Practical anaesthesia and analgesia for surgical castration and dehorning of extensively managed beef cattle

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BAnVetBioSci (Hons II Class 1)

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

The University of Sydney
Sydney School of Veterinary Science
Faculty of Science

June 2017
Declaration

This thesis is submitted to The University of Sydney in fulfillment of the requirements for the Degree of Doctor of Philosophy.

The work presented in this thesis is original except as acknowledged in the text. I hereby declare that I have not submitted this material, in either full or in part, for a degree at this or any other university.

Signature: 
Date: 28.06.2017

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With loving memory, I dedicate this thesis to my grandfathers, Maurice Colreavy and John McCarthy.
Abstract

Castration and dehorning are routine husbandry procedures commonly performed on cattle within the beef industry. Although there is extensive literature demonstrating the pain and distress resulting from these procedures, they have traditionally been performed without anaesthesia or analgesia due to the practical constraints associated with administering injections on-farm. Consumer demand for improved production animal welfare is continuously increasing. The beef industry therefore needs to address issues of concern, such as painful husbandry procedures, to ensure a sustainable future. Ceasing castration and dehorning is not an appropriate solution, as these procedures are justifiable for numerous reasons related to management, safety, production and animal welfare. There are currently no alternative options to performing castration and dehorning, therefore there is a need to incorporate pain relief into routine procedure. Recently, the practical constraints associated with conventional forms of anaesthesia and analgesia 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 absorbed through tissue in open wounds, and a buccal meloxicam (BM) gel (Ilium® Buccalgescic 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.

This thesis has aimed to assess the efficacy of TA and BM, singly and in combination, for the relief of post-operative pain caused by surgical castration and amputation dehorning of calves. This thesis has also aimed to assess the efficacy of a vapocoolant spray as a practical option for providing temporary local anaesthesia for alleviation of intra-operative pain during surgical
castration. This thesis has addressed these aims through experimentation presented as several studies incorporating multiple methods of pain assessment.

The effect of TA on the cortisol response of unweaned beef calves to surgical castration was examined for 6 hours following the procedure. Topical anaesthesia did not significantly reduce plasma cortisol concentration following castration of calves. However, there was a trend for calves treated with TA to display lower cortisol concentrations than untreated castrated calves.

The effect of TA and BM, singly and combined, on production, behaviour and wound inflammation of unweaned beef calves following surgical castration, was investigated. Results suggest that TA and BM, alone and in combination, reduced pain, as demonstrated through a reduction in some pain-related behaviours. Results indicate that BM reduced inflammation, as demonstrated through reduced maximum scrotal temperature.

To address the intra-operative pain of surgical castration in calves, the effects of a topical vapocoolant spray, applied to the scrotum and spermatic cords, and lignocaine injected into the scrotum and spermatic cords, were assessed. Results show that both the vapocoolant spray and lignocaine were inadequate to reduce pain during surgical castration of unweaned beef calves, as calf behaviour during the procedure did not differ from that of untreated, castrated calves.

Mechanical stimulation of wounds, treated with and without various TA formulations, or a cornual nerve block of lignocaine, was conducted to investigate wound sensitivity following amputation dehorning of unweaned beef calves. Results indicate that 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, with and without treatment with TA or BM, was assessed. There were no clear effects of TA and BM on pain and inflammation in this study and further work is required.

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

In conclusion, results of all studies suggest that TA and BM result in some amelioration of pain caused by castration and dehorning of calves, with indications of increased efficacy when a combination of TA and BM is 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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**McCarthys D, Lomax S, Windsor PA, Taylor C and White PJ.** Effects of topical anaesthesia and buccal meloxicam treatments on production, behaviour and inflammation of unweaned beef calves following surgical castration.

**McCarthys D, Lomax S, Windsor PA and White PJ.** Effect of lignocaine or a topical vapocoolant spray on the pain response to surgical castration in beef calves.

**McCarthys D, Lomax S, Windsor PA, Taylor C and White PJ.** Evaluating treatments with topical anaesthesia and buccal meloxicam on pain and inflammation caused by amputation dehorning of calves.
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I, Dominique McCarthy, was responsible for the study design, data collection, statistical analysis and the original preparation and editing of this manuscript.

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Chapter 3

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Chapter 5


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### Abbreviations

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<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<td>ADG</td>
<td>Average daily gain</td>
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<td>A</td>
<td>First autoregressive</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>AVP</td>
<td>Vasopressin</td>
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<td>BM</td>
<td>Buccal meloxicam</td>
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<tr>
<td><strong>B. Indicus</strong></td>
<td>Bos Indicus</td>
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<tr>
<td>BW</td>
<td>Body weight</td>
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<td>°C</td>
<td>Degrees celsius</td>
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<td>CA</td>
<td>California</td>
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<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
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<td>COX</td>
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<td>CRF</td>
<td>Corticotropin-releasing factor</td>
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<tr>
<td>CRFR1</td>
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<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
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<td>DAG</td>
<td>Diacylglycerol</td>
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<td>d.f.</td>
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<td>FAWC</td>
<td>Farm animal welfare committee</td>
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<td>Gauge</td>
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<tr>
<td>g/L</td>
<td>Grams per litre</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotropin-releasing hormone</td>
</tr>
<tr>
<td>GLMM</td>
<td>Generalised linear mixed models</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>ha</td>
<td>Hectare</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloride</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>IASP</td>
<td>International association for the study of pain</td>
</tr>
<tr>
<td>ID</td>
<td>Identification</td>
</tr>
<tr>
<td>IM</td>
<td>Intramuscularly</td>
</tr>
<tr>
<td>IP3</td>
<td>Inositol triphosphate</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenously</td>
</tr>
<tr>
<td>kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>km²</td>
<td>Square kilometres</td>
</tr>
<tr>
<td>LA</td>
<td>Local anaesthetic</td>
</tr>
<tr>
<td>LSD</td>
<td>Least significant difference</td>
</tr>
<tr>
<td>m</td>
<td>Metres</td>
</tr>
<tr>
<td>m²</td>
<td>Square metres</td>
</tr>
<tr>
<td>MA</td>
<td>Massachusetts</td>
</tr>
<tr>
<td>MC2-R</td>
<td>Melanocortin type 2 receptor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>mg/kg BW</td>
<td>Milligrams per kilogram of body weight</td>
</tr>
<tr>
<td>mg/L</td>
<td>Milligrams per litre</td>
</tr>
<tr>
<td>mg/mL</td>
<td>Milligrams per millilitre</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetres</td>
</tr>
<tr>
<td>MNT</td>
<td>Mechanical nociceptive threshold</td>
</tr>
<tr>
<td>m/s</td>
<td>Metres per second</td>
</tr>
<tr>
<td>NLIS</td>
<td>National livestock identification system</td>
</tr>
<tr>
<td>nmol/L</td>
<td>Nanomoles per litre</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>OLR</td>
<td>Ordinal logistic regression</td>
</tr>
<tr>
<td>P</td>
<td>P-value</td>
</tr>
<tr>
<td>PIC</td>
<td>Property identification code</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular nucleus</td>
</tr>
<tr>
<td>Qld</td>
<td>Queensland</td>
</tr>
<tr>
<td>QST</td>
<td>Quantitative sensory testing</td>
</tr>
<tr>
<td>REML</td>
<td>Restricted maximum likelihood</td>
</tr>
<tr>
<td>r.p.m.</td>
<td>Repetitions per minute</td>
</tr>
<tr>
<td>s</td>
<td>Seconds</td>
</tr>
<tr>
<td>SC</td>
<td>Subcutaneously</td>
</tr>
<tr>
<td>s.e.m.</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>TA</td>
<td>Topical anaesthetic</td>
</tr>
<tr>
<td><strong>USA</strong></td>
<td>United States of America</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>$V_{1b}$</td>
<td>Vasopressin $V_{1b}$ receptor</td>
</tr>
<tr>
<td><strong>VIC</strong></td>
<td>Victoria</td>
</tr>
<tr>
<td><strong>w/v</strong></td>
<td>Weight for volume</td>
</tr>
</tbody>
</table>
CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Ear tagging, ear notching, branding, dehorning, castration and spaying are routine husbandry procedures commonly performed on cattle in beef production systems. These procedures are performed for various reasons related to animal welfare, management and productivity. Although the pain of these procedures has been well documented, they have traditionally been conducted without anaesthesia or analgesia. Until recently, pain relieving pharmaceutical products have been impractical for use during routine livestock husbandry procedures due to their mode of administration being registered for use as injectable products. As industry and consumer concerns for animal welfare are continuously increasing, exploration of practical options for delivery of pain relief to cattle undergoing invasive husbandry procedures is necessary.

This review presents an overview of husbandry procedures performed on Australian beef cattle and the methods that have been used to quantify the pain of such procedures; the pharmaceutical treatments that can be used for the relief of pain caused by husbandry procedures in cattle; and the relevant limitations and constraints to pain assessment techniques and the use of pain relief in cattle. The need for investigation of practical pain relief products, such as topical anaesthetic (TA), buccal meloxicam (BM) and vapocoolant spray, for use during husbandry procedures performed on beef cattle, is highlighted throughout this review, leading to an outline of the research objectives of this thesis.
1.2 Welfare of Australian beef cattle

Animal welfare has been defined by the World Organisation for Animal Health as “how an animal copes with the conditions in which it lives”. “An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviours, and if it is not suffering from unpleasant states such as pain, fear, and distress” (World Organisation for Animal Health (OIE), 2015b).

Farm animal welfare refers to how well an animal is coping with its environment and the impact of husbandry procedures or management practices on the health, comfort and state of animals. The ‘Five Freedoms’ of animal welfare which were developed by the Farm Animal Welfare Committee (FAWC) in the United Kingdom are commonly referred to in animal welfare discussions. These five freedoms constitute good animal welfare, consisting of “freedom from thirst, hunger and malnutrition, freedom from discomfort, freedom from pain, injury and disease, freedom to display most normal patterns of behaviour and freedom from fear and distress” (Farm Animal Welfare Committee (FAWC), 2009).

The creation of farm animal welfare policies is reliant on science using validated measures to quantify the impact of the environment and husbandry procedures on the wellbeing of animals. Continuous development in animal welfare science and a growing public concern about the wellbeing of animals have contributed to an increasing focus on animal welfare in recent years (Potard, 2015).

The aspects of commercial beef cattle production systems that can impact animal welfare include disease, environment, nutrition, facilities, social environment and management. Farm animal welfare can be improved through a clear understanding of the biological, genetic and behavioural
needs of animals (Potard, 2015). In regards to surgical husbandry procedures, the World Organisation for Animal Health recommends that they be performed in a way that minimises pain and stress to the animal and should be performed at as early an age as possible (OIE, 2015a). In a production environment, the degree to which animal welfare can be improved depends on the socioeconomic capacity of the farm and product supply chains (Potard, 2015). It also depends on the type and scale of operation and the degree of input to animal management (Petherick, 2005).

In Australia, each state and territory has separate government responsibilities for animal welfare at the farm level (Potard, 2015). The Australian Animal Welfare Strategy (AAWS) is a national partnership between governments, industry and the community that has the intention of structuring a national framework to improve animal welfare. The strategy aims to address multiple points of view on animal welfare matters and promote science based outcomes (Gemmell, 2009). The Australian animal welfare standards and guidelines for cattle are a product of the AAWS which provide a foundation for development and implementation of legislation (Animal Health Australia (AHA), 2014a). Those responsible for the care of cattle can use these standards and guidelines as a reference for best practice. The standards and guidelines have been developed by researchers, government and industry and in consultation with state and territory governments, livestock industry organisations, animal welfare groups and the general community. The objective of the cattle welfare standards and guidelines in relation to painful husbandry procedures is that “castration, dehorning and spaying are only done when necessary and in a manner that minimises the risk to the welfare of cattle, particularly pain and distress”. 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. They also state that pain relief must be used when performing
flank laparotomy for spaying or webbing of cattle. The guidelines state that pain relief should be used for all surgical procedures (AHA, 2014a).

1.3 The northern Australian beef industry

Australia is in the top 10 beef producing countries in the world, and is the world’s leading beef and buffalo meat exporter (Meat and Livestock Australia (MLA), 2016). Australians consumed approximately 25.4 kg of beef per person in 2015 – 2016 and beef is currently the second most popular fresh meat consumed in Australia (MLA, 2016). The Australian red meat industry employs approximately 200,000 people and the cattle industry involves 58% of all Australian agricultural farms (MLA, 2016). The off-farm value of the Australian beef and cattle industry was $17.87 billion in 2015 – 2016 (MLA, 2016). Australia is currently free from most exotic diseases that affect other beef producing countries and is therefore a preferred global exporter (Bell et al., 2011). Australia was the world’s largest beef exporter in 2015 with the value of total beef and veal exports at $8.5 billion in 2015 – 2016 (MLA, 2016).

The northern Australian beef cattle industry is related to beef production in the areas covering the states of Queensland and the Northern Territory and the Kimberley / Pilbara region of the state of Western Australia. Beef cattle production is the main form of land utilisation in this area which encompasses approximately 4 million km² (Burns et al., 2010).

The northern Australian component of Australia’s beef industry is of primary focus due to the conditions under which northern Australian beef cattle are managed. Northern Australia contains tropic and sub-tropic areas which pose a variety of environmental stressors. Most beef production occurs on unimproved pastures in the dry tropics where rainfall is highly seasonal and variable across years and averages at approximately 450 mm per annum (Burns et al., 2010). Property and
herd sizes in northern Australia are considerably variable yet on average are large and extensive (Table 1.1) (McLean et al., 2014). Due to the environmental conditions of the northern Australian region, cattle are managed in large numbers at a low stocking rate (Burns et al., 2010), which requires extensive areas of land on which herds are managed. There are many constraints to production in northern Australia including the presence of the cattle tick (*Boophilus microplus*) and gastro-intestinal helminths (*Haemonchus placei*, *Cooperia* spp., *Trichostrongylus axei* and *Oesphagostomum radiatum*), high ambient temperatures and solar radiation, the risk of Bovine Infectious Keratoconjunctivitis and other diseases, unreliable rainfall, poor soil fertility, woody vegetation and fluctuating quantity and quality of forage (Burns et al., 2010).

**Table 1.1** Head of cattle, property size and herd size in Queensland, the Northern Territory and Western Australia

<table>
<thead>
<tr>
<th></th>
<th>Queensland</th>
<th>Northern Territory</th>
<th>Western Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head of cattle (million)</td>
<td>11.3</td>
<td>2.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Property size (managed ha)</td>
<td>39,364</td>
<td>255,923</td>
<td>238,005</td>
</tr>
<tr>
<td>Herd size (adult equivalent$^1$)</td>
<td>2727</td>
<td>8389</td>
<td>8661</td>
</tr>
</tbody>
</table>

Adapted from McLean et al. (2014).

$^1$ Adult equivalent = a *Bos taurus* steer weighing 450 kg at maintenance.

Management of most northern Australian beef systems is relatively low-input and mating is typically uncontrolled due to the range of the land on which cattle are run and the lack of fencing. Due to property distances, animal numbers and cost, it is common for cattle to be handled only
once or twice a year at weaning. Most producers practise calf weaning once in each of the periods April to July and August to September (Petherick, 2005). The common practice of uncontrolled mating results in irregular timings of births and therefore a wide range of weaning ages (between 3.5 to 10 months of age). In addition, if calves are accidentally missed during mustering, there can be a long delay before the next mustering process (Prayaga, 2007). *Bos indicus* and *Bos indicus* x *Bos taurus* are the main breeds of cattle in northern Australia as they are well adapted to cope under the pressures of previously mentioned constraints to production. Brahman and Brahman derivatives such as Droughtmaster, Santa Gertrudis, Braford, Brangus, Charbray, Simbrah and Brahmousin, are most commonly farmed (Burns et al., 2010).

### 1.4 Invasive husbandry procedures performed on cattle

Numerous husbandry procedures are routinely performed on beef cattle which have the potential to cause pain and distress including ear tagging, ear notching, branding, dehorning, castration and spaying. They are performed for reasons related to production efficiency, animal health and welfare and the safety of people working with cattle (OIE, 2015a). Despite these procedures often being performed at the same time, there is limited literature on the pain and distress of concurrent castration and dehorning (Baldrige et al., 2011, Mosher et al., 2013). Surgically performed procedures have welfare implications that are exacerbated by the nature of the northern Australian beef production context. Performing these procedures on older animals that are not accustomed to handling by humans requires greater restraint and can cause considerable stress. Dehorning, castration and spaying of older animals results in larger wounds, increased blood loss, longer healing periods and potentially higher mortalities. It also causes a significant amount of pain (Stafford and Mellor, 2015).
1.4.1 Identification

Australia has a national livestock identification system (NLIS) for identification and tracking of all Australian livestock. It is employed for biosecurity, food safety, product integrity and market access purposes. All livestock must be fitted with a NLIS device either as an ear tag or a rumen bolus accompanying an ear tag. The NLIS device holds electronic information on the animals’ property identification code (PIC) (MLA, 2015). Ear notching involves the use of ear marking pliers (MLA, 2015) which create notches in the ear tissue (Stafford and Mellor, 2015) and is used to identify the property to which the animal belongs (Stafford and Mellor, 2015). Branding is another form of identifying ownership (Stafford and Mellor, 2015) and is conducted by applying excessively hot or cold temperatures to the skin causing cell destruction and permanent marking of the pelt (Schwartzkopf-Genswein and Stookey, 1997).

1.4.2 Dehorning

Disbudding or dehorning of cattle involves the removal of horn buds or horns. This procedure is performed to reduce injury to other cattle and stockpersons, reduce damage to facilities, decrease space required for transport and housing and decrease bruising of cattle caused by injury from horns (OIE, 2015a). Bruise trim costs the Australian beef industry over $30 million annually (CSIRO, 2015) and dehorning reduces the prevalence of bruises by approximately 50 per cent (Prayaga, 2007). Although breeding of polled cattle would be ideal, this is difficult in certain breeds such as Brahman because of fewer polled animals and a relatively complex mode of inheritance (Prayaga, 2007).

The methods used to remove cattle horns work by either damaging or removing horn corium. Disbudding involves damage to horn buds via cautery, cryosurgery or application of caustic paste, or they can be excised with a knife (Figure 1.1). Dehorning involves the excision of the horn and
surrounding tissue (Figure 1.2). This procedure is performed using a variety of tools including dehorning knives, scoop dehorners, embryotomy wire, saws and guillotine shears, with the former two being the most commonly used in extensively managed beef production systems in Australia (AHA, 2014b). There is extensive literature demonstrating the pain of disbudding and dehorning in cattle (Stafford and Mellor, 2005b, Stafford and Mellor, 2011). However, there are fewer studies documenting amputation dehorning of older calves as opposed to hot-iron disbudding in younger calves (McMeekan et al., 1998b, Sylvester et al., 1998a, McMeekan et al., 1999, Sutherland et al., 2002, Sylvester et al., 2004, Coetzee et al., 2012).

**Figure 1.1** Illustration of surgical cutting during the removal of a horn bud on a young calf Sourced from AHA (2014b).

**Figure 1.2** Illustration of surgical cutting during the removal of a horn on an adult beast Sourced from AHA (2014b).
1.4.3 Castration

Castration of cattle involves the removal or irreversible damage of the testes in males (Stafford et al., 2003). Castration is performed in the beef industry to prevent unwanted breeding as well as to reduce aggression and sexual behaviour of males, facilitating handling and management (AHA, 2014b). In northern Australia, castration is important to control breeding, as separation of males and females is not feasible. Fencing is expensive and difficult to erect and maintain due to the terrain, distances involved and at times, occurrences of fires and floods (Petherick, 2005). Damage to facilities, handlers and other animals, and stress in animals are all reduced as a result of castration (AHA, 2014b). The reduction in animal injury and stress results in a lower incidence of bruised and dark cutting carcasses at slaughter (Price et al., 2003). In addition, beef yielded from steers is of a higher quality than that from bulls due to increased marbling and tenderness. Producers receive a superior price for beef from castrated cattle (Coetzee, 2013).

There are several different methods used to castrate cattle which can be classified as physical, chemical or hormonal. Physical methods, including surgical, rubber ring, latex banding and burdizzo are the most common and involve either surgical removal of the testicles or irreversible damage to the testicles via disruption of the blood supply (Coetzee, 2012). In Australia, castration via surgical cutting or rubber ring application are the most commonly practiced methods (AHA, 2014b). Chemical (Andrade Neto et al., 2014) and physical (Huxley and Whay, 2006) methods of castration have been shown to cause pain and distress in calves, measured through a variety of parameters. Immunisation against gonadotropin releasing hormone (GnRH) has been evaluated as an alternative option to conventional methods of castration (Bonneau and Enright, 1995). Immunocastration of cattle has animal welfare and production benefits over physical castration (Amatayakul-Chantler et al., 2013), however it requires multiple injections (Amatayakul-Chantler
et al., 2013, Norman and Collop, 2014) and displays variable efficacy (Bonneau and Enright, 1995, Norman and Collop, 2014).

1.4.4 Spaying

Spaying is performed on cattle in extensively managed beef production systems in northern Australia, north and south America and southern Africa (Petherick et al., 2013). Spaying is a surgical procedure involving removal of the ovaries to prevent unwanted mating and pregnancies and consequently reduce female mortalities, injuries and bruising (AHA, 2013). The common methods of spaying cattle are flank laparotomy and the dropped ovary technique. Flank laparotomy involves an incision in the flank region through the abdominal wall from which the ovaries are reached for excision. Instead of ovary excision, sometimes a portion of the oviduct from each side is excised, a method termed ‘webbing’ (McCosker et al., 2010). The dropped ovary technique does not involve flank incision as the ovaries are reached and excised through puncture of the anterior vagina.

1.5 Pain pathways and pain mechanisms

Pain, as defined by the International Association for the Study of Pain (IASP), is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Broom, 2001). More applicable to non-human animals, are the definitions of pain as “an aversive sensation and feeling associated with actual or potential tissue damage” (Broom, 2001) or “an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues” (Molony and Kent, 1997). Perception and sensation of pain serve to send an alert to the individual experiencing it. Actions can then be taken to stop or ameliorate present or potential damage (Broom, 2001). An
example of this may be the adoption of an abnormal ventral lying position in livestock following castration, to prevent stimulation of injured tissues (Prunier et al., 2013). Pain also serves to prevent future risk of injury through learned avoidance of behaviours or situations that may have painful consequences (Broom, 2001).

Pain results from chemical, mechanical or thermal stimulation of free nerve endings (Hudson et al., 2008) that contain primary afferent neurons called nociceptors (Dubin and Patapoutian, 2010, Okafor et al., 2014). These nociceptors are found within skin, muscle, joints and some viscera and can be specifically receptive to one type of stimulus or can be responsive to multiple stimuli. There are two types of nerve fibres, C and A (mostly A-δ), associated with nociceptors, which differ in anatomy and mediation of pain sensations (Table 1.2) (Dubin and Patapoutian, 2010, Okafor et al., 2014).

**Table 1.2** Differences between C and A-δ nerve fibres

<table>
<thead>
<tr>
<th></th>
<th>C nerve fibre</th>
<th>A-δ nerve fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anatomy</strong></td>
<td>*Unmyelinated axons bundled in fascicles surrounded by Schwann cells</td>
<td>*Myelinated axon</td>
</tr>
<tr>
<td></td>
<td>*Small diameter</td>
<td>*Medium diameter</td>
</tr>
<tr>
<td><strong>Pain sensation</strong></td>
<td>*Slow (0.4 – 1.4 m/s)</td>
<td>*Fast (5 – 30 m/s)</td>
</tr>
<tr>
<td></td>
<td>*Diffuse</td>
<td>*Localised</td>
</tr>
</tbody>
</table>

Adapted from Dubin and Patapoutian (2010), Okafor et al. (2014).

The pain pathway integrates nociception and emotion through a process of transduction, transmission, modulation and perception. Pain is processed by the cerebral cortex, thalamus and the limbic system (Figure 1.3) (Okafor et al., 2014). Activity from nociceptors in injured tissues (Pogatzki et al., 2002) is relayed via the spinothalamic tracts by nerve fibres with modulation often
occurring in the dorsal horn of the spinal cord (Coetzee, 2011). C and A-δ nerve fibres terminate predominantly in laminae I and V and I and II of the dorsal horn, respectively (Dubin and Patapoutian, 2010) (Figures 1.4 and 1.5). The ventrolateral part of the spinal cord conducts impulses to the brainstem and thalamus where further amplification occurs. Certain areas of the cerebral cortex are then activated via the thalamus to result in conscious recognition of pain (Hudson et al., 2008).

**Figure 1.3** Schematic diagram of pain pathway
Sourced from Okafor et al. (2014).

**Figure 1.4** Illustration of unmyelinated C-fibres terminating in laminae I and II of the dorsal horn
Sourced from Dubin and Patapoutian (2010).
Pain is highly subjective (Dubin and Patapoutian, 2010) and sensitivity varies among individuals. In humans, perception of pain can be restricted by stress or intensified by anticipation (Dubin and Patapoutian, 2010). The emotional and sensory awareness components of pain in animals are not well understood. Consequently, assessment of pain in animals is more difficult than in humans (Prunier et al., 2013).

There are two phases to the pain response; the acute phase and the inflammatory phase (Duffield, 2008). The acute phase, stimulated by tissue injury (Hudson et al., 2008), is usually brief, localised and relative to the intensity of the injury or insult. The secondary phase is prolonged, diffuse and usually results in hypersensitivity around the point of injury or insult. The secondary phase can lead to changes in the central nervous system which cause pain hypersensitivity or central sensitisation (Coetzee, 2011). This is due to production of chemicals including hydrogen and potassium ions, prostaglandins, histamine, bradykinin, nerve growth factor, cytokines and chemokines resulting from tissue damage and the associated inflammatory response. Together, these chemicals activate or sensitise nociceptors, resulting in peripheral sensitisation and primary hyperalgesia (hypersensitivity in immediate tissues). These chemicals can be spread through local

Figure 1.5 Illustration of myelinated A-fibres terminating in laminae I and V of the dorsal horn Sourced from Dubin and Patapoutian (2010).
vasodilation and plasma extravasation to result in secondary hyperalgesia (hypersensitivity in surrounding tissues). Allodynia (sensitivity to previously non-painful stimuli) is also a possible result of central pain sensitisation (Anderson and Muir, 2005). Increased hyperalgesia and allodynia has been demonstrated in calves following castration (Lomax and Windsor, 2014) and dehorning (Espinoza et al., 2013).

Nociceptive pain can be categorised as somatic or visceral, per the associated tissues. Somatic pain originates from receptors in peripheral tissues such as skin, muscles, joint and bones and is well localised. Visceral pain originates from receptors in the organs and is not well localised. Usually, somatic pain is described as being sharp, aching or throbbing whereas visceral pain is a more diffuse, dull sensation (Okafor et al., 2014). This is due to the viscera being sparsely innervated in combination with the production of referred pain. Production of referred pain is due to the spinal neurons that receive the visceral input also receiving input from other non-visceral and visceral structures. Visceral structures vary in terms of the type of nerve fibres present in the dorsal root ganglia somata. However, up to 80% have C-fibres, whereas less than 40% have A-δ fibres (Robinson and Gebhart, 2008). As a result different tissues and organs of the body vary in their sensitivity to pain, where some tissues such as mucous membranes and cornea are very sensitive and parenchymatous organs are less sensitive (Landa, 2012).

1.6 Pain assessment in cattle

Assessment of pain in animals is important for the application of appropriate analgesic therapies (Prunier et al., 2013). It is unknown whether animals and humans perceive pain in the same way, however, it is thought that pain serves the same purpose in animals as it does in humans (Landa, 2012), this purpose being “to change the animal’s physiology and behaviour to reduce or avoid
damage, to reduce the likelihood of recurrence and to promote recovery” (Molony and Kent, 1997). Assessment of pain in humans often relies on methods of self-report. As this is not possible in animals, assessment of pain is more difficult (Prunier et al., 2013). This relies on indirect evidence because direct measurement of an individual’s subjective experience is not possible (Molony and Kent, 1997). Pain responses in livestock can be broadly associated with changes to behaviour, physiology or production performance.

1.6.1 Pain responses related to behaviour

Behavioural responses to pain can be categorised as those that enable the animal to evade the painful experience in the future, those that protect the animal from further injury and pain, those that minimise pain and support healing and those that communicate for help or stop another individual from inflicting pain (Molony and Kent, 1997). Behavioural indices of distress in animals are characterised by changes in vocalisation, posture, locomotion and temperament. The presence of abnormal behaviours, as specified by their absence in control animals, are a measurable indication of distress and pain in animals. Differences in the type and method of painful procedure results in different areas and types of tissue damage and therefore different behavioural responses (Mellor et al., 2000). There is a variety of methods for assessing behaviour, of which those most relevant to this thesis are outlined below.

1.6.1.1 Ethological measurement of behaviour

Monitoring individual specified behaviours is an objective way of assessing changes or differences between treatment groups (Johnson, 2008, White et al., 2008) through analysis of the incidence and prevalence of such behaviours (Johnson, 2008). This can be done using various sampling methods classified as continuous (all-occurrences, sequence or sociometric matrix) or time sampling (one-zero, instantaneous or scan) (Table 1.3) (Lehner, 1998). Choice of which sampling
method to use depends on the research questions, the experimental design, the number and types of behavioural units being measured, the scale of measurement and practical considerations (Lehner, 1998). Continuous sampling is thought to provide the most complete and accurate data as it involves unceasing recording of occurrence, duration and sequences of behavioural states and events. However, time sampling provides an ease of recording observations and a high inter-observer reliability (Lehner, 1998).

Table 1.3 Ethological sampling methods

<table>
<thead>
<tr>
<th>Sampling method classification</th>
<th>Sampling method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>All-occurrences sampling</td>
<td>Recording all incidences of certain behaviours</td>
</tr>
<tr>
<td></td>
<td>Sequence sampling</td>
<td>Recording a chain of behaviours</td>
</tr>
<tr>
<td></td>
<td>Sociometric matrix</td>
<td>Recording interactions between pairs of individuals</td>
</tr>
<tr>
<td>Time Sampling</td>
<td>One-zero sampling</td>
<td>Recording whether a behaviour occurs during a sample period of a short duration</td>
</tr>
<tr>
<td></td>
<td>Instantaneous sampling</td>
<td>Recording behaviour of individuals at pre-determined instant time points</td>
</tr>
<tr>
<td></td>
<td>Scan sampling</td>
<td>Recording behaviour of several individuals at pre-determined instant time points</td>
</tr>
</tbody>
</table>

Adapted from Lehner, 1998.

Ethological measurement of behaviour has been utilised to assess the pain of various husbandry procedures in cattle, namely dehorning (McMeekan et al., 1999, Sylvester et al., 2004), castration (Ting et al., 2003, Petherick et al., 2015), branding (Lay et al., 1992a, Schwartzkopf-Genswein et al., 1997a) and spaying (Petherick et al., 2013), and the effects of anaesthetics and analgesics for such procedures (McMeekan et al., 1999, Ting et al., 2003, Sylvester et al., 2004). Previous studies
have demonstrated that some pain-related behaviours are influenced by the location and type of injury (Sylvester *et al.*, 2004, Petherick *et al.*, 2015). For example, post-surgical somatic pain following dehorning can be associated with increased displays of head-related behaviours (Millman, 2013), such as head shakes and ear flicks (Sylvester *et al.*, 2004). Another example is the association between post-surgical somatic and visceral pain caused by castration (Millman, 2013) and increased foot stamping, kicking and easing quarters (Molony *et al.*, 1995).

Behavioural states such as lying, grazing and ruminating, and behavioural activities such as tail shaking, ear flicking, head shaking, foot stamping and grooming have been recorded to assess the pain of scoop dehorning in calves and the effects of lignocaine (2%), and ketoprofen (10%) administered intravenously (IV) (McMeekan *et al.*, 1999). Dehorning caused changes in lying, grazing or ruminating, tail shaking and ear flicking, as compared to undehorned control calves. A combination of lignocaine and ketoprofen reduced the difference between dehorned and control groups during the first 4 h following treatment (McMeekan *et al.*, 1999). Instantaneous scan sampling has been used to monitor the difference in behaviours of undehorned control calves and calves that have been amputation dehorned, with and without lignocaine (2%) (Sylvester *et al.*, 2004). Dehorned calves displayed significantly more tail flicking, head shaking and ear flicking and significantly less rumination than control calves for the first 6 h. Dehorned calves treated with lignocaine displayed similar behaviours to control calves for the first 2 h, after which their behaviour was similar to the untreated dehorned group (Sylvester *et al.*, 2004).

Methods of castration and age groups of cattle undergoing such procedures have been compared using analysis of individual behaviours, both at the time of castration and following the procedure, to assess welfare outcomes (Petherick *et al.*, 2015). Measurement of the frequency of vocalisations, struggles, kicks and tail flicks showed that surgical castration was more painful than ring
application. On the day of castration, there were pain-related behaviours exhibited by both surgical and ring castrated calves. Active pain-related behaviours, such as walking and leg and tail movements, were more apparent in ring castrated calves as opposed to stationary behaviours, such as standing, in surgically castrated calves. Behaviours indicative of restlessness, such as vocalisation, were greater in 3-month-old calves compared to 6-month old calves, possibly because the younger calves had a higher motivation to be reunited with their mothers on whom they had a greater reliance for food (Petherick et al., 2015). Instantaneous scan sampling of surgically castrated bulls was conducted to assess the effect of ketoprofen, administrated IV once (3 mg/ kg BW at -20 min relative to castration), twice (1.5 mg / kg BW at -20 min and 1.5 mg / kg BW at 0 min relative to castration) and three times (1.5 mg / kg BW at -20 min, 1.5 mg / kg BW at 0 min and 3 mg/ kg BW at 24 h relative to castration) (Ting et al., 2003). Surgically castrated calves performed fewer lying postures, more abnormal standing activities and fewer rumination activities compared to uncastrated control calves. Ketoprofen administered twice and three times significantly lowered the incidence of abnormal standing compared to untreated castrated calves and all ketoprofen treatments significantly increased rumination so that it did not differ from control calves (Ting et al., 2003).

Hot-iron and freeze branding have been compared through analysis of the frequency of tail flicks, kicks, falls in the chute and vocalisations, with hot-iron branding resulting in significantly higher frequencies of all these behaviours compared to freeze and sham branding (Schwartzkopf-Genswein et al., 1997a). Freeze branding did not differ from sham branding, with the exception of a significantly higher number of tail flicks (Schwartzkopf-Genswein et al., 1997a). A similar study found that hot-iron branded cows kicked more than freeze branded and sham branded cows during the procedure (Lay et al., 1992a).
Two methods of spaying, the dropped ovary technique and flank laparotomy have been compared to one another and to other procedures: physical restraint only, electroimmobilisation and mock artificial insemination (Petherick et al., 2013) using scan sampling of behaviour. The occurrence of some behaviours was too infrequent for statistical analysis. Flank spayed cows and heifers stood with their heads down more than animals in all other treatment groups up to day 3, apart from no difference between the two spaying methods at the end of day 0. At the end of the day 0, fewer spayed cows and heifers were observed feeding compared to animals in other treatment groups, apart from no difference between spayed cows and cows that had undergone electroimmobilisation (Petherick et al., 2013).

Although ethological measurement of behaviour has been demonstrated as an accurate tool for assessment of pain in cattle (Lay et al., 1992a, Schwartzkopf-Genswein et al., 1997a, McMeekan et al., 1999, Ting et al., 2003, Sylvester et al., 2004, Petherick et al., 2013, Petherick et al., 2015), this method of assessment is labour intensive (Johnson, 2008, White et al., 2008). Therefore, other methods of behavioural assessment, such as scoring systems, quantitative sensory testing (QST) and accelerometry are often used, as they are less time consuming.

1.6.1.2 Scoring systems

Descriptive or numerical scoring systems are based on an observer’s subjective assessment of the degree of pain an animal appears to be experiencing. This method of pain assessment has been widely used to evaluate pain associated with disbudding and dehorning (Stilwell et al., 2010) and castration in cattle (Coetzee et al., 2014, Lomax and Windsor, 2014), and to investigate the use of pain relief for these procedures (Stilwell et al., 2010, Coetzee et al., 2014, Lomax and Windsor, 2014).
The behavioural responses of hot-iron disbudded calves following IV administration of xylazine (0.2 mg/kg BW), alone or in combination with lignocaine (2%), have been compared to sham disbudded calves (Stilwell et al., 2010). The pain of the procedure was evaluated using a numerical grading scale of 0 to 5, with grade 0 representing no behaviours exhibited and grade 5 represented all behaviours (stretching of hind and forelegs, ear flicking, head jerking and vocalisation) exhibited. Calves disbudded and treated with xylazine alone struggled the most during the procedure compared to all other treatment groups (Stilwell et al., 2010). Another study comparing different methods of dehorning (band, mechanical and tip) analysed vocalisation scores (0 to 2) during treatment and scores for depression, gait and posture, appetite and lying (0 to 3) every day following treatment for 28 days. The study found that cattle that had been band or mechanically dehorned had significantly greater vocalisation scores than un-dehorned or tip dehorned cattle. Cattle dehorned with a band had significantly greater lying scores and tended to have higher depression, gait and posture and appetite scores than the other treatments (Neely et al., 2014).

A TA formulation for application to surgical castration wounds post-procedure was evaluated through assessment of pain-related behaviour using a numerical rating scale of 0 to 3, where 0 represented no pain-related behaviour and 3 represented severe abnormalities of posture, gait or agitation. Castrated calves treated with TA expressed significantly less pain-related behaviour than untreated castrated calves (Lomax and Windsor, 2014). Another study utilised behavioural scoring at the time of surgical castration, with and without IV administration of the opioid nalbuphine (0.4 mg/ kg BW) (Coetzee et al., 2014). Vocalisation and attitude were scored on a scale of 0 to 3, where 0 represented no change from before castration and 3 represented continuous vocalisation during and immediately after castration and violent escape behaviour. Despite no difference in vocalisation scores between treated and untreated castrated calves, nalbuphine did
reduce attitude score. This study suggested that further research was required to determine optimum dosage of nalbuphine (Coetzee et al., 2014).

Compared to other methods of behavioural assessment, scoring systems have a greater potential for between-observer variability, due to previous experience and bias of the observers (Johnson, 2008). They also may be less sensitive to smaller between-treatment differences (White et al., 2008).

1.6.1.3 Quantitative sensory testing

Quantitative sensory testing (QST) is used to measure stimulus-dependent pain, namely hyperalgesia and allodynia (Keizer et al., 2008). Quantitative sensory testing in livestock involves the application of a mechanical stimulus with an increasing pressure until a withdrawal response occurs (Fitzpatrick et al., 2006). Pain withdrawal responses in cattle undergoing surgical husbandry procedures have been measured using an electronic von Frey anaesthesiometer (Lomax and Windsor, 2014, Tucker et al., 2014a, Tucker et al., 2014b), von Frey monofilaments (Espinoza et al., 2013, Lomax and Windsor, 2014) and an algometer (Heinrich et al., 2010, Mintline et al., 2013, Stock et al., 2015). These surgical husbandry procedures included disbudding and dehorning (Heinrich et al., 2010, Espinoza et al., 2013, Mintline et al., 2013, Espinoza et al., 2015, Stock et al., 2015), surgical castration (Lomax and Windsor, 2014) and hot-iron branding (Tucker et al., 2014a, Tucker et al., 2014b). Electronic von Frey anaesthesiometers and algometers automatically record the maximum pressure exerted before the occurrence of a withdrawal response (Lomax and Windsor, 2014). Von Frey monofilaments are calibrated to bend at specific pressures with the response to stimulation graded using a numerical rating scale based on the behaviour of the animal (Lomax et al., 2008).
Pain sensitivity to hot-iron branding, with and without IV administration of the NSAID flunixin meglumine (1.1 mg/kg BW) has been quantified through the use of an anaesthesiometer (Tucker et al., 2014b). Calves with brands were more sensitive to stimulation of the test site than unbranded calves throughout the study and flunixin had no effect on this. It was suggested that the force applied may have been insufficient to detect any drug-related effects on pain sensitivity (Tucker et al., 2014b). This has been demonstrated in another study where provision of the NSAID meloxicam (0.5 mg/kg BW) administered IV did not alter sensitivity of hot-iron disbudding wounds in calves when a maximum of 110 g of force was applied using von Frey monofilaments (Mintline et al., 2013). It is also possible that the size and shape of the anaesthesiometer probe may have been unsuitable for detection of NSAID-related effects on tissue sensitivity. The size and shape of probes on handheld QST devices affect the layers of tissue to which pressure is applied, and therefore the location and degree of nociceptor activation (Raundal et al., 2014). Larger probes, such as those on algometers, activate a greater number of nociceptors, predominantly from deeper tissues (Nie et al., 2009). Therefore, the smaller probe on an anaesthesiometer may not have been sufficient to detect inflammatory pain of deeper tissues where NSAIDs could potentially have an effect.

Another study tested the sensitivity of four sites on or within the vicinity of branded tissue and one site on non-branded tissue in calves using an anaesthesiometer (Tucker et al., 2014a). The centre of the brand was the most sensitive, with sensitivity decreasing the further away from the wound. This study also examined the effect of a topical cooling gel on wound sensitivity, with little effect demonstrated (Tucker et al., 2014a).

A von Frey anaesthesiometer and a von Frey monofilament (300 g) have been used to assess primary and secondary hyperalgesia of surgical castration wounds in calves and the effect of a TA
formulation on sensitivity (Lomax and Windsor, 2014). Topical anaesthetic significantly reduced primary and secondary hyperalgesia, as shown through greater nociceptive threshold values at the wound and peri-wound sites tested (Lomax and Windsor, 2014). The effects of varying TA formulations on the sensitivity of scoop dehorning wounds in calves has also been examined using von Frey monofilaments (10 g and 300 g) (Espinoza et al., 2013). Light touch stimulation with the 10 g von Frey monofilament demonstrated a presence of, and increase in, allodynia in dehorned calves. Pain stimulation with the 300 g von Frey monofilament demonstrated increasing primary and secondary hyperalgesia over time. Topical anaesthetic reduced short-term hypersensitivity of damaged tissues in this study (Espinoza et al., 2013). Another formulation of TA reduced pressure sensitivity, as measured with an algometer, up to 5 h following scoop dehorning (Espinoza et al., 2015). The NSAID ketoprofen (3 mg/ kg BW) administered intramuscularly (IM) reduced mechanical nociceptive threshold (MNT) only in the short-term (up to 2 h) following dehorning in this study, possibly due to the short half-life of ketoprofen in most species (less than 2 h) (Espinoza et al., 2015). Algometers appear to be more suitable for measurement of inflammatory-related pain, whereas anaesthesiometers and von Frey monofilaments are more suitable for measurement of surface nociception.

1.6.1.4 Accelerometry

Accelerometers are remote sensing devices designed to monitor activity continuously and non-invasively (White et al., 2008). They do so by measuring gravitational force in multiple axes (x-, y- and z-) which can then be interpreted as specific activities or postures (Theurer et al., 2013). Two-dimensional (2D) accelerometers measure gravitational force in axes that are vertical (x-) and horizontal-parallel (y-) to the body. Three-dimensional (3D) or triaxial accelerometers also measure gravitational force in the x- and y- axes, in addition to gravitational force that is
horizontal-perpendicular to the body (z-) (Figure 7). Triaxial accelerometers are therefore more accurate when studying movement in 3D space (White et al., 2008).

![Image of a three-dimensional accelerometer](image)

**Figure 1.6 A** Three-dimensional accelerometer (measuring x-, y- and z- axes) positioned on the lateral aspect of the right hind limb of a calf that is standing (A) and lying (B)

Sourced from Theurer et al. (2012).

Accelerometers have been used to record activity of cattle following surgical castration (White et al., 2008), disbudding and dehorning (Heinrich et al., 2010, Theurer et al., 2012) and concurrent dehorning and castration (Pauly et al., 2012), and the effects of analgesic protocols for such procedures (Heinrich et al., 2010, Pauly et al., 2012, Theurer et al., 2012).

Two-dimensional accelerometers have been validated for monitoring the behaviour of beef calves following surgical castration (White et al., 2008). This study showed that calves were more active and spent a greater amount of time standing following surgical castration compared to before the procedure (White et al., 2008). Another study using 3D accelerometers found that calves treated with oral meloxicam (0.5 mg/kg BW) immediately following hot-iron dehorning spent more time lying down on most days following the procedure (Theurer et al., 2012). These studies suggest that there is a reluctance to lie down and sometimes an increase in activity following castration.
and dehorning in calves. An increase in locomotion can be indicative of restlessness caused by pain (Mellor and Molony, 1991). A reluctance to move and the adoption of an immobile standing posture has also been shown to follow painful procedures, likely serving to prevent stimulation of hyperalgesic tissues (Molony and Kent, 1997). A study on concurrent castration and dehorning found contrasting results, with cattle spending more time lying following the procedures than before, as measured with 3D accelerometers (Pauly et al., 2012). As measurements were only taken between the hours of 6pm and 6am in this study, these differing results were suggested as being time-related (Pauly et al., 2012).

Accelerometers enable non-invasive, objective characterisation of animal behaviour without the presence of humans and without requiring labour intensive observation (White et al., 2008). However, this form of behavioural monitoring is generally more expensive, when measuring activity of a large number of individuals, compared to other forms of behavioural assessment, such as video observation. Another limitation to the use of accelerometers is the finite battery life and memory storage of the devices. This can limit the time allowance for data collection (Theurer et al., 2013), especially when the sampling rate is of a higher frequency (Mattachini et al., 2013), such as that necessary to detect gait patterns of calves (minimum sampling rate of 33 Hz) (Passille et al., 2010). Therefore, accelerometry is mostly useful when combined with other measures to obtain an understanding of an animal’s time budget.

1.6.2 Pain responses related to physiology

Physiological parameters measured for assessment of distress caused by pain in cattle include blood hormone and metabolite concentrations and other variables. Blood hormones measured include adrenaline, noradrenaline, corticotropin-releasing factor, adrenocorticotrophic hormone, glucocorticoids and prolactin. Blood metabolites measured include glucose, lactic acid, free fatty
acids and β-hydroxybutyrate. Other parameters measured include heart rate, respiration rate, packed cell volume, sweat production, muscle tremor, body temperature, plasma α-acid glycoprotein levels, blood leukocyte levels and cellular and humoral immune responses (Mellor et al., 2000). The physiological parameters most relevant to this thesis are outlined below.

1.6.2.1 Cortisol

Cortisol is a glucocorticoid secreted into the general blood stream by the presence of adrenocorticotropic hormone (ACTH). The secretion of ACTH into the general blood stream is induced by the release of corticotropin-releasing hormone (CRH) from the paraventricular nuclei of the hypothalamus into the hypothalamic-hypophyseal portal blood vessels following stress. Corticotrophin-releasing-hormone binds to corticotropin-releasing factor (CRF) type 1 receptor and activates cyclic adenosine monophosphate (cAMP) pathway events, leading to the release of ACTH. Vasopressin, in the presence of CRH, also affects the release of ACTH through the vasopressin V$_{1b}$ receptor. Glucocorticoid synthesis is stimulated by the binding of ACTH to melanocortin type 2 receptor (MC2-R) in the adrenal cortex. Glucocorticoids, including cortisol, regulate physiological events and negatively feed-back by acting on corticosteroid receptors in the brain and peripheral tissues (Smith and Vale, 2006) (Figure 1.7). These are the physiological responses that are part of the hypothalamic-pituitary-adrenal (HPA) axis, with changes within this multilevel system indicative of stress (Rushen et al., 2008), including emotional and physical distress. Therefore, cortisol has been widely used to measure acute pain and distress in animals (Mellor et al., 2000).
The most common procedure for measuring cortisol involves repeated blood sampling to create a concentration-time curve relative to the time of distress. The cortisol distress response is quantitatively characterised by studying the magnitude and speed of concentration change, and the duration and pattern of the response. The integrated cortisol response (the area under the cortisol curve that lies above the pre-treatment value) is also used as a quantitative measure of the cortisol response to stressors (Mellor et al., 2000). Cortisol has been shown to indicate the pain associated with castration (Petherick et al., 2015), dehorning (Sylvester et al., 1998b), ear notching (Stewart et al., 2013), branding (Schwartzkopf-Genswein et al., 1997b) and spaying (Petherick et al., 2011) in cattle. Pain associated with these procedures has been inferred through various components of the cortisol response curve including increased peak (Sutherland et al., 2002), extended duration of elevation (Sylvester et al., 1998b), slower decline from peak (Petherick et al., 2015) and greater
area under the curve (McMeekan et al., 1998a) as compared to control animals that did not undergo any procedures. Cortisol has also been used to compare the pain associated with different methods of castration (Fisher et al., 1996, Stafford et al., 2002), dehorning (Petrie et al., 1996, Sylvester et al., 1998b), branding (Lay et al., 1992a) and spaying (Petherick et al., 2011, Petherick et al., 2013) in cattle and to assess the effect of anaesthetics and analgesics on the pain response to castration (Sutherland et al., 2002) and dehorning (Webster et al., 2013).

It has been recognised that cortisol concentration indicates the overall distress of an experience, rather than solely measuring pain (Mellor et al., 2000), as its secretion occurs in response to a variety of stressors (Molony and Kent, 1997). These stressors include weaning, social isolation, transport, social mixing, novelty, restraint and handling (Stilwell et al., 2008). Diurnal changes and individual variation also influence cortisol concentration (Molony and Kent, 1997). In addition, cortisol increases in response to haemorrhage (Gann and Egdaahl, 1965), regardless of the presence of pain. As cortisol plays various roles, including maintaining blood volume, mediating the inflammatory response and facilitating wound healing (Fox et al., 1994, Hughan et al., 2001), it may not be suitable for comparing the pain of different types of tissue damage. The HPA axis responds quite slowly, meaning that determination of different degrees of distress within the first few minutes of an adverse experience is not always accurate (Mellor et al., 2000). The factors limiting the use of cortisol as a measurement of pain have been demonstrated in studies investigating the pain of freeze and hot-iron branding (Lay et al., 1992c, Lay et al., 1992b) and surgical castration (King et al., 1991, Coetzee et al., 2007, Coetzee et al., 2008). Lay et al. (1992b) did not observe any treatment differences in cortisol concentrations of sham branded, freeze branded and hot-iron branded Bradford crossbred calves at any time up to 20 min post-procedure. It was suggested that the effect of restraint may have masked or reduced the effect of branding on
cortisol. The breed of cattle was also suggested to possibly influence the cortisol response as most previous research had used European breeds (Lay et al., 1992b). When this study was replicated in dairy cows, the sham branded cows displayed significantly lower cortisol concentrations than branded cows at 5.5 min to 25.5 min post-treatment (Lay et al., 1992a). The difference between study results was attributed to dairy cattle being more accustomed to handling and restraint, which would lower the stress associated with these procedures in the experiment (Lay et al., 1992a). The other obvious difference is that the dairy cows were mature animals whereas the crossbred calves were younger animals that had only been weaned immediately before the acclimation period commenced (Lay et al., 1992b). The studies on surgical castration similarly found no significant difference between castrated and uncastrated Angus crossbred calves (Coetzee et al., 2007, Coetzee et al., 2008) and calves of mixed breed (Hereford, Angus, Charolais, Simmental and Maine-Anjou sires) (King et al., 1991). This suggests that measurement of cortisol in environments reflective of typical commercial production conditions is more representative of the stress of handling and restraint than pain (Coetzee et al., 2007). Separating unweaned calves from their mothers is also suggested to cause distress and a cortisol response independent of pain (King et al., 1991). It is possible that this distress shifts calves’ attention away from pain (Petherick et al., 2015). Despite the limitations to using cortisol as a measure of pain, it is widely used for this purpose in animals (Mellor et al., 2000) because of its objective quantification (Hart, 2012) and because the magnitude and duration of response is usually relative to the predicted noxiousness of the experience (Mellor et al., 2000). The effect of TA and BM on the cortisol response of livestock following surgical husbandry procedures has previously not been investigated.
1.6.2.2 Ocular temperature (as measured using infrared thermography)

Change in ocular temperature has been used to detect the activation of the autonomic nervous system (ANS) in response to pain. The sympathetic division of the ANS prepares the body for ‘flight or fight’ through its effect on various organs and body systems. A decrease in ocular temperature following a stressful or painful experience could be due to the reduction of blood in ocular capillaries due to vasoconstriction of arterioles following the activation of the sympathetic nervous system. A subsequent rapid increase in ocular temperature could be due to the vasodilation of blood vessels caused by the activation of the parasympathetic nervous system (Godyn et al., 2013).

Change in ocular temperature has been used to assess the pain of disbudding (Stewart et al., 2009) and castration (Stewart et al., 2010, Dockweiler et al., 2013) of calves and the effects of pain relief for these procedures (Stewart et al., 2009, Stewart et al., 2010). However, the response of ocular temperature to pain is not consistently reported in the literature, potentially due to differences in animals used, animal handling and data collection techniques (Stock et al., 2016).

The pain of surgical castration in calves, with and without lignocaine (2%), has been evaluated through assessment of ocular temperature (Stewart et al., 2010). By 2 min following castration, there was a transient but non-significant decrease in ocular temperature followed by a rapid increase in untreated castrated calves. By 5 min following castration, ocular temperature was greater than baseline for all calves and stayed at this elevated level for the entire 20-minute period following treatment. Ocular temperature of sham castrated calves increased to a level of elevation less than that of calves that had been castrated. This demonstrates that changes in ocular temperature can occur in response to other stressors independent of pain, such as fear (Stewart et al., 2008b).
Change in ocular temperature has also been used to assess the pain of hot-iron dehorning and the effects of anaesthesia and analgesia (Stock et al., 2016). A decrease in ocular temperature following dehorning with lignocaine (2%), appeared 2.5 and 3 h following the procedure, thought to be due to the anaesthetic effect wearing off. This demonstrates that ocular temperature not only changes in response to a painful procedure, but can determine the onset of pain following a procedure.

1.6.2.3 Inflammation

Tissue injury causes an inflammatory response involving vascular and cellular components that enable tissue repair. The vascular component of inflammation involves the development of a fibrin clot and coagulation. The cellular component of inflammation involves the mediation of leukocytes to the site of injury. There are numerous inflammatory mediators that are involved in acute inflammation, such as bradykinin and prostaglandins, that increase nociceptive sensitivity (Li et al., 2007). Swelling and redness at the site of injury are visible signs of inflammation, as a result of plasma extravasation, and vasodilatation respectively (Gregory, 2004). An increase in skin temperature also accompanies inflammation due to cutaneous cell metabolism and cutaneous blood flow (Wright et al., 2006).

1.6.2.3.1 Wound temperature (as measured using infrared thermography)

Infrared images of the scrotal area of beef calves have been captured to study the effects of castration on localised tissue temperature (Mintline et al., 2014, Moya et al., 2014). In one study, thermographic images of the scrotal area were captured before and after surgical and band castration, with and without ketoprofen (3 mg/ kg BW) administered IM (Moya et al., 2014). Increased temperatures due to inflammation resulting from tissue damage caused by the physical methods of castration were observed in the first week for surgical castration and 4 weeks following
the procedure for band castration. Administration of ketoprofen had no effect on scrotal temperature in this study (Moya et al., 2014). Similarly, in the other study, there was no effect of an NSAID, flunixin (1.1 mg/ kg BW) administered IV, on scrotal temperature as measured using infrared thermography (Mintline et al., 2014). Administration of lignocaine (2%), to all calves in this study was suggested to have possibly limited the ability to assess effects of flunixin. In this study, scrotal temperature peaked on days 21 and 35, which was thought to be due to revascularisation of the tissue, as this was also when the most pronounced healing occurred (Mintline et al., 2014).

Infrared thermography has been used to explore inflammation associated with branding, with and without flunixin meglumine (1.1 mg/ kg BW) administered intravenously (Tucker et al., 2014b). The surface temperature of branded tissue was higher than that of unbranded tissue on the day of the procedure and days 42, 56 and 71 following the procedure. The higher temperature seen on days 42, 56 and 71 is likely due to the loss of primary scab during the healing process. There was no effect of flunixin meglumine on tissue temperature (Tucker et al., 2014b). The effects of a cooling gel on the surface temperature of brands has also been assessed using infrared thermography (Tucker et al., 2014a). The cooling gel reduced the heat emitted from brands on the days it was applied (Tucker et al., 2014a).

Wound temperature following dehorning of calves has not previously been studied. Furthermore, the effect of LA and other types of NSAIDs on wound temperature following castration and dehorning in calves also has not been evaluated.
1.6.2.3.2 Wound morphology

Healing of dehorning (Kihurani et al., 1989, Neely et al., 2014), castration (Fisher et al., 1996, Marti et al., 2010, Mintline et al., 2014, Petherick et al., 2014a, Petherick et al., 2015) and branding (Tucker et al., 2014a, Tucker et al., 2014b) wounds in cattle has been studied for the evaluation of inflammation, pain and welfare following such procedures and the effects of administering analgesia. Wound healing has been examined through the use of descriptive, numerical rating scales (Fisher et al., 1996, Marti et al., 2010, Mintline et al., 2014, Neely et al., 2014, Petherick et al., 2014a, Tucker et al., 2014a, Tucker et al., 2014b, Petherick et al., 2015). In addition, inflammation and healing of castration wounds has been assessed by measuring scrotal size (Fisher et al., 1996, Mintline et al., 2014, Petherick et al., 2014a). The effects of three different methods of dehorning (mechanical, band and tip) on wound healing was assessed daily for 28 days following the procedures using a numerical rating scale of 0 to 3, where 0 represented a fully healed wound (Neely et al., 2014). During the first week following treatment, there was a trend for mechanically dehorned calves to have a higher score than all other groups, whereas in weeks 3 and 4, the band dehorned calves had a higher score than all other treatment groups (Neely et al., 2014).

Inflammation and wound healing following surgical castration, with and without administration of flunixin, was assessed using a numerical rating scale of 1 to 5, where 5 represented a fully healed wound (Mintline et al., 2014). Scrotal size increased on the first day following castration and peaked on day 2 to 3. The most prominent increase in healing score was seen between days 21 and 35. Flunixin had no effect on scrotal size or healing score (Mintline et al., 2014).

Healing of hot-iron branding wounds has been scored on a 6-point scale, where 6 represented an almost fully healed wound (Tucker et al., 2014a, Tucker et al., 2014b). Healing scores increased over time (Tucker et al., 2014a, Tucker et al., 2014b). Flunixin had no effect on healing (Tucker
et al., 2014b) whereas a cooling gel slowed healing rate, perhaps due to physical disruption, irritation or insulation of the wound (Tucker et al., 2014a).

The effect of TA on wound healing has been examined following mulesing in lambs (Lomax et al., 2008). The effect of BM on wound healing has also been assessed in lambs following surgical castration and tail docking (Small et al., 2014). However, there has been no previous assessment of the effects of TA and BM on wound morphology following castration and dehorning of calves.

1.6.3 Pain responses related to production performance

Pain can result in reduced feeding behaviour, stress and immune reactions that affect nutrient fluxes and utilisation and inhibition of physiological axes, such as the gonadotropic and somatotropic axes. These effects of pain can therefore influence production outputs (Prunier et al., 2013). The main production parameters used to evaluate pain in cattle are feed intake and weight gain (Coetzee, 2011).

1.6.3.1 Weight gain

Weight gain has been used to assess pain following castration and dehorning, as a reduction in this parameter can occur following these procedures (Mosher et al., 2013). Weight gain has also been used to evaluate the efficacy of analgesia. Administration of lignocaine (2%), has been shown to improve average daily gain (ADG) following surgical castration of calves (Fisher et al., 1996). Similarly, analgesic drugs, meloxicam (1 mg/kg BW) administered orally, flunixin (2.2 mg/kg BW) administered IV and gabapentin (15 mg/kg BW) administered orally, have been shown to improve weight gain following dehorning of calves (Glynn et al., 2013). Sodium salicylate and a combination of sodium salicylate, xylazine, ketamine and butorphanol improved ADG of calves following concurrent castration and dehorning (Baldridge et al., 2011).
There can be considerable individual variability in weight gain, hence larger treatment group sizes may be necessary when assessing this outcome (Webster et al., 2013). The effects of TA and BM on weight gain of calves following husbandry procedures has not yet been studied.

### 1.6.4 Multidisciplinary approach to pain assessment

There are limitations to each method of behavioural or physiological pain assessment. The main limitations to using behavioural methods of pain assessment include the aspect of subjectivity, the possibility of influence from other factors independent of pain and individual animal variability (Prunier et al., 2013). Another limitation to behavioural assessment of pain in livestock is the stoic nature of the animals as prey species, resulting in a tendency for production animals to conceal behavioural signs of pain (Landa, 2012). For example, a study investigating the effect of lignocaine (2%), and flunixin meglumine (1.1 mg/kg BW) administered IV, on the pain of surgical castration in calves, had difficulty identifying effects of these drugs due to limited behavioural expressions of pain (Webster et al., 2013). Only a small number of behavioural differences were detected between castrated calves and uncastrated control calves. This study therefore relied on measurements of cortisol in addition to behaviour to conclude that a combination of lignocaine and flunixin appeared to be an appropriate analgesic treatment for surgical castration of calves (Webster et al., 2013). The main limitation to using physiological methods of pain assessment is that most of these indices can be associated with stressful experiences or illness independent of pain (Prunier et al., 2013). A study on stress and nociception responses following cautery dehorning showed no difference between ocular temperature, heart rate, respiratory rate, cortisol and substance P of dehorned and undehorned control calves (Stock et al., 2016). This was the case, despite greater MNT values in dehorned calves, indicative of increased nociception (Stock et al.,
2016). Hence, a combination of indicators it is often necessary to improve accuracy and efficiency of pain assessment (Molony and Kent, 1997, Prunier et al., 2013).

1.7 Analgesic therapies for painful husbandry procedures in cattle

The efficacy of various forms of pain relief for cattle undergoing husbandry procedures is well documented in the literature (Stafford and Mellor, 2005b, Sutherland et al., 2011, Coetzee, 2013). Pain can be reduced using various pharmacological agents known as anaesthetics or analgesics. Anaesthesia describes an absence of sensation, whereas analgesia describes the reduction or removal of pain. Therefore, anaesthesia leads to analgesia (Schwartzkopf-Genswein et al., 2012). Analgesic pharmacological agents available for use in cattle include LA, NSAIDs, opioids and α-2 agonists (Schwartzkopf-Genswein et al., 2012). A multi-modal approach to pain management usually incorporating a combination of LA and an NSAID provides superior analgesia as it addresses both acute and inflammatory pain pathways (Mellor and Stafford, 1999, Hudson et al., 2008).

1.7.1 Local anaesthetics

Local anaesthetics diminish sensation by impeding nerve depolarisation and conduction via inhibition of sodium channels (Anderson and Muir, 2005). A widely used LA in cattle is lignocaine as it is inexpensive and has limited toxic effects (Edmondson, 2014). Lignocaine has an onset of action of 1 to 2 min and lasts 60 to 120 min. Other LA agents include mepivacaine, procaine and bupivacaine which have different pharmacodynamics. Bupivacaine has a longer time to onset of action (5 to 10 min) and a longer duration of activity (4 to 12 h) (Stafford and Mellor, 2015). The techniques for administering LA agents vary depending on their use and include topical
application, local blocks, ring blocks, selected peripheral nerve blocks and regional blocks (Anderson and Muir, 2005). There is sufficient research showing that an effective cornual nerve block reduces the pain and stress response of calves undergoing dehorning for the duration of the anaesthetic agent’s activity (Stafford and Mellor, 2005b). The results from studies examining LA injected prior to surgical castration in calves vary. Earley and Crowe (2002) found that lignocaine (2%) injected into each testicle (6 mL) and subcutaneously (SC) along each incision line of the scrotum (3 mL) 20 min prior to surgical castration significantly reduced the cortisol response of 5.5-month-old dairy calves to the procedure. In contrast, in another study, lignocaine (2%) injected into each testicle (6 mL) and SC around the spermatic cords (6 mL) 20 min prior to surgical castration was not sufficient to cause a significant reduction in the cortisol response of 2 to 3-month-old dairy calves (Webster et al., 2013).

A topical anaesthetic gel containing lignocaine and bupivacaine applied immediately following dehorning and surgical castration of calves has been shown to reduce wound sensitivity for up to 5 and 24 h, respectively (Espinoza et al., 2013, Lomax and Windsor, 2014, Espinoza et al., 2015). It has also been shown to reduce pain-related behaviour in calves for the first 4 h following surgical castration (Lomax and Windsor, 2014). This form of LA is easy and quick to administer (Lomax and Windsor, 2014), however, being applied post-operatively, is not effective for peri-operative pain relief. Topical application of LA has been suggested to extend the duration of anaesthesia in comparison to injected administration of LA, possibly due to a slower absorption rate (Brofeldt et al., 1989, Lomax et al., 2013, Lomax and Windsor, 2014). Further work is needed to examine the efficacy of TA for surgical husbandry procedures in cattle. Hence, the studies throughout this thesis address this. A potential limitation to the use of TA for dehorning is the possibility of
insufficient absorption of anaesthetic agents following excessive haemorrhage (Espinoza et al., 2013).

A practical way of addressing peri-operative pain of minor surgical procedures is through the use of cryo-anaesthesia (Lomax et al., 2017). This is a form of local anaesthesia which involves disruption of local sensory nerve function through reduction of tissue temperature (Griffith et al., 2016). Topical vapocoolants can be used to reduce tissue temperature through the rapid evaporation of volatile liquid from the skin surface to which they are applied (Collado-Mesa et al., 2015). In beef calves, a topical vapocoolant has been shown to reduce the peri-operative pain response to ear tagging and ear notching (Lomax et al., 2017). The efficacy of topical vapocoolant for other husbandry procedures in cattle has not previously been investigated.

1.7.2 Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs reduce inflammation by inhibiting the enzyme cyclo-oxygenase (COX) which synthesizes prostaglandins and other inflammatory mediators (Hudson et al., 2008). The analgesic effect of NSAIDs is mediated by their anti-inflammatory action, reducing nociceptor sensitisation within injured tissue (Stafford and Mellor, 2005b). There are a variety of NSAIDs available for use in cattle, each with different pharmacokinetic and pharmacodynamic properties (Anderson and Muir, 2005). For example, in addition to peripheral analgesic effects, some NSAIDs also have various central analgesic effects, including inhibition of neurotransmitter synthesis, increasing nerve membrane potentials and reducing synaptic output (Stafford and Mellor, 2005b). NSAIDs commonly used in cattle include meloxicam, ketoprofen, carprofen, flunixin meglumine and tolfenamic acid (Hudson et al., 2008). Unlike LA, NSAIDs act
systemically and can be administered orally, IV or intramuscularly (IM) (Stafford and Mellor, 2015).

There is extensive literature on the use of various NSAIDs for the relief of pain caused by dehorning and surgical castration, with differing conclusions on their efficacy (Stock et al., 2013). Ketoprofen (3 mL) administered IV 15 to 20 min prior to scoop dehorning of 3 to 4-month-old dairy calves has been shown to have a minimal effect on the initial cortisol response, however reduces the later phase of the cortisol response, suggesting it may ameliorate inflammatory pain (McMeekan et al., 1998b). Similarly, meloxicam (0.5 mg/kg BW) administered IV to 16 to 20-week-old dairy calves immediately prior to scoop dehorning was demonstrated to have no effect on the cortisol response in the acute post-operative period. However, it did result in longer term physiological, behavioural and performance effects indicative of analgesia (Coetzee et al., 2012).

Oral administration of meloxicam has been recognised as having certain advantages over injectable administration, due to its differing pharmacokinetic profile. Although oral meloxicam has a greater ‘time-to-peak-concentration’ than meloxicam administered IV (Coetzee et al., 2009), its mean plasma half-life is also greater, indicating that oral administration of the drug may result in longer lasting analgesia (Coetzee et al., 2009). In addition, oral administration allows the drug to be delivered via feed, eliminating the requirement for restraint (Olson et al., 2016). It also removes the risk of injection site reactions as well as needle related injuries (Olson et al., 2016), and requires less operator skill (Small et al., 2014). Oral meloxicam (1 mg/kg BW) administered to 4 to 5-month-old Holstein calves approximately 2 h prior to surgical and band castration has been shown to significantly reduce physiological and behavioural responses to procedural pain (Olson et al., 2016). Similarly, yearling beef bulls exhibited a reduced acute inflammatory response following surgical castration with concurrent treatment of meloxicam bolus (1.1 mg/kg
administered directly into the rumen via esophageal intubation (Roberts et al., 2015). Although NSAIDs administered orally can be effective, there are concerns about slow absorption rate when administered this way (Small et al., 2014). Drugs delivered by oral trans-mucosal absorption offer some advantages over drugs delivered orally, including abundant blood supply and improved bioavailability due to avoidance of degradation in the gastrointestinal tract and hepatic first-pass metabolism (Habib et al., 2011). Recently, a buccal meloxicam (BM) gel has been developed for use in calves and lambs undergoing surgical husbandry procedures. When administered into the buccal cavity (0.5 mg/kg BW) immediately prior to surgical castration and tail docking in lambs, BM reduced pain-related behaviours in the first 8 h following the procedures (Small et al., 2014). There is currently no published literature on the pharmacokinetics or efficacy of BM in cattle.

1.7.3 *α*-2 agonists

*α*-2 agonists activate *α*-2-adrenoreceptors in the central and peripheral ANS producing analgesic and sedative effects by negatively affecting sympathetic activity and the release of noradrenaline (Hudson et al., 2008). Side effects include decreased cardiac output, reduced respiratory rate, relaxation of muscles and depressed gastrointestinal motility. Xylazine is the most commonly used *α*-2 agonist in cattle (Coetzee, 2011).

Xylazine (0.2 mg/kg BW), in combination with ketoprofen (3 mg/kg BW), both administered IM, has been shown to reduce struggling behaviour during hot-iron disbudding of calves and reduce heart rate following the procedure (Caray et al., 2015). The sedative effects of xylazine could explain the reduced struggling during the procedure, however it could also be indicative of analgesia. The reduction in heart rate was due to the induction of bradycardia through peripheral and central effects of xylazine. From this study, it was suggested that xylazine improves the
welfare of calves undergoing hot-iron disbudding (Caray et al., 2015). Another study suggested that xylazine administered alone was insufficient to relieve pain during hot-iron disbudding, based on the degree of struggling behaviour, and for the first 40 min following the procedure, based on the frequency of ear flicks and head shakes (Stilwell et al., 2010).

1.7.4 Opioids

Opioids exhibit analgesic effects by binding to spinal and supraspinal μ, κ and δ receptors and consequently decreasing propagation of the nociceptive signal by activating receptor linked potassium channels and inhibiting voltage-gated sodium channels. Administration of opioids can result in side effects including respiratory depression, decreased gastrointestinal motility, increased appetite, sedation, euphoria and nausea. To reduce these side effects, partial or mixed receptor opioids have been developed (Coetzee, 2011). Butorphanol is the most commonly used opioid in cattle (Anderson and Muir, 2005). Opioids may act synergistically with α-2 agonists and therefore these compounds are often administered in combination (Coetzee, 2011).

Treatment with xylazine (0.05 mg/ kg BW), ketamine (0.1 mg/kg BW) and butorphanol (0.025 mg. kg BW) administered IM has been shown to result in a reduced chute exit velocity and reduced electrodermal activity following concurrent castration and dehorning of calves, likely due to the sedative effects of this drug combination (Baldridge et al., 2011). In this study, treatment with xylazine, ketamine and butorphanol only reduced serum cortisol for less than 1 h following the procedures, which is related to the short-lived effects of all the drugs in that combination treatment. There was no effect of this combination treatment alone on weight gain (Baldridge et al., 2011). A combination of butorphanol (0.07 mg/kg BW) and xylazine (0.02 mg/kg BW) administered IV to weanling bulls at the time of castration did not affect indicators of stress and did not improve performance, as measured through analysis of chute exit velocity, cortisol, haptoglobin, weight
gain, feed intake, mortality and morbidity (Faulkner et al., 1992). The apparent lack of analgesia was suggested possibly due to the dose rate or timing of administration (90 s before castration) (Faulkner et al., 1992).

1.7.5 Constraints to the use of anaesthesia and analgesia in cattle undergoing routine husbandry procedures

α-2 agonists and opioids are not well suited for use on-farm due to the cost associated with these drugs and their sedative effects (Anderson and Muir, 2005, Hudson et al., 2008). Therefore, most research has focused on LA and NSAIDs for the relief of castration and dehorning in calves, administered mostly SC, IV or IM prior to the procedures (Stafford and Mellor, 2005b, Coetzee, 2011, Stock et al., 2013), and it has been established that a combination of LA and an NSAID is best practice (Mellor and Stafford, 1999, Hudson et al., 2008). However, the uptake and use of analgesic therapies for husbandry procedures in commercially produced livestock largely depends on their cost, ease of administration and time to effectiveness (Mellor and Stafford, 1999). In addition, the requirement for veterinary or expert supervision and registration of pharmaceuticals affect the uptake of analgesic treatments on-farm (Mellor et al., 2008). Reasons provided by veterinarians for their limited use of pain relief include delivery of anaesthesia and analgesia being too time consuming, doubt that the general public will be willing to pay extra costs, difficulty in assessing pain in animals, concern for residues in food producing animals, lack of long acting, cost-effective analgesic treatments for farm animals, and uncertainty on withdrawal periods for opioids and dissociative anaesthetics in farm animals (Cornish et al., 2016).

In relation to northern Australian beef systems, the extra costs and time required to administer conventional forms of pain relief to large numbers of cattle is a major hindrance to the adoption of
such forms of analgesia. Further, these cattle are unaccustomed to human handling and hence, longer restraint times or doubling handling could pose additional animal welfare issues (Petherick, 2005). Topical anaesthetic and BM are practical forms of analgesia for use in such extensive settings, therefore there is a need to investigate their efficacy for castration and dehorning of calves. As multi-modal analgesia is most effective, a combination of TA and BM would be likely to provide superior pain relief than TA or BM administered alone.

1.8 Consumer and producer attitudes to animal welfare and pain

Public concern for animal welfare has continuously increased over recent decades as most people recognise animals, especially vertebrates, as sentient beings that have the ability to experience pain and distress (Cornish et al., 2016). The Australian public regards animal welfare as an important issue and this is associated with related community behaviours such as donating to animal welfare groups and writing to the media. This can affect the economics and sustainability of livestock production industries as it influences actions from retailers, regulators and legislators (Coleman, 2008). There is limited, specific data on the attitudes of Australians towards painful husbandry procedures performed on livestock. However, in general, the Australian public believe animals should not have to experience unnecessary pain (Coleman, 2008). As the public becomes more aware of the pain and distress caused by certain husbandry procedures performed on livestock, it is likely that the requirement for pain relief will be greater (Mellor et al., 2008).

An Australian survey of farmers, government officials, animal welfare scientists, animal welfare advocates, veterinarians and livestock transport representatives identified spaying and dehorning to be of high importance, and castration and methods of identification to be of below average importance in relation to animal welfare (Phillips et al., 2009). The provision of pain relief was
seen as more important than the particular methods used for performing these invasive procedures (Phillips et al., 2009).

1.9 Summary and research objectives

Pain has been identified as an important component of animal welfare and there is a growing expectation that appropriate analgesic therapies be utilised in farm animals. Existing studies provide thorough understanding of the efficacy of a range of anaesthetics and analgesics for invasive husbandry procedures performed on cattle. However, most of this research focuses on SC, IV, or IM administration of pharmaceuticals, of which practical constraints have limited their widespread adoption by producers.

In northern Australia, dehorning, castration, spaying, and methods of identification are often performed on older animals, exacerbating welfare implications of such procedures. The northern Australian beef industry is a significant contributor to Australia’s agricultural income. As consumer preference for ethically produced food continues to grow, improvements to current practices conducted in northern Australian beef production systems are required to ensure sustainability of the industry. As practical limitations to using anaesthetics and analgesics are particularly an issue for large-scale, extensively managed cattle operations, there is a need for other modes of delivering pain relief to animals produced under such conditions.

Therefore, the aim of this thesis was to examine the efficacy of practical analgesic therapies that are more likely to be adopted by cattle producers for use during routine surgical husbandry procedures. The specific objectives that were addressed throughout the studies within this thesis were:
1. To examine the effect of TA on the cortisol response of unweaned calves following surgical castration.

2. To examine the effects of TA and BM, alone and in combination, on production, behaviour and inflammation following surgical castration of unweaned calves.

3. To examine the effect of a vapocoolant spray on behaviour during surgical castration of unweaned calves.

4. To examine the effects of three formulations of TA on wound sensitivity following amputation dehorning of unweaned calves.

5. To examine the effects of TA and BM, on behaviour and inflammation following amputation dehorning of unweaned calves.

6. To examine the effects of TA and BM, alone and in combination, on production and behaviour following concurrent surgical castration and amputation dehorning of weaned calves.
CHAPTER 2: EFFECT OF A TOPICAL ANAESTHETIC FORMULATION ON THE CORTISOL RESPONSE TO SURGICAL CASTRATION OF UNWEANED BEEF CALVES

This chapter appears as the following published paper in the international peer reviewed scientific journal, Animal (citation below). Only the format has been changed for the purposes of consistency of style in this thesis.

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2.1 Abstract

Impracticality and cost of existing pain management strategies during surgical castration of beef cattle have limited their widespread implementation on-farm. A farmer-applied topical anaesthetic formulation, originally developed and used commercially to mitigate the pain of mulesing in lambs, was investigated for its potential use for managing pain in surgically castrated calves. This formulation contained lidocaine, bupivacaine, adrenalin and cetrimide. In this study, 24 Angus bull calves were randomly allocated to (1) surgical castration (C, n = 8), (2) surgical castration with the post-operative application of topical anaesthetic (CTA, n = 8) and (3) sham castration/control (CON, n = 8). The experiment was conducted over 2 days, with treatment groups evenly represented across each day. Calves were habituated to handling before the experiment and blood samples were collected for plasma cortisol measurement at defined time periods before, at and post treatment, (at −0.5, 0 h, then +0.5, 1, 1.5, 2, 4 and 6 h). There was a significant effect of time on cortisol concentrations across all treatment groups (P < 0.01), with lowest concentrations at −0.5 and 6 h and peak concentration at 0.5 h being significantly higher than the cortisol response at 0 h. The effect of treatment was not significant (P = 0.077), however, there was a trend for CON calves to display lower cortisol concentrations than C and CTA calves and CTA calves to display lower cortisol concentrations than C calves. The mean area under the curve (AUC) of CON calves was significantly lower than those of C and CTA calves (P = 0.04), however, there was no significant difference between the AUCs of CTA and C calves. Immediate application of topical anaesthetic after surgical castration did not significantly reduce plasma cortisol concentrations. However, the trend for CTA calves to display lower cortisol concentrations than C calves warrants further investigation into the use of TA for pain relief of surgically castrated beef calves.
2.2 Implications

This study investigated the use of a topical anaesthetic (TA) formulation for post-operative pain relief of castrated calves, which offers a practical option for producers to provide pain relief on-farm. In this study, TA had no significant effect on the cortisol response to surgical castration of unweaned Angus calves. However, there was a trend for calves treated with TA to have lower cortisol concentrations than untreated castrated calves at some time points after castration. No conclusions can be drawn from the current study regarding the effectiveness of TA to ameliorate pain during castration and further research is required.

2.3 Introduction

Castration of male calves is an important management practice routinely performed in beef cattle (Earley and Crowe, 2002, Coetzee, 2011) to prevent unwanted breeding, facilitate fattening (Puig et al., 2011) and improve meat quality (Coetzee, 2013). Castration also reduces aggression and mounting behaviours that cause injury and stress to other cattle (Earley and Crowe, 2002). Pain and suffering in animals used in agriculture is of increasing concern to consumers of livestock products (Earley and Crowe, 2002). Although the pain of castration in cattle has been well documented (Fisher et al., 1996, Coetzee, 2011), the procedure is commonly performed without analgesic or anaesthetic intervention. Considerable research on pain alleviation in castrated calves has been published (Fisher et al., 1996, Earley and Crowe, 2002). However, the practicality and cost-effectiveness of these pain management strategies are a major limitation to their implementation (Petherick, 2005). To address these issues, a farmer-applied ‘spray-on’ topical anaesthetic (TA) was recently studied in calves undergoing castration (Lomax and Windsor, 2014). This TA was shown to reduce pain-related behaviours and sensitivity of wounds and surrounding
tissue for at least 24 h post-procedure (Lomax and Windsor, 2014). This followed previous studies demonstrating successful pain management during mulesing and castration of lambs (Lomax et al., 2008, Lomax et al., 2010, Lomax et al., 2013). The TA (Tri-Solfen®; Bayer Animal Health, Pymble, NSW, Australia) used in these studies and the current study consists of lidocaine (40.6 g/l), bupivacaine (4.2 g/l), adrenalin (24.8 mg/l) and cetrimide (5.0 g/l). This product is currently only registered for use in lambs undergoing mulesing. Experimental use of the product in cattle is conducted under a research permit issued by the Australian pesticides and veterinary medicines authority.

Assessment of cortisol concentration has been widely used as a measure of acute distress in animals. Cortisol concentration as a measure of pain-induced distress is used extensively because the response magnitude and duration, as measured by peak height and integrated cortisol response, usually accord with the predicted noxiousness of certain procedures (Mellor et al., 2000). Measurement of cortisol has been used in cattle to quantify the effects of different painful procedures such as dehorning (Sylvester et al., 1998a), branding (Lay et al., 1992a) and castration (Fisher et al., 1996).

The aim of this study was to investigate the effect of TA on the cortisol response to surgical castration of beef calves and evaluate the effectiveness of TA as an option for pain relief. It was hypothesised that provision of TA would reduce the post-operative cortisol response of calves following surgical castration.
2.4 Materials and methods

2.4.1 Animals and housing

A total of 24 unweaned, Angus bull calves (3 months old) were sourced from a commercial herd at the University of Sydney property ‘Arthursleigh’ (Marulan, NSW, Australia) in November 2013. The experimental protocol was approved by the institutional animal ethics committee (Approval No. 5832). Calves had not previously undergone any husbandry procedures. 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, New Zealand). Calf BW ranged from 77 to 102 kg. 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, 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.
2.4.2 Experimental design and treatments

The experiment was conducted over 2 days, with treatment groups evenly represented across each day. Maximum daily temperatures for these days were 26.4 and 31.0°C. 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. Calves were randomly assigned 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 TA (CTA, n = 8). Four calves from each treatment group were treated each day. 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). Castration was performed using a technique that required initial transverse excision of the distal third of the scrotal skin with a sterilised knife. Each testis was manually exteriorised by pulling from the tunica vaginalis, and the spermatic cord cut ~12 cm proximal to the head of the epididymis. This method ensured that all
tissue that had been handled or contaminated was exteriorised and removed from the calf, reducing
the chance of infection of retracted material. For CTA calves, before removal of each testis, the
exposed testicular tissue was coated with Tri-Solfen® by inserting the applicator nozzle along the
spermatic cord inside the tunica vaginalis, into the inguinal cavity and applying 3 ml of TA. A
quantity (2 to 3 ml) of TA was also applied to the cut skin edge of the scrotum. This application
of TA aimed to provide maximum coverage of exposed cut tissue and ensured the retracted
spermatic cord was covered in a pool of anaesthetic within the inguinal canal.

2.4.2.1 Blood sample collection

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®, 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.
2.4.2.2 Plasma cortisol determination

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.

2.4.2.3 Statistical analysis

The program GenStat® (VSN International Ltd, Hemel Hempstead, UK) was used to conduct all statistical analyses and generate LSD values. Data on cortisol concentrations were subjected to residual maximum likelihood for repeated measures. The fixed effects of the model were treatment (CON, C, CTA), time (−0.5, 0, 0.5, 1, 1.5, 2, 4, 6 h), day (1, 2) and BW (covariate). 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 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.

2.5 Results

There was no significant interaction between time and treatment (\( F = 0.99; \) d.f. = 14, 147; \( P = 0.463, \) Table 2.1). 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 2.1 Mean cortisol concentration (nmol/L ± s.e.m.) of calves in each treatment group over time

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

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).
Table 2.2 Mean cortisol concentration (nmol/L ± s.e.m.) of all calves over time

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Cortisol concentration (nmol/L)</th>
<th>s.e.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.5</td>
<td>29.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.17</td>
</tr>
<tr>
<td>0</td>
<td>62.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.47</td>
</tr>
<tr>
<td>0.5</td>
<td>77.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.91</td>
</tr>
<tr>
<td>1</td>
<td>72.01&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>9.27</td>
</tr>
<tr>
<td>1.5</td>
<td>66.79&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>9.87</td>
</tr>
<tr>
<td>2</td>
<td>66.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.42</td>
</tr>
<tr>
<td>4</td>
<td>44.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.49</td>
</tr>
<tr>
<td>6</td>
<td>23.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.99</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d</sup> Values within a column with different superscripts differ significantly at \( P < 0.05 \).

Descriptive statistics are based on predicted means (± s.e.m.). A significant effect was found (\( P < 0.001 \)).

2.6 Discussion

The results of this study did not support our 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. This was the first time that the cortisol response of castrated calves treated with TA had been studied.

In this study we elected to use cortisol as an indirect measure of pain associated with castration as 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, despite this, 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 other variables, such as diurnal changes and individual variation that influence cortisol concentration. This can implicate interpretation of experimental results (Molony and Kent, 1997). The results of the current study highlight the responsiveness of cortisol to factors other than pain. While it is ideal to combine multiple physiological, neuroendocrine and behavioural measures to reduce the impact of non-painful factors on results, this usually requires using separate groups of animals for each measure. As we only utilised a single group of calves for this study, our options for obtaining other data in addition to cortisol concentrations were limited due to the constant movement and repeated handling of the calves for blood sampling. In addition, other measurements could have caused distress which would have affected our cortisol findings. However, a previous study conducted by the same 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). The 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 this study, an increase in plasma cortisol from −0.5 to 0 h was apparent for all treatment groups (Tables 2.1 and 2.2). This may reflect the stress of separation from mothers (Loberg et al., 2008),
handling through a race, and restraint in a head bale (Cooke et al., 2009) before 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 2.1). 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 tissue damage, stimulation of nociceptors (Earley and Crowe, 2002) and haemorrhage (Gann and Egdahl, 1965).

This study is 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 is not suitable to explain the lack of difference between the integrated cortisol response of CTA and C calves in the current study. The TA in the current study 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 component of TA has been suggested to slow the rate of systemic absorption of lidocaine and bupivacaine, thereby prolonging their duration of action (Lomax et al., 2013). Another 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. It was suggested that the relatively high dose rate and the injection into the testes rather than the spermatic cords may have caused 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 the current study, the castration procedure and the mode of anaesthetic application differs 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 was applied postoperatively, directly onto exposed, injured tissue. Therefore, a more likely explanation for the lack of difference between C and CTA calves in the current study is that the castration procedure itself caused tissue damage, inflammation, stimulation of nociceptors, and haemmorhage, all of which can induce a rise in cortisol (Gann and Egdahl, 1965, Earley and Crowe, 2002). This explanation is also applicable when comparing the results of the current study to contrasting results from previous literature. In some studies, local administration of 2% lidocaine HCl has been shown to significantly reduce the acute cortisol response to castration of Friesian calves, though not completely eliminating it (Ting et al., 2003, Stewart et al., 2010). In these studies, lidocaine HCl was injected 10 (Stewart et al., 2010) or 20 min (Ting et al., 2003) before castration. Pre-operative administration of lidocaine HCl would have ensured amelioration of both peri-operative and acute post-operative pain. TA, being applied postoperatively, had no effect on peri-operative pain, which may induce a rise in cortisol (Mellor et al., 2000). In addition, the study by Ting et al. (2003) employed the burdizzo method for castration, which restricts blood flow to the testes, causing
necrosis. This prevents haemorrhage (Stafford and Mellor, 2005a), of which cortisol secretion is a concomitant (Gann and Egdahl, 1965).

It is important to note that in the previously mentioned studies (Stafford et al., 2002, Webster et al., 2013), cortisol concentrations of uncastrated calves were significantly lower than those of untreated castrated calves. In the current 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 the current study, Coetzee et al. (2008) used Angus crossbred calves and habituation for the experiment consisted 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 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 the current study include separation from their mothers, novel exposure to human handling, and restraint. Other studies have habituated calves to intensive handling and holding facilities for 3 weeks before experimentation commenced (Ting et al., 2003, Stewart et al., 2010). This extensive habituation reduced the effect of handling on treatment outcomes, resulting in a significant effect of castration on cortisol response (Ting et al., 2003, Stewart et al., 2010). The calves used in the current study underwent a less intensive, shorter habituation process. Hence, 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 also inserted indwelling jugular catheters 1 day before experimentation to facilitate intensive blood sampling and minimise animal handling (Ting et al., 2003). Calves in one of these studies were held in individual pens for the duration of the trial. For that reason, manual restraint for each blood sample was possible (Ting et al., 2003). In the other study, blood samples were only taken −20, −10, 15 and 20 min relative to castration, which meant that each calf could be restrained one at a time in a squeeze chute for the duration of sample collection (Stewart et al., 2010). Therefore, in both of these studies, access to the catheter did not require movement or head restraint of calves. Due to the design of the current study, calves needed to be 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 pulled out by this form of restraint meant that jugular venipuncture was a more practical option. Furthermore, there are contradictory results in the literature on the effects of venipuncture on cortisol, with some suggestions that it has no effect (Alam and Dobson, 1986) and some suggestions that it causes an increase in cortisol (Veissier and Leneindre, 1988). Hopster et al. (1999) suggest that jugular venipuncture may induce an increase in cortisol concentration, but it seemingly relates to the handling experience of cattle. In the current study, manual restraint for sampling, and jugular venipuncture, may have increased cortisol concentrations. Further, in the current study, calves had never experienced separation from their mothers before the experimental days, where 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.,
The calves used in the current study were unweaned beef calves that before experimentation had minimal exposure to humans. Studies reporting an effect of castration on cortisol (Ting et al., 2003) used dairy calves, which under commercial conditions 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 ~6 months of age, hence the period of separation from mothers in the current study likely caused distress and hence a major cortisol response.

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 and the long acting TA was the same product as that used in the current study (Sutherland et al., 2010). Sutherland et al. (2010) suggests that as TA is applied postoperatively, the pain of the castration procedure itself may have overshadowed any effect of TA on cortisol. It was also suggested that the anaesthetic or application method was inadequate (Sutherland et al., 2010). These limitations can be applied to the current study. A study investigating the pain relieving effects of the same TA for mulesing and tail docking in lambs found that TA significantly, yet only moderately, reduced the peak cortisol response to the procedure and it had no effect on the AUC (Paull et al., 2007). Paull et al. (2007) found that combining this TA 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. Therefore, the effect of TA in combination with an NSAID on the cortisol response of calves to castration is worth future investigation.
2.7 Conclusion

In this 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. 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. Future studies should incorporate more extensive habituation of calves to reduce the impact of stress on results.

2.8 Conflicts of interest

The authors declare there are no conflicts of interest.

2.9 Acknowledgements

The authors gratefully acknowledge the financial support of Meat and Livestock Australia and Bayer Animal Health Australia. The authors are grateful for the technical assistance of Steve Burgun and his staff at Arthursleigh, Marulan, NSW, and honours students Charissa Harris and Samantha Faber from the University of Sydney. The authors thank Kim Heasman from the University of Sydney for assistance with laboratory work. Statistical advice provided by Peter Thomson from the University of Sydney is greatly appreciated.
CHAPTER 3: EFFECTS OF TOPICAL ANAESTHESIA AND BUCCAL MELOXICAM TREATMENTS ON PRODUCTION, BEHAVIOUR AND INFLAMMATION OF UNWEANED BEEF CALVES FOLLOWING SURGICAL CASTRATION

This chapter is currently under review with the international peer reviewed scientific journal Animal. Only the format has been changed for the purposes of consistency of style in this thesis.

McCarthy D, Lomax S, Windsor PA, Taylor C and White PJ. Effects of topical anaesthesia and buccal meloxicam treatments on production, behaviour and inflammation of unweaned beef calves following surgical castration.
3.1 Abstract

To assess the effects of treatments with topical anaesthetic (TA) and buccal meloxicam (BM) on production, behaviour and inflammation following surgical castration of beef calves, 50 unweaned Angus bull calves were randomly allocated to: (1) sham castration (SHAM, n = 10); (2) surgical castration (C, n = 10); (3) surgical castration with pre-operative buccal meloxicam (CBM, n = 10); (4) surgical castration with post-operative topical anaesthetic (CTA, n = 10); and (5) surgical castration with pre-operative buccal meloxicam and post-operative topical anaesthetic (CBMTA, n = 10). Video footage of the calves was captured for 5 h following treatment. Each calf was later observed for 5 min every hour and the frequency and duration of specific behaviours displayed during these focal periods was recorded. To calculate average daily gain (ADG) following treatment, body weight data was obtained from each calf immediately prior to treatment and 1, 2 and 6 days following treatment. Scrotal diameter measurements and infrared and digital photographs of scrotal wounds were collected from all castrated calves 1, 2 and 6 days following treatment to evaluate inflammation and wound healing. Infrared photographs were used to identify the maximum surface temperature within the scrotal area. Digital photographs were used to score wounds based on visual signs of inflammation and healing on a numerical rating scale of 1 to 5, with signs of inflammation increasing and signs of healing decreasing with progressive scores. SHAM calves displayed significantly less, and C calves displayed significantly more foot stamps than all other calves (P = 0.005). Observations on the duration of time that calves displayed a hypometric 'stiff gait' locomotion, indicated that SHAM calves tended to spend no time, C calves tended to spend the greatest time, and all other calves tended to spend an intermediate time displaying this behaviour (P = 0.06). CBM and CBMTA calves had significantly lower maximum scrotal temperatures than C and CTA calves 2 days following treatment (P = 0.004). There was no
significant effect of treatment on ADG ($P = 0.71$), scrotal diameter ($P = 0.091$) or wound morphology score ($P = 0.521$). These results suggest that TA and BM, alone and in combination, reduced pain and BM reduced inflammation following surgical castration of calves.

### 3.2 Implications

Research shows that local anaesthetics and non-steroidal anti-inflammatory drugs can improve the welfare of calves undergoing castration. However, much of this research does not consider the practical issues associated with administration of injections. Topical anaesthetic and buccal meloxicam offer practical options for producers to improve the welfare of calves undergoing castration. As assessed through behaviour, topical anaesthetic and buccal meloxicam appear to reduce post-operative pain associated with surgical castration of calves. Buccal meloxicam also appears to have an anti-inflammatory effect on surgical castration wounds in calves, observed as reduced maximum wound temperature.

### 3.3 Introduction

Castration of cattle is a common procedure performed in the beef industry to manage unwanted aggression and sexual behaviour (Fisher et al., 1996) resulting in a reduced incidence of stressed and injured cattle and a lower risk of dark cutting carcasses at slaughter (Fisher et al., 1996, Earley and Crowe, 2002, Coetzee, 2013). The meat from castrated cattle (steers) is of a higher quality than that from bulls due to superior tenderness and marbling, hence steers provide a premium price for producers (Coetzee, 2013). Castration facilitates handling, simplifying management and increasing the safety of stockpersons (Petherick, 2005, Amatayakul-Chantler et al., 2013) and is
especially useful in systems where separation of males and females is not always feasible in order to prevent unwanted breeding (Petherick, 2005).

Numerous studies have shown that castration causes pain and distress, as indicated by physiological, neuroendocrine and behavioural changes (Olson et al., 2016). The Australian Animal Welfare Standards and Guidelines for cattle state that pain relief should be used for all surgical procedures (AHA, 2014a), although this is not legislated. Despite this recommendation, castration of Australian beef cattle is generally performed without any form of pain relief, mainly due to the additional time required for administration of conventional drugs (Petherick, 2005, Coetzee, 2011). This is accentuated in northern Australia where the properties and herd sizes are extremely large and highly seasonal rainfall results in infrequent musters of cattle. In this setting, routine husbandry procedures, including castration, are conducted on large numbers of cattle that are unaccustomed to confinement and handling. The use of standard injectable pain relief drugs for these procedures would increase restraint and handling times and could pose additional welfare issues for such cattle (Petherick, 2005).

An anaesthetic gel designed for topical application to wounds, Tri-Solfen® (Bayer Animal Health, Pymble NSW Australia), and a meloxicam gel, Ilium® Buccalgesic OTM (Troy Laboratories, Glendenning NSW Australia), designed for oral administration into the buccal cavity, have recently been registered for use on calves undergoing surgical castration. Topical anaesthetic (TA) applied during surgical castration has been shown to reduce post-surgical wound sensitivity and pain-related behaviours in beef calves (Lomax and Windsor, 2014). Buccal meloxicam (BM) has been shown to reduce pain-related behaviours following knife castration and hot-iron tail docking in lambs (Small et al., 2014).
The aim of this study was to evaluate the effects of pre-operative BM and peri-operative TA, alone or in combination, on production, behaviour and wound inflammation, following surgical castration of beef calves. Observations included: body weights to determine average daily gain (ADG) as a measure of production; changes in calf behavior to assess pain; and scrotal diameter, maximum scrotal temperature and scrotal morphology were recorded to assess inflammation.

3.4 Materials and methods

The experimental protocol was approved by the Animal Ethics Committee of the University of Sydney (Approval No. 5832).

3.4.1 Animals

A total of 50 unweaned Angus bull calves (2 – 4 months old) requiring routine castration were used in the trial. These calves were sourced from a commercial herd on a property in the southern highlands of NSW, Australia, owned by The University of Sydney. 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 for data collection. Calves were released back into the paddock with their mothers upon conclusion of data collection each trial day.

3.4.2 Experimental design and treatments

The trial was conducted across 7 days, with observations recorded on days 0, 1, 2 and 6 relative to treatment. Prior to data collection and treatment on each day of the trial, calves were held in a holding pen (4 m x 10 m) that led into two smaller holding pens (3 m x 3 m each) adjacent to the
cattle race (Figure 3.1). Approximately 5 to 10 calves were moved through these smaller holding pens and into the cattle race for data collection and treatment. On day 0, calves were drafted through the race twice. The initial draft included collection of BW data and administration of BM to CBM and CBMTA calves. Calves were also spray painted with an identification number (1 to 50) on both sides and the back of the body. On the second draft, calves were sham castrated or castrated when CTA and CBMTA calves were treated with TA. This enabled administration of BM 25 min prior to castration as per label instructions. On other days, calves were drafted through the race once for data collection.
Prior to data collection and treatment on each day of the trial, calves were held in a holding pen (A) that led into two smaller holding pens (B) adjacent to the cattle race (C). Calves were weighed using cattle scales (D) on each day of the trial. Calves were restrained in lateral recumbency in a swing-away calf cradle (E) for treatment and data collection on day 0, and for data collection on days 1, 2 and 6. Calves were placed in an observation yard (G) adjacent to the cattle handling facilities immediately following treatment on day 0 for collection of behavioural data.

(A = holding yard (4 m x 10 m); B = smaller holding yards (3 m x 3 m each); C = cattle race; D = weigh scales; E = cattle crush; F = calf cradle; G = observation yard (10 m x 25 m)). Blue diamonds represent video cameras set up on the fence of the yard.

The calves were randomly allocated to one of five treatment groups by use of computer generated random numbers (Microsoft Excel 2007, Microsoft Corporation, International): (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).

Sham castration was performed by physically manipulating the scrotum without surgery. Castration technique involved an initial transverse excision of the distal third of the scrotum with a sharpened, sterilised knife, then applying downward pressure to expose the testes and spermatic cord from the tunica vaginalis. The spermatic cord was incised approximately 12 cm proximal to the head of the epididymis, using a scraping motion.

The BM used was a gel formulation containing 10 mg / mL meloxicam. It was administered (0.5 mg / kg BW) via a hook nozzle placed into the oral cavity adjacent to the upper molar teeth for absorption through the buccal mucosa. CBM and CBMTA calves were treated with BM 25 min prior to castration.

The TA used was a gel formulation containing lignocaine (40.6 g / L), bupivacaine (4.2 g / L), cetrimide (5 g / L) and adrenaline (24.8 mg / L). This product was applied directly to the wound via a spray nozzle. In CTA and CBMTA calves, TA was applied following initial exposure of the testes by inserting the nozzle into the tunica vaginalis and delivering approximately 2 mL of the product into the inguinal canal. Approximately 2 mL of TA was also applied to the cut skin edge of the scrotum. This method of application aimed to cover all incised tissue with TA, including the spermatic cord prior to retraction.

To facilitate animal handling, the calves were restrained in lateral recumbency in a swing-away calf cradle (Arrow Farmquip, Australia) for procedure and treatment on day 0, and for data collection on days 1, 2 and 6.
3.4.3 Measurements and observations

3.4.3.1 Average daily gain (ADG)
Calves were weighed in the cattle crush using weigh scales and a data recorder, W810 (Gallagher Group Ltd, Hamilton New Zealand), prior to restraint in the calf cradle. ADG 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.

3.4.3.2 Behaviour
Calves were placed in 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), were attached at various points around the yard to capture video recordings of the calves from numerous angles. The videos were later analysed using continuous recording of the frequency or duration of specified behaviours displayed by each calf within a 5-minute focal period. This was repeated every hour for 5 h following treatment. 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, International), 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 3.1); state behaviours were recorded as the total duration of time (s) and point behaviours were recorded as the total frequency.
<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>States</strong></td>
<td></td>
</tr>
<tr>
<td>Walk</td>
<td>Walking forwards or backwards in any style at any pace (the sum of ‘walk</td>
</tr>
<tr>
<td>relaxed’</td>
<td>relaxed’, ‘walk with a stiff gait’, and ‘walk with a limp’)</td>
</tr>
<tr>
<td>Walk relaxed</td>
<td>Walking with muscles relaxed</td>
</tr>
<tr>
<td>Walk with a stiff</td>
<td>Walking slowly with muscles stiff</td>
</tr>
<tr>
<td>gait</td>
<td></td>
</tr>
<tr>
<td>Walk with a limp</td>
<td>Walking slowly with a limp</td>
</tr>
<tr>
<td>Stand</td>
<td>Standing in any style (the sum of ‘stand relaxed’ and ‘stand statue’)</td>
</tr>
<tr>
<td>Stand relaxed</td>
<td>Standing passively or actively with head held relaxed and muscles relaxed</td>
</tr>
<tr>
<td>Stand statue</td>
<td>Standing stationary with muscles stiff and head held below brisket</td>
</tr>
<tr>
<td>Lie</td>
<td>Lying down completely on the ground in any style (the sum of ‘lie normal’</td>
</tr>
<tr>
<td></td>
<td>and ‘lie abnormal’)</td>
</tr>
<tr>
<td>Lie normal</td>
<td>Lying in a normal posture (ventral position and no extension of limbs)</td>
</tr>
<tr>
<td>Lie abnormal</td>
<td>Lying in an abnormal posture (lateral recumbency, one or both hind limbs</td>
</tr>
<tr>
<td></td>
<td>extended &gt; 90°, both forelimbs extended</td>
</tr>
<tr>
<td>Arch back</td>
<td>Curving of the spine</td>
</tr>
<tr>
<td>Scratch</td>
<td>Raising a hind leg and scratching part of the body or scratching body against</td>
</tr>
<tr>
<td></td>
<td>the yard fence</td>
</tr>
<tr>
<td>Lick</td>
<td>Turning head back and licking body with lips or tongue, or both</td>
</tr>
<tr>
<td>Eat</td>
<td>Ingesting lucerne hay</td>
</tr>
<tr>
<td>Drink</td>
<td>Ingesting water</td>
</tr>
<tr>
<td><strong>Points</strong></td>
<td></td>
</tr>
<tr>
<td>Lick wound</td>
<td>Licking of scrotal area whilst lifting a hind limb</td>
</tr>
<tr>
<td>Stamp</td>
<td>Lifting front or hind foot and forcefully placing it on the ground</td>
</tr>
<tr>
<td>Kick</td>
<td>Kicking backward or towards the belly with a hind limb</td>
</tr>
</tbody>
</table>
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.

### 3.4.2.3 Scrotal diameter

Scrotal diameters (mm) of all castrated calves were measured at the base of the scrotum on days 1, 2 and 6 of the trial using Budget 150 mm digital vernier calipers (Jaycar Electronics, Australia) to evaluate oedema as an indicator of inflammation.

### 3.4.2.4 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, FLIRE50 (FLIR Systems, Inc., International), 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. 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., International). 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 (Figure 3.2).

![Infrared image of a castration wound analysed for maximum surface temperature](image)

**Figure 3.2** Infrared image of a castration wound analysed for maximum surface temperature

A thermal imaging software program, FLIR Tools Software (FLIR Systems, Inc., International) was used to calculate maximum scrotal surface temperature within a square drawn inside a cardboard frame which was held over each wound for each photograph.

### 3.4.2.5 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 (Table 3.2).

**Table 3.2** Customised numerical rating scale used to score wound morphology

<table>
<thead>
<tr>
<th>Score</th>
<th>Example</th>
<th>Wound description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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.
### 3.4.4 Statistical analysis

Data on ADG, each state behaviour (Table 3.1), scrotal diameter and maximum scrotal temperature were subjected to restricted maximum likelihood (REML) for repeated measures using the linear mixed models procedure in Genstat® 17th Edition statistical software (VSN International Ltd, Hemel Hempstead UK). Data on each point behaviour (Table 3.1) was subjected to REML using the generalised linear mixed models (GLMM) procedure with a poisson distribution in Genstat®. For ADG, the fixed effect of the model was treatment x day. For each behaviour (Table 3.1), the fixed effects of the model were treatment x time-point and BW (day 0). Data on ambient temperature and ambient humidity was subjected to a Spearman’s rank correlation using the nonparametric correlations procedure of Genstat®. 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, BW (day 0) and ambient temperature. The random effect of all models was calf ID. Due to the significance of BW in the models for scrotal diameter and maximum scrotal temperature, data on BW and scrotal diameter or maximum scrotal temperature were subjected to a Spearman’s rank correlation using the nonparametric correlations procedure of Genstat®. Wound appearance scores were subjected to ordinal logistic regression (OLR) in ASReml® 3.0 statistical software (VSN International, Hemel Hempstead UK). The fixed effects of the model were treatment x day and BW (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. 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 \( \leq 0.05 \) were considered statistically significant and \( P \) values \( > 0.05 \) and \( \leq 0.075 \) were considered trends.

### 3.4 Results

#### 3.4.2 Animals and environment

Calves had a mean initial BW of 107.68 ± 26.3 kg. Mean ambient temperature and humidity during data collection on days 1, 2 and 6 were 33.99°C, 15.37%; 24.32°C, 48.73%; and 38.76°C, 18.66%, respectively.

#### 3.4.3 Average daily gain

There was a significant effect of day \((P < 0.001)\), with ADG lower on day 1 than on days 2 and 6 (Table 3.3). There was no significant effect of treatment \((P = 0.71)\).

**Table 3.3** Average daily gain (ADG) of all calves on days 1, 2 and 6 following treatment

<table>
<thead>
<tr>
<th>Day (relative to treatment)</th>
<th>Mean ADG (kg) ± s.e.m</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.29(^a) ± 0.56</td>
</tr>
<tr>
<td>2</td>
<td>0.75(^b) ± 0.56</td>
</tr>
<tr>
<td>6</td>
<td>0.94(^b) ± 0.56</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Values within a column with different superscripts differ significantly at \( P \leq 0.05 \).

Descriptive statistics are based on predicted means (± s.e.m.). A significant effect was found \((P < 0.001)\).
3.5.3 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, and C calves displaying more foot stamps than all other calves (Table 3.4). There was a trend for treatment to have a significant effect on the duration of time spent walking with hypometria, observed as a 'stiff gait' \((P = 0.06)\), with SHAM calves spending no time, C calves spending the greatest duration of time and all other treatment groups spending an intermediate duration of time walking with a stiff gait (Table 3.5). 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.

Table 3.4 Mean frequency of foot stamps displayed by calves in each treatment group within a 5-minute focal sample

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Frequency ± s.e.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>0.1914± 0.03</td>
</tr>
<tr>
<td>C</td>
<td>1.1093± 0.20</td>
</tr>
<tr>
<td>CBM</td>
<td>0.8459± 0.15</td>
</tr>
<tr>
<td>CTA</td>
<td>0.678± 0.12</td>
</tr>
<tr>
<td>CBMTA</td>
<td>0.5622± 0.10</td>
</tr>
</tbody>
</table>

SHAM = Sham castration / control; 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.

\(^{a, b, c}\) 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 \((P = 0.005)\).
Table 3.5 Mean duration of time spent walking with a stiff gait by calves in each treatment group within a 5-minute focal sample

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean duration (s) ± s.e.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>0 ± 0.934</td>
</tr>
<tr>
<td>C</td>
<td>4.078 ± 0.999</td>
</tr>
<tr>
<td>CBM</td>
<td>0.988 ± 0.922</td>
</tr>
<tr>
<td>CTA</td>
<td>1.182 ± 0.974</td>
</tr>
<tr>
<td>CBMTA</td>
<td>1.846 ± 0.985</td>
</tr>
</tbody>
</table>

SHAM = Sham castration / control; 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.

Descriptive statistics are based on predicted means (± s.e.m.). A statistical trend was found ($P = 0.06$).

3.5.4 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 ($P = 0.091$), day ($P = 1$) or ambient temperature ($P = 1$).

3.5.5 Maximum scrotal temperature

There was a significant treatment x day interaction ($P = 0.004$). 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 3.6). 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 ($P = 0.823$).

### Table 3.6 Mean maximum scrotal temperature of castrated calves in each treatment group on days 1, 2 and 6 following treatment

<table>
<thead>
<tr>
<th>Day</th>
<th>C</th>
<th>CBM</th>
<th>CTA</th>
<th>CBMTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$39.6^{Aa} \pm 0.19$</td>
<td>$39.55^{Aa} \pm 0.20$</td>
<td>$39.46^{Aa} \pm 0.19$</td>
<td>$39.85^{Aa} \pm 0.19$</td>
</tr>
<tr>
<td>2</td>
<td>$39.63^{Aa} \pm 0.19$</td>
<td>$38.82^{Bb} \pm 0.19$</td>
<td>$39.47^{Aa} \pm 0.19$</td>
<td>$38.72^{Bb} \pm 0.19$</td>
</tr>
<tr>
<td>6</td>
<td>$40.21^{Ba} \pm 0.19$</td>
<td>$39.83^{Aa} \pm 0.19$</td>
<td>$40.3^{Ba} \pm 0.19$</td>
<td>$40.02^{Aa} \pm 0.19$</td>
</tr>
</tbody>
</table>

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 ($\pm$ s.e.m.). A significant effect was found ($P = 0.004$).

### 3.5.6 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 (Figure 3.3). There was no significant effect of treatment ($P = 0.521$) or BW ($P = 0.487$).
Figure 3.3 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 \( (P < 0.001) \).

### 3.5 Discussion

This study evaluated 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. The results of this study 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-hour 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 1 was most likely due to the long (5 h) separation of calves from their mothers on day 0 and therefore reduced feed intake and increased stress experienced (Perez-Torres et al., 2016). Significantly reduced weight gain following surgical castration has been shown to occur in calves (Fisher et al., 1996, Ting et al., 2003, Bretschneider, 2005, Petherick et al., 2015). However, this is not always the case, as demonstrated in this study and other previous studies (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 nutrients, and therefore did not need to actively source feed. This may be why there was no effect of castration on ADG. In addition, calves may have increased suckling behaviour as a response to pain, as 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 could explain why there was no apparent effect of castration on ADG. Further, larger treatment group sizes may have been necessary to demonstrate potential differences due to individual variability of weight gain as an outcome (Webster et al., 2013).

Ethological measurement of behaviour has been used extensively to evaluate pain following castration of calves (Ting et al., 2003, 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 is indicative of irritation, possibly due to pain sensation, 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 likely to reduce 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 a 5-hour 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). TA has been shown to reduce pain-related behaviour following surgical castration of beef calves (Lomax and Windsor, 2014) and combined surgical castration and tail docking of lambs (Lomax et al., 2010), as scored using a numerical rating scale. Buccal meloxicam has previously reduced the amount of time lambs spent standing in a normal posture, standing in a hunched posture, standing in a stretched posture and walking with a stiff gait following combined surgical castration and tail docking. It also reduced the amount of time spent in combined abnormal postures and behaviours (Small et al., 2014). In the current study, post-treatment differences between groups were not detected for many behaviours, considered likely due to the overall limited expression of these during the observation period. Temporary separation of calves from their mothers during the experimental period may have attenuated pain-related behavioural responses as calves were likely to have been motivated in reuniting with their mothers, possibly shifting their 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., 2014a, 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, flunixin (1.1 mg / kg BW), on scrotal size (Mintline et al., 2014). However, in another previous study, oral meloxicam (1 mg / kg BW) 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 dosage rate and route may have contributed to the contrasting results. Meloxicam has been shown to have a longer lasting effect when administered orally compared to injectable administration (Olson et al., 2016). It is possible that oral administration of meloxicam at the higher dose rate of 1 mg / kg BW (Olson et al., 2016) may have resulted in a longer lasting effect compared to trans-mucosal absorption through the buccal cavity at a dose rate of 0.5 mg/ kg BW. It is also possible that differences in methodology could have affected results, with the previous study investigating oral meloxicam measuring the mid-scrotal diameter whilst calves were standing (Olson et al., 2016), as opposed to measuring of the base of the scrotum whilst calves were in lateral recumbency, as done in the current study. With calves standing, it could be assumed that gravity would have caused swollen tissue and fluid to collect towards the distal scrotum and perhaps provide a measurement of standing rather than recumbent scrotal oedema. This may also explain why differences in scrotal size were not detected across experimental days in the current study. The effect of body weight on scrotal diameter showed heavier calves to have a larger scrotal diameter, likely due to 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 2 days following surgical castration. This may be attributable to the NSAID used; previous studies 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 at 2 days following castration. The increase in maximum scrotal temperature on day 6 from days 1 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 (Figure 3.3). 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., 2014a, Petherick et al., 2015). Wound healing was investigated in the 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., 2014a) and flunixin (Mintline et al., 2014) on wound appearance 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, 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, both alone and in combination, suggesting both alleviated pain to some degree during the acute post-operative period. Buccal meloxicam reduced maximum scrotal temperature 2 days following surgical castration, consistent with an anti-inflammatory effect.

3.6 Conflicts of interest

The authors declare no conflicts of interest.
3.7 Acknowledgements

The authors gratefully acknowledge the financial support of Meat and Livestock Australia and the provision of topical anaesthetic from Bayer Animal Health Australia and buccal meloxicam from Troy Laboratories Pty Ltd. The authors thank Steve Burgun and his staff at ‘Arthursleigh Farm’ and students from the University of Sydney, including Charissa Harris, Anna Cooper, Sarah Legge and Esteban Martinez, for their technical assistance. Statistical advice provided by Peter Thomson and Evelyn Hall from the University of Sydney is greatly appreciated.
CHAPTER 4: EFFECT OF LIGNOCAINE OR A TOPICAL VAPOCOOLANT SPRAY ON THE PAIN RESPONSE TO SURGICAL CASTRATION IN BEEF CALVES

This chapter is currently under review with the international peer reviewed scientific journal *PLoS ONE*. Only the format has been changed for the purposes of consistency of style in this thesis.

**McCarthy D, Lomax S, Windsor PA and White PJ.** Effect of lignocaine or a topical vapocoolant spray on the pain response to surgical castration in beef calves.
4.1 Abstract

To assess the efficacy of lignocaine or a topical vapocoolant spray to provide local anaesthesia for calves during surgical castration, 40 Angus bull calves were randomly allocated to: (1) sham castration (SHAM, n = 10); (2) surgical castration (CAST, n = 10); (3) surgical castration with pre-operative local anaesthetic lignocaine (LIG, n = 10); and (4) surgical castration with peri-operative vapocoolant spray (VAPO, n = 10). Peri-operative behavioural response to the sham castration or castration procedure was scored on a numerical rating scale from 0 (no response) to 3 (severe response). Each stage of the castration procedure (excision of distal scrotum, extrusion of right spermatic cord with excision and removal of the right spermatic cord and testicle, extrusion of left spermatic cord with excision and removal of the left spermatic cord and testicle) was scored individually. Maximum ocular temperature was measured three times: immediately following restraint in a calf cradle; 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 were significant effects of treatment ($P < 0.001$) and stage of procedure ($P < 0.001$) on calf behavioural response to the procedure. SHAM calves were more likely to display less severe responses compared to all other calves and LIG calves were more likely to display less severe responses than VAPO calves. However there were no significant differences between CAST calves and either LIG or VAPO calves. There was a significant effect of time ($P < 0.001$) on ocular temperature, with an increase in temperature following sham castration or castration for all calves. Lignocaine administered 5 min prior to castration or vapocoolant spray applied for 3 s to the scrotum and spermatic cords immediately prior to excision, had no effect on the peri-operative pain response to surgical castration.
4.2 Introduction

Castration of beef cattle is performed to prevent unwanted breeding (Petherick, 2005), improve meat quality and reduce aggressive behaviour (Seideman et al., 1982, Cohen et al., 1990, Zobell et al., 1993). Most male calves in Australia are castrated using a surgical method due to practicality and low cost (Fordyce et al., 1989). This method involves calf restraint, incision of the scrotum, withdrawal of the testis, incision of the mediastinum testis and vas deferens, then removal of the testis by extraction or incision of the spermatic cord (Fordyce et al., 1989, McCarthy et al., 2016b).

Surgical castration causes pain and distress in calves, measured by a range of parameters, including behaviour (Currah et al., 2009), plasma cortisol (Bretschneider, 2005), weight gain (Petherick et al., 2015), inflammatory mediators (Brown et al., 2015), substance P (Coetzee et al., 2008), ocular temperature (Stewart et al., 2010) and heart rate (Olson et al., 2016). Local anaesthetics including lignocaine can be used to block nerve conduction at the incision site of the scrotum and in the spermatic cord to provide pre-operative, peri-operative and acute post-operative pain relief (Clarke and Trim, 2014). Reports on its efficacy for surgical castration in calves vary, with some studies suggesting that local anaesthetic alone does not effectively relieve the pain and distress of the procedure (Stafford et al., 2002, Webster et al., 2013). For large commercial beef operations as occur in northern Australia, additional handling time required for administration of local anaesthetic is a major constraint (Petherick, 2005).

Topical vapocoolant sprays have been used in recent years to provide local anaesthesia prior to injections or minor surgical procedures in humans (Fjordbakk and Haga, 2011). They have been shown to decrease pain associated with 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).
Vapocoolant sprays have also been shown to reduce pain responses in horses during arthrocentesis (Fjordbakk and Haga, 2011) and in calves during ear tagging and notching (Lomax et al., 2017). Topical vapocoolants result in local anaesthesia by causing a drop in temperature due to rapid evaporation of volatile liquid from the skin surface (Collado-Mesa et al., 2015). This temporary epidermal hypothermia interrupts initiation and conduction of neural impulses in local sensory nerves, resulting in reduced pain sensation (Griffith et al., 2016). Vapocoolant spray could be a practical option for farmers to provide pre-operative anaesthesia as it is low cost, easily administered, rapid to effect (Cohen et al., 2009) and painless (Griffith et al., 2016). The aim of this study was to assess the efficacy of pre-operative injected lignocaine or a topical vapocoolant spray to provide local anaesthesia during surgical castration of calves.

4.3 Materials and methods

4.3.1 Animals

The experimental protocol was approved by the Animal Ethics Committee of The University of Sydney (Approval No. 5832). Forty unweaned Angus bull calves, weighing 103.5 ± 30.2 kg, were randomly selected for the trial from a commercial herd on a University of Sydney farm in the southern highlands of NSW, Australia. Surgical castration is undertaken as a routine farm management procedure at this property and the calves were routinely ear tagged and notched one week prior to the trial.

4.3.2 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, W810 (Gallagher Group Ltd, Hamilton, New Zealand) within the race and then restrained in right lateral recumbency in a calf cradle (Arrow Farmquip, 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, NSW, Australia) (LIG, n = 10); and (4) surgical castration following a pre-operative application of topical vapocoolant spray (Animal Ethics Pty Ltd, VIC, Australia) comprising a hydrocarbon propellant in an aerosol canister (VAPO, n = 10).

For SHAM calves, testes were physically manipulated for 30 s with no surgical intervention. Surgical castration was performed by transversely excising the distal third of the scrotum with a sharpened sterilised knife, then application of downward pressure to the scrotum above the testicle enabled extrusion of each testis and spermatic cord from the tunica vaginalis. Following extrusion, each spermatic cord was excised with the knife using a scraping motion. For LIG calves, 3 mL of lignocaine HCl (2%) were injected into each side of the scrotum 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 VAPO calves, vapocoolant spray was applied to the distal scrotum immediately prior to incision and then to each exposed spermatic cord following extrusion, prior to excision. Vapocoolant spray was applied for 3 s from a distance of 10 cm to the scrotum and spermatic cords, as this application method has been shown to be effective for ear tagging and notching (Lomax et al., 2017). The castration procedure took approximately 30 to 40 s.

4.3.3 Behavioural scoring
A video camera mounted on a tripod was used to film the responses of each calf to the procedures. Ear tag numbers were visible in the videos, allowing for individual animal identification. Videos were later scored by two trained observers at the same time. Both observers agreed on allocated scores for each calf. Behavioural responses were scored on a numerical rating scale of 0 to 3, taken from a previous study on the behavioural response to ear tagging and notching (Lomax et al., 2017) 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) excision of right spermatic cord; (4) extrusion of left spermatic cord; and (5) excision 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.

4.3.4 Ocular temperature

Infrared photographs of the left eye were captured from calves using a handheld infrared camera, FLIRE50 (FLIR Systems, Inc., International), 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 1 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., International). 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.

4.3.5 Statistical analysis

Behavioural score data were subjected to ordinal logistic regression (OLR) in ASReml® 3.0 statistical software (VSN International, Hemel Hempstead, UK). The fixed effects of this model were treatment (SHAM, C, CLIG, CVS) x stage of procedure (1 to 5) and BW (variate). Maximum ocular temperature data was subjected to restricted maximum likelihood (REML) for repeated measures using the mixed models procedure of Genstat® 17th Edition statistical software (VSN International Ltd, Hemel Hempstead, UK). 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 (SHAM, C, CLIG, CVS) x time-point (1, 2, 3), BW (variate) and ambient temperature (variate). 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 \( \leq 0.05 \) were considered statistically significant and \( P \) values \( \leq 0.1 \) were considered statistical tendencies.

### 4.4 Results

#### 4.4.1 Behavioural scoring

There was no significant effect of BW (\( P = 1 \)). 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 display lower pain response scores to stages 2, 3, 4 and 5. There was also a trend for LIG calves to display lower pain response scores to stages 2 and 4 (extrusion of the testes) compared to CAST and VAPO calves (Figure 4.1). There was a significant effect of treatment (\( P < 0.001 \)). SHAM calves displayed significantly lower pain response scores than all castrated calves. LIG calves displayed significantly lower pain response scores compared to VAPO calves (Figure 4.2). There was a significant effect of stage of procedure (\( P < 0.001 \)). Pain response scores to stage 2 (extrusion of the first testis and spermatic cord) were significantly greater than pain response scores to all other stages. Pain response scores to stage 4 (extrusion of the second testis and spermatic cord) were significantly greater than pain response scores to stage 5 (excision of second spermatic cord) (Figure 4.3).
Figure 4.1 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 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 \(P \leq 0.05\).

A significant effect was found \((P < 0.001)\).
Figure 4.3 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 \( (P < 0.001) \).

4.4.2 Ocular temperature

There were no significant effects of treatment or body weight \( (P = 0.739 \text{ and } P = 0.479, \text{ respectively}) \). There were significant effects of time-point and ambient temperature \( (P = 0.002 \text{ and } P < 0.001, \text{ respectively}) \). Maximum ocular temperature was greater at time-point 3 (following sham castration or castration) \( (38.69 \pm 0.09 \, ^\circ C) \) than at time-points 1 and 2 \( (38.44 \pm 0.09 \, ^\circ C \text{ and } 38.49 \pm 0.09 \, ^\circ C, \text{ respectively}) \). A low positive relationship between ambient temperature and maximum ocular temperature was identified \( (R = 0.30) \).
4.5 **Discussion**

This may be the first study to investigate the use of a topical vapocoolant spray to provide local anaesthesia during surgical castration of calves, with results suggesting it is not an effective form of pain relief for such a procedure. The results of this study also add to the contrasting information on the efficacy of lignocaine for surgical castration of calves, showing minimal effects following administration by the technique used into the neck of the scrotum 5 min before the procedure was performed. This study may also be the first to compare pain associated with different stages of the surgical castration operation in calves, with extrusion of the testes and pulling of spermatic cords eliciting the greatest pain response.

Descriptive or numerical behavioural scoring systems have previously been used to assess the peri-operative pain of ear tagging and 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 the greatest proportion of score 0 responses. This contrasts with all castrated calves, mostly displaying scores of 1 to 3 (Figure 4.2). There was no difference between pain response scores of CAST calves and either LIG or VAPO calves. However, there was a difference between pain response scores of LIG and VAPO calves (Figure 4.2). In this study, there appeared to be a minimal degree of local anaesthesia induced by the injected lignocaine, with responses not significantly different from CAST calves. Surprisingly, the application of vapocoolant spray appeared to slightly increase behavioural response of the calves during castration. 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).

There are contrasting results regarding the efficacy of lignocaine for pre and peri-operative pain relief of surgical castration in calves. 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 during surgical castration of calves. However, other studies that used cortisol as 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, Webster et al., 2013). These differences may relate to 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, as cortisol responses are induced by other factors independent of pain including tissue damage, inflammation and haemorrhage (McCarthy et al., 2016a). In the current study, lignocaine was administered 5 min prior to castration as this is a more realistic representation of use for routine husbandry procedures performed on cattle in a commercial setting. Previous studies administered lignocaine 10 (Stewart et al., 2010), 15 (Fisher et al., 1996) or 20 (Earley and Crowe, 2002, Stafford et al., 2002, Webster et al., 2013) min prior to castration. This 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 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. However, 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 a product with a short application duration (≤ 3 s) is 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 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 the pulling stages of castration would still not be alleviated, as this involves 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, vapocoolant sprays appear ineffective for use during surgical castration of calves.

There was a trend ($P = 0.051$) for an interaction between treatment and stage of procedure, with SHAM calves displaying lower pain response scores to stages 2, 3, 4 and 5 compared to all castrated calves (Figure 4.1). At stage 1 of sham castration or castration, all calves reacted similarly, suggesting that the first handling of the testes initiated a behavioural distress response. This may have overshadowed a potential 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 assumed pain sensation associated with excision of the scrotum is of a mild intensity. There appeared to be a tendency for LIG calves to display lower pain response scores to extrusion of the spermatic cords (stages 2 and 4) (Figure 4.1), suggesting lignocaine may have reduced the pain associated with this component of the castration procedure.

This study may be the first to present on 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) where results are similar to those of the current study. In piglets, initial restraint, washing of the ano-genital area, incision of the scrotum, and pulling / incision of the spermatic cords were all compared, with pulling / incision of the spermatic cords evoking the greatest degree of vocalisations (Taylor and Weary, 2000). Scrotal incision and pulling / incision of the spermatic cords affect different tissues, 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). Pulling of the spermatic cords likely results in sensation along the length of the spermatic cords and into the inguinal canal and beyond, likely resulting in greater pain responses compared to the rapid excision stage of the castration procedure (Taylor and Weary, 2000). In the current study, there appeared to be an effect of the order of stages, with extrusion of the second spermatic cord (stage 4) resulting in lower pain response scores than extrusion of the first spermatic cord (stage 2). Similarly, the last stage of the procedure resulted in the lowest behavioural response overall (Figure 4.3). 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). This could be the case in the current study, with the pain associated with pulling the first spermatic cord likely startling calves and therefore resulting in a greater pain response.

Measurement of ocular temperature has been used to evaluate stress and pain associated with disbudding (Stewart et al., 2008a, 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 this 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. These results contrast from those of some previous studies where differences in ocular temperature have been detected between control calves and calves undergoing disbudding (Stewart et al., 2008a, Stewart et al., 2009) or castration (Stewart et al., 2010). However, as in the current 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 the current study. Whereas a difference has 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 exact location of where maximum temperature was detected, which was 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, the current 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 the current study and it appears that stress, rather than pain, had the dominant effect on ocular temperature, which has also been previously suggested (Stock et al., 2016) and should be considered for future studies.
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 provide adequate anaesthesia. Lignocaine administered 5 min prior to castration also did not provide adequate peri-operative anaesthesia for surgical castration of beef calves. Future research should consider analgesic interventions that address the pain associated with pulling of the spermatic cords, as this stage of the surgical castration procedure appears to be the primary source of distress in beef calves.

4.6 Conflicts of interest

The authors declare there are no conflicts of interest.

4.7 Acknowledgements

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CHAPTER 5: EFFECT OF TOPICALLY APPLIED ANAESTHETIC FORMULATION ON THE SENSITIVITY OF SCOOP DEHORNING WOUNDS IN CALVES

This chapter appears as the following published paper in the international, peer-reviewed, scientific *PloS One* (citation below). The format has been changed for the purposes of consistency of style in this thesis.


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5.1 Abstract

The post-operative effects of three formulations of topical anaesthetic and a cornual nerve block on the sensitivity of scoop dehorning wounds in calves were compared in two trials. In Trial 1, 21 female Holstein dairy calves aged 8 to 24 weeks were randomly allocated to two groups: (1) scoop dehorning with a post-operative application of a novel topical anaesthetic powder (DTAP, n = 10); and (2) scoop dehorning with a post-operative application of a novel topical anaesthetic ethanol liquid (DTAE, n = 11). In Trial 2, 18 castrated male and 18 female Hereford beef calves aged 16 to 20 weeks were randomly allocated to four groups: (1) scoop dehorning with a pre-operative cornual nerve block of lignocaine (DCB, n = 9); (2) scoop dehorning with a post-operative application of the novel topical anaesthetic ethanol liquid from Trial 1 (DTAE, n = 9); (3) scoop dehorning with a post-operative application of a topical anaesthetic gel (DTAG, n = 9); and (4) sham dehorning (CON, n = 9). Sensitivity was assessed by scoring the behavioural response of calves to stimulation of the wound or skin at time points before and after treatment. In Trial 1, DTAP calves had a greater probability of displaying more severe responses than DTAE calves at 90 and 180 min ($P < 0.001$). In Trial 2, at 1 h, DTAG calves had a greater probability of displaying more severe responses than CON calves. At 2 h onwards, all dehorned calves had a greater probability of displaying more severe responses than CON calves ($P < 0.001$). There were no differences between the responses of DCB, DTAG and DTAE calves at any time point. Topical anaesthetic formulations result in almost immediate but temporary anaesthesia of the wound following scoop dehorning in calves and may provide a practical option for pain relief on-farm.


5.2 Introduction

Dehorning of cattle is painful, yet remains a commonly performed procedure in horned breeds (Petherick, 2005) as it reduces injuries from social contact, improves safety for stock-persons (Kupczynski et al., 2014), dehorned cattle require less space during feeding and transport (Stafford and Mellor, 2005b) and dehorning reduces bruise trim at slaughter by approximately 50% (Prayaga, 2007). As bruising of cattle costs the Australian beef industry approximately $30 million annually (CSIRO, 2015), retention of horns has major economic implications.

The northern beef industry in Australia is very extensive, with average property size reported at 23,436 ha for 2014–2015 (Martin, 2015). The majority of cattle breeds in this region are Bos indicus or B. indicus crosses and most calves are born with horns. Animal management on these properties differs greatly from the smaller southern Australian properties and from that of most other beef producing nations in that cattle are handled infrequently during the year. The age at which beef calves are ‘marked’ (that is, animals are mustered and subjected to husbandry management procedures including ear tagging, branding, vaccination and dehorning) varies, but is usually in excess of 3.5 months of age (Petherick, 2005). The horn tissue at this stage is more developed than in dairy calves that are usually dehorned within 1 to 3 months of birth. In northern Australia, most beef calves are dehorned by horn amputation, using scoop, cup or knife dehorning tools (Irwin and Walker, 1998). This method of dehorning involves removal of the horn as well as a small area of surrounding skin (Stafford and Mellor, 2015) and has been found to cause significant pain and distress (Sylvester et al., 1998a, Sutherland et al., 2002, Sylvester et al., 2004). Numerous studies have shown that a cornual nerve block using local anaesthetics (LA) such as lignocaine is effective at alleviating the intra-operative and acute post-operative pain caused by dehorning (Petrie et al., 1996, McMeekan et al., 1998a, Sylvester et al., 1998a, Sutherland et al.,
2002). However, the impracticality of injectable drug administration in this environment has prevented widespread adoption of this technique by Australian producers (Petherick, 2005).

Topically applied LA may provide an alternative option to address the post-operative pain of dehorning wounds as administration does not involve extra handling time or a high level of skill. Previous studies have demonstrated the efficacy of a topical anaesthetic formulation (Tri-Solfen®, Bayer Animal Health, Pymble NSW Australia) for use during mulesing (Lomax et al., 2008), tail docking and surgical castration (Lomax et al., 2010) in lambs and surgical castration in beef calves (Lomax and Windsor, 2014). Preliminary studies have shown that modified formulations of Tri-Solfen® applied to dairy calves during scoop dehorning significantly reduced the sensitivity of wounds up to 1.5 h (Espinoza et al., 2013) and 5 h (Espinoza et al., 2015) following the procedure.

The overall aim of the previous studies (Espinoza et al., 2013, Espinoza et al., 2015) and the current study, are to investigate options for providing pain relief to calves undergoing scoop dehorning that are both effective and practical to use on-farm.

5.3 Materials and methods

5.3.1 Animals and housing

Experimental protocols were approved by the Animal Ethics Committee of The University of Sydney (Approval No. 5832). The study involved two trials on separate groups of calves to investigate the effect of three different topical anaesthetic products on wound sensitivity post dehorning. In trial 1, calves were sourced from a commercial dairy, “Schofields”, in the Southern Highlands, NSW, Australia. In trial 2, calves were sourced from a commercial beef herd, “Ayrston”, in the Central Tablelands of NSW, Australia. At the conclusion of the trials, calves remained on the properties and continued to be used as commercial livestock.
5.3.1.1 Trial 1
Twenty-one female horned Holstein-Friesian dairy calves aged 8 to 24 weeks undergoing routine dehorning were used in this trial. Prior to experimentation, calves were moved from group pens into a holding pen adjacent to the cattle handling facilities, 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.

5.3.1.2 Trial 2
Eighteen castrated male and 18 female unweaned horned Hereford beef calves aged 16 to 20 weeks undergoing routine dehorning were used in this trial. 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.

5.3.1.3 Topical anaesthetic products
Tri-Solfen® is a registered and commercially available local anaesthetic and antiseptic gel for topical application immediately post mulesing of merino lambs. It contains 40.6 g/L lignocaine, 4.2 g/L bupivacaine, 5 g/L cetrimide and 24.8 mg/L adrenalin. We have been studying the efficacy of this product for pain relief during a number of surgical interventions in farm animals (Windsor and Lomax, 2016), including various modifications of the original formula to enhance adherence and efficacy during scoop dehorning (Espinoza et al., 2013, Espinoza et al. 2015).
Trial 1 compared the practicality and efficacy of two novel topical anaesthetic agents developed for dehorning (Bayer Animal Health, Pymble NSW Australia). Both formulations contained 20% w/v lignocaine and 4% w/v bupivacaine. These 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. The most effective formulation from this trial was used in the second trial comparing the practicality and efficacy with that of Tri-Solfen1 and a cornual nerve block (2% w/v lignocaine).

In both trials, practicality was assessed through observations of product application and efficacy was evaluated through assessment of skin and wound sensitivity.

5.3.2 Experimental design and treatments

5.3.2.1 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 computer generated random numbers (Microsoft Excel 2007, Microsoft Corporation): (1) scoop dehorning with a post-operative application of a novel topical anaesthetic powder (Bayer Animal Health, Pymble NSW Australia) (DTAP, n = 10); and (2) scoop dehorning with a post-operative application of a novel topical anaesthetic ethanol liquid (Bayer Animal Health, Pymble NSW Australia) (DTAE, n = 11). Approximately 5 to 10 g of powder was applied to DTAP calf wounds with a measuring spoon and approximately 4 mL of the ethanol liquid was applied to DTAE calf wounds using a household spray bottle. The amount of ethanol liquid sprayed from the bottle was calibrated using a 3 mL syringe. The liquid was sprayed into a cup and the
syringe used to measure the liquid and it was determined there was 2 mL of product released per spray. The products were applied immediately after dehorning so as to completely cover the wound and cut skin edge. Dehorning was performed by a single, experienced technician using a medium size scoop dehorning device (Bainbridge Barnes Dehorner, The Farm Store, Melbourne, VIC, Australia). Dehorning was performed by placing the dehorner over the horn and pulling apart the handles to excise the horn and surrounding skin. 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.

5.3.2.2 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 computer generated random numbers (Microsoft Excel 2007, Microsoft Corporation): (1) scoop dehorning with a pre-operative cornual nerve block of lignocaine (Ilum Lignocaine 201, Troy Laboratories, Glendenning NSW Australia) (DCB, n = 9); (2) scoop dehorning with a post-operative application of the ethanol liquid from Trial 1 (Bayer Animal Health, Pymble NSW Australia) (DTAE, n = 9); (3) scoop dehorning with a post-operative application of a topical anaesthetic gel (Tri-Solfen1, 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. An 18 G needle was 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 (Clarke and Trim, 2014, Edmondson, 2014). Lignocaine (5 mL)
was injected into the tissue in the vicinity of the cornual nerve of each horn and anaesthesia of the horn area was confirmed by the pinprick test with an 18 G needle immediately prior to dehorning. For calves in the DTAE and DTAG groups, approximately 4 mL of product was applied to each wound immediately after dehorning so as to completely cover the wound and cut skin edge. Dehorning was performed as described for Trial 1. The ethanol spray and the gel were applied using household spray bottles and the amount applied per spray was calibrated as described for Trial 1. The procedure of dehorning and applying the ethanol liquid or the gel took approximately 15 s per animal. Sham dehorning was performed by placing the dehorner over the horn bud and applying light pressure to the surrounding skin, without excising any tissue. 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.

5.3.3 Assessment of skin and 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, 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) (Figure 5.1). 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.

**Figure 5.1** Sites subjected to sensory testing
Grey stars represent sites on the edge of the horn base or wound (Area 1) and black stars represent sites on the skin surrounding the horn or wound (Area 2).

### 5.3.4 Statistical analysis

Sample size calculation was based on the following assumptions: the cornual nerve block and sham treatments will have response scores of 0 or 1, where the topical anaesthetic groups will have median scores 1 or 2, with an assumed standard deviation (SD) of 1.8. Using the equation \((a + b)^2 \times 2(SD)^2/(\text{mean}_1 - \text{mean}_2)^2\) and assuming a Type 1 error (a) of 5% and a Type 2 error (b) of 80%,
8.13 animals per group would be required. Therefore a minimum of 9 animals per group were included to allow for error in estimates of the means and SD. All data was analysed using ordinal logistic regression (OLR) in ASReml13.0 statistical software (VSN International Ltd, Hemel Hempstead 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 was included as a random effect. In Trial 2, Calf, 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 \( \leq 0.05 \) were considered statistically significant.

5.4 Results

There were no adverse clinical effects registered for any of the animals following treatment.

5.4.1 Trial 1

There was a significant Time x Treatment interaction \( (P < 0.001) \) (Figure 5.2).

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 5.2 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 ($P < 0.001$) (Figure 5.3). Calves had a greater probability of displaying more severe responses for Area 1 than for Area 2.
Figure 5.3 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.

5.4.2 Trial 2

There was a significant Time x Treatment interaction ($P < 0.001$) (Figure 5.4). 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.

Figure 5.4 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).

There was a significant effect of Area (P < 0.001) (Figure 5.5). Calves had a greater probability of displaying more severe responses for Area 1 than for Area 2.
Figure 5.5 Probability of calves from Trial 2 displaying responses \((Y; 0 = \text{no response}, 1 = \text{mild}, 2 = \text{moderate}, 3 = \text{severe})\) to stimulation of different Areas

Results combine the effect of both von Frey monofilaments, all treatment groups and all time points.

There was a significant effect of von Frey \((P = 0.007)\) (Figure 5.6). 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.
Figure 5.6 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.

5.5 Discussion

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., 1998a, 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 Tri-Solfen for dehorning in calves (Espinoza et al., 2013, Espinoza et al., 2015), comparing the effects of three formulations of topical anaesthetic 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 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 topical anaesthetic gel for surgical tail-docking wounds in lambs (Lomax and Windsor, 2014). In this study 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 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 topical anaesthetic in this study.

Trial 1 demonstrated that the ethanol spray was more effective than the powder, as shown by greater anti-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 waning effect of the powder after 90 min 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 antinociceptive responses (score 0) to stimulation at all time points, indicating an absence of pain or hypersensitivity in the intact tissue. The increase in response severity seen in all dehorned calves over time demonstrated a hyperalgesic progression associated with the pain escalation response of skin incisions or open wounds (Redua et al., 2002, Lomax et al., 2008). There was no change in sensitivity from before treatment to 1 h post treatment in DCB calves, suggesting effective local anaesthesia. From 2 h onwards, response severity of DCB calves increased at each time point, indicating diminishing efficacy. Similar responses have been reported in previous work investigating the efficacy of a lignocaine cornual nerve block on cortisol (Sylvester et al., 1998a) 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, however 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. 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 topical anaesthetic gel formulation up to 24 h post treatment has been observed in mulesed sheep (Brofeldt et al., 1989) and castrated calves (Lomax and Windsor, 2014). The vasoconstrictive properties of adrenaline in the formulation may contribute to slowing
the rate of systemic absorption of the anaesthetic agents, therefore concentration at the wound site is protracted. Additionally, the effect of a wound barrier created by the gel base is 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). Extension of the observation period beyond 6 h should be considered in future studies on the duration of efficacy of topical anaesthetic 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 1 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 which again could be attributed to a slower rate of absorption, as mentioned previously (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 1 h and 2 h post treatment (Figure 5.4), 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 (Figure 5.6).

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., 1998a, 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 response 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. Secondary hyperalgesia develops in the tissue surrounding the site of injury and is due to central sensitisation (Meyer et al., 2005). Secondary hyperalgesia is a consequence of primary hyperalgesia and therefore 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 use on 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 topical anaesthetic for calves undergoing scoop dehorning as its post-operative ease of administration was superior to a cornual nerve block and apparent efficacy in desensitising dehorning wounds was comparable to that of a lignocaine cornual nerve block.

### 5.6 Conflicts of interest

The authors declare there are no conflicts of interest.
5.7 Acknowledgements

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CHAPTER 6: EVALUATING TREATMENTS WITH TOPICAL ANAESTHESIA AND BUCCAL MELOXICAM ON PAIN AND INFLAMMATION CAUSED BY AMPUTATION DEHORNING OF CALVES

This chapter is currently under review with the international peer reviewed scientific journal *PLoS ONE*. Only the format has been changed for the purposes of consistency of style in this thesis.

**McCarthy D**, Lomax S, Windsor PA, Taylor C and White PJ. Evaluating treatments with topical anaesthesia and buccal meloxicam on pain and inflammation caused by amputation dehorning of calves.
6.1 Abstract

To assess the effects of a topical anaesthetic (TA) and buccal meloxicam (BM) on behaviour, wound temperature and wound morphology following amputation dehorning of beef calves, 48 unweaned Hereford calves were randomly allocated to: (1) sham dehorning / control (CON, n = 12); (2) amputation dehorning (D, n = 12); (3) amputation dehorning with pre-operative buccal meloxicam (DBM, n = 12); and (4) amputation dehorning with post-operative topical anaesthetic (DTA, n = 12). Videos of the calves were captured for 3 h following treatment. Each calf was later observed for 5 min every hour and the frequency and duration of specific behaviours displayed during these focal periods was recorded. To evaluate inflammation and wound healing, infrared and digital photographs of dehorning wounds were collected from all dehorned calves on days 1, 3 and 7 following treatment. Infrared photographs were used to identify the maximum temperature within the wound area. Digital photographs were used to score wounds based on visual signs of inflammation and healing, using a numerical rating scale of 1 to 3, with signs of inflammation increasing and signs of healing decreasing with progressive scores. CON calves displayed fewer head shakes than all dehorned calves at 2 and 3 h following treatment ($P = 0.025$). CON and DTA calves displayed less head turns than DBM calves at 2 h following treatment ($P = 0.036$). CON calves displayed fewer combined point behaviours than all dehorned calves at 2 h following treatment ($P = 0.037$). All dehorning wounds had a greater maximum temperature on days 3 and 7 compared to day 1 ($P = 0.003$). All wound morphology scores decreased from day 1 to day 3 and wound morphology scores of DBM and DTA calves increased from day 3 to day 7 ($P = 0.03$). Although fly-strike and potentially acute sinusitis may have confounded these observations, no clear effects of TA or BM on pain and inflammation following dehorning of calves were observed.
and further research is required to evaluate the efficacy of these products for amputation dehorning of calves.

6.2 Introduction

It is well recognised that amputation dehorning of cattle causes pain and distress (McMeekan et al., 1998a, McMeekan et al., 1998b, Sylvester et al., 1998a, McMeekan et al., 1999, Sylvester et al., 2004, Coetzee et al., 2012). Despite this, it remains a common procedure as it reduces the risk of injury to cattle and people, damage to infrastructure, space requirements for housing and transport, and the incidence of both hide damage and bruised carcasses at slaughter (OIE, 2015a).

The Australian animal welfare guidelines for cattle state that surgical procedures should be performed with pain relief (AHA, 2014a). However, the additional time, skills and expense required for administration of injected forms of anaesthesia is a major barrier to uptake by the majority of producers (Petherick, 2005). These constraints are a particular issue for cattle industries where polled animals are rare, such as the northern Australian beef production systems, where horned Brahman cattle dominate on properties where herd numbers are large and raised under very extensive pastoral conditions (McLean et al., 2014). In these systems, calves are ‘marked’ (mustered and subjected to husbandry management procedures including ear tagging, ear notching, branding and dehorning) only once or twice a year, resulting in dehorning of large numbers of animals of various ages (Petherick, 2005).

In recent years, the practical limitations to using anaesthesia and analgesia during surgical husbandry procedures in livestock have been addressed through the development and registration of ‘farmer applied’ products. A topical anaesthetic (TA) gel, Tri-Solfen® (Bayer Animal Health, NSW Australia), originally developed for spray-on application to open wounds induced during
mulesing of lambs, has recently been registered for tail docking of lambs and surgical castration of lambs and calves (Lomax et al., 2010, Lomax and Windsor, 2014). This TA has also been shown to provide post-operative anaesthesia of dehorning wounds comparable to that provided by a cornual nerve block of lignocaine (McCarthy et al., 2016b). In addition to TA, a gel containing the non-steroidal anti-inflammatory drug (NSAID) meloxicam, Ilium® Buccalgesic OTM (Troy Laboratories Ltd Pty, NSW Australia), was developed for administration into the buccal cavity for oral trans-mucosal delivery. A previous study using buccal meloxicam (BM) demonstrated a reduction in pain-related behaviours following surgical castration and tail docking in lambs (Small et al., 2014).

The aim of this study was to investigate the effects of TA and BM on behaviour, wound temperature and wound appearance following amputation dehorning in beef calves. Calf behavior was measured to assess pain, and changes in wound temperature and morphology were recorded to assess inflammation.

6.3 Materials and methods

6.3.1 Animals and treatments

The experiment was approved by the Animal Ethics Committee of The University of Sydney (Approval No. 5832). Fifty unweaned Hereford beef calves (6 to 8 months old) were sourced from a commercial property on the southern tablelands of NSW, Australia. Of the 50 calves, 21 were steers and 29 were heifers. Steers had been castrated at 3 to 4 months of age. All castration wounds were fully healed by the time this study commenced.
Calves were blocked by sex and randomly allocated to one of five treatment groups by use of computer generated random numbers, Microsoft Excel 2007 (Microsoft Corporation, International): (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, NSW Australia) (DBM, n = 12); and (4) dehorned with post-operative application of topical anaesthetic (Tri-Solfen®, Bayer Animal Health Australia, 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.

Dehorning was performed by a single, experienced technician using a yearling cup dehorner, Dominion Yearling Cup (The Farm Store, VIC Australia) designed for use on cattle up to 18 months of age. Dehorning was performed by placing the open cup over the horn, applying downward pressure and closing the handles in a scissor-like action to excise the horn and immediate surrounding tissue. Sham dehorning was performed by placing the open cup over the horn and applying light downward pressure without closing the handles so that no physical injury occurred.

The TA contained lignocaine (40.6 g / L), bupivacaine (4.2 g / L), cetrimide (5g / L) and adrenaline (24.8 mg / L) in a gel formulation. For DTA calves, 4 mL of product was applied by spraying each wound immediately post dehorning, covering the entire wound and immediate surrounding skin. The product was applied using a household spray bottle and the amount applied per spray (2 mL) was calibrated by spraying the TA into a cup and using a 3 mL syringe to measure the volume.
The BM contained meloxicam (10 mg / mL) in a gel formulation. For DBM calves, 1 mL / 20 kg body weight of BM was administered into the buccal cavity between the dorsal molar teeth and the buccal mucosa of the oral cavity, using a drench-like gun applicator, delivering meloxicam at a dose rate of 0.5 mg / kg body weight.

6.3.2 Experimental design

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. 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 buccal meloxicam 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 for fly control, Extinosad Aerosol for Wounds (Elanco Animal Health, NSW Australia) following data collection.
6.3.3 Observations and measurements

6.3.3.1 Behaviour

On day 0, the calves were observed immediately following treatment for 3 hours of behavioural observations. This was conducted in two round yards (80 m² each) adjacent to the cattle handling facilities with 3 video cameras, HD 1080p Sports Action Cam (Sony Australia Ltd, 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). Each observer recorded the behaviour of 6 or 7 calves from each treatment group, to minimise any potential effect of observer bias. An ethogram was designed using this software whereby behaviours were categorised as states or points (Table 6.1). 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 6.1 Ethogram developed for behavioural observations conducted on calves following treatment

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

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.

### 6.3.3.2 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, FLIRE50 (FLIR Systems, Inc., International), 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., International). 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 (Figure 6.1).

![Figure 6.1 Infrared image of a dehorning wound analysed for maximum surface temperature](image)

A thermal imaging software program (FLIR Tools Software, FLIR Systems, Inc., International) was used to calculate maximum surface temperature within a circle drawn inside a cardboard frame which was held over each wound for each photograph.
6.3.3.3 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 (Table 6.2).
### Table 6.2 Customised numerical rating scale used to score wound appearance

<table>
<thead>
<tr>
<th>Score</th>
<th>Example</th>
<th>Wound description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Image" /></td>
<td>Focal non-suppurative lesion characterised by a dried thickened fibrinous exudate and adequate closure of the wound surface</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Image" /></td>
<td>Focal mildly suppurative lesion characterised by a mildly moist thickened serous exudate, and mildly inadequate closure of the wound surface</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Image" /></td>
<td>Focal moderate to severe suppurative lesion characterised by a moist thickened suppurative exudate and inadequate closure of the wound surface</td>
</tr>
</tbody>
</table>
6.3.4 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® 17th Edition statistical software (VSN International Ltd, Hemel Hempstead 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® 3.0 statistical software (VSN International, Hemel Hempstead UK). For each behaviour and combined point behaviours (Table 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.
6.4 Results

6.4.1 Animals and environment
Calves weighed 235.67 ± 44.83 kg. 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.

6.4.2 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 6.3). 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 ($P > 0.05$).
Table 6.3 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

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>P – value</th>
<th>Time-point (h)</th>
<th>Mean frequency (± s.e.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CON</td>
</tr>
<tr>
<td>Head shakes</td>
<td>0.025</td>
<td>1</td>
<td>0.94&lt;sup&gt;Aa&lt;/sup&gt; ± 0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.13&lt;sup&gt;Ba&lt;/sup&gt; ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0.17&lt;sup&gt;Ba&lt;/sup&gt; ± 0.16</td>
</tr>
<tr>
<td>Head turns</td>
<td>0.036</td>
<td>1</td>
<td>2.33&lt;sup&gt;Aa&lt;/sup&gt; ± 0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1.25&lt;sup&gt;Aa&lt;/sup&gt; ± 0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>2.10&lt;sup&gt;Aa&lt;/sup&gt; ± 0.52</td>
</tr>
<tr>
<td>Combined point behaviours</td>
<td>0.037</td>
<td>1</td>
<td>1.70&lt;sup&gt;Aa&lt;/sup&gt; ± 1.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.88&lt;sup&gt;Ba&lt;/sup&gt; ± 0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1.32&lt;sup&gt;Aba&lt;/sup&gt; ± 0.98</td>
</tr>
</tbody>
</table>

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

<sup>a, b</sup> Values within a row with different superscripts differ significantly at <i>P ≤ 0.05</i>.

<sup>A, B</sup> Values within a column with different superscripts differ significantly at <i>P ≤ 0.05</i>.

Descriptive statistics are based on predicted means (± s.e.m.).
6.4.3 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 6.4). 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>
<thead>
<tr>
<th>Day</th>
<th>Mean maximum wound temperature (°C) ± s.e.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.83$^a$ ± 0.42</td>
</tr>
<tr>
<td>2</td>
<td>40.43$^b$ ± 0.35</td>
</tr>
<tr>
<td>6</td>
<td>40.30$^b$ ± 0.40</td>
</tr>
</tbody>
</table>

$^a,b$ 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 ($P = 0.003$).

6.4.3 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 (Figure 6.2).

![Figure 6.2 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](image)

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 \((P < 0.03)\).

### 6.5 Discussion

The aim of this study was to evaluate the effects of TA and BM on pain and inflammation following amputation dehorning of calves. 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, suggesting these behaviours were pain-related. It also suggests an escalation in pain over time in dehorned calves, likely due to a progression in 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 the current 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). Dairy calves, already separated from their mothers, were used in similar previous studies (McMeekan et al., 1999, Sylvester et al., 2004) where 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 1, 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 flystrike. The presence of infection may also explain the increase in wound morphology score from day 3 to day 7 (Figure 6.2), as the role of the inflammatory response is to ensure all bacteria and debris is cleared (Harper et al., 2014). Neither TA nor BM affected maximum wound temperature on 1, 3 or 7 days following treatment, suggesting that these products may not have had any 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 suitable 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., 2014a, Petherick et al., 2015) and branding (Tucker et al., 2014a, Tucker et al., 2014b). Results from this study showed an increase in wound morphology score from day 1 to day 3 for all calves, suggesting a development in 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 infection caused by flystrike or acute sinusitis. It is difficult to say whether this was a causative effect as although it was noted, there was no formal recording of the presence of flystrike or infection. It was noted that there was
a sequential substantial increase in the number of flies and maggots on or within the wound and sinus throughout the study that would likely have confounded normal wound healing results. Topical anaesthesia has been found to improve wound healing 2 and 4 weeks following mulesing in lambs. However, 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). However, flystrike appeared to confound the results in this study, which is probably also the case for the current study.

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, flystrike and infection. This was likely a major confounding factor when examining pain and inflammation with and without TA and BM. Therefore, further research is needed to draw conclusions on the efficacy of these products for amputation dehorning of calves. This study identified flystrike and sinusitis as potential animal welfare issues following dehorning of calves which should be addressed through controlled timing of husbandry procedures if possible.

6.6 Conflicts of interest

The authors declare there are no conflicts of interest.

6.7 Acknowledgements

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provided by Peter Thomson and Evelyn Hall from the University of Sydney. This work was funded by Meat and Livestock Australia (http://www.mla.com.au/Home). The grant number for this funding is P.PSH.0654. Topical anaesthetic was supplied by Bayer Animal Health Australia (https://www.bayer.com.au/en/about/profile-and-organisation/animal-health/) and buccal meloxicam was supplied by Troy Laboratories, Pty Ltd (http://www.troylab.com.au/).
CHAPTER 7: EFFECTS OF TOPICAL ANAESTHESIA AND BUCCAL MELOXICAM TREATMENTS ON PRODUCTION AND BEHAVIOUR OF WEANED BEEF CALVES FOLLOWING CONCURRENT CASTRATION AND DEHORNING

This chapter is to be submitted to the international peer reviewed scientific journal *Applied Animal Behaviour Science*. Only the format has been changed for the purposes of consistency of style in this thesis.

**McCarthy D.,** White PJ, Thomson, P, Eldridge, E and Lomax, S. Effects of topical anaesthesia and buccal meloxicam treatments on production and behaviour of weaned beef calves following concurrent castration and dehorning.
7.1 Abstract

To evaluate the effect of a topical anaesthetic and buccal meloxicam on weight gain and behaviour of beef weaner calves following concurrent surgical castration and amputation dehorning, two experiments were conducted using *Bos indicus* or *Bos indicus* crossbred bull weaner calves randomly allocated to one of five treatment groups; (1) no castration and dehorning / positive control (CONP); (2) castration and dehorning / negative control (CONN); (3) castration and dehorning with pre-operative buccal meloxicam (BM); (4) castration and dehorning with post-operative topical anaesthetic (TA); and (5) castration and dehorning with pre-operative buccal meloxicam and post-operative topical anaesthetic (BMTA). The first experiment investigated weight gain and lying activity of 250 calves (n=50 per treatment). Calves were weighed immediately prior to and 6 days post treatment. Accelerometers were attached to a subset of these calves (n=10 per treatment group) immediately prior to treatment to monitor whether animals were lying or standing on the day of and for 2 days following treatment. The second experiment assessed the post-operative behaviour of 57 calves (n=11 or 12 per treatment). Calves were videotaped for 6 h following treatment. The frequency and duration of specific behaviours displayed by the calves was later recorded in 5-minute focal samples per hour. Results from experiment 1 showed that CONP and BMTA calves had significantly greater weight gain than CONN calves ($P < 0.001$) and that CONN calves spent a lower proportion of time lying compared to BMTA calves on all days ($P < 0.001$). Results from experiment 2 showed dehorned and castrated calves to spend a greater duration of time walking ($P = 0.024$) and a lower duration of time eating ($P < 0.001$) compared to CONP calves. Experiment 2 also showed a trend for CONP calves to spend the greatest duration of time standing and CONN calves to spend the least duration of time standing ($P = 0.059$). There were also trends for the frequency of head turns to be lowest in CONP and BMTA calves ($P =$
0.098) and tail flicks to be highest in CONN and BM calves ($P = 0.061$). The results of this study suggest that production benefits are associated with administration of TA and BM when castrating and dehorning weaner calves. There are also behavioural indications of reduced pain experienced by calves following castration and dehorning when TA and BM has been administered, however, further research is required to clarify this.

### 7.2 Introduction

Dehorning and castration are routine procedures performed on northern Australian beef calves, which are predominantly of *Bos indicus* breed. Dehorning is still a necessary procedure in northern Australia as there are low numbers of polled animals to breed with (Petherick, 2005) and the mode of inheritance of the poll gene in *Bos indicus* breeds is complex (Prayaga, 2007). Castration is especially important on northern Australian beef properties as the extensive nature of farming practices makes separation of males and females unfeasible (Petherick, 2005). On these properties, it is common for calves to be mustered only once or twice a year for weaning and ‘marking’ (that is, ear notching, branding, dehorning and castrating) (Petherick, 2005). The infrequency of mustering results in large numbers of calves being processed for surgical husbandry procedures at various ages, up to 10 months old (Prayaga, 2007). The usual practice at this time is to separate cows and calves and hold the calves in yards for a length of time before marking, then moving weaned calves to a paddock separate from their mothers (Petherick, 2005). Pain associated with castration and dehorning is a significant welfare issue in the beef industry, particularly when experienced by older calves. However, injected anaesthetics and analgesics are not currently used for these procedures due to their practical constraints (Petherick, 2005).
The need for practical pain relief in livestock systems has been recognised in recent years, with a topical anaesthetic gel, Tri-Solfen® (Bayer Animal Health, NSW Australia), now available for various husbandry procedures in lambs and calves. Tri-Solfen® is registered for application to mulesing and tail docking wounds in lambs, and for surgical castration wounds in both lambs and calves. Similarly, for practical reasons, a non-steroidal anti-inflammatory (NSAID) meloxicam gel, Ilium® Buccalgesic OTM (Troy Laboratories, NSW Australia), is registered for surgical castration of lambs and calves and tail docking of lambs. The topical anaesthetic (TA) is applied using a spray applicator and is absorbed across open wounds and mucosal tissue. The buccal meloxicam (BM) is administered using a gun applicator and is absorbed through the oral mucosa. Both methods of anaesthetic and analgesic delivery relieve the need for injections.

This study aimed to assess the effects of TA and BM, alone and in combination, on weight gain and behaviour following concurrent surgical castration and amputation dehorning of Bos indicus weaner calves in an extensively managed system.

7.3 Materials and methods

7.3.1 Animals

The experimental protocol was approved by the Animal Ethics Committee of the University of Sydney (Approval No. 5832). Two experiments were conducted using Bos indicus or Bos indicus crossbred weaner bulls (approximately 6 – 8 months of age). All animals were sourced from a commercial beef herd in Queensland, Australia and were undergoing routine weaning and ‘marking’ (ear tagging, ear notching, branding, dehorning and castration). One week prior to commencement of experiment 1, all calves were mustered, separated from their mothers and held
in a set of yards with *ad libitum* access to water and lucerne hay. This was done as a process of ‘yard weaning’, a commonly employed method of weaning in northern Australian beef herds.

### 7.3.2 Treatments and experimental design

For both experiments calves were randomly allocated to one of five treatments by use of computer generated random numbers (Microsoft Excel 2007, Microsoft Corporation): (1) no castration or dehorning / positive control (CONP); (2) castration and dehorning / negative control (CONN); (3) castration and dehorning with pre-operative buccal meloxicam (BM); (4) castration and dehorning with post-operative topical anaesthetic (TA); and (5) castration and dehorning with pre-operative buccal meloxicam and post-operative topical anaesthetic (BMTA). There were 50 calves per treatment group for experiment 1. A subset of these calves was fitted with accelerometers (10 per treatment group). In experiment 2, there were 12 calves in the CONP and BMTA treatment groups and 11 calves in the CONN, BM and TA 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, Qld Australia). They were restrained in a head bale for ear tagging and ear notching. BM and BMTA calves were treated with BM at this point. Calves were then moved through a separate race to a weaner cradle (Morrissey & Co Calves Handling Equipment, Qld Australia) where they were restrained in left lateral recumbency. Commercially produced tri-axial accelerometer loggers, HOBO Pendant G Acceleration data Logger (Onset Computer Corporation, 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 adhesive bandage and gaffer tape. 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 (Figure 7.1). All calves except CONP calves were castrated and dehorned and TA and BMTA calves were treated with TA, as described below, whilst still restrained in the cradle. Calves were released into another holding yard (300 m²) where they remained until the last animal was processed (refer to Figure 7.2 for detailed description of holding yards and handling facilities). The whole process commenced at 07:30 and concluded at 17:00. When all calves had been processed, they were moved into a laneway (700 m²) where they remained until 06:00 the following day. At this point, they were moved to a large paddock (619 ha) where they remained for 6 full days. During this time, calves had *ad libitum* access to pasture and water. On day 6 at 06:00, calves were mustered back into the holding yards adjacent to the handling facilities and processed through the first race. Whilst in the race, accelerometer units were removed, then the calves were weighed in the cattle crush and released.

![Figure 7.1](image.png) Figure 7.1 A three-dimensional accelerometer (measuring x-, y- and z- axes) was positioned on the lateral aspect of the right hind leg of each calf, proximal to the fetlock. Image (Theurer et al., 2013) shows the orientation of each axis when the calves were (A) standing and (B) lying.
Figure 7.2 Diagram of cattle handling facilities and yards

In experiment 1, calves were moved through two holding yards (A and B) and into a round yard (C) before being processed through a race (D) and cattle crush and weigh scales (E) for weighing, ear tagging and notching and treatment with buccal meloxicam. Calves were then moved through an observation yard (F) and into the round yard (C) before being processed through a second race (G) and a weaner calf cradle (H) for attachment of accelerometers and GPS units, branding, castration, dehorning and treatment with topical anaesthetic. Calves were then moved back into the first two holding yards (A and B).

In experiment 2, calves were moved through two holding yards (A and B) and into a round yard (C) before being processed through a race (D) and cattle crush and weigh scales (E) for weighing, ear tagging and notching and treatment with buccal meloxicam. Calves were then moved through an observation yard (F) and into the round yard (C) before being processed through a second race (G) and a weaner calf cradle (H) for branding, castration, dehorning and treatment with topical anaesthetic. Calves were then moved through one of the first holding yards (B) and then through the round yard (C) into the second holding yard (F) for collection of behavioural data.

(A = holding yard (75 m$^2$); B = holding yard (70 m$^2$); C = round yard; D = cattle race; E = cattle crush and weigh scales; F = observation yard (104 m$^2$); G = cattle race; H = weaner cradle). Blue diamonds represent video cameras set up on the fence of the yard.

Experiment 2 was conducted over 3 days, with 20 calves (4 per treatment group) treated each day. Each day, calves were processed as per experiment 1. Calves were numbered from 1 to 20 on both sides and the back of the body with spray paint while in the race. Following treatment, calves were released into a holding yard (104 m$^2$) for behavioural recording, as described below (refer to Figure
7.2 for detailed description of holding yards and handling facilities). This whole process commenced at 07:30 and concluded at 08:30.

7.3.3 Castration and dehorning

Castration and dehorning were performed by experienced technicians. Castration was performed by pushing the testicles to the distal end of the scrotum and incising the scrotum and tunica dartos from the base and up each side with a scalpel blade. The testicles were extruded through the openings to expose the spermatic cords which were severed approximately 10 cm proximal to the head of the epididymis using the scalpel blade. Dehorning was performed using a yearling cup dehorner, Dominion Yearling Cup (The Farm Store, VIC Australia). Dehorning was conducted by opening the cup, placing it over the horn, applying downward pressure and closing the handles to excise the horn tissue and immediate surrounding skin. The scalpel blade and the cup dehorner were sterilised between use on each animal.

7.3.4 Analgesic products

The BM, Ilium Buccalgescic® (Troy Laboratories, NSW Australia), is a gel formulation containing meloxicam (10 mg / mL). It was administered (0.5 mg / kg BW, rounded up to the nearest 50 kg BW) via a hook nozzle into the buccal pouch for absorption through the oral mucosa. Buccal meloxicam was administered 1 to 2 and 0.5 to 1 h prior to castration and dehorning, for experiments 1 and 2, respectively.

The TA, Tri-Solfen® (Bayer Animal Health, NSW Australia), is a gel formulation containing lignocaine (40.6 g / L), bupivacaine (4.2 g / L), cetrimide (5 g / L) and adrenaline (24.8 mg / L). It was applied via a spray applicator where approximately 4 mL was applied for castration and another 4 mL for dehorning. For castration, it was applied following extrusion of the testes and
prior to severing the spermatic cords, by inserting the nozzle into the tunica vaginalis and delivering the product into the inguinal canal. For dehorning, it was applied directly onto the wounds immediately following the procedure. The method of application aimed to cover all injured tissue, including the spermatic cords which retract into the inguinal canal following excision.

7.3.5 Outcomes measured

7.3.5.1 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.

7.3.5.2 Lying activity (Experiment 1)
The loggers were pre-programmed using Onset HOBOware software (Onset Computer Corporation, MA USA) to record the g-force on the x-, y- and z-axes every 10 s from 10:00 on day 0. The loggers recorded until the memory was filled at 22:13 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 (Microsoft Corporation) 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 on day 0 were removed as the last accelerometer unit was attached at 11:45. 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 logger on the leg of the animal.
7.3.5.3 Behaviour (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, 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 or duration of behaviours were recorded by a single, trained observer using the observational data software package, The Observer® XT 12 (Noldus Information Technology, International). The observer was blinded to treatment, although it was clear which calves were CONP 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 7.1). 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.*, 2003, Sylvester *et al.*, 2004, Petherick *et al.*, 2015).
<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>States</strong><em>1</em></td>
<td></td>
</tr>
<tr>
<td>Walk</td>
<td>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’)</td>
</tr>
<tr>
<td>Walk relaxed</td>
<td>Walking with muscles relaxed</td>
</tr>
<tr>
<td>Walk with a stiff gait</td>
<td>Walking slowly with muscles stiff</td>
</tr>
<tr>
<td>Walk with a limp</td>
<td>Walking slowly with a limp</td>
</tr>
<tr>
<td>Stand</td>
<td>Standing in any style (the sum of ‘stand relaxed’ and ‘stand statue’)</td>
</tr>
<tr>
<td>Stand relaxed</td>
<td>Standing passively or actively with head held relaxed and muscles relaxed</td>
</tr>
<tr>
<td>Stand statue</td>
<td>Standing stationary with muscles stiff and head held below brisket</td>
</tr>
<tr>
<td>Lie</td>
<td>Lying down completely on the ground in any style (the sum of ‘lie normal’ and ‘lie abnormal’)</td>
</tr>
<tr>
<td>Lie normal</td>
<td>Lying in a normal posture (ventral position and no extension of limbs)</td>
</tr>
<tr>
<td>Lie abnormal</td>
<td>Lying in an abnormal posture (lateral recumbency, one or both hind limbs extended &gt; 90°, both forelimbs extended)</td>
</tr>
<tr>
<td>Head down</td>
<td>Holding head below brisket</td>
</tr>
<tr>
<td>Eat</td>
<td>Ingesting lucerne hay</td>
</tr>
<tr>
<td>Drink</td>
<td>Ingesting water</td>
</tr>
<tr>
<td><strong>Points</strong><em>2</em></td>
<td></td>
</tr>
<tr>
<td>Head shake</td>
<td>Rapid shaking of the head around a rostral to caudal axis</td>
</tr>
<tr>
<td>Head turn</td>
<td>Rapid turning of the head to either side of the body</td>
</tr>
<tr>
<td>Head paw</td>
<td>Lifting of hind leg and contacting the head</td>
</tr>
<tr>
<td>Kick</td>
<td>Kicking backward or towards the belly with a hind limb</td>
</tr>
<tr>
<td>Stamp</td>
<td>Lifting front or hind foot and forcefully placing it on the ground</td>
</tr>
<tr>
<td>Ear flick</td>
<td>Rapid movement of one or both ears</td>
</tr>
<tr>
<td>Tail flick</td>
<td>Sideways movement of the tail from vertical to return to vertical</td>
</tr>
</tbody>
</table>

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

7.3.6 Statistical analysis

All data were subjected to restricted maximum likelihood (REML) using Genstat® 17th Edition statistical software (VSN International Ltd, Hemel Hempstead 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 and observed state behaviours. A generalised linear mixed models (GLMM) procedure with a binomial distribution was used to analyse the total lying activity generated from accelerometer readings. A macro was used in 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, the fixed effect of the model was treatment. For total lying activity, frequency of lying bouts and average duration of lying bouts the fixed effects of the model were treatment x day + BW. For each observed behaviour (Table 7.1), the fixed effects of the model were treatment x time-point + day + BW. 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 $\leq 0.05$ were considered statistically significant.
7.4 Results

7.4.1 Animals and environment

For experiment 1, calves weighed 198.77 ± 36.39 kg at the beginning of the trial. Daily maximum temperatures throughout this trial were 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.

Average weight of calves in experiment 2 was 206.88 ± 40.23 kg. Daily maximum temperature throughout this trial were 23.8°C, 19.7°C and 19.9°C for days 1, 2 and 3, respectively.

7.4.2 Weight gain

There were 10 data points excluded as 3 (1 x CONN, 1 x TA and 1 x BMTA) were missing upon the second weighing and 7 (1 X CONP, 2 x CONN, 1 x BM and 3 x BMTA) were identified as outliers within their treatment groups using the boxplot procedure of Genstat®.

There was a significant effect of treatment on weight gain ($P < 0.001$). CONP and BMTA calves had significantly greater weight gain values than CONN calves. CONP calves also had significantly greater weight gain values than BM and TA calves (Table 7.2).

Table 7.2 Mean weight gain of calves in each treatment group over 6 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean weight gain (kg) ± s.e.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONP</td>
<td>-3.69$^a$ ± 0.77</td>
</tr>
<tr>
<td>CONN</td>
<td>-8.30$^c$ ± 0.77</td>
</tr>
<tr>
<td>BM</td>
<td>-6.62$^{bc}$ ± 0.76</td>
</tr>
<tr>
<td>TA</td>
<td>-6.59$^{bc}$ ± 0.76</td>
</tr>
</tbody>
</table>
CONP = no castration and dehorning / positive control; CONN = castration and dehorning / negative control; BM = castration and dehorning with pre-operative buccal meloxicam; TA = castration and dehorning with post-operative topical anaesthetic; and BMTA = castration and dehorning with pre-operative buccal meloxicam and post-operative topical anaesthetic.

Values with different superscripts differ significantly at $P \leq 0.05$.

Descriptive statistics are based on predicted means ($\pm$ s.e.m.). A significant effect was found ($P < 0.001$).

### 7.4.3 Lying activity

For total lying activity, there was a significant interaction between treatment and day ($P < 0.001$). CONN calves spent the least proportion of time lying and BMTA calves spent the greatest proportion of time lying on all days. All other calves spent an intermediate proportion of time lying compared to CONN and BMTA calves on all days. The proportion of time spent lying increased from day 1 to day 2 for all calves and again from day 2 to day 3 for all calves except CONP calves (Table 7.3).

There was no significant effect of body weight on total lying activity ($P = 0.724$).

### Table 7.3 Proportion of time spent lying by calves in each treatment group on days 0, 1 and 2

<table>
<thead>
<tr>
<th>Day</th>
<th>Proportion of time spent lying down (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONP</td>
</tr>
<tr>
<td>0</td>
<td>30.09$^{Aab}$ ± 0.37</td>
</tr>
<tr>
<td>1</td>
<td>50.57$^{Bab}$ ± 0.26</td>
</tr>
<tr>
<td>2</td>
<td>49.19$^{Bab}$ ± 0.27</td>
</tr>
</tbody>
</table>

Descriptive statistics are based on predicted means ($\pm$ s.e.m.). A significant effect was found ($P < 0.001$).
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). The effect of day alone is neither presented nor discussed as the duration of recording intervals differed across days.

### 7.4.4 Behaviour

There were 6 missing focal samples due to calves being unidentified in the video footage. Of these missing samples, there were 1 from time point 1 (1 x BMTA calf), 1 from time point 2 (1 x BMTA calf) and 4 from time point 6 (1 x CONP, 1 x BM and 2 x TA calves). Behaviours influenced by time only are neither presented nor discussed. The behaviours ‘walk with a stiff gait’, ‘walk with a limp’, ‘stand statue’ and ‘lie abnormal’ occurred infrequently. Therefore, it was decided that the analysis would be simplified by only analysing 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 also occurred too infrequently for statistical analysis.

There was a significant effect of treatment x time on the frequency of ear flicks \((P = 0.006)\) displayed by calves. The frequency of ear flicks was significantly greater in TA calves than in CONP, CONN and BMTA calves at 120 min, and significantly greater in BM calves than in TA calves at 240 min (Table 7.4).
Table 7.4 Mean frequency of ear flicks, head turns and tail flicks displayed by calves in each treatment group within a 5-minute focal sample at each time-point

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Effect and P-value</th>
<th>Time (min)</th>
<th>CONP</th>
<th>CONN</th>
<th>BM</th>
<th>TA</th>
<th>BMTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear flicks</td>
<td>Treatment x Time</td>
<td>40</td>
<td>0.53&lt;sup&gt;ABA&lt;/sup&gt; ± 0.31</td>
<td>1.84&lt;sup&gt;AA&lt;/sup&gt; ± 0.71</td>
<td>0.66&lt;sup&gt;AA&lt;/sup&gt; ± 0.35</td>
<td>1.59&lt;sup&gt;BA&lt;/sup&gt; ± 0.61</td>
<td>0.50&lt;sup&gt;ABA&lt;/sup&gt; ± 0.30</td>
</tr>
<tr>
<td></td>
<td>(P = 0.006)</td>
<td>80</td>
<td>0.20&lt;sup&gt;AA&lt;/sup&gt; ± 0.18</td>
<td>0.80&lt;sup&gt;AA&lt;/sup&gt; ± 0.42</td>
<td>0.86&lt;sup&gt;ABA&lt;/sup&gt; ± 0.41</td>
<td>0.25&lt;sup&gt;AA&lt;/sup&gt; ± 0.20</td>
<td>0.14&lt;sup&gt;AA&lt;/sup&gt; ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>0.27&lt;sup&gt;AA&lt;/sup&gt; ± 0.21</td>
<td>0.56&lt;sup&gt;AA&lt;/sup&gt; ± 0.34</td>
<td>0.72&lt;sup&gt;ABab&lt;/sup&gt; ± 0.37</td>
<td>3.24&lt;sup&gt;Bb&lt;/sup&gt; ± 1.05</td>
<td>0.48&lt;sup&gt;ABA&lt;/sup&gt; ± 0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180</td>
<td>0.53&lt;sup&gt;ABA&lt;/sup&gt; ± 0.31</td>
<td>0.80&lt;sup&gt;AA&lt;/sup&gt; ± 0.42</td>
<td>1.78&lt;sup&gt;ABA&lt;/sup&gt; ± 0.66</td>
<td>0.89&lt;sup&gt;ABA&lt;/sup&gt; ± 0.41</td>
<td>0.41&lt;sup&gt;ABA&lt;/sup&gt; ± 0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>240</td>
<td>0.47&lt;sup&gt;ABab&lt;/sup&gt; ± 0.28</td>
<td>1.36&lt;sup&gt;Ab&lt;/sup&gt; ± 0.58</td>
<td>2.57&lt;sup&gt;Ab&lt;/sup&gt; ± 0.87</td>
<td>0.38&lt;sup&gt;AA&lt;/sup&gt; ± 0.25</td>
<td>0.55&lt;sup&gt;ABA&lt;/sup&gt; ± 1.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>360</td>
<td>1.12&lt;sup&gt;BA&lt;/sup&gt; ± 0.50</td>
<td>0.72&lt;sup&gt;AA&lt;/sup&gt; ± 0.39</td>
<td>3.31&lt;sup&gt;BA&lt;/sup&gt; ± 1.09</td>
<td>2.14&lt;sup&gt;BA&lt;/sup&gt; ± 0.96</td>
<td>0.68&lt;sup&gt;BA&lt;/sup&gt; ± 0.36</td>
</tr>
<tr>
<td>Head turns</td>
<td>Treatment (P = 0.049)</td>
<td>52&lt;sup&gt;a&lt;/sup&gt; ± 0.15</td>
<td>0.97&lt;sup&gt;ab&lt;/sup&gt; ± 0.24</td>
<td>1.04&lt;sup&gt;ab&lt;/sup&gt; ± 0.26</td>
<td>1.42&lt;sup&gt;b&lt;/sup&gt; ± 0.33</td>
<td>0.57&lt;sup&gt;a&lt;/sup&gt; ± 0.28</td>
<td></td>
</tr>
<tr>
<td>Tail flicks</td>
<td>Treatment (P = 0.04)</td>
<td>2.95&lt;sup&gt;c&lt;/sup&gt; ± 0.92</td>
<td>7.73&lt;sup&gt;c&lt;/sup&gt; ± 2.16</td>
<td>9.65&lt;sup&gt;c&lt;/sup&gt; ± 2.65</td>
<td>3.95&lt;sup&gt;ab&lt;/sup&gt; ± 1.21</td>
<td>6.13&lt;sup&gt;bc&lt;/sup&gt; ± 1.67</td>
<td></td>
</tr>
</tbody>
</table>

CONP = no castration and dehorning / positive control; CONN = castration and dehorning / negative control; BM = castration and dehorning with pre-operative buccal meloxicam; TA = castration and dehorning with post-operative topical anaesthetic; and BMTA = castration and dehorning with pre-operative buccal meloxicam and post-operative topical anaesthetic.

<sup>a, b, c</sup> Values within a row with different superscripts differ significantly at P ≤ 0.05.

<sup>A, B</sup> Values within a column with different superscripts differ significantly at P ≤ 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. CONP calves displayed significantly fewer head turns than TA calves. CONP and TA calves displayed significantly fewer tail flicks than CONN and BM calves (Table 7.4). 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 lower in CONP calves than in CONN and BMTA calves and significantly greater in BMTA calves than in BM and TA calves. The duration of time spent eating was significantly greater in CONP calves than in all other calves and significantly lower in TA calves than in BMTA calves. The duration of time spent drinking was significantly greater in CONP calves than in BMTA calves (Table 7.5). Treatment did not have a significant effect on the duration or frequency of any other behaviours.

Table 7.5 Mean duration of time (s) spent walking, eating and drinking by calves in each treatment group within a 5-minute focal sample

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Effect and P value</th>
<th>CONP</th>
<th>CONN</th>
<th>BM</th>
<th>TA</th>
<th>BMTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking</td>
<td>Treatment (P = 0.024)</td>
<td>23.82 ± 6.62</td>
<td>47.09 ± 6.90</td>
<td>36.89 ± 6.92</td>
<td>32.78 ± 6.93</td>
<td>53.45 ± 6.64</td>
</tr>
<tr>
<td>Eating</td>
<td>Treatment (P &lt; 0.001)</td>
<td>127.64 ± 14.00</td>
<td>33.01 ± 14.55</td>
<td>48.73 ± 14.63</td>
<td>18.98 ± 14.71</td>
<td>67.88 ± 14.08</td>
</tr>
<tr>
<td>Drinking</td>
<td>Treatment (P = 0.002)</td>
<td>9.43 ± 1.86</td>
<td>5.30 ± 1.92</td>
<td>6.39 ± 1.95</td>
<td>2.65 ± 1.96</td>
<td>1.20 ± 1.87</td>
</tr>
</tbody>
</table>

CONP = no castration and dehorning / positive control; CONN = castration and dehorning / negative control; BM = castration and dehorning with pre-operative buccal meloxicam; TA = castration and dehorning with post-operative topical anaesthetic; and BMTA = castration and dehorning with pre-operative buccal meloxicam and post-operative topical anaesthetic.

a, b, c Values within a row with different superscripts differ significantly at P ≤ 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 7.6). 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 7.6). Day did not have a significant effect on the duration or frequency of any other behaviours.

Table 7.6 Mean duration of time (s) spent drinking and mean frequency of head shakes, head turns, stamps, ear flicks and tail flicks displayed by calves on each day within a 5-minute focal sample

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>P – value</th>
<th>Outcome</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking</td>
<td>P &lt; 0.001</td>
<td>Duration time (s) of</td>
<td>12.43 ± 1.56</td>
<td>2.43 ± 1.45</td>
<td>0.12 ± 1.44</td>
</tr>
<tr>
<td>Head shakes</td>
<td>P &lt; 0.001</td>
<td>Frequency</td>
<td>1.44 ± 10.61</td>
<td>0.46 ± 3.37</td>
<td>0.28 ± 2.10</td>
</tr>
<tr>
<td>Head turns</td>
<td>P &lt; 0.001</td>
<td>Frequency</td>
<td>1.62 ± 0.30</td>
<td>0.60 ± 0.13</td>
<td>0.61 ± 0.13</td>
</tr>
<tr>
<td>Stamps</td>
<td>P = 0.022</td>
<td>Frequency</td>
<td>0.21 ± 0.07</td>
<td>0.17 ± 0.06</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>Ear flicks</td>
<td>P &lt; 0.001</td>
<td>Frequency</td>
<td>1.57 ± 0.34</td>
<td>0.52 ± 0.13</td>
<td>0.51 ± 0.13</td>
</tr>
<tr>
<td>Tail flicks</td>
<td>P &lt; 0.001</td>
<td>Frequency</td>
<td>11.45 ± 2.47</td>
<td>5.39 ± 1.18</td>
<td>2.79 ± 0.68</td>
</tr>
</tbody>
</table>

\(^{a,b}\) 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.

7.5 Discussion

Practical issues with injected anaesthetics and analgesics have prevented their widespread uptake by producers. However, ‘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, alone and in combination, on weight gain, lying activity and behaviour following concurrent castration and dehorning of Bos indicus weaner calves. A combination of TA and BM improved short-term weight gain and increased lying activity following castration and dehorning, suggesting this analgesic treatment was effective. There were behavioural trends to suggest TA and BM reduced pain to some degree.
Assessment of production parameters following invasive husbandry procedures in livestock is important due to its relevance to producers (Coetzee, 2011). Weight gain has been used as a production parameter, alongside measures of stress and pain, to evaluate animal welfare following castration and dehorning in calves (Fisher et al., 1996, Baldridge et al., 2011, Glynn et al., 2013). In farm animals, pain can lead to reduced feeding behaviour, stress and immune reactions that affect nutrient fluxes and utilisation and inhibition of physiological axes, such as the gonadotropic and somatotropic axes, all of which can affect production parameters, such as weight gain (Prunier et al., 2013). For example, increased nociceptor activity increases sympathetic tone and adrenal secretions which potentially inhibits gastric centres, causing a decrease in rumen motility (Coetzee et al., 2012). Therefore, a reduction in weight gain is generally expected to follow castration and dehorning (Mosher et al., 2013), which suggests poor animal welfare and economic losses resulting from such procedures (Glynn et al., 2013). In the current study, all calves, including CONP calves, appeared to lose weight over the 6 days following treatment. This may have been partly due to differences in feed allocation and gut fill between days 0 and 6, as calves were kept in holding yards with access to feed and water 1 week before day 0 and were then moved to a large paddock to feed on available pasture on days 1 to 6. Weight loss was greatest in CONN calves and lowest in CONP calves, which aligns with previous findings showing concurrent castration and dehorning to negatively impact average daily gain (ADG) (Baldridge et al., 2011, Mosher et al., 2013). Weight change of BMTA calves did not differ significantly from that of CONP calves. A combination of TA and BM therefore appears to provide superior pain relief than TA or BM alone. This finding is consistent with the literature which recommends a combination of LA and NSAIDs to target both acute and inflammatory phases of the pain response (Mellor and Stafford, 1999, Hudson et al., 2008). The weight gain results of the current study support previous research
findings, where calves had significantly greater ADG values for the first 13 days following concurrent castration and dehorning when administered sodium salicylate or a combination of sodium salicylate, xylazine, ketamine, and butorphanol, compared to no analgesic treatment (Baldridge et al., 2011). Similarly, surgically castrated calves that received lignocaine had greater ADG values than untreated calves during a 7-day period following the procedure (Fisher et al., 1996). Likewise, dehorned calves given meloxicam, flunixin, gabapentin or a combination of meloxicam and gabapentin gained more weight than untreated dehorned calves during a 7-day post-operative period (Glynn et al., 2013). In Australia, beef cattle producers are generally paid a monetary value per kg live-weight (lwt) or carcase weight (cwt). The results of the current study therefore demonstrate that a combination of TA and BM can be a cost-effective addition to routine practice, whilst improving animal welfare (Baldridge et al., 2011). For example the current average price for yearling steers in central Qld is approximately $3.50 / kg lwt (Meat and Livestock Australia (MLA), 2017). In the current trial, to administer TA and BM cost approximately $5 per calf at retail price. CONN calves lost 2.9 kg BW more than BMTA calves, which equates to $10.15 in value. Therefore, the price of pain relief was less than the product value gained through its use.

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). An increase or decrease in lying activity is not a direct measure of pain and therefore should be interpreted as compared to what is normal, in the absence of pain (Coetzee et al., 2012). As lying activity exhibits a significant degree of individual variability in cattle (Coetzee et al., 2012), it is likely that inter-animal comparisons from before to after treatment are a more sensitive measurement than between-animal comparisons. However, inter-animal comparisons from before to after treatment would have required an additional round

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of mustering in the current study. Hence, between-animal comparisons were used in this study for practical reasons. Although insignificant, the trend for less lying activity seen in CONN calves compared to CONP calves, suggests that this is indicative of greater discomfort or pain. This 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., 2003, White et al., 2008, Heinrich et al., 2010, Coetzee et al., 2012, 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, which is less significant or not apparent when meloxicam has been administered (Coetzee et al., 2012, Theurer et al., 2012).

In the current study, it is unknown why BMTA calves spent more time lying than CONP calves. One theory is that BMTA calves may have been comfortable enough to lie rather than stand, though grazing activity may have been restricted compared to CONP calves. This theory is supported by previous research showing grazing activity to be reduced following surgical castration in calves (Robertson et al., 1994, Fisher et al., 2001). Increased grazing activity in CONP calves would have been accompanied with increased standing activity which potentially differed from the standing activity of CONN calves, in regards to whether it was ‘immobile’ or ‘mobile / walking’. It is probable that CONP calves spent more time in immobile standing positions to graze and that CONN calves spent more time walking, due to the discomfort of the injuries. Again, this theory is supported by previous research showing calves to be more active following castration (White et al., 2008) and dehorning (Heinrich et al., 2010) without analgesic intervention as compared to control calves (White et al., 2008) or calves treated with pain relief (Heinrich et al., 2010). This is suggested due to transient pain (White et al., 2008) and a greater
degree of restlessness (Heinrich et al., 2010), which may be reflected by increased locomotion (Petherick et al., 2014b). This was also observed through behavioural observations in the current study, showing castrated and dehorned calves to spend more time walking than CONP calves. In future research, it would 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, subsequently reducing the memory storage, therefore limiting the time period for data collection. The increase in lying activity seen in all calves from day 0 to day 1 can be explained by the restriction of calves to the holding yards and laneway on day 0. The calves may have been less inclined to lie down in this environment compared to a paddock environment, as ground cover in the laneway mainly consisted of dirt. In addition, there were humans present near the laneway during daytime hours on day 0, which may have deterred the calves from resting. The increase in lying activity from day 1 to day 2 was only seen in castrated and dehorned calves. Therefore, this 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., 2003, Petherick et al., 2015), 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., 2003, 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 lower duration of time eating compared to CONP 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.,
It is unclear why BMTA calves spent more time walking compared to BM and TA calves. As mentioned above, expression of behaviour is largely variable between individual animals (Coetzee et al., 2012, Webster et al., 2013) 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, CONP calves spent more time eating than all other calves and there was a trend for BMTA calves to spend more time eating than CONN calves, suggesting a reduction in pain with a combination of TA and BM. These results follow a similar trend to the weight gain results of experiment 1 in regards to treatment. Therefore, the effect of treatment on eating behaviour could explain the effect of treatment on weight gain. In experiment 2 of the current study, calves that had been castrated and dehorned tended to display a greater frequency of tail flicks than CONP calves, which has previously been observed for these procedures performed both singularly (Fisher et al., 2001, Sylvester et al., 2004) and combined (Sutherland et al., 2013), and therefore suggested to be due to irritation or pain (Fisher et al., 2001, Sylvester et al., 2004, Sutherland et al., 2013). TA calves did not differ from CONP calves in their display of tail flicks and there was a trend for BMTA calves to display fewer tail flicks in comparison to CONN and BM calves. This suggests that TA may have reduced pain to some degree. 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. However, there was no clear trend in the data. Again, potential variation between individual animals in regards to expression of these behaviours may have influenced these results. In regards to ear flicks, it is possible that the procedures of ear tagging and notching may have
confounded these results. In addition, the display of certain behaviours seemed to be influenced by other factors independent of pain. This is evident in the significant effect of day on some behaviours, namely the duration of time 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 around the calves treated on day 1 compared to those treated on days 2 and 3. This is likely due to differences in weather, with day 1 being hotter and less overcast than days 2 and 3. Therefore, the effect of day on behaviour is likely due to differences in weather and environmental factors. As discussed above, there were some behaviours that appeared to be associated with pain, as demonstrated through a difference between CONN and CONP calves. However, 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 relief of pain (Olson et al., 2016). Dairy calves have a more prominent response to painful procedures and pain relief interventions where beef cattle, especially when they are larger, display little demonstrations of pain (Olson et al., 2016). The calves used in this study may therefore have had a strong tendency to hide signs of pain. Majority of previous literature on the behavioural response to castration and dehorning of cattle has used younger dairy calves (Stafford and Mellor, 2005b, Coetzee, 2013), while less research has been conducted using older, weaned Bos Indicus beef calves (Petherick et al., 2014b). 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). Therefore, the results of this study provide novel information on the behaviour of weaned Bos Indicus calves following concurrent castration and dehorning.

This was the first time that the effects of TA and BM have been investigated following concurrent castration and dehorning of weaner calves. In this study, 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, so that there was no difference between CONP and BMTA calves. This study also found a combination of TA and BM increased lying activity in the first few days following treatment, suggesting a reduction in pain. There were trends for TA and the combination of TA and BM to reduce pain-related behaviours during a 6-hour period following castration and dehorning that warrant further investigation. 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 important for large, extensive beef production systems that are seeking practical options for improving animal welfare.

### 7.6 Conflicts of interest

The authors declare there are no conflicts of interest.

### 7.6 Acknowledgements

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CHAPTER 8: GENERAL DISCUSSION
8.1 Introduction

There are numerous surgical husbandry procedures performed on livestock which cause pain and distress. Among these are castration and dehorning of calves. There is a growing consumer demand for improved welfare of production animals. Hence, castration and dehorning of calves are a significant animal welfare concern. The management and production benefits of surgical husbandry procedures outweigh the negative impacts on animal welfare. The cost and practicality constraints of conventional forms of anaesthetic and analgesic have prevented their widespread commercial uptake for routine husbandry procedures. Anaesthesia and analgesia that can be easily and quickly administered to livestock at the time of processing for husbandry procedures would be ideal if shown to be effective.

This thesis presents results from six studies investigating practical anaesthesia and analgesia for surgical castration and amputation dehorning in beef cattle. The objectives of this thesis were to examine the efficacy of a vapocoolant spray to provide intra-operative local anaesthesia during surgical castration of cattle and to examine the efficacy of topical anaesthetic (TA) and buccal meloxicam (BM) formulations for post-operative local anaesthesia and analgesia following surgical castration and amputation dehorning of cattle. The overall aim of the thesis was to evaluate pain relief that can be practically incorporated into routine husbandry procedures conducted in large, extensively managed beef production systems. Firstly, castration and dehorning were examined separately in smaller scale trials in southern Australia to examine the efficacy of TA and BM for singular procedures and to refine methodology. A larger scale trial was then conducted investigating the effects of TA and BM for concurrent castration and dehorning of older weaner calves. This was important as the research is especially relevant for larger scale, northern Australian beef production systems. The results of the studies presented in this thesis suggest that
TA and BM result in some reduction of the pain caused by castration and dehorning in calves. Some results also indicated an improvement in efficacy when a combination of TA and BM was administered to calves undergoing concurrent castration and dehorning. However, the results suggest that a single treatment with these products does not completely abolish pain. Therefore, further work is needed to develop a solution to the animal welfare issues associated with castration and dehorning of calves. The results presented in this thesis also highlight the complexity of understanding pain in animals and emphasises the need for multiple and varied measures of pain.

8.2 Studies on castration

Castration is known to cause significant pain and distress in cattle. However, it is routinely performed on beef calves for various reasons related to management and production. Most previous research has investigated injected local anaesthetics (LA) and non-steroidal anti-inflammatory drugs (NSAIDs) for the relief of pain caused by surgical castration, of which their practical constraints have been outlined throughout this thesis.

Following the development and demonstrated efficacy of TA (Tri-Solfen®, Bayer Animal Health Australia) for post-operative pain relief of mulesing in lambs, the extension of the use of TA for other procedures and species has been investigated. A previous study found that TA reduced wound sensitivity following surgical castration in calves, as measured using an electronic von Frey anaesthesiometer and von Frey monofilaments. This study also found TA to reduce pain-related behaviours, subjectively assessed by scoring individual calves on a numerical rating scale (Lomax and Windsor, 2014). The first study in this thesis aimed to add to this information by objectively evaluating the physiological effects of TA on the pain response, following surgical castration of calves. In the literature, measurement of cortisol is used as the ‘gold standard’ approach to
identifying and quantifying pain in animals. Cortisol has been used extensively in previous research to objectively quantify pain and the effect of anaesthesia and analgesia following surgical castration. Results from this study indicated a trend for TA to reduce cortisol at some time points following castration, however this was not significant. This study highlighted the effects of handling and restraint on the cortisol response of unweaned calves. The need for intensive habituation to remove the impact of these factors on results was emphasised through this study. Cortisol was influenced by non-painful stressors including separation of calves from their mothers, processing through handling facilities and restraint in a head bale, as indicated by the cortisol response of uncastrated control calves. The impact of these stressors on cortisol was apparent regardless of a habituation period of 4 days where calves were handled and restrained in a head bale twice daily. Based on these results, subsequent studies in this thesis did not use cortisol to evaluate pain due to its limitations, especially when using unweaned beef calves managed under commercial conditions.

The overall aim of this thesis was to evaluate ‘farmer applied’ pain relief products for castration and dehorning of calves. Hence, a meloxicam gel formulation (Ilium® Buccalgesic OTM, Troy Laboratories), designed for oral trans-mucosal absorption from the buccal cavity, was investigated following its registration for use in calves undergoing castration. It is well documented that multimodal analgesia using a combination of LA and an NSAID provides superior analgesia following castration in calves. Therefore, the second study in this thesis compared the effects of TA and BM singly and in combination. Average daily gain (ADG), behaviour and inflammation were evaluated in response to surgical castration. Results indicated that both TA and BM reduced pain in the acute post-operative period following castration as demonstrated through a reduction in some pain-related behaviours. As in the first study presented in this thesis, the use of unweaned
calves unaccustomed to humans and handling facilities seemed to limit the ability to evaluate pain, as there was very little expression of most pain-related behaviours. It is suggested that the motivation of calves to reunite with their mothers shifted their focus from the pain caused by castration. In addition, the calves were likely stressed before, during and after the castration procedure due to the relative novelty of being handled by humans and enclosed in a yard. It is possible that these stressors overshadowed some pain of the castration procedure as part of the ‘flight or fight’ response. Alternatively, the calves may have displayed less pain-related behaviours as a means of hiding their vulnerability in the presence of humans. The unweaned status of calves may also have contributed to the lack of a treatment effect on ADG. Unweaned calves have a readily available source of feed which does not require movement around the paddock associated with grazing activity. It is also possible that calves may have suckled milk as a pain-coping mechanism. In addition, production parameters may require larger treatment group numbers to demonstrate statistical differences (Webster et al., 2013). In this study, inflammation at the wound site was also assessed, as it can be used as an indirect measure of pain. Infrared thermography was used to measure maximum scrotal temperature of castrated calves, assuming higher temperatures are associated with acute inflammation. This method of assessment detected a treatment effect, with BM treated calves exhibiting lower maximum scrotal temperatures 2 days post castration. Meloxicam inhibits production of inflammatory mediators and has a half-life of 19.97 to 43.29 hours. Hence this reduction in scrotal temperature at 2 days following castration was likely due to the anti-inflammatory effect of meloxicam. Topical anaesthetic had no apparent effect on inflammation, although this is not surprising as it does not contain anti-inflammatory agents. However, the efficacy of TA for reducing pain following castration of calves has previously been
shown (Lomax and Windsor, 2014) and was further demonstrated in the research presented in this thesis through reduced pain-related behaviour.

Topical anaesthetic and BM have the advantage of being administered quickly and easily, however they do not address intra-operative pain. Previous studies have identified cryo-anaesthesia as a potential practical option for alleviation of procedural pain in animals (Fjordbakk and Haga, 2011, Lomax et al., 2017). A vapocoolant spray comprising of a hydrocarbon propellant in an aerosol canister was shown to effectively reduce the pain of ear tagging and ear notching when applied immediately prior to these procedures (Lomax et al., 2017). The third study presented in this thesis investigated the application of this spray to the scrotum and spermatic cords during castration for intra-operative pain management. The vapocoolant spray did not reduce intra-operative pain of surgical castration. Surgical castration is a more invasive procedure than ear tagging and notching, involving damage to deeper visceral tissues. Information on the pain of each stage of the surgical castration procedure in calves was gained through this trial, with pulling of the spermatic cords being identified as the most painful component. This is important as appropriate pain relief should predominantly target the type of pain caused by pulling the spermatic cords. Until an effective ‘farmer applied’ option for intra-operative pain relief is developed, the current best practice involves injection of lignocaine into the scrotum, testes and spermatic cords 15 to 20 min prior to castration. In addition, the practice should be conducted quickly using sharp tools and at as young an age as possible.

8.3 Studies on dehorning

Amputation dehorning is a significant animal welfare issue for the northern Australian beef industry as the procedure causes pain and distress and is often performed on animals up to 12
months of age without any form of pain relief. The majority of previous research has focused on dairy calves that are usually disbudded or dehorned within 1 to 3 months of age. There is less research on amputation dehorning of older calves or weaned cattle and hence the studies in this thesis were of importance to the Australian beef industry. Local anaesthesia injected near the cornual nerve is currently the best practice for alleviating the intra-operative and acute post-operative pain of the procedure in older calves undergoing amputation dehorning (Sylvester et al., 1998a, Sylvester et al., 2004). However as noted throughout the thesis, the practical constraints of this form of pain relief has prevented its widespread use in commercial beef production systems, particularly in the northern Australian industry.

Previous studies have investigated modified formulations of the topical anaesthetic Tri-Solfen® for application to amputation dehorning wounds in 2 to 3-month-old dairy calves (Espinoza et al., 2013, Espinoza et al., 2015). Adherence of TA to knife tail docking wounds in lambs (Lomax et al., 2010) and scoop dehorning wounds was observed to be reduced due to arterial haemorrhage. Therefore in the Espinoza et al. studies (2013, 2015), the concentration of anaesthetic agents, adrenaline and the viscosity of the formulation were increased in an effort to improve adherence to the wound and absorption of anaesthetic agents in the presence of arterial haemorrhage (Espinoza et al., 2013, Espinoza et al., 2015). Results indicated limited efficacy and adherence of these modified formulations to the dehorning wounds. The first experiment on dehorning within this thesis aimed to address the knowledge gaps from this research. Therefore, novel carrier bases for topical delivery of anaesthetic agents to dehorning wounds were investigated. The original formulation of Tri-Solfen® was also investigated as there was no published data on its application for dehorning. The first study on dehorning outlined in this thesis examined two novel formulations of TA, a powder and an evaporative ethanol spray, that both contained the active ingredients
lignocaine and bupivacaine. As the ethanol spray appeared to be more effective and practical than the powder, it was then compared to the original formulation of Tri-Solfen® and a cornual nerve block of lignocaine. Wound sensitivity testing showed that the ethanol spray and Tri-Solfen® resulted in post-operative wound anaesthesia comparable to a lignocaine cornual nerve block for 6 h following dehorning. Changes within each treatment group over time indicated that the TA formulations may result in extended anaesthesia of the wound beyond 2 h when the effects of the cornual nerve block begin to wane. No further exploration of the ethanol spray was conducted as there were no apparent improvements to efficacy compared to Tri-Solfen®, which is already a commercially available product.

Based on the results of the first study on dehorning, the original formulation of Tri-Solfen® was investigated in the second study on dehorning. This study aimed to evaluate pain and inflammation following amputation dehorning, with and without TA and BM, as per the study on castration. In contrast to the castration study, 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, however it is worthwhile examining in future studies on 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 of the castration and dehorning studies within this thesis could be due to multiple factors. Firstly, the location and type of tissue damage is different. Castration likely causes an initial nociceptor barrage due to excision of the scrotum followed by a visceral pain response originating in the peritoneal cavity. Amputation
dehorning also causes an initial nociceptor barrage, 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 involves damage to the frontal
bone and in many cases, exposure of the frontal sinus (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 more efficient when applied to castration wounds
than to dehorning wounds due to a greater tissue surface and less haemorrhage. Although there
were some pain-related behaviours expressed by dehorned calves, as compared to undehorned
control calves, there was no reduction in the expression of these behaviours when TA or BM was
used. Some factors likely limiting these results include the relatively short behavioural data
collection period (3 h), a result of practical constraints associated with conducting research on a
commercial farm, and little overall expression of pain-related behaviours displayed by unweaned
calves unaccustomed to human handling, as also noted in the castration study. As seen in the
second dehorning study within this thesis, sinusitis is commonly associated with amputation
dehorning where the frontal sinus is open and exposed to the environment (Mosher et al., 2013).
The presence of sinusitis and flystrike in the calves used for this thesis affected the ability to assess
the effects of TA and BM on inflammation. The effects of body weight and ambient temperature
on wound temperature are other potential constraints to interpretation of these results. Additional
studies investigating the effects of TA and BM for dehorning alone may be required to clarify their
effects on pain caused by this procedure.

Cryo-anaesthesia was not explored as an option for intra-operative pain relief of amputation
dehorning due to its lack of efficacy for surgical castration. Like castration, amputation dehorning
is a significantly more invasive procedure than ear tagging and notching, with involvement of
damage to bone and deeper tissues. Hence, the assumption was made that a topical vapocoolant spray would be too superficial for alleviation of procedural pain associated with amputation dehorning.

8.4 Study on concurrent castration and dehorning

The final study in this thesis investigated the effects of TA and BM, alone and in combination, on weight gain and behaviour following concurrent castration and dehorning of weaner cattle. This study was a true representation of an extensively managed beef system in northern Australia, where *Bos indicus* or *B. indicus* crossbred cattle undergo ‘marking’ (that is ear tagging, ear notching, branding, castrating and dehorning) at an older age than calves in other beef production systems. Practical methods for alleviating pain caused by husbandry procedures is imperative in these large-scale systems, hence it was important to investigate the effects of TA and BM in this setting. There is limited literature on castration and dehorning performed concurrently, despite this being common practice. Findings from the previous studies on TA and BM in this thesis were considered during the design of this final study. Weaned cattle were used to examine weight gain. In addition, larger treatment group numbers (*n* = 50) were used for this outcome. Weaned cattle were also used for behavioural observations to eliminate the effect of potential 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. This study showed that a combination of TA and BM significantly improved weight gain in marked calves. This is an important finding, as it reflects a potential economic benefit of TA and BM for producers in addition to an improvement in animal welfare. A combination of TA and BM increased the amount of time spent lying on the day of treatment and for 2 days following
treatment, which suggested these animals were less restless, interpreted as due to reduced pain. A more detailed exploration of animal time budget is required to understand why the calves treated with the combination of TA and BM spent more time lying than the unmarked control calves. This may be due to a difference in time spent standing stationary as opposed to time spent exhibiting active standing behaviours, such as walking. Therefore, it is necessary to monitor active standing activity separately from stationary standing activity to achieve a better understanding of behaviour. However, this requires a greater sampling rate which consequently fills the storage space on the accelerometer devices at a faster rate, reducing the time period for data collection.

It was hypothesised that weaned calves in the current study would have a greater expression of pain-related behaviours compared to the previous studies using unweaned calves, as they would not be distracted by their motivation to reunite with their mothers. However, there was no significant effect of treatment on the expression of most behaviours observed. Overall, there was limited expression of pain-related behaviours in calves, as in the earlier studies using unweaned calves. This could be due to large inter-animal variation and the stoic response of cattle, as a prey species, to pain (Currah et al., 2009). Perhaps older, weaned calves may have a more developed prey response and therefore tend to hide signs of pain more than younger, unweaned calves. This may have been especially exacerbated in the environment of this trial, as prior to the weaning and experimental periods, calves had no or minimal interactions with humans, handling facilities and yards. For some of the behaviours that were significantly affected by treatment, the trends were unclear. This could be reflective of behavioural responses to factors independent of pain, such as the presence of flies and crows. However, there were some behavioural indications that concurrent castration and dehorning caused post-operative pain, which seemed to be relieved to some degree when TA or a combination of TA and BM was used.
8.5 Summary and recommendations

The results of the studies outlined in this thesis demonstrate the difficulty in assessing pain in cattle and therefore the challenges associated with evaluating anaesthesia and analgesia. There is extensive literature that also demonstrates these challenges. Therefore, the need for measurement of multiple outcomes to assess pain in animals has been extensively acknowledged in the literature and is emphasised here. A reliance on data trends in addition to statistically significant results was necessary in this thesis to infer findings on TA and BM for pain relief following castration and dehorning. These results, along with knowledge on the mode of anaesthetic and analgesic action of lignocaine, bupivacaine and meloxicam, leads to the conclusion that TA and BM are having some effect on pain following castration and dehorning of calves.

In this thesis, quantitative sensory testing (QST) using von Frey monofilaments and behavioural scoring appeared to be the most useful measurements for assessment of local anaesthesia. The use of an anaesthesiometer rather than von Frey monofilaments would provide objective results and should be considered for future studies. In this thesis, assessment of analgesia was difficult using the chosen measurable outcomes. It is recommended that future studies employ the use of additional physiological measurements such as heart rate, respiratory rate and inflammatory mediators. It would be beneficial for future studies to correlate wound temperature and inflammatory mediators to determine any potential relationship between the two measures of inflammation. The behavioural outcomes used in this thesis to measure pain could be improved in future studies with some additions and amendments to equipment and methodology. The use of higher grade accelerometers, such as IceTags (IceRobotics Ltd, Edinburgh, Scotland, UK) could provide more accurate, descriptive data on animal activity monitored remotely. For example, IceTags allow for logging data at more frequent intervals over a longer period of time. They
therefore provide more detailed and accurate information on the animals’ time budget, with recording of multiple activities such as walking, grazing and ruminating. In terms of behavioural observations, the use of a continuous sampling method where all occurrences of specified behaviours are monitored for the entire acute period following treatment would be ideal. Global positioning system technology is a behavioural assessment tool which may be worthwhile investigating in future studies. In cattle, GPS tracking has previously mainly been used to monitor grazing behaviour (Turner et al., 2000, Schlecht et al., 2004, Gonzalez et al., 2014). More relative to livestock welfare is the use of GPS tracking to identify ewes that are close to lambing or have lambed (Dobos et al., 2014) and to identify sheep that are resistant to internal parasites (Falzon et al., 2013). In regards to pain assessment, GPS tracking has been shown to successfully differentiate between healthy dogs and dogs with osteoarthritis and osteoarthritic dogs before and after treatment with an oral NSAID, carprofen (Bruno et al., 2015). Therefore GPS technology has the potential to be useful for assessment of pain in cattle.

The analgesic treatments investigated in this thesis have the potential to improve the welfare of many cattle; some effectiveness has been demonstrated and their practical administration means they are likely to be readily incorporated into routine marking procedures. In addition, an improvement to production, and a willingness of consumers to pay more for ‘better welfare’ animal products, may offset economic constraints of employing these products into routine operations. However, the degree of their efficacy is questionable and perhaps worth further investigation. This thesis therefore poses some questions: Is a one-off treatment with TA, an NSAID or both, although an improvement, a solution to the welfare implications caused by castration and dehorning in cattle? Should changes be made to current management regimes to ensure a true ‘best practice’ approach to animal welfare?
Perhaps the results presented in these studies should lead to a re-evaluation of the approach to pain management within livestock systems. Practices that were once viewed as acceptable are now being re-assessed in light of new knowledge and changing attitudes. With increasing consumer preferences for better welfare products, recent years have seen continuous improvements to various livestock industries, despite the challenges this may present. For example, many years of research into alternative housing methods for sows has resulted in the commitment of the Australian pork industry to voluntarily phase out sow stalls by 2017. Similarly, a demand from consumers for a consistent definition and labelling system has led to a new national standard for eggs sold as ‘free range’. Until recently, there was no legally enforceable standard of eggs sold as ‘free range’, creating difficulty for consumers wanting to make informed ethical decisions on their purchase of animal products. These examples demonstrate consumer demand for improved production animal welfare and the ability of industry to respond to this.

It is recommended that research into improved pain management for cattle undergoing castration and dehorning be continued. An approach to pain management incorporating both intra-operative and continuous post-operative anaesthesia and analgesia would be ideal. 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 multiple treatment or *ad libitum* delivery of analgesia may be the next step in addressing pain management whilst continuing to consider practical constraints. In addition to pain relief, consideration of management practices is important. Ideally, procedures should be performed at as young an age as possible and at times of the year when risk of flystrike and infection is lowest. Perhaps it is necessary to incorporate routine monitoring of animals during the healing period following castration and dehorning to allow for appropriate treatment of any subsequent health and welfare
issues. The feasibility of incorporating these recommendations on-farm will depend on the scale of the operation, with larger production systems facing greater practical constraints than smaller production systems. Topical anaesthetic and BM are appropriate treatments for the relief of pain caused by castration and dehorning in calves in the interim until a better solution is available. Ultimately, a solution which ends the need to perform castration and dehorning of calves will be the best outcome for the beef industry.
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