after the distal third of the scrotum was excised and the testes were exposed. TA formulation was applied postoperatively, directly onto exposed, injured tissue. Therefore, a more likely explanation for the lack of difference between C and CTA calves in our study is that the castration procedure causing tissue damage, inflammation, stimulation of nociceptors, and haemmorhage, led to a rise in cortisol (Gann and Egdahl 1965; Earley and Crowe 2002). This explanation is also applicable when comparing the results of our study to contrasting results from previously published studies. In some studies, local administration of 2% lidocaine HCl has been shown to significantly reduce but not eliminate the acute cortisol response to castration of Friesian calves (Ting *et al.* 2003a; Stewart *et al.* 2010). In these studies, lidocaine HCl was injected either 10 (Stewart *et al.* 2010) or 20 min (Ting *et al.* 2003a) before castration. Pre-operative administration of lidocaine HCl would have ensured amelioration of both peri-operative and acute post-operative pain. Provision of a TA formulation applied postoperatively, had no effect on peri-operative pain and thus may induce a rise in cortisol (Mellor *et al.* 2000). Of note, the study by Ting *et al.* (2003a) employed the burdizzo method for castration and as this restricts blood flow to the testes,

causing necrosis and preventing haemorrhage (Stafford and Mellor 2005b), of which cortisol secretion is a concomitant (Gann and Egdahl 1965).

It is important to note that in the previous studies (Stafford *et al.* 2002; Webster *et al.* 2013), cortisol concentrations of uncastrated calves were significantly lower than those of untreated castrated calves. In our study, although the integrated cortisol response of CON calves was significantly lower than that of C and CTA calves, there was no significant difference between the mean cortisol concentrations of any treatment group. These findings have been demonstrated previously in a study comparing plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration (Coetzee *et al.* 2008). In this study, mean cortisol concentrations of castrated and uncastrated calves were not significantly different at any point up to 4 h following the procedure. In addition, the mean cortisol response of castrated and uncastrated calves was similar regardless of whether castrated calves vocalised or displayed aversive behaviour during the procedure. Similar to our study, Coetzee *et al.* (2008) used Angus crossbred calves and habituation for the experiment, consisting of restraint in a head bale and a rope halter for 15 to 30 min daily for 5 days. It was proposed that non-painful stressors, such as animal handling, had an effect on cortisol that was disproportional to that of the nociceptive stimulus of castration (Coetzee *et al.* 2008). Non-painful stressors experienced by the calves in our study included separation from their mothers, novel exposure to human handling, and physical restraint.

Other studies have habituated calves to intensive handling and holding facilities for 3 weeks before experimentation commenced (Ting *et al.* 2003a; Stewart *et al.* 2010). This extensive habituation reduced the effect of animl handling on treatment outcomes, resulting in a significant effect of castration on cortisol responses (Ting *et al.* 2003a; Stewart *et al.* 2010). As the calves used in our study underwent a less intensive, shorter habituation process, the intensity and duration of habituation may not have been sufficient to eliminate the stress caused by handling and restraint in a head bale. Furthermore, previous studies inserted indwelling jugular catheters 1 day before experimentation to facilitate intensive blood sampling and minimise animal handling (Ting *et al.* 2003a), with calves in one study held in individual pens for the duration of the trial, enabling manual restraint for collection of each blood sample (Ting *et al.* 2003a). In another study, blood samples were only taken −20, −10, 15 and 20 min relative to castration, with each calf restrained individually in a squeeze chute for the duration of sample collection (Stewart *et al.* 2010). In both of these studies, access to the catheter did not require movement or head restraint of calves. In our study, calves were moved through the race and into the crush and restrained in a head bale in order to collect blood samples, regardless of indwelling catheter or jugular venipuncture. The risk of the catheters being damaged or even lost by this form of restraint meant that jugular venipuncture was a more practical option. Further, there are contradictory results in the literature on the effects of venipuncture on cortisol;some suggest that it has no effect (Alam and Dobson 1986), some suggest that it causes an increase in cortisol (Veissier and Leneindre 1988), with another suggesting that jugular venipuncture may induce an increase in cortisol concentration but it seemingly relates to the handling experience of cattle (Hopster *et al.* (1999). In our study, manual restraint for sampling, and jugular venipuncture, may have increased cortisol concentrations.

In our stdy, the calves had not previously experienced separation from their mothers prior to the experimental days, when they were separated for a period of 7 to 8 h. Studies investigating the stress of weaning have found that separating calves from their mothers

(Lay *et al.* 1998; Loberg *et al.* 2008; O'Loughlin *et al.* 2014), and additionally, altering normal milk intake (Lay *et al.* 1998), results in an elevated cortisol response (Lay *et al.* 1998; Loberg *et al.* 2008). The calves used in the current study were unweaned beef calves that prior to experimentation, had had minimal exposure to humans. Studies reporting an effect of castration on cortisol (Ting *et al.* 2003a) used dairy calves that in typical commercial situations are permanently separated from their mothers and artificially reared by humans within hours of their birth (Budzynska and Weary 2008). Commercial beef production systems typically wean calves at between 4 and 8 months of age, hence the period of separation from mothers in the current study likely caused distress and hence would have contributed to a major elevation in plasma cortisol. .

The effect of TA on the cortisol response to painful husbandry procedures has been explored in other production animal species. A study investigating a short acting TA, and a long acting TA found that both formulations were unsuccessful at reducing the cortisol response to castration in piglets. The short acting TA contained 14% Benzaine, 2% Butamben and 2% Tetracaine hydrochloride, whereas and the long acting TA formulation was the same product as used in our current study (Sutherland *et al.* 2010). It was suggested that as TA is applied postoperatively, the pain of the castration procedure itself may have overshadowed any effect of TA on cortisol and it was also suggested that the anaesthetic or application method was inadequate (Sutherland *et al.* 2010). These limitations can be applied to our current study. Of interest, a study investigating the pain relieving effects of the same TA formulation for mulesing and tail docking in lambs found that the product significantly, yet only moderately, reduced the peak cortisol response to the procedure and it had no effect on the AUC (Paull *et al.* 2007). It was also found that combining this TA formulation with the non-steroidal anti-inflammatory drugs (NSAIDs) carprofen and flunixin, resulted in a greater decrease in peak cortisol than TA alone, as well as a significant reduction in AUC Paull *et al.* (2007). Therefore, the effect of TA in combination with an NSAID on the cortisol response of calves to castration is worthy of future investigation.

In our study, there was no significant effect of treatment on the cortisol response of unweaned beef calves. It is likely that an insufficient habituation period, in addition to separation of calves from their mothers, may have caused an increase in calf cortisol concentrations independent of pain and that this may have masked any effects of TA on the pain of castration. The tendency for castrated calves treated with TA to have reduced cortisol concentrations at some time-points after castration and a reduced integrated cortisol response compared with untreated castrated calves, warrants further investigation. Based on the results of this tial and several other studies conducted by our team and others using cortisol estimations, it was decided that subsequent studies in this project should not continue to use cortisol in the evaluation of pain due to its many limitations, especially when using unweaned beef calves managed under commercial conditions.

5.4 Effects of topical anaesthesia and buccal meloxicam on average daily gain, behaviour and inflammation of unweaned beef calves following surgical castration

It is well documented that multimodal analgesia using a combination of LA and an NSAID provides superior analgesia following castration in calves. Therefore, this study compared the efficacy of TA and BM as single treatments and in combination. The effects of TA and

BM formulations, alone and in combination, on ADG, behaviour, scrotal diameter, maximum scrotal temperature and wound appearance score following surgical castration in beef calves were evaluated. The results suggest that TA and BM reduced post-operative pain and that BM reduced wound inflammation of calves that had been surgically castrated. This was demonstrated through a reduction in some pain-related behaviours within a 5 h period following castration when TA, BM or a combination of TA and BM had been administered. The anti-inflammatory effect of BM was shown through reduced maximum scrotal temperature 2 days following treatment when BM or a combination of TA and BM had been administered.

In this study, there was no effect of treatment on ADG, suggesting the low ADG on day one was most likely due to the long (5 h) separation of calves from their mothers on day 0 and therefore reduced milk intake and increased stress experienced (Perez-Torres *et al.* 2016). In addition, as these calves were unaccustomed to handling by humans, the processing of animals through handling facilities may have been an additional stressor that potentially contributed to reduced ADG (Petherick *et al.* 2009). Significantly reduced weight gain following surgical castration has been shown to occur in calves (Fisher *et al.* 1996; Ting *et al.* 2003a; Bretschneider 2005; Petherick *et al.* 2015). However, this is not always the case (Molony *et al.* 1995; Stafford *et al.* 2002; Webster *et al.* 2013). As the calves in the current study were unweaned, they had a readily available source of quality nutrients provided in their mothers' milk, and did not need to actively source feed and particularly protein, through grazing. This may explain why the impact of the castration procedure had no effect on ADG. In addition, as calves may have suckled more in response to the presence of pain, and suckling of milk in mammals has been suggested to have an analgesic effect via activation of the endogenous opioid system (Noonan *et al.* 1994; Landa 2003), this may also explain why there was no apparent effect of castration on ADG. Further, larger treatment group sizes may have been necessary to demonstrate potential differences between treatment groups, reflecting individual variability of weight gain as an outcome (Webster *et al.* 2013).

Behaviour has been used extensively to evaluate pain following castration of calves (Ting *et al.* 2003a; Petherick *et al.* 2015). As in the current study, an increase in the frequency of foot stamps has previously been observed in surgically castrated cattle (Fisher *et al.* 2001; Sutherland *et al.* 2013) and this display is considered indicative of irritation, possibly due to the sensation of pain following the surgical castration procedure (Fisher *et al.* 2001). Abnormal standing and walking have also been shown to occur following surgical castration of calves (Molony *et al.* 1995; Webster *et al.* 2013) and this behaviour is considered as a 'protective response' aimed at reducing the stimulation of injured tissue (Prunier *et al.* 2013). In our study, there were behavioural indications that both TA and BM reduced the pain of surgical castration during the 5-h post-operative period, observed as a significant reduction in the frequency of foot stamps and a tendency for reduced duration of time spent walking with a stiff gait. This is consistent with previous findings in beef calves (Lomax and Windsor 2014) and lambs (Lomax *et al.* 2010; Small *et al.* 2014). Use of TA formulation peri-operatively has been shown to reduce pain-related behaviour for at least 4 h following surgical castration of beef calves (Lomax and Windsor 2014) and for at least 5 h following combined surgical castration and tail docking of lambs (Lomax *et al.* 2010), as scored using a numerical rating scale. BM has previously been shown to reduce the amount of time lambs spend standing in a normal posture, standing in a hunched posture, standing in a

stretched posture and walking with a stiff gait in an 8 h period following combined surgical castration and tail docking, plus reduce the amount of time lambs spent in combined abnormal postures and behaviours during the 8-h period (Small *et al.* 2014).

In our current study, post-treatment differences between groups were not detected for many behaviours, with limited expression of these during the observation period. As in the study investigating the effect of TA formulation on the cortisol response to surgical castration in unweaned beef calves (outlined in sections 3.4, 4.3 and 5.3), the use of unweaned calves unaccustomed to humans and handling facilities may have limitted the ability to evaluate pain, as there was very little expression of most pain-related behaviours.Temporary separation of calves from their mothers during the experimental period may have attenuated pain-related behavioural responses, with the calves appearing to be motivated to reunite with their mothers, as indictaed by the increased vocalisation expressed during the behavioural observation period. This motivation to reunite with their mothers possibly shifted the calves' attention from the experience of pain (Petherick *et al.* 2015).

Scrotal size has been used to measure wound inflammation and healing following surgical castration (Mintline *et al.* 2014; Petherick *et al.* 2014b; Petherick *et al.* 2015; Olson *et al.* 2016), as extravasation of blood and onset of inflammation following surgical tissue injury is often observed visually (Gregory 2004). Physiological processes following tissue injury include hyperaemia, due to production of vasoactive metabolites and the release of histamine from mast cells, causing vasodilatation, an increase in vascular permeability, and extravasation of plasma and inflammatory cells into the extracellular space surrounding the wound to prevent infection and regulate wound healing (Harper *et al.* 2014). In the current study, there was no significant effect of treatment on scrotal size. This was also the case for a previous study that found no effect of the NSAID and flunixin, on scrotal size (Mintline *et al.* 2014). However, in another previous study, oral meloxicam was shown to reduce the increase in scrotal diameter of calves for the first 3 days following surgical castration (Olson *et al.* 2016). It is possible that differences in methodology may have contributed to the contrasting results. The previous study investigating oral meloxicam measured the mid-scrotal diameter whilst calves were standing (Olson *et al.* 2016), as opposed to measurement of the base of the scrotum whilst calves were in lateral recumbency in our study. With calves standing, it could be assumed that gravity would have caused sera and inflammatory fluids to cause odema and swelling of tissues in the distal scrotum, perhaps providing a measurement indicative that calves were standing rather than the more even distribution of sera and inflammatory fluids in the scotum when calves were in recumbency. This approach may also explain why differences in scrotal size were not detected across experimental days in the current study. The effect of BW on scrotal diameter showed heavier calves to have a larger scrotal diameter and is likely to represent a pre-treatment difference in scrotal and testicular development.

Infrared thermography has been used as a non-invasive, indirect measure of inflammation (Wright *et al.* 2006; Celeste *et al.* 2013). Skin temperature is influenced by cutaneous cell metabolism and blood flow, with an increase in temperature considered reflective of an increase in these factors (Celeste *et al.* 2013). Infrared thermography has previously been used to correlate an increase in scrotal temperature due to the presence of inflammation caused by surgical and band castration in beef calves (Moya *et al.* 2014). The effect of NSAIDs on scrotal temperature following castration has also previously been investigated (Mintline *et al.* 2014; Moya *et al.* 2014), with no effect found. In the current study, BM

reduced maximum scrotal temperature on day 2 following surgical castration. This may be attributable to the NSAID being used. Previous studies have used ketoprofen (Moya *et al.* 2014), with a half-life of 0.42 h (Coetzee 2011), or flunixin (Mintline *et al.* 2014), with a half-life of 3 to 8 h (Coetzee 2011). In comparison, meloxicam is considered to have an extended half-life of 27 h (range 19.97 to 43.29 h) (Coetzee *et al.* 2009; Coetzee 2011) and may explain the apparent reduction in inflammation on day 2 following castration. The increase in maximum scrotal temperature on day 6 from days one and 2 in C and CTA calves and from day 2 in CBM and CBMTA calves may be due to loss of the initial scab or re-vascularisation of the tissue, as day 6 was when lower wound morphology scores were detected (Fig. 4.4.1). This correlation between greater surface temperatures and healing has previously been shown for castration wounds in beef calves (Mintline *et al.* 2014) and cutaneous wounds in horses (Celeste *et al.* 2013). Although the present study found an effect of BW on scrotal temperature, the correlation was weak with no obvious trend identified. This may require further research to clarify individual animal effects on wound surface temperature.

Wound inflammation and healing following surgical castration of calves has previously been assessed using numerical rating scales based on visual assessment (Mintline *et al.* 2014; Petherick *et al.* 2014b; Petherick *et al.* 2015). Wound healing was investigated in our current study, as an increased rate of contraction in wound surface area over an extended period of healing has been shown to occur following application of TA to mulesing wounds in lambs (Lomax *et al.* 2008). In the current study, TA did not appear to affect inflammation and healing of surgical castration wounds in calves, as assessed visually over a 6-day period. Buccal meloxicam also had no effect on this outcome and is consistent with findings from previous studies showing no effect of the NSAIDs ketoprofen (Petherick *et al.* 2014b) and flunixin (Mintline *et al.* 2014) on wound morphology following surgical castration in calves. In the present study, the effect of day on wound morphology scores was consistent with the stages of wound inflammation and healing. At early stages from one to 3 days post-wounding, lesions are characterised by formation of a fibrin-blood clot, activation of epidermal edges, and influx of inflammatory cells dominated by neutrophils. From 4 to 7 days post-wounding, lymphocytes and macrophages are present, epidermal edges migrate, granulation tissue commences to proliferate, and a scab begins to form (Braiman-Wiksman *et al.* 2007). This latter stage of healing was observed for most calves by day 6, hence the improvement in wound morphology scores.

In conclusion, surgical castration resulted in an increased frequency of foot stamps and a tendency for an increased duration of time spent walking with a stiff hypometric gait. The frequency or duration of these behaviours was reduced by TA and BM, alone and in combination, suggesting both alleviated pain to some degree during the post-operative period. Buccal meloxicam reduced maximum scrotal temperature 2 days following surgical castration, consistent with an anti-inflammatory effect.

5.5 Effect of topically applied anaesthetic formulation on the sensitivity of scoop dehorning wounds in calves

Amputation dehorning is widely used in the Australian beef industry, causing an open wound and haemorrhage, plus pain and distress to calves (Petherick 2005; Stafford and Mellor 2009). Currently, there are no commercially available, farmer-applied anaesthetic or analgesic options for pain management for dehorning in Australia. While a cornual nerve block has been shown to effectively minimise acute pain associated with the procedure

(Sylvester *et al.* 1998; Sylvester *et al.* 2004), it has limitations for use in an extensive setting (Petherick 2005). The current study extended previous studies on the efficacy of modified formulations of TA formulations (including modifications of Tri-Solfen®) for dehorning in calves (Espinoza *et al.* 2013; Espinoza *et al.* 2015), comparing the effects of three formulations of TA and a cornual nerve block on wound sensitivity in scoop dehorned calves.

Firstly, the effects of two novel topical anaesthetics designed for use on scoop dehorning wounds were compared, to assess which formulation was more effective at providing wound anaesthesia. The ease of application was also observed to evaluate the practicality of these products for use in a commercial farm setting. These novel formulations were designed to improve absorption of anaesthetic agents in the presence of arterial haemorrhage resulting from the scoop dehorning procedure (Stafford and Mellor 2009). This issue was identified in a previous study investigating the use of the TA formulation for surgical tail-docking wounds in lambs where it was noted that arterial bleeding associated with surgical tail removal may have prevented effective adherence of the product to the wound, resulting in reduced TA efficacy (Lomax *et al.* 2010). Although modified formulations of Tri-Solfen® have shown some efficacy for scoop dehorning of cattle (Espinoza *et al.* 2013; Espinoza *et al.* 2015), compromised adherence of a gel product to the wound may still be an issue (Espinoza *et al.* 2013). Hence the investigation of the powder and ethanol / water base carriers of TA in this study.

Trial 1 demonstrated that the ethanol spray was more effective than the powder, as shown by greater amelioration of nociceptive responses to wound stimulation at 90 and 180 min post treatment. Responses of all calves increased in severity from 1 to 90 min after dehorning, indicating heightened sensitivity of the wound. Wound sensitivity continued to increase in DTAP calves from 90 to 180 min, suggesting a diminution of the effect of the powder after 90 min when compared to the ethanol spray.

The efficacy and practicality of the ethanol spray, a topical anaesthetic gel and a cornual nerve block were then compared in trial 2. Sham dehorned calves had the greatest probability of displaying minimal nociceptive responses (score 0) to stimulation at all time-points, indicating an absence of pain or hypersensitivity in the intact tissue. The increase in severity of nociceptive repsonses observed in all dehorned calves over time, demonstrated a progression of hyperaesthesia associated with the escalation of pain responses in skin incisions or open wounds (Redua *et al.* 2002; Lomax *et al.* 2008). There was no change in sensitivity from before treatment to one h post treatment in DCB calves, suggesting effective local anaesthesia. From 2 h onwards, the severity of responses in DCB calves increased at each time-point, indicating diminishing efficacy of the treatment. Similar responses have been reported in previous work investigating the efficacy of a cornual nerve block with lignocaine on prevention of cortisol (Sylvester *et al.* 1998) and behavioural responses (Sylvester *et al.* 2004) of dehorned calves. The response score severity of DTAG and DTAE calves increased up to 2 and 4 h, respectively, although did not change up to 6 h. This suggests a delayed anaesthetic effect for the spray-on formulations which persisted longer than the cornual nerve block. The ethanol spray contained a much higher concentration of anaesthetic agents compared to the other treatments and this could explain the extended duration of anaesthesia compared to the cornual nerve block. In addition, topical application of the anaesthetic agents may have impacted the rate of absorption, as suggested in previous studies (Brofeldt *et al.* 1989; Lomax *et al.* 2013; Lomax and Windsor 2014).

Extended duration of topical lignocaine applied to burn wounds in humans has been reported (Brofeldt *et al.* 1989). It was suggested that the gradual absorption of the lignocaine from a cream base resulted in extended anaesthesia. Prolonged efficacy of the TA gel formulation up to 24 h post treatment has been observed in mulesed sheep (Lomax *et al.* 2013) and castrated calves (Lomax and Windsor 2014). The vasoconstrictive properties of adrenaline in the TA formulation may contribute to slowing the rate of systemic absorption of the anaesthetic agents, enablinh concentration of the TA actives at the wound site to be protracted. In addition, the effect of a wound barrier created by the gel base has been suggested to attenuate pain, possibly by covering damaged nerve endings and protecting the wound from exposure to the environment and stimulation (Lomax *et al.* 2013) or from delaying absorption and metabolism of the TA actives. Extension of the observation period beyond 6 h should be considered in future studies examining the duration of efficacy of TA formulation for dehorning wounds.

There were no treatment differences in responses of DCB, DTAG or DTAE calves at any time-point, suggesting that the post-operative efficacy of all anaesthetic treatments was similar up to 6 h. DCB and DTAE calves responded similarly to CON calves at one h post treatment, indicating effective local anaesthesia at this time. DTAG calves tended to have more mild and moderate responses compared to CON calves at this time-point; again this may be attributed to a slower rate of absorption, as previously described (Lomax *et al.* 2013; Lomax and Windsor 2014).

There were very few severe response scores displayed by any dehorned calves at all time-points, particularly at one h and 2 h post treatment (Fig. 4.5.3), suggesting an anaesthetic effect. Alternatively this could indicate that the 300 g/f was not eliciting a noxious pain stimulus, resulting in less severe responses. In trial 2, although there was a greater probability of calves having more severe responses to stimulation with the 300 g/f von Frey than the 75 g/f, this was only marginal (Fig. 4.5.5).

Conclusions from this study are limited by the lack of a comparison to an untreated dehorned group of calves, omitted due to welfare concerns for such animals. However, the comparable results of the post operative spray-on formulations to the injected LA in the cornual nerve block, demonstrates efficacy of these products (Petrie *et al.* 1996; Sylvester *et al.* 1998; Sylvester *et al.* 2004).

Von Frey stimulation of both area 1 and 2 produced nociceptive responses from calves in both trials 1 and 2. The effect of Area is a reflection of primary and secondary hyperalgesia, with stimulation of area 1 (wound site) eliciting greater severity of responses from calves than that of area 2 (uncut surrounding tissue) within the 180 min (trial 1) and 6 h (trial 2) observation periods. Primary hyperalgesia develops at the site of injury due to sensitised nociceptors inducing local hyperaesthesia. Secondary hyperalgesia develops in the tissue surrounding the site of injury and is due to central sensitisation (Meyer *et al.* 2005), although is a consequence of primary hyperalgesia and thus tends to develop at a slower rate (Lomax and Windsor 2014), when accumulation of inflammatory mediators, initiated from wound injury, results in depolarisation of terminal nerve endings and excitation of nociceptors. The inflammatory mediators take time to accumulate and a pain response is only initiated when an excitation threshold is reached, resulting in gradual or delayed development of secondary hyperalgesia (Gregory 2004).

This is the first time that these current formulations of topical anaesthetic have been investigated for treatment of the wounds of dehorned calves and compared to a cornual nerve block for postoperative pain relief. The results of this study warrant further investigation into the pain relieving effects of TA for calves undergoing scoop dehorning, particularly as the ease of administration was superior to administration of a cornual nerve block. Further, the apparent efficacy of TA in desensitising dehorning wounds was comparable to that of a lignocaine cornual nerve block. No further exploration of the ethanol spray was conducted following this study as there were no apparent improvements to efficacy compared to Tri-Solfen®, a commercially available product now registered for use in Australian cattle.

5.6 Evaluating treatments with topical anaesthesia and buccal meloxicam on pain and inflammation caused by amputation dehorning of calves

The aim of this study was to evaluate the effects of TA and BM on pain and inflammation following amputation dehorning of calves (with similarities to the study on castration as see above in sections 3.5, 4.4 and 5.4). Although these products were investigated as their modes of administration are considered practical for on-farm administration, this study did not demonstrate any clear effects of TA or BM on pain or inflammation when administered alone. No conclusions can be made on the efficacy of these products for the relief of pain caused by dehorning in calves and further research is required.

In this study, pain was assessed using objective behavioural observations. There were behavioural differences detected between undehorned and dehorned calves but there were no significant effects of TA or BM on these behaviours. Dehorned calves displayed more head shakes at 2 and 3 h following treatment and more combined point behaviours at 2 h following treatment compared to CON calves ; indicating that these behaviours were pain-related and suggesting an escalation in pain over time in dehorned calves, likely due to a progression of inflammation (Coetzee 2011). This aligns with previous studies that have found head shaking to occur more frequently in dehorned than undehorned calves (Grondahl-Nielsen *et al.* 1999; Sylvester *et al.* 2004; Stilwell *et al.* 2010; Huber *et al.* 2013). Similarly, combined pain-related behaviours have been shown to occur more frequently in dehorned compared to undehorned calves (Stilwell *et al.* 2010). Although at 2 h following treatment, CON and DTA calves displayed fewer head turns than DBM calves, as there were no significant difference between CON, DTA and D calves at this point, it is unclear if this is indicative of effective pain relief from the TA. This behavior may be associated with other irritating factors such as the presence of blood from the wound running into the eyes of the calves, or flies on the wound or body.

A previous study found that the combination of lignocaine and ketoprofen had a significant effect on lying, grazing or ruminating, tail shaking and ear flicking between control and dehorned calves during the first 4 h following treatment, whereas this was not as evident when lignocaine or ketoprofen were administered alone (McMeekan *et al.* 1999). This suggests that a combination of TA and BM may have had a greater effect on the number of head shakes or combined point behaviours following dehorning in the current study. A combination of TA and BM was not assessed due to limited animal numbers for inclusion in the study and should be investigated in future research. In our study, there was little expression of pain-related behaviours overall and many behaviours were seemingly unaffected by treatment. The behavioural results from the current study may have been

affected by the calves being unweaned and potentially focused mainly on reuniting with their mothers (Petherick *et al.* 2015), as was also noted in the study investigating the effects of TA and BM on castration (see above in section 5.4). Dairy calves, already separated from their mothers, were used in similar studies previously (McMeekan *et al.* 1999; Sylvester *et al.* 2004) and treatment differences were more detectable. However, there are studies that have also found little or no difference in post-operative behaviour of dehorned and undehorned control animals (Doherty *et al.* 2007), hence the findings of the present study are not unusual.

Maximum wound temperature was greater on days 3 and 7 than on day one, reflecting the progression of inflammation and consistent with previous studies on wound temperature following surgical castration (Moya *et al.* 2014) and branding (Schwartzkopf-Genswein and Stookey 1997). Increased surface temperature resulting from hoof lesions (Alsaaod and Buscher 2012) and mammary gland infections (Colak *et al.* 2008) in dairy cattle, ear lesions in lambs (Karakus *et al.* 2015) and castration in beef calves (Moya *et al.* 2014) has previously been detected through the use of infrared thermography. In the initial stage following tissue injury, vasoactive metabolites are released, causing vasodilation of arterial vasculature and hyperthermia. Histamine release from mast cells, additionally increases vasodilation and vascular permeability to allow inflammatory cells to enter the perivascular space in the vicinity of the wound. Inflammation leads to wound healing, although it persists until bacteria and debris are cleared. It is characterised by an influx of neutrophils, macrophages and lymphocytes (Harper *et al.* 2014) and increased cutaneous cell metabolism and blood flow, observed as increased cutaneous temperature (Celeste *et al.* 2013).

The elevated temperatures observed on days 3 and 7 could be due to the presence of inflammation associated with bacterial infection and this may also explain the increase in wound morphology score from day 3 to day 7 (Fig. 4.6.1). The role of the inflammatory response is to ensure all bacteria and necrotic tissue debris is cleared (Harper *et al.* 2014). Neither TA nor BM affected maximum wound temperature on one, 3 or 7 days following treatment, suggesting that these products may not have had any or only minimal effect on inflammation at these time-points. This may be expected with TA, although was unexpected with meloxicam as this NSAID compound is known to inhibit production of inflammatory mediators and has a half-life of 19.97 to 43.29 h (Coetzee *et al.* 2009; Coetzee 2011). An alternative explanation for the lack of a treatment effect may be that infrared thermography is not sufficiently sensitive or appropriate to detect drug induced changes in dehorning wound inflammatory status. Previous studies have found no differences in wound surface temperature of calves castrated with and without the NSAIDs ketoprofen (Moya *et al.* 2014) or flunixin (Mintline *et al.* 2014). Flunixin also has been shown to have no effect on surface temperature of hot-iron brands in cattle (Tucker *et al.* 2014b). Future research should include alternative measures of inflammation, such as analysis of acute phase proteins.

Wound morphology scores have been used to assess inflammation and healing associated with dehorning (Neely *et al.* 2014), castration (Marti *et al.* 2010; Mintline *et al.* 2014; Petherick *et al.* 2014b; Petherick *et al.* 2015) and branding (Tucker *et al.* 2014a; Tucker *et al.* 2014b). Results from our study showed an increase in wound morphology score from day 1 to day 3 for all calves, consistent with expected progress towards wound healing. From day 3 to day 7, a reduction in wound morphology score was seen for DBM and DTA calves, possibly due to a progression in localised infection, fly worry and flystrike, plus potentially

sinusitis. As there was no formal recording of the presence and degree of local infection, flies or sinusitis, it is speculative to suggest if these developments may have impaired wound healing, although it was noted that there was a substantial increase in the number of flies on or within the wound and sinuses throughout the study that may have confounded normal wound healing. TA has been found to improve wound healing 2 and 4 weeks following mulesing in lambs. However, photographic measurement of wound contraction rather than visual scoring was used to assess healing in this study (Lomax *et al.* 2008). Buccal meloxicam has been found to worsen wound conditions 4 and 7 days following surgical castration and 7 days following tail docking in lambs, as measured using a visual scoring system (Small *et al.* 2014), although flystrike may have confounded the results in that study, with fly worry probably contributing to delayed healing in our current study.

In contrast to the study investigating the effects of TA and BM on castration (see sections 3.5, 4.4 and 5.4), there was no apparent amelioration of pain or inflammation by TA or BM following dehorning. This indicates that these products may not alter wound temperature, wound morphology or behaviour following amputation dehorning in older calves (6 to 8 months of age), when administered singly. A combination of TA and BM was not examined in this study due to limited animal numbers, although it is considered appropriate to examine in future studies of the single procedure of dehorning. Combined administration of LA and an NSAID has been shown to reduce pain-related behaviours following dehorning, despite no effect when either LA or NSAID is used alone (McMeekan *et al.* 1999).

The difference between the results in the castration and dehorning studies conducted in here could be due to multiple factors. Firstly, the location and type of tissue damage is very different. Castration likely causes an initial extensive stimulation of scrotal and cord nociceptors due to excision of the scrotum followed by a visceral pain response from extraction of the spermatic cords, Amputation dehorning also causes an initial stimulation of nociceptors in the skin. However this is likely followed by continued nociceptor impulses at the wound site arising from stimulation by inflammatory mediators (Sutherland *et al.* 2013). Amputation dehorning of older calves where the horn is attached to the frontal bone causes damage to the periosteum and underlying tissues with exposure of the frontal sinuses (Kihurani *et al.* 1989). The nature of this wound differs greatly from surgical castration wounds where damage occurs to internal viscera and scrotal tissue. Absorption of TA is likely to be more efficient when applied to castration wounds than to dehorning wounds due to a greater soft tissue surface and presumably less haemorrhage (spermatic arterial verss corneal arterial bleeding).

In this study, there was no effect of TA or BM on pain and inflammation following dehorning of calves, as measured through analysis of behaviour, wound temperature and wound morphology. The timing of the study in summer meant that there were many flies present, resulting in irritation of the wound, fly worry and infection. This was likely a major confounding factor when examining pain and inflammation with and without TA and BM. Further research is needed to draw conclusions on the efficacy of these products for amputation dehorning, particularly in older calves. This study identified likely bacterial infection, fly worry, and potentially flystrike and sinusitis as probable animal welfare issues following dehorning of calves and this needs to be addressed through controlled timing of husbandry procedures and preventative treatments for flies where possible.

5.7 Effects of topical anaesthetic and buccal meloxicam treatments on concurrent castration and dehorning of beef calves

Use of injectable anaesthetics and analgesics on Australian farms is challenging, preventing the widespread uptake of these approaches to pain management by Australian beef producers. However, as 'farmer applied' pain relief products are now commercially available for use on calves undergoing surgical husbandry procedures, this study investigated the effects of TA and BM, separately and in combination, on weight gain, individual behaviours including lying activity, following concurrent castration and dehorning of *Bos indicus* weaner calves. TA allows delivery of lignocaine and bupivacaine via absorption at the wound site and BM is absorbed through the mucosa of the buccal cavity. There are few previous studies investigating the effects of surgical husbandry procedures and pain relief on welfare of *Bos indicus* cattle (McCosker *et al.* 2010; Petherick *et al.* 2011; Petherick *et al.* 2013; Petherick *et al.* 2014a, 2014b; Laurence *et al.* 2016; Musk *et al.* 2017). In our study, results of experiments 1 and 2 have not been directly compared due to differences in animal numbers, dehorning and castration technicians and experimental environments and timeframes. The findings show a combination of TA and BM improved short-term weight gain and increased lying activity following castration and dehorning, suggesting this combination of treatments was likely effective in improving the welfare of *Bos indicus* weaner calves. There were also behavioural trends suggesting TA and BM reduced pain following castration and dehorning of *Bos indicus* weaner calves.

This study aimed to represent procedures on an extensively managed beef system in northern Australia, where *Bos indicus* or *B. indicus* crossbred cattle undergo 'marking' (including ear tagging, ear notching, branding, castrating and dehorning) at a much wider range of ages than calves in temperate beef production systems. Practical methods for alleviating pain caused by husbandry procedures is increasingly important in all production systems, with TA and BM offering a potentially convenient approach to providing this in large-scale systems. . There is limited literature on castration and dehorning performed concurrently, despite this being an increasingly common practice. Findings from previous studies on TA and BM in this project (see above sections 4.3, 4.4, 4.5 and 4.6) were considered during the design of this study. Weaned cattle were used to examine weight gain involving larger treatment group numbers (n = 50). Weaned cattle were also used for behavioural observations to eliminate or at least minimise the effect of potentially competing motivational states on expression of pain-related behaviour in the yards. Accelerometers were used to monitor lying behaviour in a large paddock, to provide more realistic representation of behaviour following marking on commercial properties.

Assessment of production parameters following invasive husbandry procedures in livestock is important and of relevance to producers seeking to optimise welfare and production. Weight gain and various measures of stress and pain have been used to evaluate the impact of castration and dehorning in calves on welfare and production (Fisher *et al.* 1996; Baldridge *et al.* 2011; Glynn *et al.* 2013). In farm animals, pain can reduce feeding behaviour and invoke stress responses and immune reactions that affect production parameters, including weight gain (Prunier *et al.* 2013). For example, increased nociceptor activity increases sympathetic tone and adrenal secretions, potentially inhibiting gastric control centres, causing decreased rumen motility (Coetzee *et al.* 2014). A reduction in weight gain is expected to follow castration and dehorning (Mosher *et al.* 2013), suggesting poor animal

welfare and economic losses occur from such procedures (Glynn *et al.* 2013). In our study, all calves, including CON calves, appeared to lose weight over the 6 days following treatment. This may have partially been due to differences in feed allocation and gut fill prior to and following the interventions. Between days 0 and 6, the calves were weaned and kept in holding yards with access to feed and water for the week prior to the procedure, and were then moved to a large paddock to feed on available pasture on days one to 6 following treatment. Weight loss following treatment was greatest in CD calves and lowest in CON calves. This result aligns with previous findings showing concurrent castration and dehorning to negatively impact on average daily gain (ADG) (Baldridge *et al.* 2011; Mosher *et al.* 2013). Weight change of CDBMTA calves did not differ significantly from that of CON calves, indicating that a combination of TA and MEL may provide superior pain relief than when TA or MEL is provided alone. This finding is consistent with published literature that recommends a combination of LA and NSAIDs to target both the acute nociceptive and following inflammatory phases of the pain response (Mellor and Stafford 1999; Hudson *et al.* 2008). The weight gain results in our study support previous research findings, where calves had greater ADG values for the first 13 days following concurrent castration and dehorning when administered pain relief in the form of sodium salicylate or a combination of sodium salicylate, xylazine, ketamine and butorphanol, compared to no analgesic treatment (Baldridge *et al.* 2011).

In Australia, beef cattle producers are generally paid a monetary value per kg body weight or carcass weight (cwt). Our results demonstrate that a combination of TA and BM can be a cost-effective addition to routine practice, in addition to improving animal welfare (Baldridge *et al.* 2011). For example, the current price of beef is approximately $3.50/kg live-weight. In this trial, the administration of combined TA and BM cost approximately $5 per calf (using the retail price of the therapeutics). CD calves lost 2.9 kg BW more than CDBMTA calves, equating to a loss of $10.15 in value, indicating that the price of providing pain relief was less than the gain in product value from its use (with a potential benefit to cost ratio of 2).

In cattle, GPS technology has been mainly used to monitor grazing behaviour (Turner *et al.* 2000; Schlecht *et al.* 2004; Gonzalez *et al.* 2014). This study attempted to use GPS location to identify possible changes in calf behaviour during paddock utilisation in response to pain. In this case, paddock utilisation was measured through calculation of 95% MCPs. The MCP is a frequently used technique for home-range calculation, which identifies a restricted area within which an animal moves when performing normal activities (Harris *et al.* 1990). The MCP technique has mostly been used in wildlife habitat studies and to date, there has been minimal use of this technique in livestock studies (Perotto-Baldivieso *et al.* 2012). In our study, there was no effect of treatment on 95% MCP values, suggesting concurrent castration and dehorning may have had no impact on the ability for calves to access and utilise available pasture resources across their landscape in the days following the procedures. It is likely that paddock utilisation was similar between all animals because of a social influence of peer activity on individual calf behaviour (Launchbaugh and Howery 2005).

Pain may have had an effect on other behavioural measures, including speed of movement, distance travelled and distance to peers, with these variables having been used to evaluate welfare in other species. In sheep, GPS technology has been used to document lambing behaviour (Dobos *et al.* 2014). A decrease in daily speed and hourly speed following lambing and an increase in distance to peers during lambing was identified (Dobos *et al.* 2014). In addition, GPS technology has been used in sheep to show a positive linear

relationship between faecal egg count and distance moved per time step, suggesting that an increase in parasite load may result in animals grazing for longer periods or travelling to water more frequently (Falzon *et al.* 2013). In dogs, GPS technology has been used to distinguish between healthy dogs and dogs with osteoporosis through differences in performance measures (Bruno *et al.* 2015). Velocity, acceleration and deceleration were all reduced in dogs with osteoporosis compared to healthy dogs (Bruno *et al.* 2015). In addition, an improvement to these performance measures was shown in dogs with osteoporosis when oral carprofen was administered (Bruno *et al.* 2015). These studies reinforce the potential for GPS technology to identify production and welfare improvements in animals. In our study, the total number of data points that were removed as part of the 'cleaning' procedures prior to analysis was 8.4%, suggesting the accuracy of the positional fixes may not have been high. An estimation of paddock utilisation may require less accuracy than measurements of fine-scale dynamics of movement (Forin-Wiart *et al.* 2015). Hence in our study we chose to use 95% MCP as a measure of paddock utilisation due to the likeliness that the GPS positional fixes were not highly accurate. Future studies should employ the use of suitable GPS units to accurately measure other variables including speed and distance travelled, to assess potential effects of pain and pain relief in cattle.

Accelerometers have been used to record activity of calves following surgical castration (White *et al.* 2008), disbudding and dehorning (Heinrich *et al.* 2010; Theurer *et al.* 2012) and concurrent castration and dehorning (Pauly *et al.* 2012). As an increase or decrease in lying activity is not a direct measure of pain, such observations should be interpreted with caution. Lying activity exhibits a significant degree of individual variability in cattle (Coetzee *et al.* 2012b) and it is likely that inter-animal comparisons from what is normal in the absence of pain (Coetzee *et al.* 2012b) before treatment, compared to after treatment, may be a more sensitive measurement than between-animal comparisons. However, as inter-animal comparisons from before to after treatment would have required an additional round of mustering in the current study, between-animal comparisons were used for practical reasons. Although the analysis failed to find a significant difference between CD and CON calves for lying activity, the overall results and trends for this outcome suggest that less lying may be indicative of greater discomfort or pain. This finding agrees with the results of previous studies using accelerometers or behavioural observations to monitor lying activity of calves undergoing castration or dehorning (Ting *et al.* 2003a; White *et al.* 2008; Heinrich *et al.* 2010; Coetzee *et al.* 2012b; Theurer *et al.* 2012). Surgically castrated calves have previously been shown to spend more time standing following the procedure, compared to pre-operatively, as measured using accelerometers (White *et al.* 2008). Similarly, accelerometer measurements have shown that dehorning in calves reduces lying activity. The administration of meloxicam dimishes or removes this reduction in lying behaviour following dehrning (Coetzee *et al.* 2012b; Theurer *et al.* 2012). In future research, it could be beneficial to further classify standing activity as 'immobile' or 'mobile/walking,' as this could highlight potential differences between treatment groups that were unknown in the current study. However, this would require a higher sampling rate, challenging the memory storage of recording devices and limiting the time period for data collection. The increase in lying activity seen in all calves from day 0 to day one can be explained by the restriction of calves to the holding yards and laneway on day 0 and the increased sampling time on day one. The calves may have been less inclined to lie down in this environment compared to a paddock environment due to the absence of ground cover in the laneway.. In addition, there were humans present near the laneway during daytime hours on day 0, potentially deterring the

calves from resting. As the increase in lying activity from day one to day 2 was only seen in castrated and dehorned calves, it may indicate a reduction in discomfort or pain over time.

Observation of individual behaviours has previously been used to measure pain following castration (Ting *et al.* 2003a), dehorning (McMeekan *et al.* 1999; Sylvester *et al.* 2004) and concurrent castration and dehorning (Sutherland *et al.* 2013). These studies have also used the analysis of individual behaviours to evaluate the efficacy of local anaesthesia and analgesia for these procedures (McMeekan *et al.* 1999; Ting *et al.* 2003a; Sylvester *et al.* 2004; Sutherland *et al.* 2013). In experiment 2, calves that had been castrated and dehorned spent a significantly greater duration of time walking and a significantly less duration of time eating compared to CON calves. Excessive locomotion, as demonstrated in this study through increased time spent walking, is recognised as a pain-related behaviour (Prunier *et al.* 2013; Petherick *et al.* 2014a). It is unclear why CDBMTA calves spent more time walking compared to CDBM and CDTA calves. Pain-related behaviour and behavioural responses to certain procedures is variable between individual animals (Coetzee *et al.* 2012b; Webster *et al.* 2013; Laurence *et al.* 2016) and may explain this finding. Pain in animals has the potential to reduce eating behaviour in animals (Prunier *et al.* 2013). A previous study showed that control calves spent more time eating than castrated and dehorned calves and that a combination of lignocaine and flunixin meglumine, increased the amount of time spent eating (Sutherland *et al.* 2013). In experiment 2 of the current study, CON calves spent more time eating than all other calves and there was a trend for CDBMTA calves to spend more time eating than CD calves, suggesting a reduction in pain with a combination of TA and MEL.

In experiment 2 of the current study, calves that had been castrated and dehorned tended to display a greater frequency of tail flicks than CON calves. An increased frequency of tail flicks has previously been observed for these procedures performed both singularly (Fisher *et al.* 2001; Sylvester *et al.* 2004) and in combination (Sutherland *et al.* 2013) and has been suggested due to irritation or pain (Fisher *et al.* 2001; Sylvester *et al.* 2004; Sutherland *et al.* 2013). CDTA calves did not differ from CON calves in their display of tail flicks and there was a trend for CDBMTA calves to display less tail flicks in comparison to CD and CDBM calves. This finding suggests that TA may have reduced pain. There was a significant interaction between treatment and time on the frequency of ear flicks and a significant effect of treatment on the duration of time spent drinking and the frequency of head turns, although there was no clear trend in this data. Again, potential variation in expression of these behaviours between individual animals, may have influenced these results. With ear flicks, it is likely that the procedures of ear tagging and notching, may have confounded these results, particularly as notching has been shown to cause substantial pain (Lomax *et al.* 2017). In addition, the display of certain behaviours appeared to be influenced by other factors independent of pain. This was evident in the significant effect of day on some behaviours, including the duration of time that calves spent drinking and the frequency of head shakes, head turns, stamps, ear flicks and tail flicks. It was noted that more crows and flies were present in the vicinity of the calves treated on day one compared to those treated on days 2 and 3. Differences in weather conditions are likely to explain this observation, with day one being hotter and less overcast than days 2 and 3.

As discussed above, although there were some behaviours that appeared to be associated with pain, as demonstrated through a difference between CD and CON calves, overall, there was limited expression of pain-related behaviours displayed by the calves in this study. It

has been suggested that the age and breed of animals influences their behavioural demonstration of pain and thus affects observations on methods for relief of pain (Olson *et al.* 2016). Dairy calves appear to display more prominent responses to painful procedures and pain relief interventions compared to beef cattle, particularly when the beef calves are from environments where predation occurs commonly and animals quickly learn to minimise demonstration of pain (Olson *et al.* 2016). The calves in our study are likely to have had a strong tendency to hide their expression of pain. The majority of the previous literature on the behavioural response to castration and dehorning of cattle has used younger dairy calves (Stafford and Mellor 2005a; Coetzee 2013), with minimal research having been conducted using older *Bos Indicus* beef calves (Petherick *et al.* 2014a; Laurence *et al.* 2016; Musk *et al.* 2017). In addition, there is very little research that has examined the behavioural response to castration and dehorning of calves, when performed concurrently (Sutherland *et al.* 2013). Ther results of this study provide novel information on the behaviour of weaned *Bos Indicus* calves following concurrent castration and dehorning.

This study may be the first documented examination the effects of TA and BM following concurrent castration and dehorning of weaner calves. It is likely that the stressful experiences of handling, weaning and concurrently performed surgical procedures would have influenced the results in study, especially as these calves had none or very little prior interaction with humans and handling facilities. The importance of conducting studies that closely represent current and changing industry practices has been previously acknowledged (Laurence *et al.* 2016) and was an emphasis in the design of the current study. The difficulty in obtaining consistent results across all measures of pain and for all treatments is a common issue in studies on animal pain and may be especially apparent in studies on extensively-raised *Bos indicus* cattle where the animals are usually unaccustomed to humans and handling (Laurence *et al.* 2016; Musk *et al.* 2017).

In experiment one, a significant improvement in weight gain was seen following castration and dehorning when a combination of TA and BM had been administered at the time of marking, resulting in no difference between CON and CDBMTA calves. This experiment also found a combination of TA and BM increased lying activity in the first few days following treatment, suggesting a reduction in pain. In experiment 2, there were trends for TA and the combination of TA and BM to reduce pain-related behaviours during a 6 h period following castration and dehorning that warrant further investigation. Overall, an improvement in weight gain, an increase in lying activity and behavioural trends indicative of efficacy demonstrate the potential for TA and BM to improve welfare and production following castration and dehorning of beef calves. This is an important finding for large, extensive tropical beef production systems that are seeking practical options for improving animal welfare.

5.8 Investigating analgesia for control of pain and haemorrhage following spaying via the willis dropped ovary technique in cattle

This study aimed to investigate the effects of TA and MI, singularly and in combination, on pain and haemorrhage following WDOT spaying in beef cattle. The outcomes were to measure post-operative behaviour as an indication of pain and to measure change in TPP and PCV from before to after treatment as indications of post-surgical haemorrhage. In addition, we monitored change in body weight from before to after treatment and incidence

and cause of mortalities within the first 7 days following treatment. Analysis of behavioural data is continuing. As there was no significant effect of treatment on change in TPP and PCV, our findings on post-surgical haemorrhage were inconclusive, although there were 2 mortalities, determined at necropsy to be due to severe internal haemorrhage from the surgical sitewith no trend of a causative effect of treatment. There was also no effect of treatment on change in body weight.

Previous research has measured morbidity, mortality, body weight gain and physiological responses as potential indiatiors of welfare following surgical spaying via flank incision and WDOT (McCosker *et al.* 2010; Petherick *et al.* 2011; Petherick *et al.* 2013). Previous studies have identified that both flank and WDOT spaying cause pain, morbidity, mortality (McCosker *et al.* 2010; Petherick *et al.* 2011; Petherick *et al.* 2013) and reduced weight gains (McCosker *et al.* 2010) with flank spaying resulting in longer-term pain and higher incidences of morbidity and mortality compared to WDOT spaying (McCosker *et al.* 2010; Petherick *et al.* 2011; Petherick *et al.* 2013). Following this, our study focused on improving welfare following WDOT spaying as a potential approach towards achieving best practice outcomes.

One of the aims of our study was to investigate the efficacy of TA and MI for alleviation of post-operative pain following WDOT spaying in cattle. As analysis of behavioural data is yet to be finalised, discussion on the analgesic efficacy of TA and MI will be provided in an appendix to this report.

Morbidity resulting from post-surgical haemorrhage as confirmed through post-mortem examination, has been identified as a welfare and producton concern following spaying of cattle (McCosker *et al.* 2010). Therefore, another aim of our study was to investigate whether the haemostasis provided by a TA formulation containing adrenalin administered to the vicinity of the tissue during the WDOT spaying procedure in cattle, was sufficiently efficacious to prevent mortalities. As post-surgical haemorrhage results in a depletion of blood components including erythrocytes and protein, changes in TPP and PCV can be used as a measure of acute blood loss (Thrall *et al.* 2012). The techniques used for measuring TPP and PCV are simple, inexpensive and feasible to conduct in the field (Fielding and Magdesian 2011). Measurement of TPP and PCV has been used in previous studies investigating the physiological responses to haemorrhage in goats (Abdalla and Abdelatif 2008; Abdelatif and Abdalla 2009; Abdalla and Abdelatif 2010), dogs (Okrasinski *et al.* 1992; McGowan *et al.* 2017) and horses (Schmall *et al.* 1990). In goats, decreased PCV has been shown to occur by 6 h following haemorrhage, with restoration of normal values occurring from days to weeks, depending on the percentage of blood lost (Abdalla and Abdelatif 2008; Abdelatif and Abdalla 2009; Abdalla and Abdelatif 2010). Serum total protein concentration has also been shown to decrease following haemorrhage in goats, with restoration of normal values occurring from 6 h onwards (Abdalla and Abdelatif 2008; Abdelatif and Abdalla 2009; Abdalla and Abdelatif 2010).

In our study, as there was no effect of treatment on change in PCV or TPP, the haemostatic efficacy of TA for WDOT spaying in cattle could not be evaluated. As the degree of haemorrhage influences any changes in TPP and PCV, mild to moderate levels of blood loss may have minimal effect on these outcomes in healthy animals with an adequate reserve of blood stored in the spleen and eslwehere that can be readily recruited to the peripheral bloodstream. One of the animals that had a marked decrease in PCV outlying the

normal range of the data, died the next day and necropsy confirmed that with the cause of death was severe haemorrhage from the internal surgical site. . This finding suggests that a marked decrease in PCV at 24 h following WDOT spaying in cattle may only occur in cases where severe haemorrhage is occurring. Single measures of TPP and PCV may not provide sufficient information for determination of the degree of haemorrhage as these parameters may be normal or only changing slowly following acute blood loss (Fielding and Magdesian 2011). In future work, it may be useful to collect serial blood samples for analysis of PCV and TPP or analyse additional haemolytic or physiological variables indicative of haemorrhage, including blood or plasma lactate, central venous pressure, central venous oxygen saturation and oxygen extraction ratios (Fielding and Magdesian 2011). Unfortunately the use of many of these variables is challenging when conducting field studies as they as not as readily assessed as TPP and PCV (Fielding and Magdesian 2011).

It has been mentioned that pain may reduce feed intake and therefore affect body weight changes from reduced gut fill (Prunier *et al.* 2013). In addition, it is possible for the presence of stress-related constituents in blood, including aldosterone, catecholamines, vasopressin and cortisol to influence fluid loss from tissue and consequently reduce body weight (Browning and Leite-Browning 2013) Therefore, change in body weight over a 24 hour period was examined in our study as a potential indicatior of pain and morbidity. However, our assessment of short-term change in body weight found no effect of treatment on this outcome. In future studies it may be beneficial to increase the sample size and in addition, measure longer-term changes in weight gain to evaluate the effects of TA and MI following WDOT spaying in cattle.

TWith only one S cow and one STA heifer dying due to severe haemorrhage, no trend was observed on the effect of treatment on mortality. However, the veterinarian performing the spay procedure considered that the mortality rate in our study (~ 2%) was greater than the mortality rate normally observed (~ 0.5%). The cause of this this observation is unknown, although on reflection, it was noted that the tip on the head of the modified ovariotome used in our study was more rounded than the tip on the head of the ovariotome that is usually used by the veterinarian when spaying cattle. The suggestion was that this may have led at least to the prolonged incision in the cervical wall musculature and subsequent haemorrhage during ovariotome penetration, as revealed on necropsy examination. Although unrelated to the objectives of our study, this is an important observation worthy of future research, with additional work required on the design of the modified ovariotome that enables delivery of TA actives to the internal surgical site.

In our study, TPP and PCV values were not indicative of haemorrhage, except in cases of severe to extreme blood loss. Mortality occurring from severe internal haemorrhage within 48 h following WDOT spaying is a welfare issue that has previously been identified (McCosker *et al.* 2010). Our current analysis of cattle behaviour will likely add useful to information on assessment and improvement of animal welfare following WDOT spaying, with and without TA and MI.

5.9 Success in achieving objectives

5.9.1 Objective 1: Examine the efficacy of topical anaesthetic, cryoanaesthetic and non-steroidal anti-inflammatory drugs alone and in various combinations, during single routine surgical procedures including dehorning, castration, ear notching, branding and spaying, using behavioural scores, pressure algometry, thermography and physiological parameters including cortisol to enable quantification of pain responses

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The outcomes measured for each trial were chosen based on practicality, research objectives and results of previous studies. For logistical reasons, the initial studies in this project were conducted in NSW, where horned cattle are less common than in northern Australia. For this reason, there were some difficulties sourcing sufficiently larger numbers of horned cattle in NSW in reasonable proximity to the university campus, resulting in limited animal numbers for the initial studies. Further, As branding is not a commonly performed procedure in NSW and for the research conducted in northern Australia, time and resources for studies investigating pain relief for concurrent castration and dehorning of weaner cattle and spaying of female cattle were prioritised. Therefore, the efficacy of TA and meloxicam for post-operative pain relief of branding was not investigated.

The efficacy of cryoanaesthesia via topical vapocoolant spray to the skin of both sides of the ear for intra-operative pain relief of ear tagging and notching was examined using analysis of behavioural scores. The efficacy of cryoanaesthetic via topical vapocoolant spray to the skin of the scrotum for intra-operative pain relief of surgical castration was examined using analysis of ocular temperature and behaviour scores. Based on the results of this study, the efficacy of cryoanaesthetic was not examined for any other husbandry procedures.

The efficacy of TA for post-operative pain relief of castration was examined using analysis of cortisol concentration. The efficacy of TA and BM was examined, singularly and in combination, for post-operative pain relief of castration using analysis of frequency and duration of specific, individual behaviours, wound temperature and wound morphology scores.

The efficacy of TA for post-operative pain relief of dehorning was examined using analysis of wound sensitivity scores. The efficacy of TA and BM for post-operative pain relief of dehorning was examined using analysis of frequency and duration of specific, individual behaviours, wound temperature and wound morphology scores.

The efficacy of TA and MI was examined, singularly and in combination, for post-operative pain relief of spaying, using analysis of frequency of specific behaviours.

5.9.2 Objective 2: Examine the efficacy of topical anaesthetic, cryoanaesthetic and non-steroidal anti-inflammatory drugs alone and in various combinations for multiple combinations of routine husbandry practices as practised routinely on northern beef properties, as directed by results from Objective 1.

As directed by results of Objective 1, in Objective 2, the efficacy of TA and BM was examined for post-operative pain relief of concurrent castration and dehorning of weaner

calves using analysis of frequency and duration of specific individual behaviours, paddock utilisation and lying activity.

5.9.3 Objective 3: Monitor the effect of analgesia provided during aversive procedures on production parameters including weight gain, morbidity and mortality.

The effect of TA and BM on short-term change in body weight was examined for castration, dehorning and concurrent castration and dehorning.

Change in body weight, wound morphology scores, paddock utilisation and lying activities, as mentioned in sections 5.9.1 and 5.9.2, were considered indications of morbidity in the studies investigating the efficacy of TA and BM for castration, dehorning and concurrent castration and dehorning. Change in body weight, TPP and PCV values were considered indications of morbidity in the study investigating the efficacy of TA and MI for spaying.

Mortality was monitored in the study investigating the efficacy of TA and BM for concurrent castration and dehorning. However, there were no incidences of mortality and the effect of TA and BM on this outcome could not be evaluated. Mortality was also monitored in the study investigating the efficacy of TA and MI for spaying.

6 Conclusions/Recommendations

6.1 Pain assessment

The results of the studies conducted throughout this project demonstrate the complexity of assessing pain in cattle and for this reason, it is challenging to evaluate the efficacy of anaesthesia and analgesia. There is extensive literature that also demonstrates the difficulty associated with assessing pain in livestock. Hence, the findings of this project further emphasise the need for multiple measurement outcomes to assess pain and the efficacy of pain relief in cattle. Further, this project highlights the difficulty associated with pain assessment and the need for improved methods for assessing pain in livestock.

6.2 Efficacy of cryoanaesthetic, topical anaesthetic and buccal meloxicam

The results of the studies on cryoanaesthesia suggest that it is a convenient and effective way to address intra-operative pain of ear tagging and ear notching, yet it is probably not appropriate for procedures of a more invasive nature, such as castration and dehorning. Although ear tagging and ear notching are clearly less invasive procedures than castration and dehorning, there remain animal welfare concerns that need to be considered with these important procedures. Cryo-anaesthesia with a topical vapocoolant spray is a quick, easy, safe, affordable way to reduce pain and distress of calves undergoing ear tagging and ear notching. Research into practical options for providing intra-operative pain relief during more invasive husbandry procedures should continue.

The results of the studies on TA and BM suggest that both these products result in some reduction of the pain caused by castration and dehorning in calves. Further, the results of the study on concurrent castration and dehorning suggested improved efficacy when a combination of TA and BM was administered. A reliance on data trends in addition to

statistically significant results was necessary throughout this project to infer findings on TA and BM for pain relief following husbandry procedures. Study results together with knowledge on the mode of anaesthetic and analgesic action of lignocaine, bupivacaine and meloxicam leads to the conclusion that TA and BM when used together, do reduce pain following castration and dehorning of calves. However, the degree of pain reduction is difficult to deduce. The findings also indicate that a single treatment with these products, although effective to some degree, does not completely abolish pain. This suggests that continued research should be conducted to develop a solution to the animal welfare issues associated with the prolonged pain from routine husbandry procedures performed on cattle.

The analgesic treatments evaluated throughout this project have the potential to improve the welfare of many cattle as some efficacy has been demonstrated and their practicality for use on-farm means they can be easily incorporated into routine procedures. In addition, the economic constraints of employing these products into routine operations could potentially be offset by improvements to production and a willingness of consumers to pay more for 'better welfare' animal products.

6.3 Management practices

Ideally, 'marking' procedures should be performed on calves at as young an age as possible and at times of the year when risk of flystrike and infection is lowest. Monitoring of animals during the healing period following calf 'marking' procedures and spaying of female cattle should be incorporated within a considered 'calendar of routine operations plan' to allow for administration of appropriate pain relief and treatment of any subsequent health and welfare issues. The feasibility of incorporating these recommendations on-farm depends on many factors, including available environemnetal, financial and labour resources and the scale of the operation. There are clearly greater practical and managerial constraints for the very large extensive tropical beef cattle properties, including those that participated in this research.

6.4 Future research and development

As mentioned throughout sections 5 and 6.1, accurately assessing pain in cattle is difficult. Future research should focus on developing a robust pain model to aid in the measure of both pain and the level of analgesia provided by various therapeutic agents. Reliance on behavioural changes and increased cortisol as indicators of pain only provides a crude binary measurement of absence or presence of pain. The use of alternative measures such as electrophysiology (EEG and ECG), thermography and novel pian biomarkers may improve the measurement and allow ranking of pain and pain relief.

Although some pain relief was shown for the analgesic treatments investigated throughout this project, the degree of their efficacy was not clear. Further investigation of these analgesic treatments for husbandry procedures in cattle would be beneficial for understanding the degree of improvement to animal welfare through their use. In addition, a one-off treatment with TA, an NSAID or both, although a major step forward in improvement of animal welfare compared to current practices where no pain relief os provided, should not necessarily be considered the 'best practice' solution to the welfare implications caused by husbandry procedures in cattle. Continued support of research to examine more efficacious methods for improved pain relief and welfare management within our various livestock

systems is necessary. For example, an approach to pain management incorporating both intra-operative and continuous post-operative anaesthesia and analgesia may be more appropriate approach. Recent research in sheep has shown that provision of analgesia through feed may be an alternative, practical method of providing longer-term analgesia (Marini *et al.* 2016). Research into methods for more prolonged analgesia or *ad libitum* delivery of analgesia may be the next step in addressing pain management whilst continuing to consider practical constraints. In addition, methods or products to control haemorrhage and infection following spaying and dehorning is worthy of further research.

7 Key Messages

'Farmer applied' analgesic products, TA (Trisolfen ®), BM (Buccalgesic®) and MI (Metacam ®), are now available for use in cattle undergoing painful husbandry procedures. Research indicates that these products have the potential to improve the welfare of cattle undergoing painful procedures, especially when TA is administered in combination with meloxicam, to address both acute and inflammatory pain. All products have practical modes of administration with TA administered topically to wounded tissue, BM administered into the buccal cavity and IM administered subcutaneously. To achieve current best practice welfare standards for cattle undergoing painful husbandry procedures, it is recommended to use TA in addition to either BM or IM, depending on preference for ease of administration. Regular monitoring of cattle during the healing period following husbandry procedures is recommended so that subsequent health and welfare issues can be addressed. Adoption of pain management will strengthen consumer satisfaction with the beef industry and potentially offset losses in production. These outcomes offer economic, social and sustainability benefits to beef producers and processors.

8 Bibliography

Abdalla, SE, Abdelatif, AM (2008) Effects of haemorrhage on thermoregulation, heart rate and blood constituents in goats (Capra hircus). *Pakistan Journal of Biological Sciences* **11**, 1194-1203.

Abdalla, SE, Abdelatif, AM (2010) Physiological responses of goats (Capra hircus) to haemorrhage as influenced by splenectomy. *American-Eurasian Journal of Scientific Research* **5**, 76-87.

Abdelatif, AM, Abdalla, SE (2009) Physiological responses of goats (Capra hircus) to haemorrhage as influenced by age. *Global Veterinaria* **3**, 151-164.

AHA, 2014. Australian animal welfare standards and guidelines for cattle. Animal Health Australia (AHA), Deakin, ACT.

Alam, MGS, Dobson, H (1986) Effect of various veterinary procedures on plasma-concentrations of cortisol, luteinizing-hormone and prostaglandin-F2-alpha metabolite in the cow. *Veterinary Record* **118**, 7-10.

Allen, KA, Coetzee, JF, Edwards-Callaway, LN, Glynn, H, Dockweiler, J, KuKanich, B, Lin, H, Wang, C, Fraccaro, E, Jones, M, Bergamasco, L (2013) The effect of timing of oral meloxicam administration on physiological responses in calves after cautery dehorning with local anesthesia. *Journal of Dairy Science* **96**, 5194-5205.

Alsaaod, M, Buscher, W (2012) Detection of hoof lesions using digital infrared thermography in dairy cows. *Journal of Dairy Science* **95**, 735-742.

Anderson, DE, Muir, WW (2005) Pain management in cattle. *Veterinary Clinics of North America, Food Animal Practice* **21**, 623-635.

Anil, L, Anil, SS, Deen, J (2005) Pain detection and amelioration in animals on the farm: Issues and options. *Journal of Applied Animal Welfare Science* **8**, 261-278.

Australian Bureau of Agricultural and Resource Economics and Sciences [ABARES] (2017) 'Beef farms.' Available at http://www.agriculture.gov.au/abares/Pages/Beef.aspx#physical-characteristics [Accessed 01 March 2018].

Baldridge, SL, Coetzee, JF, Dritz, SS, Reinbold, JB, Gehring, R, Havel, J, KuKanich, B (2011) Pharmacokinetics and physiologic effects of intramuscularly administered xylazine hydrochloride-ketamine hydrochloride-butorphanol tartrate alone or in combination with orally administered sodium salicylate on biomarkers of pain in Holstein calves following castration and dehorning. *American Journal of Veterinary Research* **72**, 1305-1317.

Bateson, P (1991) ASSESSMENT OF PAIN IN ANIMALS. *Animal Behaviour* **42**, 827-839.

Baumans, V, Brain, PF, Brugere, H, Clausing, P, Jeneskog, T, Perretta, G (1994) Pain and distress in laboratory rodents and lagomorphs - report of the federation-of-European-laboratory-animal-science-associations (FELASA) working group on pain and distress accepted by the FELASA board of management November 1992. *Laboratory Animals* **28**, 97-112.

Bortolussi, G, McIvor, JG, Hodgkinson, JJ, Coffey, SG, Holmes, CR (2005) The northern Australian beef industry, a snapshot. 2. Breeding herd performance and management. *Australian Journal of Experimental Agriculture* **45**,

Braiman-Wiksman, L, Solomonik, I, Spira, R, Tennenbaum, T (2007) Novel insights into wound healing sequence of events. *Toxicologic Pathology* **35**, 767-79.

Bretschneider, G (2005) Effects of age and method of castration on performance and stress response of beef male cattle: a review. *Livestock Production Science* **97**, 89-100.

Brofeldt, BT, Cornwell, P, Doherty, D, Batra, K, Gunther, RA (1989) Topical lidocaine in the treatment of partial-thickness burns. *The Journal of Burn Care and Rehabilitation* **10**, 63-68.

Brown, AC, Powell, JG, Kegley, EB, Gadberry, MS, Reynolds, JL, Hughes, HD, Carroll, JA, Sanchez, NCB, Thaxton, YV, Backes, EA, Richeson, JT (2015) Effect of castration timing and oral meloxicam administration on growth performance, inflammation, behavior, and carcass quality of beef calves. *Journal of Animal Science* **93**, 2460-2470.

Browning, R, Jr., Leite-Browning, ML (2013) Comparative stress responses to short transport and related events in Hereford and Brahman steers. *Journal of Animal Science* **91**, 957-969.

Bruno, EA, Guthrie, JW, Ellwood, SA, Mellanby, RJ, Clements, DN (2015) Global positioning system derived performance measures are responsive indicators of physical activity, disease and the success of clinical treatments in domestic dogs. *Plos One* **10**,

Budzynska, M, Weary, DM (2008) Weaning distress in dairy calves: Effects of alternative weaning procedures. *Applied Animal Behaviour Science* **112**, 33-39.

Bursac, Z, Gauss, CH, Williams, DK, Hosmer, DW (2008) Purposeful selection of variables in logistic regression. *Source code for biology and medicine* **3**, 17-17.

Calenge, C (2006) The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling* **197**, 516-519.

Celeste, CJ, Deschesne, K, Riley, CB, Theoret, CL (2013) Skin temperature during cutaneous wound healing in an equine model of cutaneous fibroproliferative disorder: kinetics and anatomic-site differences. *Veterinary Surgery* **42**, 147-153.

Coetzee, JF (2011) A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the united states. *Applied Animal Behaviour Science* **135**, 192-213.

Coetzee, JF (2013) Assessment and management of pain associated with castration in cattle. *Veterinary Clinics of North America, Food Animal Practice* **29**, 75-101.

Coetzee, JF, Edwards, LN, Mosher, RA, Bello, NM, O'Connor, AM, Wang, B, KuKanich, B, Blasi, DA (2012a) Effect of oral meloxicam on health and performance of beef steers relative to bulls castrated on arrival at the feedlot. *Journal of Animal Science* **90**, 1026-1039.

Coetzee, JF, KuKanich, B, Mosher, R, Allen, P (2009) Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. *Veterinary Therapeutics* **10**, unpaginated.

Coetzee, JF, Lechtenberg, KF, Stock, ML, Kukanich, B (2014) Pharmacokinetics and effect of intravenous nalbuphine in weaned Holstein calves after surgical castration. *Journal of Veterinary Pharmacology and Therapeutics* **37**, 169-177.

Coetzee, JF, Lubbers, BV, Toerber, SE, Gehring, R, Thomson, DU, White, BJ, Apley, MD (2008) Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. *American Journal of Veterinary Research* **69**, 751-762.

Coetzee, JF, Mosher, RA, KuKanich, B, Gehring, R, Robert, B, Reinbold, JB, White, BJ (2012b) Pharmacokinetics and effect of intravenous meloxicam in weaned Holstein calves following scoop dehorning without local anesthesia. *BMC Veterinary Research* **8**, (1 September 2012).

Cohen, LL, MacLaren, JE, DeMore, M, Fortson, B, Friedman, A, Lim, CS, Gangaram, B (2009) A Randomized Controlled Trial of Vapocoolant for Pediatric Immunization Distress Relief. *Clinical Journal of Pain* **25**, 490-494.

Colak, A, Polat, B, Okumus, Z, Kaya, M, Yanmaz, LE, Hayirli, A (2008) Early detection of mastitis using infrared thermography in dairy cows. *Journal of Dairy Science* **91**, 4244-4248

Coleman, G (Eds DJ Mellor, PM Thornber, DAC Bayvel, S Kahn (2008) 'Public perceptions of animal pain and animal welfare.' (OIE (World Organisation for Animal Health): Paris, France)

Collado-Mesa, F, Net, JM, Arheart, K, Klevos, GA, Yepes, MM (2015) Application of a topical vapocoolant spray decreases pain at the site of initial intradermal anaesthetic injection during ultrasound-guided breast needle biopsy. *Clinical Radiology* **70**, 938-942.

Cooke, RF, Arthington, JD, Austin, BR, Yelich, JV (2009) Effects of acclimation to handling on performance, reproductive, and physiological responses of Brahman-crossbred heifers. *Journal of Animal Science* **87**, 3403-3412.

Cornish, A, Raubenheimer, D, McGreevy, P (2016) What We Know about the Public's Level of Concern for Farm Animal Welfare in Food Production in Developed Countries. *Animals : an open access journal from MDPI* **6**,

Costello, M, Ramundo, M, Christopher, NC, Powell, KR (2006) Ethyl vinyl chloride vapocoolant spray fails to decrease pain associated with intravenous cannulation in children. *Clinical Pediatrics* **45**, 628-632.

Cruz, AM, Naylor, JM (1993) A retrospective study of frostbite injuries in calves (1982-1991). *Canadian Veterinary Journal* **34**, 306-308.

Currah, JM, Hendrick, SH, Stookey, JM (2009) The behavioral assessment and alleviation of pain associated with castration in beef calves treated with flunixin meglumine and caudal lidocaine epidural anesthesia with epinephrine. *Canadian Veterinary Journal* **50**, 375-382.

Dobos, RC, Dickson, S, Bailey, DW, Trotter, MG (2014) The use of GNSS technology to identify lambing behaviour in pregnant grazing Merino ewes. *Animal Production Science* **54**,

Dockweiler, JC, Coetzee, JF, Edwards-Callaway, LN, Bello, NM, Glynn, HD, Allen, KA, Theurer, ME, Jones, ML, Miller, KA, Bergamasco, L (2013) Effect of castration method on neurohormonal and electroencephalographic stress indicators in Holstein calves of different ages. *Journal of Dairy Science* **96**, 4340-4354.

Doherty, TJ, Kattesh, HG, Adcock, RJ, Welborn, MG, Saxton, AM, Morrow, JL, Dailey, JW (2007) Effects of a concentrated lidocaine solution on the acute phase stress response to dehorning in dairy calves. *Journal of Dairy Science* **90**, 4232-4239.

Douglas, WW, Malcolm, JL (1955) THE EFFECT OF LOCALIZED COOLING ON CONDUCTION IN CAT NERVES. *Journal of Physiology-London* **130**, 53-71.

Earley, B, Crowe, MA (2002) Effects of ketoprofen alone or in combination with local anaesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. *Journal of Animal Science* **80**, 1044-1052.

Espinoza, C, Lomax, S, Windsor, P (2013) The effect of a topical anesthetic on the sensitivity of calf dehorning wounds. *Journal of Dairy Science* **96**, 2894-2902.

Espinoza, CA, McCarthy, D, White, PJ, Windsor, PA, Lomax, SH (2015) 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. *Animal Production Science* 1512-1519.

Evans, PJD, Lloyd, JW, Green, CJ (1981) CRYOANALGESIA - THE RESPONSE TO ALTERATIONS IN FREEZE CYCLE AND TEMPERATURE. *British Journal of Anaesthesia* **53**, 1121-1127.

Falzon, G, Schneider, D, Trotter, M, Lamb, DW (2013) A relationship between faecal egg counts and the distance travelled by sheep. *Small Ruminant Research* **111**,

Farion, KJ, Splinter, KL, Newhook, K, Gaboury, I, Splinter, WM (2008) The effect of vapocoolant spray on pain due to intravenous cannulation in children: a randomized controlled trial. *Canadian Medical Association Journal* **179**, 31-36.

Fielding, CL, Magdesian, KG (2011) 'Review of packed cell volume and total protein for use in equine practice.'

Fisher, AD, Crowe, MA, Varga, MEAdl, Enright, WJ (1996) Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. *Journal of Animal Science* **74**, 2336-2343.

Fisher, AD, Knight, TW, Cosgrove, GP, Death, AF, Anderson, CB, Duganzich, DM, Matthews, LR (2001) Effects of surgical or banding castration on stress responses and behaviour of bulls. *Australian Veterinary Journal* **79**, 279-284.

Fjordbakk, CT, Haga, HA (2011) Effect of topical vapocoolant spray on response to arthrocentesis and intravenous catheterization in unsedated horses. *American Journal of Veterinary Research* **72**, 746-750.

Forin-Wiart, MA, Hubert, P, Sirguey, P, Poulle, ML (2015) Performance and Accuracy of Lightweight and Low-Cost GPS Data Loggers According to Antenna Positions, Fix Intervals, Habitats and Animal Movements. *Plos One* **10**, 21.

Friend, TH, Lay, DC, Jr., Bushong, DM, Pierce, DW (1994) Wisconsin's "stale calf" issue and a study designed to resolve some of the animal welfare concerns. *Journal of Animal Science* **72**, 2260-2263.

Gann, DS, Egdahl, RH (1965) Responses of adrenal corticosteroid secretion to hypotension and hypovolemia. *Journal of Clinical Investigation* **44**, 1-&.

Glynn, HD, Coetzee, JF, Edwards-Callaway, LN, Dockweiler, JC, Allen, KA, Lubbers, B, Jones, M, Fraccaro, E, Bergamasco, LL, KuKanich, B (2013) The pharmacokinetics and effects of meloxicam, gabapentin, and flunixin in postweaning dairy calves following dehorning with local anesthesia. *Journal of Veterinary Pharmacology and Therapeutics* **36**, 550-561.

Godyn, D, Herbut, E, Walczak, J (2013) Infrared thermography as a method for evaluating the welfare of animals subjected to invasive procedures - a review. *Annals of Animal Science* **13**, 423-434.

Gonzalez, LA, Bishop-Hurley, G, Henry, D, Charmley, E (2014) Wireless sensor networks to study, monitor and manage cattle in grazing systems. *Animal Production Science* **54**,

Gregory, NG (2004) Pain. In 'Physiology and behaviour of animal suffering.' (Blackwell Science: Oxford, UK)

Grondahl-Nielsen, C, Simonsen, HB, Lund, JD, Hesselholt, M (1999) Behavioural, endocrine and cardiac responses in young calves undergoing dehorning without and with use of sedation and analgesia. *Veterinary Journal* **158**, 14-20.

Habib, F, Shaltout, SE-D, Azeem, MA, Fetih, G, Safwat, M (2011) MUCOADHESIVE BUCCAL PATCHES OF LORNOXICAM: II-IN-VIVO EVALUATION AND CLINICAL EFFICACY. *Bulletin of Pharmaceutical Sciences* **34**, 21-30.

Harper, D, Young, A, McNaught, C-E (2014) The physiology of wound healing. *Surgery (Oxford)* **32**, 445-450.

Harris, S, Cresswell, WJ, Forde, PG, Trewhella, WJ, Woollard, T, Wray, S (1990) HOME-RANGE ANALYSIS USING RADIO-TRACKING DATA - A REVIEW OF PROBLEMS AND TECHNIQUES PARTICULARLY AS APPLIED TO THE STUDY OF MAMMALS. *Mammal Review* **20**, 97-123.

Heglund, NC, Taylor, CR (1988) SPEED, STRIDE FREQUENCY AND ENERGY-COST PER STRIDE - HOW DO THEY CHANGE WITH BODY SIZE AND GAIT. *Journal of Experimental Biology* **138**, 301-318.

Heinrich, A, Duffield, TF, Lissemore, KD, Millman, S (2010) The effect of meloxicam on behavior and pain sensitivity of dairy calves following cautery dehorning with a local anesthetic. *Journal of Dairy Science* **93**, 2450-2457.

Hopster, H, van der Werf, JTN, Erkens, JHF, Blokhuis, HJ (1999) Effects of repeated jugular puncture on plasma cortisol concentrations in loose-housed dairy cows. *Journal of Animal Science* **77**, 708-714.

Huber, J, Arnholdt, T, Mostl, E, Gelfert, CC, Drillich, M (2013) Pain management with flunixin meglumine at dehorning of calves. *Journal of Dairy Science* **96**,

Hudson, C, Whay, H, Huxley, J (2008) Recognition and management of pain in cattle. *In Practice* **30**, 126, 128-134.

Ito, K, Weary, DM, Keyserlingk, MAGv (2009) Lying behavior: assessing within- and between-herd variation in free-stall-housed dairy cows. *Journal of Dairy Science* **92**, 4412-4420.

Karakus, F, Duzgun, A, Karakus, M, Aslan, L (2015) Can infrared thermography be used to predict ear tags infections in lambs? *Scientific Papers, Series D. Animal Science* **58**, 205-208.

Kihurani, DO, Mbiuk, SM, Ngatia, TA (1989) Healing of dehorning wounds. *British Veterinary Journal* **145**, 580-585.

Kupczynski, R, Budny, A, Spitalniak, K, Tracz, E (2014) Dehorning of calves - methods of pain and stress alleviation - a review. *Annals of Animal Science* **14**, 231-243.

Landa, L (2003) The effect of milk suckling from the dam or glucose administration on the behavioural responses to tail docking in lambs. *Acta Veterinaria Brno* **72**, 175-182.

Launchbaugh, KL, Howery, LD (2005) Understanding landscape use patterns of livestock as a consequence of foraging behavior. *Rangeland Ecology & Management* **58**, 99-108.

Laurence, M, Barnes, A, Collins, T, Hyndman, T, Musk, GC (2016) Assessing and mitigating post-operative castration pain in Bos indicus cattle. *Animal Production Science* -.

Lay, DC, Friend, TH, Randel, RD, Bowers, CL, Grissom, KK, Neuendorff, DA, Jenkins, OC (1998) Effects of restricted nursing on physiological and behavioral reactions of Brahman calves to subsequent restraint and weaning. *Applied Animal Behaviour Science* **56**, 109-119.

Lay, DC, Jr, Friend, TH, Bowers, CL, Grissom, KK, Jenkins, OC (1992a) A comparative physiological and behavioral study of freeze and hot-iron branding using dairy cows. *Journal of Animal Science* **70**, 1121-1125.

Lay, DC, Jr, Friend, TH, Randel, RD, Bowers, CL, Grissom, KK, Jenkins, OC (1992b) Behavioral and physiological effects of freeze or hot-iron branding on crossbred cattle. *Journal of Animal Science* **70**, 330-336.

Lay, DCJ, Friend, TH, Grissom, KK, Bowers, CL, Mal, ME (1992c) Effects of freeze or hot-iron branding of Angus calves on some physiological and behavioral indicators of stress. *Applied Animal Behaviour Science* **33**, 137-147.

Loberg, JM, Hernandez, CE, Thierfelder, T, Jensen, MB, Berg, C, Lidfors, L (2008) Weaning and separation in two steps - a way to decrease stress in dairy calves suckled by foster cows. *Applied Animal Behaviour Science* **111**, 222-234.

Lomax, S, Dickson, H, Sheil, M, Windsor, PA (2010) Topical anaesthesia alleviates short-term pain of castration and tail docking in lambs. *Australian Veterinary Journal* **88**, 67-74.

Lomax, S, Sheil, M, Windsor, PA (2008) Impact of topical anaesthesia on pain alleviation and wound healing in lambs after mulesing. *Australian Veterinary Journal* **86**, 159–168.

Lomax, S, Sheil, M, Windsor, PA (2013) Duration of action of a topical anaesthetic formulation for pain management of mulesing in sheep. *Australian Veterinary Journal* **91**, 160-167.

Lomax, S, Windsor, PA (2014) Topical anesthesia mitigates the pain of castration in beef calves. *Journal of Animal Science* **91**, 4945-52.

Lomax, S, Witenden, E, Windsor, P, White, P (2017) Effect of topical vapocoolant spray on perioperative pain response of unweaned calves to ear tagging and ear notching. *Veterinary Anaesthesia and Analgesia* **44**, 163-172.

Mace, SE (2016) Prospective, randomized, double-blind controlled trial comparing vapocoolant spray vs placebo spray in adults undergoing venipuncture. *American Journal of Emergency Medicine* **34**, 798-804.

Marini, D, Pippia, J, Colditz, IG, Hinch, GN, Petherick, CJ, Lee, C (2016) Palatability and pharmacokinetics of flunixin when administered to sheep through feed. *PeerJ* **4**, e1800.

Marti, S, Velarde, A, Torre, JLdl, Bach, A, Aris, A, Serrano, A, Manteca, X, Devant, M (2010) Effects of ring castration with local anesthesia and analgesia in Holstein calves at 3 months of age on welfare indicators. *Journal of Animal Science* **88**, 2789-2796

Mattachini, G, Riva, E, Bisaglia, C, Pompe, JCAM, Provolo, G (2013) Methodology for quantifying the behavioral activity of dairy cows in freestall barns. *Journal of Animal Science* **91**, 4899-4907.

McCarthy, D, Lomax, S, Windsor, PA, White, PJ (2016) Effect of a topical anaesthetic formulation on the cortisol response to surgical castration of unweaned beef calves. *Animal* **10**, 150-6.

McCosker, K, Letchford, P, Petherick, JC, Meyer, D, McGowan, M (2010) Morbidity, mortality and body weight gain of surgically spayed, yearling Brahman heifers. *Australian Veterinary Journal* **88**, 497-503.

McGowan, EE, Marryott, K, Drobatz, KJ, Reineke, EL (2017) Evaluation of the use of shock index in identifying acute blood loss in healthy blood donor dogs. *Journal of Veterinary Emergency and Critical Care* **27**, 524-531.

McLean, I, Holmes, P, Counsell, D, 2014. The northern beef report 2013 northern beef situation analysis. Meat and Livestock Australia Limited, Sydney.

McMeekan, C, Stafford, KJ, Mellor, DJ, Bruce, RA, Ward, RN, Gregory, N (1999) Effects of a local anaesthetic and a non-steroidal anti-inflammatory analgesic on the behavioural responses of calves to dehorning. *New Zealand Veterinary Journal* **47**, 92-96.

Mellor, D, Stafford, K (1999) Assessing and minimising the distress caused by painful husbandry procedures in ruminants. *In Practice* **21**, 436-446.

Mellor, DJ, Cook, CJ, Stafford, KJ, 2000. Quantifying some responses to pain as a stressor. The biology of animal stress: basic principles and implications for animal welfare. 171-191.

Mellor, DJ, Fisher, MW, Stafford, KJ (Eds DJ Mellor, PM Thornber, DAC Bayvel, S Kahn (2008) 'A cost-benefit analysis of pain relief for farm animals.' (OIE (World Organisation for Animal Health): Paris, France)

Meyer, H, Starke, A, Kehler, W, Rehage, J (2007) High caudal epidural anaesthesia with local anaesthetics or alpha 2-agonists in calves. *J Vet Med A* **54**, 384-389.

Meyer, RA, Ringcamp, M, Campbell, JN, Raja, SN (2005) Neural Mechanisms of hyperalgesia after tissue injury. *Johns Hopkins APL Technical Digest* **26**, 56-66.

Mickey, RM, Greenland, S (1989) THE IMPACT OF CONFOUNDER SELECTION CRITERIA ON EFFECT ESTIMATION. *American Journal of Epidemiology* **129**, 125-137.

Millis, DL (2004) Getting the dog moving after surgery. *Journal of the American Animal Hospital Association* **40**, 429-436.

Millman, ST (2013) Behavioral responses of cattle to pain and implications for diagnosis, management, and animal welfare. *Veterinary Clinics of North America, Food Animal Practice* **29**, 47-58.

Mintline, EM, Stewart, M, Rogers, AR, Cox, NR, Verkerk, GA, Stookey, JM, Webster, JR, Tucker, CB (2013) Play behavior as an indicator of animal welfare: Disbudding in dairy calves. *Applied Animal Behaviour Science* **144**, 22-30.

Mintline, EM, Varga, A, Banuelos, J, Walker, KA, Hoar, B, Drake, D, Weary, DM, Coetzee, JF, Stock, ML, Tucker, CB (2014) Healing of surgical castration wounds: a description and an evaluation of flunixin. *Journal of Animal Science* **92**, 5659-5665.

Mitchell, KD, Stookey, JM, Laturnas, DK, Watts, JM, Haley, DB, Huyde, T (2004) The effects of blindfolding on behavior and heart rate in beef cattle during restraint. *Applied Animal Behaviour Science* **85**, 233-245

Molony, V, Kent, JE (1997) Assessment of acute pain in farm animals using behavioral and physiological measurements. *Journal of Animal Science* **75**, 266-272.

Molony, V, Kent, JE, Robertson, IS (1995) Assessment of acute and chronic pain after different methods of castration of calves. *Applied Animal Behaviour Science* **46**, 33-48.

Mosher, RA, Wang, C, Allen, PS, Coetzee, JF (2013) Comparative effects of castration and dehorning in series or concurrent castration and dehorning procedures on stress responses and production in Holstein calves. *Journal of Animal Science* **91**, 4133-4145.

Moya, D, Gonzalez, LA, Janzen, E, Caulkett, NA, Fireheller, E, Schwartzkopf-Genswein, KS (2014) Effects of castration method and frequency of intramuscular injections of ketoprofen on behavioral and physiological indicators of pain in beef cattle. *Journal of Animal Science* **92**, 1686-1697.

Musk, GC, Jacobsen, S, Hyndman, TH, Lehmann, HS, Tuke, SJ, Collins, T, Gleerup, KB, Johnson, CB, Laurence, M (2017) Objective measures for the assessment of post-operative pain in Bos indicus bull calves following castration. *Animals* **7**, 76.

Neely, CD, Thomson, DU, Kerr, CA, Reinhardt, CD (2014) Effects of three dehorning techniques on behavior and wound healing in feedlot cattle. *Journal of Animal Science* **92**, 2225-2229.

Noonan, GJ, Rand, JS, Priest, J, Ainscow, J, Blackshaw, JK (1994) Behavioural observations of piglets undergoing tail docking, teeth clipping and ear notching. *Applied Animal Behaviour Science* **39**, 203-213.

O'Loughlin, A, McGee, M, Doyle, S, Earley, B (2014) Biomarker responses to weaning stress in beef calves. *Research in Veterinary Science* **97**, 458-463.

OIE (2015) Animal welfare and beef cattle production systems.

Okafor, ROS, Remi-Adewunmi, BD, Fadason, ST, Ayo, JO, Muhammed, SM (2014) Pathophysiologic mechanisms of pain in animals - a review. *Journal of Veterinary Medicine and Animal Health* **6**, 123-130.

Okrasinski, EB, Krahwinkel, DJ, Sanders, WL (1992) TREATMENT OF DOGS IN HEMORRHAGIC-SHOCK BY INTRAOSSEOUS INFUSION OF HYPERTONIC SALINE AND DEXTRAN. *Veterinary Surgery* **21**, 20-24.

Olson, ME, Ralston, B, Burwash, L, Matheson-Bird, H, Allan, ND (2016) Efficacy of oral meloxicam suspension for prevention of pain and inflammation following band and surgical castration in calves. *BMC Veterinary Research* **12**, (13 June 2016).

Pairis-Garcia, MD, Johnson, AK, Stalder, KJ, Karriker, LA, Coetzee, JF, Millman, ST (2014) Measuring the efficacy of flunixin meglumine and meloxicam for lame sows using nociceptive threshold tests. *Animal Welfare* **23**, 219-229.

Paull, DR, Lee, C, Colditz, IG, Atkinson, SJ, Fisher, AD (2007) The effect of a topical anaesthetic formulation, systemic flunixin and carprofen, singly or in combination, on cortisol and behavioural responses of Merino lambs to mulesing. *Australian Veterinary Journal* **85**, 98-106.

Pauly, C, White, BJ, Coetzee, JF, Robert, B, Baldridge, S, Renter, DG (2012) Evaluation of analgesic protocol effect on calf behavior after concurrent castration and dehorning. *International Journal of Applied Research in Veterinary Medicine* **10**, 54-61.

Perez-Torres, L, Orihuela, A, Corro, M, Rubio, I, Alonso, MA, Galina, CS (2016) Effects of separation time on behavioral and physiological characteristics of Brahman cows and their calves. *Applied Animal Behaviour Science* **179**, 17-22

Perotto-Baldivieso, HL, Cooper, SM, Cibils, AF, Figueroa-Pagan, M, Udaeta, K, Black-Rubio, CM (2012) Detecting autocorrelation problems from GPS collar data in livestock studies. *Applied Animal Behaviour Science* **136**, 117-125.

Pesenhofer, G, Palme, R, Pesenhofer, RM, Kofler, J (2006) Comparison of two methods of fixation during functional claw trimming - walk-in crush versus tilt table - in dairy cows using faecal cortisol metabolite concentrations and daily milk yield as parameters. *Wiener Tierarztliche Monatsschrift* **93**, 288-294.

Petherick, JC (2005) Animal welfare issues associated with extensive livestock production: The northern australian beef cattle industry. *Applied Animal Behaviour Science* **92**, 211-234.

Petherick, JC, Doogan, VJ, Venus, BK, Holroyd, RG, Olsson, P (2009) Quality of handling and holding yard environment, and beef cattle temperament: 2. Consequences for stress and productivity. *Applied Animal Behaviour Science* **120**, 28-38.

Petherick, JC, McCosker, K, Mayer, DG, Letchford, P, McGowan, M (2011) Preliminary investigation of some physiological responses of Bos indicus heifers to surgical spaying. *Australian Veterinary Journal* **89**, 131-137.

Petherick, JC, McCosker, K, Mayer, DG, Letchford, P, McGowan, M (2013) Evaluation of the impacts of spaying by either the dropped ovary technique or ovariectomy via flank laparotomy on the welfare of Bos indicus beef heifers and cows. *Journal of Animal Science* **91**, 382-394.

Petherick, JC, Small, AH, Mayer, DG, Colditz, IG, Ferguson, DM, Stafford, KJ (2014a) A comparison of welfare outcomes for weaner and mature Bos indicus bulls surgically or tension band castrated with or without analgesia: 1. Behavioural responses. *Applied Animal Behaviour Science* **157**, 23-34.

Petherick, JC, Small, AH, Mayer, DG, Colditz, IG, Ferguson, DM, Stafford, KJ (2014b) A comparison of welfare outcomes for weaner and mature Bos indicus bulls surgically or tension band castrated with or without analgesia: 2. Responses related to stress, health and productivity. *Applied Animal Behaviour Science* **157**,

Petherick, JC, Small, AH, Reid, DJ, Colditz, IE, Ferguson, DM (2015) Welfare outcomes for 3- and 6-month-old beef calves in a tropical environment castrated surgically or by applying rubber rings. *Applied Animal Behaviour Science* **171**, 47-57.

Petrie, NJ, Mellor, DJ, Stafford, KJ, Bruce, RA, Ward, RN (1996) Cortisol responses of calves to two methods of disbudding used with or without local anaesthetic. *New Zealand Veterinary Journal* **44**, 4-8.

Prayaga, KC (2007) Genetic options to replace dehorning in beef cattle - a review. *Australian Journal of Agricultural Research* **58**, 1-8.

Prunier, A, Mounier, L, Neindre, Pl, Leterrier, C, Mormede, P, Paulmier, V, Prunet, P, Terlouw, C, Guatteo, R (2013) Identifying and monitoring pain in farm animals: a review. *Animal* **7**, 998-1010.

R Core Team (2017) 'R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.' Available at http://www.R-project.org

Redua, MA, Valadao, CAA, Duque, JC, Balestrero, LT (2002) The pre-emptive effect of epidural ketamine on wound sensitivity in horses tested by using von Frey filaments. *Veterinary Anaesthesia and Analgesia* **29**, 200-206.

Reichl, M, Quinton, D (1987) Comparison of 1% lignocaine with 0.5% bupivacaine in digital ring blocks. *Journal of Hand Surgery* **12**, 271-278.

Robinson, DR, Gebhart, GF (2008) Inside Information: The Unique Features of Visceral Sensation. *Molecular Interventions* **8**, 242-253.

Schlecht, E, Hulsebusch, C, Mahler, F, Becker, K (2004) The use of differentially corrected global positioning system to monitor activities of cattle at pasture. *Applied Animal Behaviour Science* **85**,

Schmall, LM, Muir, WW, Robertson, JT (1990) HEMATOLOGICAL, SERUM ELECTROLYTE AND BLOOD-GAS EFFECTS OF SMALL VOLUME HYPERTONIC SALINE IN EXPERIMENTALLY INDUCED HEMORRHAGIC-SHOCK. *Equine Veterinary Journal* **22**, 278-283.

Schwartzkopf-Genswein, KS, Stookey, JM (1997) The use of infrared thermography to assess inflammation associated with hot-iron and freeze branding in cattle. *Canadian Journal of Animal Science* **77**, 577-583.

Schwartzkopf-Genswein, KS, Stookey, JM, Passille, AMd, Rushen, J (1997a) Comparison of hot-iron and freeze branding on cortisol levels and pain sensitivity in beef cattle. *Canadian Journal of Animal Science* **77**, 369-374.

Schwartzkopf-Genswein, KS, Stookey, JM, Welford, R (1997b) Behavior of cattle during hot-iron and freeze branding and the effects of subsequent handling ease. *Journal of Animal Science* **75**, 2064-2072.

Small, AH, Belson, S, Holm, M, Colditz, IG (2014) Efficacy of a buccal meloxicam formulation for pain relief in Merino lambs undergoing knife castration and tail docking in a randomised field trial. *Australian Veterinary Journal* **92**, 381-388.

Stafford, KJ, Mellor, DJ (2005a) Dehorning and disbudding distress and its alleviation in calves. *Veterinary Journal* **169**, 337-349.

Stafford, KJ, Mellor, DJ (2005b) The welfare significance of the castration of cattle: a review. *New Zealand Veterinary Journal* **53**, 271-278.

Stafford, KJ, Mellor, DJ (2009) Painful husbandry procedures in livestock and poultry. In 'Improving animal welfare: a practical approach.' (Ed. T Grandin.) pp. 88-114. (CABI: Wallingford, UK)

Stafford, KJ, Mellor, DJ (2015) Painful husbandry procedures in livestock and poultry. In 'Improving animal welfare: a practical approach.' (Ed. T Grandin.) pp. 96-124. (CABI: Wallingford, UK)

Stafford, KJ, Mellor, DJ, Todd, SE, Bruce, RA, Ward, RN (2002) Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. *Research in Veterinary Science* **73**, 61-70.

Stewart, M, Shepherd, HM, Webster, JR, Waas, JR, McLeay, LM, Schutz, KE (2013) Effect of previous handling experiences on responses of dairy calves to routine husbandry procedures. *Animal* **7**, 828-833.

Stewart, M, Stafford, KJ, Dowling, SK, Schaefer, AL, Webster, JR (2008) Eye temperature and heart rate variability of calves disbudded with or without local anaesthetic. *Physiology & Behavior* **93**, 789-797.

Stewart, M, Stookey, JM, Stafford, KJ, Tucker, CB, Rogers, AR, Dowling, SK, Verkerk, GA, Schaefer, AL, Webster, JR (2009) Effects of local anesthetic and a nonsteroidal antiinflammatory drug on pain responses of dairy calves to hot-iron dehorning. *Journal of Dairy Science* **92**, 1512-1519.

Stewart, M, Verkerk, GA, Stafford, KJ, Schaefer, AL, Webster, JR (2010) Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. *Journal of Dairy Science* **93**, 3602–3609.

Stilwell, G, Carvalho, RC, Carolino, N, Lima, MS, Broom, DM (2010) Effect of hot-iron disbudding on behaviour and plasma cortisol of calves sedated with xylazine. *Research in Veterinary Science* **88**, 188-193.

Stock, ML, Baldridge, SL, Griffin, D, Coetzee, JF (2013) Bovine dehorning: assessing pain and providing analgesic management. *Veterinary Clinics of North America, Food Animal Practice* **29**, 103-133.

Stock, ML, Barth, LA, Engen, NKv, Millman, ST, Gehring, R, Wang, C, Voris, EA, Wulf, LW, Labeur, L, Hsu, WH, Coetzee, JF (2016) Impact of carprofen administration on stress and nociception responses of calves to cautery dehorning. *Journal of Animal Science* **94**, 542-555

Sutherland, MA, Ballou, MA, Davis, BL, Brooks, TA (2013) Effect of castration and dehorning singularly or combined on the behavior and physiology of Holstein calves. *Journal of Animal Science* **91**, 935-942.

Sutherland, MA, Davis, BL, Brooks, TA, McGlone, GA (2010) Physiology and behavior of pigs before and after castration: effects of two topical anesthetics. *Animal* **4**, 2071-2079.

Sylvester, SP, Mellor, DJ, Stafford, KJ, Bruce, RA, Ward, RN (1998) Acute cortisol responses of calves to scoop dehorning using local anaesthesia and/or cautery of the wound. *Australian Veterinary Journal* **76**, 118-122.

Sylvester, SP, Stafford, KJ, Mellor, DJ, Bruce, RA, Ward, RN (2004) Behavioural responses of calves to amputation dehorning with and without local anaesthesia. *Australian Veterinary Journal* **82**, 697-700.

Tagawa, M, Okano, S, Sako, T, Orima, H, Steffey, EP (1994) Effect of change in body position on cardiopulmonary function and plasma cortisol in cattle. *Journal of Veterinary Medical Science* **56**, 131-134

Taylor, AA, Weary, DM (2000) Vocal responses of piglets to castration: identifying procedural sources of pain. *Applied Animal Behaviour Science* **70**, 17-26.

Theurer, ME, White, BJ, Coetzee, JF, Edwards, LN, Mosher, RA, Cull, CA (2012) Assessment of behavioral changes associated with oral meloxicam administration at time of dehorning in calves using a remote triangulation device and accelerometers. *BMC Veterinary Research* **8**, (30 April 2012).

Thrall, MA, Weiser, G, Allison, R, Campbell, TW (2012) 'Veterinary hematology and clinical chemistry.' (Wiley: Hoboken)

Ting, STL, Earley, B, Crowe, MA (2003a) Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growth, and behavior. *Journal of Animal Science* **81**, 1253-1264.

Ting, STL, Earley, B, Crowe, MA (2003b) 'Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growth, and behavior.' Available at <Go to ISI>://CABI:20033102069 [Accessed 5].

Tucker, CB, Mintline, EM, Banuelos, J, Walker, KA, Hoar, B, Drake, D, Weary, DM (2014a) Effect of a cooling gel on pain sensitivity and healing of hot-iron cattle brands. *Journal of Animal Science* **92**, 5666-5673.

Tucker, CB, Mintline, EM, Banuelos, J, Walker, KA, Hoar, B, Varga, A, Drake, D, Weary, DM (2014b) Pain sensitivity and healing of hot-iron cattle brands. *Journal of Animal Science* **92**, 5674-5682.

Turner, LW, Udal, MC, Larson, BT, Shearer, SA (2000) Monitoring cattle behavior and pasture use with GPS and GIS. *Canadian Journal of Animal Science* **80**,

Veissier, I, Leneindre, P (1988) Cortisol responses to physical and pharmacological stimuli in heifers. *Reproduction Nutrition Development* **28**, 553-562.

Waibel, KH, Katial, RK (2005) Effect of topical vapocoolant spray on skin test wheal, flare, and pain responses. *Annals of Allergy, Asthma, & Immunology* **95**, 149-153.

Webster, HB, Morin, D, Jarrell, V, Shipley, C, Brown, L, Green, A, Wallace, R, Constable, PD (2013) Effects of local anesthesia and flunixin meglumine on the acute cortisol response, behavior, and performance of young dairy calves undergoing surgical castration. *Journal of Dairy Science* **96**, 6285-6300.

Weiss, RA, Lavin, PT (2009) Reduction of Pain and Anxiety Prior to Botulinum Toxin Injections With a New Topical Anesthetic Method. *Ophthalmic Plastic and Reconstructive Surgery* **25**, 173-177.

White, BJ, Coetzee, JF, Renter, DG, Babcock, AH, Thomson, DU, Andresen, D (2008) Evaluation of two-dimensional accelerometers to monitor behavior of beef calves after castration. *American Journal of Veterinary Research* **69**, 1005-1012.

Wright, CI, Kroner, CI, Draijer, R (2006) Non-invasive methods and stimuli for evaluating the skin's microcirculation. *Journal of Pharmacological and Toxicological Methods* **54**, 1-25.

9 Appendix

9.1 Metadata Storage

All metadata associated with this project is digitally stored by the University of Sydney on a password protected drive.