Qualitative Behavioural Assessment of *Bos indicus* cattle

following surgical castration

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for the degree of Master of Philosophy by

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Declaration

I declare this thesis is my own account of my research, and contains as its main content work that has not previously submitted for a degree at any university.

Thinza Vindevoghel
Abstract

Surgical castration of cattle is common however providing analgesia in the Australian cattle industry is rare. The aims of this study were to: 1) describe the behaviour of cattle post-castration using quantitative and qualitative methodology and 2) determine the effectiveness of analgesia for surgical castration. Forty-eight *Bos indicus* (Brahman) calves were divided into six groups (*n* = 8): 1) castrated without analgesia (C), 2) non-castrated (NC), 3) castrated with Meloxicam pre-castration (*CM*<sub>pre</sub>), 4) castrated with Meloxicam post-castration (*CM*<sub>post</sub>), 5) castrated with Lignocaine (CLA) and 6) castrated with Lignocaine and Meloxicam post-castration (*CLM*<sub>post</sub>). Cattle were filmed in the paddock and feeder yard on days -1 to 13, and were assessed in the crush at surgery and as they exited the crush. Video footage was shown to volunteered observers using Qualitative Behavioural Assessment (QBA). In addition, quantitative behaviour scoring by one observer was conducted. There was good inter-observer reliability for the behavioural expression of cattle. Results showed significant Treatment groups x Day interaction effects (*P* < 0.05) for the paddock and feeder yard context. Correlations between qualitative and quantitative measures of behaviour were also identified.

The behavioural responses of cattle varied; in Generalised Procrustes Analysis 1 (GPA 1), cattle in castrated (C) group in the feeder yard were described as relatively more ‘agitated’ and ‘anxious’ on day-1 and more ‘calm’ and ‘relaxed’ on day +1. Some of the behaviour was unexpected regarding the treatment; however, the behaviour can be explained by factors such as the environment or the innate characteristics of livestock. This study demonstrated that *Bos indicus* bulls are likely to benefit from the administration of analgesia at castration,
however we recommend the importance of careful interpretation of behaviour when assessing the effects of analgesia.
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“Thank you to the Brahman cattle for letting us study them!”

“You’re not just sitting in the paddock watching cows, you’re learning from them and forming bonds”
1 General Introduction

Castration of male calves is a routine husbandry procedure commonly performed without anaesthesia or analgesia on cattle herds around the world (Lomax and Windsor, 2013). Castration has been well-documented as painful (Molony et al., 1995; Fisher et al., 1996), but it is considered necessary for economic, safety and management reasons (Lomax and Windsor, 2013). There is controversy about which method of castration causes the least distress and several factors contribute to it, including, age of animals, and methods of rearing (Stafford et al., 2000). Surgical castration has proven to cause more distress than castration using rubber ring or clamp or chemical castration (Fell et al., 1986; Cohen et al., 1990; King et al., 1991). However, other authors found that band castration elicited the maximum cortisol response (Stafford et al., 2002) and “surgical cut” castration was not significantly greater than that of control group (Fisher et al., 1996). Growing public concern about farm animal welfare makes it increasingly important to find a more ethical and welfare appropriate methods of conducting routine husbandry procedures by incorporating practical and affordable methods of pain relief into routine surgical procedures (Lomax and Windsor, 2013).

The aim of this research was to identify whether there were behavioural indicators of pain in cattle during castration. We used composite behaviour scoring for quantitative measurements, as well as applying Qualitative Behavioural Assessment (QBA). Quantitative analysis using different types of behaviour scoring on cattle whilst in crush / out of crush on the day of surgery and in the paddock. A specific list of behaviour were observed whilst cattle were in the paddock called Composite behaviour scoring (CBS). QBA is a technique that allows observers to use descriptive words to define how the animal is behaving rather than what the animal is doing. This form of assessment has proved to be as effective in measuring
behavioural expression of various species of livestock. This is the first study to use QBA to assess painful behaviour in cattle. This literature review will cover concepts of cattle behaviour, the need to mitigate painful husbandry procedures such as surgical castration, various types of welfare assessment including QBA methodology as a tool in assessing pain in cattle.

1.1 Animal welfare and husbandry
The definition of animal welfare is not straightforward. As Webster (2005) stated, it is up to those who study animal welfare to create their own definitions. Dawkins (2004) argued that animal welfare should be directed at answering two key questions: 1) Are the animals healthy? 2) Do they have what they want? The concept of animal welfare has evolved from focusing primarily on an animal’s physical health and their ability to cope with their environment to recognising that animals are sentient beings capable of experiencing positive and negative emotions (Wemelsfelder and Mullan, 2014). It is those who are directly concerned with the management of animals that have the responsibility to promote their welfare through the practice of good husbandry (Webster, 2005).

Australia is among the world’s largest and most successful producers of commercial livestock and a leader in the export of red meat and livestock with an estimated value of A$16 billion (MLA, 2011). As a nation that has a significant economic reliance on the livestock industry, it is paramount to ensure animal welfare guidelines are adhered to. In addition to the ethical issues regarding animal welfare, livestock production can be negatively impacted by poor animal welfare (Dobson and Smith, 2000) as it can result in increased mortality, reduced meat quality, reduced milk production, reduce growth rate and increased incidence of diseases (Broom, 1986). For example, Kauppinen et al. (2012) found that providing piglets with
favourable environments increased the number of weaned piglets, and farmers with positive attitudes to animals gained more piglets per litter. Similarly, lambs placed in an enriched environment showed higher average daily gain, heavier carcass weight and higher fattening scores than those placed in a conventional pen (Aguayo-Ulloa et al., 2014). Correct stocking densities of cattle, sheep and pigs are also crucial to ensure animals have sufficient space to stand, lie down, turn and access to food and water to prevent unnecessary injuries and stress that can compromise their welfare (Weeks, 2008).

In addition to good husbandry practices, the attitudes of stockmen directly involved with the animals can also affect production rates. For example, higher milk yield was reported in farms with positive indicators of human-animal interaction (Breuer et al., 2000). Weeks (2008) stated that dairy cattle exposed to loud noises made by humans not only showed greater reactivity and increased heart rates, but also reduced milk production.

Certain husbandry procedure such as surgical castration and dehorning can create severe negative effects on welfare as pain is inflicted upon the animals (Petherick, 2005).

1.2 Pain

Pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IAFP, 1979). Painful procedures performed on animals are among the most emotive of public concerns about animal welfare (Weary et al., 2006). Assessing pain in animals can be difficult but it is an important part of animal welfare research (Rutherford, 2002). Routine husbandry procedures such as surgical castration of male beef cattle is part of normal management on cattle stations in Australia, yet there is ample evidence that castration causes pain (Stafford and Mellor, 2005a). Castration is performed on bull calves to reduce aggression, prevent physical danger
to both handlers and other animals, prevent unwanted breeding, and to improve meat
tenderness (del Campo et al., 2014). In Australia, there is no legal requirement for managing
pain for cattle under 6 months of age and there are few analgesic drugs approved for pain
relief in cattle and these must be administered under veterinary supervision. Castration
without local or general anaesthetic should be confined to calves under six months old, or at
their first muster prior to weaning (PISC, 2004). Although the use of analgesia is widespread
in companion animal medicine (Lascelles et al., 1999), studies have shown that farm animals
often receive no analgesia for painful procedures (Hewson et al., 2007a). Similar to Australia,
procedures performed on piglets are painful, however pain mitigation is not provided to the
animal reared in swine production in the USA due to economic barriers, impracticality,
historical precedent, uncertainty about need, legality the use of analgesic drugs and its
efficacy (O'Connor et al., 2014). Millman (2013) quoted an Australian study that livestock
producers, transporters, veterinarians, animal welfare scientists, and animal welfare
advocates generally agreed that provision of pain relief for invasive procedures, such as
castration, dehorning, and tail docking, was of greater importance than the techniques used
to perform these procedures. However, providing analgesia to cattle on extensive rangeland
properties presents challenges as cattle are commonly mustered by helicopter once annually,
not habituated to people, and infrequently handled due to large distances and inadequate
yards for monitoring.

To determine if the use of analgesia is required for surgical castration, researchers need to
show that the welfare of these animals is improved by provision of analgesia. This can be done
via various types of animal welfare assessments. Research to date on pain assessments in
animals has tended to use one of three approaches: measures of body functioning (such as
food and water intake or weight gain), measures of physiological responses (such as plasma
cortisol concentrations), and measures of behaviour (such as vocalisation) (Weary et al., 2006). When assessing behavioural changes induced by pain, as pain is a complex multidimensional phenomenon and the responses of animals to it are also complex, qualitative observation by an experienced observer is argued to be the only method truly capable of capturing this complexity (Rutherford, 2002). Studies have shown that surgically castrated calves struggled and kicked during the procedure (Fell et al., 1986) and escape behaviours were seen at castration but not afterwards (Mellor et al., 2000). Researchers have used a combination of behavioural and physiological measures to assess pain as it is generally thought to be more comprehensive than either alone.

1.3 Analgesia

Local anaesthetics (LA) such as Lignocaine are the most commonly prescribed pre-emptive drugs used in food animal practice along with systemic analgesic non-steroidal anti-inflammatory (NSAID) such as Meloxicam or Ketoprofen (Stafford et al., 2002; Coetzee, 2013b). Both LAs and NSAIDs have been proven to reduce cortisol responses in cattle undergoing castration and alleviate the associated stress response (Earley and Crowe, 2002; Stafford et al., 2002). However, various factors serve as a disincentive for producers to provide routine pre-emptive analgesia to cattle. In Australia, there is no legal requirement for managing pain for cattle under 6 months, however the cattle model code of practice stipulates that castration should be conducted by a veterinarian (PISC, 2004).

1.4 Animal Welfare Assessment

Conditions that compromise animal health and/or put them at high risk of dying are bad for welfare. However, good health is a necessary requirement for good welfare (Dawkins, 2003). There is a growing need to monitor the health and welfare of livestock efficiently and reliably (Wemelsfelder et al., 2001). It is generally accepted that welfare assessment requires the
combination of several indicators based on health, longevity, productive and reproductive success, changes to behaviour and physiology, and measures of animal preferences or motivations (Broom and Johnson, 1993; Duncan and Fraser, 1997). Various types of physiological measurements will be discussed, followed by behaviour measurements with a focus on both quantitative methods and qualitative behaviour assessment (QBA).

1.4.1 Physiological measures

Physiological measures commonly include short term responses such as increases in heart rates, body temperature, respiratory rates, circulating corticosteroids, glucose, lactate and free fatty acids (Broom and Johnson, 1993). Longer term responses include enlargement of adrenal glands and depression of immune function (Moberg, 1985).

Physiological measurements alone can cloud the difference between bad and good welfare without knowing the type of environmental condition the animal is in. For example, hunted deer have higher serum levels of creatine kinase, aspartate aminotransferase, lactate dehydrogenase, plasma glucose, lactate, sodium, cortisol and endorphins than those that were not being hunted (Bateson and Bradshaw, 1997), which could simply reflect differences between deer that have been running and those that were not (Dawkins, 2003). Other physiological indicators, such as heart rate and respiratory rate can be caused by physical activities or arousal such as during coitus, exercise and anticipation for food (Dawkins, 2003). Physiological indicators alone to assess animal welfare can therefore produce discrepancies and misinterpretation of what the animals are experiencing, especially regarding their emotional state.
1.4.2 Behavioural measures

Dawkins (2004) pointed out that behaviour is crucial in gauging what animals want, in their choices and preferences and emphasized that behaviour will become even more important that it has been as there is a growing need to find a more reliable, less invasive methods of welfare assessment. Using behaviour in the assessment of animal welfare has several major advantages as it can be less invasive or intrusive (Dawkins, 2004). Behaviour is already used widely in the clinical assessment of animal health and in the assessment of pain (Rutherford, 2002). Veterinarians traditionally use behaviour as components of assessment performed during clinical examination (Millman, 2013) for example, the use of gait scores to assess lameness in dairy cattle (Kestin et al., 1992) and repetitive leg and tail movements were reported to be associated with pain from castration (Molony et al., 1995; Fisher et al., 2001). The advantage of using behaviour to score the ability of a farmed chicken walking gives a quick, on-farm method of assessment even though the underlying pathology can vary from cartilage abnormalities to joint infection or distorted bone growth (Thorp, 1994; Bradshaw et al., 2002). Behavioural choices can also indicate what animals want. For example, a link between chicken with poor gait scores and those that choose to self-administer analgesic drugs showed that the birds wanted a reduction in pain (Weeks et al., 2000).

Different methods of on-farm monitoring have been developed and tested, some focus on physical health, better management of animals and others on assessing animal welfare (Wemelsfelder et al., 2001). Quantifying behaviour has been performed in various cattle studies such as lameness in dairy cattle and penned and free range young cattle (Ishiwata et al., 2008; Walker et al., 2008; Gomez and Cook, 2010). It is a traditional method of studying animal behaviour ranging from large carnivore predatory behaviour (MacNulty et al., 2007)
to emotionality in growing pigs (Rutherford et al., 2012). In this study, a list of behavioural patterns were collated from the published literature (Sylvester et al., 2004; Thüer et al., 2007; Napolitano et al., 2012; Sutherland et al., 2013; Petherick et al., 2014) to develop a suitable list of behaviours for scoring cattle behaviour before, during and after castration. Dawkins (2004) emphasized that behaviour will become even more important that it has been, as there is a growing need to find a more reliable, less invasive methods of welfare assessment.

1.5 Qualitative Behavioural Assessment (QBA)
One method of monitoring behaviour that has the potential to improve animal health and welfare is assessing the ‘whole animal’, using called Qualitative Behavioural Assessment (QBA). This qualitative assessment of behaviour integrates and summarises the different aspects of an animal’s dynamic style of interaction with the environment, which reflects on the animal’s experience of a situation. This experience is directly relevant to evaluations of welfare (Wemelsfelder, 1997). Observers were asked to score animals against a set of qualitative descriptors such as ‘calm’, ‘anxious’, ‘excited’, ‘annoyed’. This approach can use either a fixed lists of descriptive terms, or have observers generate their own list of terms under ‘Free-Choice Profiling’ (FCP), a method developed and tested in food sciences (Oreskovich et al., 1991). Observers using this method were less biased as they generate their own descriptive words according to their judgment of what the animals might be experiencing (Wemelsfelder et al., 2001). Clarke et al. (2016a) demonstrated that regardless of whether observers were given fixed terms or allowed to generate their own terms, observers score sow body language in a similar way. QBA has previously been applied to cattle during transport (Stockman et al., 2011), assessing cattle behaviour before slaughter (Stockman et al., 2012) as well as dairy cow under various social conditions (Rousing and Wemelsfelder,
which all revealed high agreement in how observers assessed the animals regardless of context.

Each of these methods has benefits and limitations in terms of how they can be applied to welfare assessments, and careful review of the methods will help clarify their use (Fleming et al., 2016).

QBA has been used in a variety of studies including social behaviour of dairy cows, sheep transport, cattle temperament and pigs’ emotion, and assessing horses’ demeanour during endurance races. Animals can execute a behaviour in several different ways; for example, they can be walking in a relaxed or tensed manner (Fagen et al., 1997). Therefore, when focusing on the whole animal, behaviour is not just a physical movement, but it is evaluated in a larger context and acquires an expressive, psychological quality (Bavidge and Ground, 1994; Wemelsfelder, 1997; Segerdahl et al., 2005).

Some scientists initially were apprehensive that QBA might be anthropomorphic judgements of uncertain validity as some characterize qualitative assessment as ‘subjective’ assessment (Stevenson-Hinde et al., 1980; Kennedy, 1992; Heyes, 1993). To answer the above argument, research with pigs has shown spontaneous qualitative behaviour assessments made by untrained observers to be reliable and repeatable, irrespective of observer background (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001; Wemelsfelder and Lawrence, 2001). Observers could identify differences in behavioural expression between cattle that were naïve versus habituated to road transport (Stockman et al., 2011) and these differences were supported by physiological measurements. Studies conducted on sheep and dairy cattle indicated that the qualitative assessment procedure is a reliable method (Dungey, 2003; Rousing and Wemelsfelder, 2006).
Various professionals working with animals routinely use expressive terms to discuss and manage their animal’s health and welfare state. Therefore, QBA has the potential of becoming an on-farm welfare assessment tool (Wemelsfelder, 1997).

1.6 General aims

The aims of this thesis are to determine:

1). To determine any variation in the behaviour of *Bos indicus* cattle following surgical castration

2). To determine if there is consensus in how observers use Qualitative behavioural analysis (QBA) to interpret cattle behaviour following surgical castration

3). To determine if effective analgesia could be achieved following surgical castration in *Bos indicus* cattle
1.7 Thesis outline

**Chapter 1** – contains a literature review on animal welfare, pain and types of analgesia available for cattle. It also covers animal welfare assessment and the use of QBA on cattle behaviour.

**Chapter 2** – describe QBA methodology, treatment groups, and castration method.

**Chapter 3** - describe an analysis of composite behaviour scoring (CBS) developed for behaviour in three different contexts (paddock, feeder yard and crush) on cattle with and without analgesia.

**Chapter 4** – describes QBA on treatment groups during castration (in crush) and immediately after castration (exit crush) to determine if there was an effect with analgesia. Quantification of behaviour was performed for correlation with QBA.

**Chapter 5** – applies QBA to distinguish castrated cattle from non-castrated and analgesia controls on pre-and-post castration days in two different contexts (paddock and feeder yard). CBS was performed to check for correlation with QBA.

**Chapter 6** – includes a final discussion about the results, general questions and future research.
2 General Methods and Materials

This chapter describes the general materials and methods used in this study however some additional methods are described in the relevant chapters. All experimental procedures were reviewed and approved by the animal ethics committee (Permit Number R2551/13) at Murdoch University (Perth, Australia) and the survey procedures were approved by the human ethics committee (Permit Number 2008/021) at Murdoch University (Perth, Australia).

2.1 Animals

Forty-eight Brahman bull calves (mean weight 165 ± 17.5kg) from an extensive cattle station in the north-west region of Australia (Pilbara region) were transported to Murdoch University vet farm for the project duration. They were transported from the farm of origin 8 days prior to the surgery day to allow for acclimatisation to the Murdoch University farm. The cattle had not been handled by the farmer and were not used to humans. Access to hay and water was allowed *ad lib* and EasyBeef pellets were fed daily (Milne AgriGroup Pty Ltd, Welshpool, WA, Australia) at approximately 3% of bodyweight. The day after arrival the cattle were brought into the race for identification. An ear tag was placed in the right pinna and the same number was painted onto each rump for easy identification from a distance. A pedometer was fitted with a strap to the left hind leg just above the fetlock joint for another study (Laurence et al., 2016). The following day the cattle were once again brought into the race for prophylactic parasite treatment (Moxidectin 5g/L Cydectin Pour-on, Virbac). Blood samples were collected from the tail vein for antigen capture enzyme linked immunosorbent assay testing for Bovine Viral Diarrhoea Virus.

Cattle were randomly divided into six equal groups (n=8 for each group):
1) **NC** – non-castrated (negative control). Calves were passed through the race and then held in the crush (restrained via a head bail) for the equivalent amount of time as those held for surgery (5 min)

2) **CLA** – castrated with local anaesthetic Lignocaine (2mg/kg, Lignocaine 20, Ilium, Troy Laboratories, Glendenning, NSW, Australia) injected into the testicle and subcutaneously on the incision site 5 min prior to surgery (whilst in the crush)

3) **CM_{pre}** – castrated with NSAID Meloxicam (0.5mg/kg, Meloxicam 20) administered subcutaneously 30 min prior to surgery (whilst in the race)

4) **CM_{post}** – castrated with NSAID Meloxicam (0.5mg/kg, Meloxicam 20) administered subcutaneously immediately post-surgery (whilst in the crush)

5) **CLM_{post}** – castrated with both Lignocaine administered subcutaneously 30 min prior to surgery (whilst held in the race) and Meloxicam (administered subcutaneously immediately post-surgery whilst held in the crush)

6) **C** – castrated without analgesia (positive control) in the crush

All animals were handled in the same manner and held in the race and crush for equivalent periods of time.

### 2.2 Analgesia and Surgical Procedures

Analgesia was provided by either Lignocaine (L) (2 mg/kg, Lignocaine 20) or Meloxicam (M) (0.5 mg/kg, Meloxicam 20) or a combination of both. Each Lignocaine injection was split into approximately 6ml intra-testicularly and the remaining 2ml subcutaneously into the scrotal skin. All Lignocaine injections were carried out in the same manner by the veterinarian who
castrated the cattle 5min prior to surgery. Meloxicam was injected subcutaneously in the right lateral neck area 30min prior to surgery.

Surgical castration was performed by two veterinarians with extensive experience using the open technique. The animal was restrained in the crush and head bail and the scrotum was cleaned with dilute chlorhexidine solution. One testicle was held against the bottom of the scrotal skin and a firm incision was made using a scalpel blade along the scrotum allowing the testicle to be exteriorised. The sperm duct and fibrous tissue was cut, and the testicle pulled away. The procedure was repeated for the second testicle.

2.3 Pain assessment

Pain was assessed using several measures, and the focus of this thesis, was the behavioural responses to castration, which formed a part of a larger study. Physiological and behavioural pain assessment strategies were employed at specific time points (Table 2.1): live weight, serum cortisol levels, nociceptive threshold testing, pedometer readings, balk score and crush score were taken for another study (Laurence et al., 2016; Musk et al., 2016).

Table 2.1 Timeline of sampling throughout the experiment

<table>
<thead>
<tr>
<th>Day</th>
<th>QBA</th>
<th>Quantitative (CBS)</th>
<th>Weight</th>
<th>Cortisol</th>
<th>Balk score</th>
<th>Crush score</th>
<th>NTT</th>
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*QBA – Qualitative Behavioural Assessment *CBS – Composite Behavioural Scoring
*NTT – Nociceptive Threshold Testing
2.4 Behavioural measurements

2.4.1 Composite Behaviour Scoring (CBS)

Quantifying behaviour has been performed in various cattle studies such as lameness in dairy cattle and penned and free-range behaviour of young cattle (Ishiwata et al., 2008; Walker et al., 2008; Gomez and Cook, 2010). In this study, a list of behavioural activities were collated from published literature (Sylvester et al., 2004; Thüer et al., 2007; Napolitano et al., 2012; Sutherland et al., 2013; Petherick et al., 2014) to develop a suitable list of behaviour for quantifying cattle behaviour before, during and after castration. Our method of quantification is called composite behaviour scoring (CBS), which is slightly different from the traditional ethogram and time-budget methodologies.

All observations were conducted by one observer without the knowledge of individual calf treatment group.

Quantitative measurements were taken whilst cattle were being castrated (in crush) and post-castration (exit crush) based on two (2) lists of behavioural display (Table 3.1 – Chapter 3). Observations were done on the cattle facial expression during castration as most of their body was covered by the crush and only the head was visible. Upon exiting the crush, entire body demeanour was observed.

A different list of behavioural display was used while cattle were in the paddock (Table 7.1).

Composite behaviour scoring (CBS) was conducted each morning at sunrise for good visibility (approximately at 7:30am) in the paddock for a maximum of 2 minutes on each animal on pre- and post-castration days. Cattle were sampled from left to right of the paddock. For the day of castration (Day 0), scoring was done in the afternoon, after the cattle had returned to their home paddock. Eight common types of behaviour in cattle were chosen for scoring. The
first four behaviours: Position in Group, Grazing, Ruminating and Social Behaviour (included grooming each other, sniffing or licking) were scored as follow: 0 = isolated/nil display of behaviour, 1 = semi-isolated/intermittent and 2 = positioned together/constant display of behaviour. The next four behaviours: Weigh Shifting, Hindleg Stomping, Scrotal Area Grooming and Tail Swishing were scored as follow: 2 = nil display of behaviour, 1 = intermittent and 0 = constant. The last four behaviours were thought to be abnormal or indicative of pain (Petherick et al., 2014a) and were scored low (2) if behaviour was not present and high (0) if behaviour was constantly shown. Positioning in group, grazing, ruminating and social behaviour (SB) were expected to be normal behaviour for grazing animals. (Appendix 7.1)

CBS data were analysed using Repeated Measures Analysis of Variance (RM-ANOVA) (Statistica) to check for significant differences between treatment groups and day.

2.4.2 Qualitative Behavioural Analysis (QBA)

Cattle were filmed immediately post CBS, scanning animals left to right in the paddock. The video clips captured their whole-body demeanour and using the zoom to allow animal viewing at close range. They were also filmed in the feeder yard after surgery, and in the crush at times of castration and as they exited the crush immediately after castration. While in the feeder yard, video footage was recorded the same way as paddock. During castration, only the head and face of the cattle were shown to observers so not to reveal what type of procedure was taking place. As cattle exited the crush, observers were again able to view the whole body of the animal. Approximately 3 min of video footage was recorded for each calf in the paddock using a handheld Panasonic digital video camera at 7 -7:30 am on pre- and post-surgery days. Footage was edited using, Adobe Premiere Pro CS3 and Adobe After Effects
CS3 to produce 40 - 50 seconds clip of each calf that was shown to observers for scoring. All clips were edited in similar way, without knowing the calves’ treatment groups and selecting a similar start and end time point on each footage. Some clips duration were longer than others as calves were moving constantly or hiding behind one another. Hence editing of the clips were necessary to capture the whole-body view and of similar time frame for every clip.

Volunteer observers that scored the videos were recruited via flyers, posters advertisements and through the Murdoch University social media page. There were 20 recruited for the first session and 30 for the following session. Observers included university staff members, students, primary producers and the general public. Each observer was required to complete four sessions via correspondence or on campus. Before scoring cattle, observers were asked to complete a questionnaire regarding their demographic background, experience with cattle and their attitudes and opinions towards cattle behaviour and animal welfare (Appendix 7.4 to 7.10). Observers were given detailed instructions on completing the four QBA sessions but were unaware of the treatment groups.

2.4.3 Term generation session

Observers were shown 15 video clips of individual or groups of cattle in the paddock, holding yard or feeder area demonstrating a range of behaviour to allow observers to describe as many aspects of cattle behaviour as possible. After watching each clip, observers wrote down terms that they thought described that animal’s behavioural expression. There was no limit imposed to the number of descriptive terms an observer could generate, but terms needed to describe not what the animal was doing (i.e. physical descriptions of the animal such as eating or walking), but how the animal was doing it (i.e. emotional descriptions of the animal such as relaxed or anxious). Each descriptive term was attached to a 100-mm visual analogue
scale (minimum=0 to maximum=100) in an excel worksheet. The list of terms was arranged alphabetically, although ensuring that terms with a similar meaning were not listed together.

2.4.4 Viewing sessions

Before session commencement observers were given detailed instructions on how to score each animal’s expression using the visual analogue scale: they were told to think of the distance between the zero-point and their mark on the scale as reflecting the intensity of the animal’s expression. Observers viewed and scored video clips of individual animals using their own unique list of descriptive terms. In session 1, observers viewed 32 clips of individual cattle in the paddock on pre- and post-surgery days (Day-1 vs. Day +1) for treatment groups castrated (C) vs. non-castrated (NC). In session 2, observers viewed 32 clips of individual cattle in the paddock on pre- and post-surgery days (Day-1 vs. Day+1) for treatment groups castrated (C) vs. castrated with local anaesthetic and post-surgery meloxicam (CLMpost). In session 3, observers viewed 48 clips of individual cattle in the crush on day of surgery (Day 0) for all six treatment groups. The same number of sessions were repeated for feeder yard and exit crush viewing sessions: Session 1 with 32 clips, C vs. NC, Session 2 with 32 clips, C vs CLMpost and 48 clips as cattle exited the crush immediately after castration.

2.4.5 Statistical analysis for Qualitative Behavioural Analysis

The distance from the start of the visual analogue scale to where the observer had made a mark was automatically measured in millimetres into Excel (Microsoft Excel 2003, North Ryde, NSW, Australia) files. These data were submitted to statistical analysis with Generalised Procrustes Analysis (GPA) as part of a specialised software package written for Françoise Wemelsfelder (Wemelsfelder et al., 2000; GenStat, 2008). GPA calculates a consensus or ‘best
fit’ profile between observer assessments through complex pattern matching. GPA provides a statistic (the Procrustes Statistic) that indicates the level of consensus (i.e. the percentage of variation explained between observers) that was achieved. This procedure rearranges at random each observer’s scores and produces new permutated data matrices. By applying GPA to these permutated matrices, a ‘randomised’ profile is calculated. This procedure is repeated 100 times, providing a distribution of the Procrustes Statistic indicating how likely it is to find an observer consensus based on chance alone. Subsequently a one-way t-test was used to determine whether the actual observer consensus profile falls significantly outside the distribution of randomised profiles.

Through Principle Components Analysis (PCA), the number of dimensions of the consensus profile is reduced to several main dimensions (usually 2 or 3) explaining the variation between animals. Each animal receives a quantitative score on each of these dimensions, so that the animal’s position in the consensus profile can be graphically represented in two- or three-dimensional plots. GPA dimensions are interpreted by correlating the animals’ scores to the observers’ individual scoring patterns, producing individual observer word charts that describe the consensus dimensions through their association with each individual observer’s terms. These word charts can then be compared for linguistic consistency. From these word charts, a list of terms describing the consensus dimensions was produced, by selecting terms for each observer that correlated strongly with those dimensions. To compare treatments, the GPA scores for each dimension were analysed using repeated- measures ANOVA for session 1 and 2, and one-way ANOVA for session 3.
3 Applying Composite behaviour scoring (CBS) to determine castration pain in cattle

3.1 Abstract
Castration of male cattle is traditionally performed for docility, to enhance carcass quality, as well as to prevent unwanted breeding. There have been various studies conducted to investigate ways to minimise pain in livestock and to improve their welfare. We examined behaviour displayed in response to surgical castration in cattle to identify indicators of pain. Forty-eight Brahman bull calves were divided into six treatment groups, with eight animals in each group ($n = 8$). There were two control groups: 1. castrated without analgesia (C), and 2. non-castrated (NC) and four analgesic groups: 1. castrated with local anaesthesia - Lidocaine (CLA), 2. castrated with post-surgery non-steroidal anti-inflammatory (NSAID) – Meloxicam ($CM_{post}$), 3. castrated with pre-surgery NSAID – Meloxicam ($CM_{pre}$), and 4. castrated with combined Lidocaine and post-surgery Meloxicam ($CLM_{post}$). Behaviour was recorded from footage collected under three contexts: in the paddock on pre- and post-castration days, of the animal’s head as it was held in the crush during castration, and as animals were released from the crush into a pen immediately after castration. There were no significant differences amongst treatment groups evident for footage collected in the paddock or during castration, but differences in behaviour were evident immediately after castration. This study revealed that the timing of behavioural observations and the context under which they are filmed are important in determining whether pain associated with castration is likely to be detectable. The context under which animals are filmed influences parts of the animal that can be visualised as well as the degree of movement and activity or body language. Controlling these contextual factors would allow clearer comparisons over
time with future studies. Such information will be valuable for further investigations on pain relief for cattle.

**Keywords:** Cattle, Surgical castration, Analgesia, Pain, Composite behaviour scoring (CBS)
3.2 Introduction

Castrating young male cattle is a common practice, performed on properties usually by the farmer. Reasons for doing so are for docility, enhanced carcass quality and to prevent unwanted breeding (Nielsen and Thamsborg, 2005). Various techniques have been adopted throughout the years and devices developed to make the process as humane as possible. Physical castration is the most invasive, yet most widely used compared to hormonal or chemical methods and welfare concerns are driving research into minimising the side effects (Stafford and Mellor, 2005b). Nielsen and Thamsborg (2005) and Bretschneider (2005) believed that there were no major welfare variations between the methods, because they all result in comparable situations involving pain. Behaviour has been used as a measure of welfare state and apparently can also be an indicator of pain (Stafford and Mellor, 2005b). Surgically castrated cattle have been observed to stomp their hind-legs, swish their tails and graze the least compared to non-castrated cattle (Stafford and Mellor, 2005b). Rubber-ring castrated calves assumed various stances and tried all means to reach their testicles to remove the agent of irritation for weeks post-castration (Stafford and Mellor, 2005b) which can be interpreted as being less quiet than surgically castrated cattle. However, surgically castrated cattle were interpreted as quieter post-castration, as it could be pathological pain that would be aggravated by excessive locomotion (Stafford and Mellor, 2005b).

Cattle have quite an extensive repertoire of behaviour (Kilgour, 2012) and it is important to be able to identify normal versus abnormal behaviour to assess pain and discomfort associated with negative stimuli. For example, grazing, ruminating and resting are typical of normal cattle behaviour, which makes up 90-95% of their day (Kilgour, 2012). Disturbances to their normal rage of behaviour may therefore be evident as reduced time spent grazing,
ruminating and resting, while increases in agitation or locomotion may become evident for animals experiencing physical or mental pain.

The aim of this study was to identify cattle behaviour in response to surgical castration, comparing between different analgesia treatments and recording their behaviour under three different contexts: undisturbed in the home paddock (paddock), locked in the head bail while in the crush during surgery (in crush) and exiting the crush immediately after surgery (exit crush). We expected to see treatment effects for all three contexts, in particular for C vs. NC and C vs CLMpost treatment groups.

3.3 Method
This study was approved by Murdoch University Animal Ethics (Permit Number R2551/13) and Human Ethics (Permit Number 2008/021) committees.

3.3.1 Animals and Quantitative Behaviour Analysis

Forty-eight (48) *Bos indicus* (Brahman) bull calves of six (6) to eight (8) months of age with a mean body mass of 165.5 (±17.5) kg. They were transported to Murdoch University farm from an extensive cattle station in the north-west region of Australia during the winter months.

Calves were placed in the same paddock eight (8) days prior to castration for acclimation. Calves were handled daily by the same staff members, with the calves moved through a race as a group, placed in a crush individually for weighing, blood sampling for baseline measurement of blood cortisol, nociceptive threshold testing and pedometer readings for another study (Laurence et al., 2016; Musk et al., 2016). They were randomly divided into six treatment groups (n = 8):
1). **NC** – non-castrated

2). **CLM_{post}** – castrated with Meloxicam post-castration and Lidocaine

3). **CLA** – castrated with Lidocaine

4). **CM_{pre}** – castrated with Meloxicam pre-castration

5). **CM_{post}** – castrated with Meloxicam post-castration

6). **C** – castrated

Quantification of behaviour was carried out for calves under three contexts; undisturbed in the home paddock (paddock), locked in the head bail while in the crush during surgery (in crush) and exiting the crush immediately after surgery (exit crush). Behaviour scores were counted by one observer each morning (07:00-08:00) from left to right direction of the paddock on each calf for two (2) min on pre- and post-castration days (Day -6, -1, +1, +2, +3, +6, +10, +13). Eight sets of cattle behaviours were put together for observation in the paddock, called Composite Behaviour Scoring (CBS) Scoring chart – Appendix 7.1). Calves were observed from a distance using binoculars to avoid any disturbance to their behaviour. Scores of 0, 1 or 2 were assigned to each behaviour to distinguish any variation in behavioural display.

In each context, two categories of behaviour were scored; i) states that were mutually exclusive and measured as a proportion of time, and ii) events that were activities carried out for less than five (<5) seconds each, and measured by counts (Table 3.1). On the day of castration, in the crush, video footage was taken by a stationary camera mounted on a railing located approximately two (2) metres in front of the exit gate. Exit crush footage was collected with a handheld camera from an observer approximately three (3) metres on the side of the crush. The same observer viewed the recorded footage of cattle during (in crush) and immediately after (exit crush) castration for the scoring the behaviour (Table 3.1). The
CBS performed on cattle in crush, and exit crush, recorded 11 states and 11 events, respectively.

Data analysis was performed by repeated measures ANOVA (RM-ANOVA), Statistica (StatSoft, 2007) and Paleontological Statistics (PAST) (Hammer, 2014) using One-way analysis of similarities (ANOSIM) and similarity percentage (SIMPER) to check for significant differences between treatment groups.

3.4 Results
In the paddock using CBS, there were no significant differences between behaviour pre and post-surgery days (Day -6, -1, +1, +2, +3, +6, +10, +13) in all 6 treatment groups ($P = 0.866$) when analysed with RM-ANOVA. Therefore, Day -1 and Day +1 were selected to determine differences between treatments (Figure 3.1). There were significant differences between the two days in terms of positioning in group, grazing, ruminating, social behaviour and weight shifting ($P = 0.004$). Three behaviours (hind leg stomping, scrotal area grooming and tail swishing) were not detected in the paddock. Further analysis comparing pre-and post-surgery days (Day -1 vs. Day +1) on three pairs of treatment groups: C vs. NC (Figure 3.2), C vs. CLM$_{post}$ (Figure 3.2), and CM$_{pre}$ vs. CM$_{post}$ (Figure 3.3) were conducted. Weight shifting was detected in C treatment group on Day -1 but in both C and NC treatment groups on Day +1. However, there were no significant differences in treatment effect between C and NC groups on Day +1 ($P = 0.810$). No variance in other behaviour was seen. Comparison of weight shifting between C and CLM$_{post}$, and between CM$_{pre}$ vs CM$_{post}$ treatment groups showed no significant differences ($P = 0.810$). One-way ANOSIM analysis found no significant differences found between treatment groups on all behaviour ($P = 0.251$) during castration (in crush). On exiting the crush after castration, there were significant treatment effect found between two pairs
using one-way ANOSIM C vs NC \( (P = 0.024) \) and \( C_{\text{post}} \) vs NC \( (P = 0.044) \). SIMPER analysis was then applied on the two pairs of treatment groups (C vs NC) and \( C_{\text{post}} \) vs NC to check for behaviour that showed significant differences. In percentage of time of calves running upon release for NC (9%), \( C_{\text{post}} \) (7%) and C (0%) (Figure 3.5). Proportions of calves that walked (forward and backwards) were, NC (53%), \( C_{\text{post}} \) (54%) and C (60%) and stood were, NC (38%), \( C_{\text{post}} \) (39%) and C (39%) (Figure 3.6). Proportions of calves’ weight shifted were, NC (100%), \( C_{\text{post}} \) (45%) and C (94%), tail swishing was only seen in \( C_{\text{post}} \) (45%). Urinate/defecate was identified in C (6%) and \( C_{\text{post}} \) (9%) respectively. There were no significant differences found between groups \( (P = 0.251) \) for behaviour recorded during castration.

### 3.5 Discussion

Significant differences were evident between treatment groups as cattle exited the crush after castration. However, there was little significant difference in time-budgets measurements between treatment groups for cattle observed while in the paddock or during castration. This study therefore reveals that the context in which cattle are observed is important in showing evidence of pain. Identifying when pain is most likely to be evident would aid our understanding of the efficacy of analgesia.

Previous studies have shown that castration in cattle is painful. For instance, pain can be associated with decreases in activity, eating and ruminating (Anderson and Muir, 2005), as well as abnormal gait and postures (Stafford and Mellor, 2005a; Coetzee, 2011). In our study, there was no difference in gait or activity whilst in the paddock. One explanation would be that the cattle were masking the pain. Livingston (2010), described that such masking is a result of the behavioural evolution of prey species to avoid overt displays of abnormal behaviour that may make them vulnerable to predators. Calves might behave cautiously as
they were exposed to various environmental elements being out in the open paddock and they may also have been mimicking the behaviour of one another as being amongst other cattle is crucial for their safety. Cohesive relationships are important in herd animals and cattle often display social grazing behaviour as they follow each other independently (Reinhardt and Reinhardt, 1981).

Fear while being held in the crush (head bail), can overpower other behavioural display, especially as calves had to be so close to humans. Therefore, no treatment differences were detected during castration. Cattle, by nature, are fearful animals and aware of their surroundings and the presence of people. Studies have suggested that cows alter their vigilance according to their degree of fearfulness toward people and toward different environment (Welp et al., 2004). Therefore, it is important to be able to separate the effects of pain from the effects of human presence.

There were some differences as cattle exited the crush after castration. None of the C calves ran out of the crush, which suggests that castration may cause more discomfort than controls (NC and CM_post). Walking was seen more in C and CM_post group than NC, which might be explained by castrated groups experiencing most discomfort and therefore less likely to run.

The analgesic effect was unlikely to have been effective for a least thirty (30) minutes post-administration since Meloxicam therapeutic plasma concentration is from thirty (30) minutes to forty-eight (48) hours post-administration (Dumka and Srivastava, 2004). Hence, CM_post and C would be similar in their sensitivity to pain as they exited the crush. CM_post and C groups demonstrated this through their display of forward walking behaviour and less running compared to NC calves.
NC showed the most weight shifting upon release from the crush, which could be due to them trying to move away from humans as they were fearful of the recent restraint in the crush. Some of the behavioural displays that indicate distress and fear are standing/escape or avoidance movements, pacing and restlessness (Mellor et al., 2000). However, these behavioural types could also mean the cattle were in pain. Less weight shifting in C and CMpost was seen as they were released from the crush, which could be a sign of pain as movement in the hind legs would likely aggravate the wound region. Tail swishing was seen only in CMpost group but not in C as they exited the crush. It was the expectation that both castrated groups have similar behavioural displays. However, both C and CMpost showed signs of nervousness as they urinated /defecated upon exiting the crush unlike the NC group. The procedure most likely aggravated the already fearful cattle. This may be why these cattle were more stoic and minimal display of expected behaviour changes. The NC group were placed in the crush and blood sampled. Therefore, they would still be in a state of fear that may look like the behaviour of castrated cattle. Prior to this study, these cattle had little to no exposure to humans their behavioural display indicated they remained reactive to humans throughout the study. There is evidence that cattle can be trained or habituated to humans and become less fearful (Boissy and Bouissou, 1988; Pajor et al., 2000; Hemsworth and Coleman, 2011). Repeated exposure to aversive handling procedures can result in cattle becoming more reactive and fearful of people (Grandin, 1997; Hemsworth and Coleman, 2011).

Timing of monitoring of these cattle may have been problematic as they might need close monitoring during the first forty-eight (48) hours post-procedure to capture any treatment effects. Another issue might be the number of cattle used for each treatment group. Sample size is crucial as to obtain the most accurate results.
3.6 Conclusion

Behaviour is an apparent indicator of pain (Stafford and Mellor, 2005a) and any type of behaviour should be considered within a broader perspective of the animals’ environment as some behavioural display might be conflicting. Our study showed unexpected findings, as we did not see a marked difference in calf behaviour in the castrated vs non-castrated groups regardless of the crush or paddock context. Hence, we can conclude either that surgery is not painful for this species or that in the context of our study, the calves did not exhibit overt pain behaviour. It was expected that there would be changes in behaviour in cattle before and after castration (Day -1 vs Day +1) and treatment effects during and immediately after castration. However, in this study, the provision of pain relief did not appear to significantly alter the behaviour of the cattle during or after surgery. We believe the latter is more likely and overridden by the fear/stress of handling and blood sampling for the cattle in and around the crush, masking any pain response. In the paddock, we can explain the lack of treatment difference as cattle were with their herd mates and may have displayed similar behaviour to one another. With prey species, such as cattle, strong fearful instincts and subtle behavioural displays provide only a small window of opportunity to witness changes in behaviour display and to understand their expression, which requires additional time and patience. We know surgical procedures cause pain and this study has shown difficulties in identifying the overt expression of pain in cattle not habituated to humans. An overwhelming fear of being in a confined crush and close to humans may have significantly shifted their demeanour. Hence, fear may replace any display of painful behaviour.
### 3.7 Appendix

**Table 3.1** The list of behaviour developed for scoring across three contexts (paddock, in crush and out of crush).

<table>
<thead>
<tr>
<th>Category</th>
<th>Behaviour</th>
<th>Description</th>
<th>Measure</th>
<th>Paddock (during castration)</th>
<th>In crush (after castration)</th>
<th>Exist crush (after castration)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>States</strong></td>
<td>Stand (still)</td>
<td>Stationary - torso upright position</td>
<td>Proportion of time</td>
<td>✔</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stand (hunched)</td>
<td>Stationary – shoulder slightly lowered</td>
<td>Proportion of time</td>
<td>✔</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stand (struggling)</td>
<td>Not stationary – torso moving vigorously</td>
<td>Proportion of time</td>
<td>✔</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Walk (forward)</td>
<td>Forward locomotion</td>
<td>Proportion of time</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Walk (backwards)</td>
<td>Backwards locomotion</td>
<td>Proportion of time</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stand</td>
<td>No movement of body, stationary</td>
<td>Proportion of time</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Run</td>
<td>Continuous motion of forward movement</td>
<td>Proportion of time</td>
<td></td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>Position in group</td>
<td>With another cow or being alone</td>
<td>Proportion of time</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grazing</td>
<td>Eating grass</td>
<td>Proportion of time</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ruminating</td>
<td>Chewing cud</td>
<td>Proportion of time</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SB* grooming (licking / sniffing)</td>
<td>Grooming another cow</td>
<td>Proportion of time</td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ears flick</td>
<td>Movement of one or both ears</td>
<td>Counts</td>
<td></td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>Swallowing saliva</td>
<td>Visible swallowing reflex</td>
<td>Counts</td>
<td></td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>Drooling</td>
<td>Producing clear oral secretion</td>
<td>Counts</td>
<td></td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>Head (shaking / struggling)</td>
<td>Vigorously moving head</td>
<td>Counts</td>
<td></td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>Activity</td>
<td>Description</td>
<td>Count</td>
<td>Presence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------</td>
<td>----------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyes widening</td>
<td>Widening of eyelids / sclera visible</td>
<td>Counts</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head (lowering to ground)</td>
<td>Head below brisket, almost touching ground while standing</td>
<td>Counts</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scrotal area grooming</td>
<td>Licking scrotal area</td>
<td>Counts</td>
<td>✓ ✓ ✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hind leg stomping</td>
<td>Stomping of either hind foot on ground</td>
<td>Counts</td>
<td>✓ ✓ ✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight shifting</td>
<td>Lifting either foot while maintaining balance</td>
<td>Counts</td>
<td>✓ ✓ ✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail swishing</td>
<td>Movement of tail from side to side</td>
<td>Counts</td>
<td>✓ ✓ ✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinate / Defecate</td>
<td>Excretion of bodily fluid / solid</td>
<td>Counts</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.1. Composite Behaviour Scoring (CBS) of cattle in the paddock showing 5 behavioural display, which had significant differences ($P = 0.004$).
Figure 3.2. Castrated (C) vs. Non-castrated (NC) cattle on pre-and post-surgery (Day-1 vs. Day+1)

Figure 3.3. Castrated (C) vs. Castrated with Local anaesthetic and post-surgery Meloxicam (CLM_post) cattle on pre-and post-surgery (Day-1 vs. Day+1)

Figure 3.4. Castrated with pre-surgery Meloxicam (CM_pre) vs. Castrated cattle with post-surgery Meloxicam (CM_post) on pre-and post-surgery (Day-1 vs. Day+1)
Figure 3.5. Treatment group comparison of behavioural states observed as calves exited the crush after castration.
Figure 3.6 Treatment group comparison of behavioural events observed as calves exited the crush after castration.
4 Using Qualitative and Quantitative behavioural assessment on cattle undergoing surgical castration with different analgesic protocols

4.1 Abstract
Castration is a painful procedure that is routinely performed on livestock. Identifying best practice for castration requires objective measures of pain assessment. In this study, the behavioural expression of Brahman calves during and immediately after surgical castration was examined using qualitative and quantitative methods. The objective was to compare the behaviour of control cattle with those subjected to different analgesic protocols using a non-steroidal anti-inflammatory drug (Meloxicam) administered pre- or post-surgical castration and a local anaesthetic (Lignocaine) while the animal was held in a crush. Forty-eight Brahman bull calves were divided into six treatment groups (n=8): 1) no castration (NC), 2) castration with no analgesia (C), 3) castration with Meloxicam administered pre-castration (CM$_{pre}$), 4) castration with Meloxicam administered post-castration (CM$_{post}$), 5) castration with Lidocaine (CLA) and 6) castration with Lidocaine and Meloxicam post-castration (CLM$_{post}$). Video footage recorded cattle (a) during surgical castration (facial features visible whilst restrained in the crush) and (b) post-castration (whole animal visible upon release from the crush) and shown to volunteer observers for analysis by Qualitative Behavioural Assessment (QBA) in two separate viewing sessions. The consensus profile explained 44.5% and 35.36% of the variation in observer’s scoring patterns for the two viewing sessions, respectively. There were significant differences between treatment groups for both sessions ($P < 0.001$) where castrated cattle (C) showed the differences from groups given analgesia. Descriptive terms such as ‘agitated’, ‘uneasy’ and ‘sore’ used by observers suggested that castrated cattle (C) experienced discomfort from the procedure. Cattle that received both forms of pain control
\( (CLM_{post}) \) were described as ‘calm’, relaxed’ and ‘contented’. Spearman correlations between QBA and quantitative analysis supported the interpretation that the observers scored cattle behaviour consistently with the interpretation that the cattle demonstrated differing degrees of pain.

**Keywords:** Castration, Cattle, Qualitative behavioural assessment (QBA), Pain, Analgesia, Quantitative analysis
4.2 Introduction

Castration is a painful procedure that is routinely performed on livestock. There is ample evidence that castration causes pain (Stafford and Mellor, 2005a; Coetzee, 2011). The most common methods of castration currently used are band or rubber ring, surgical castration and using a Burdizzo clamp (Stafford et al., 2000). Surgical castration is the most common method used, even though it increases the cortisol response (Earley and Crowe, 2002; Sutherland et al., 2013). Tension banding castration has gained favour as it is relatively simple to perform and promoted by retailers of the banders as a humane method of castration (Petherick et al., 2014). Comparing these methods to guide best practice requires objective measures of pain, an area of growing research.

Given the growing concern for animal welfare amongst the public, it is appropriate to determine whether effective analgesia can be achieved (Weary and Fraser, 2004). Several pain mitigating strategies have been proven to be effective in mitigating pain during castration including the use of local anaesthetics and systemic non-steroidal anti-inflammatory drugs (NSAID). Petherick et al. (2014) found that the NSAID, Ketoprofen appeared effective in moderating pain-related behaviour in the three (3) hours following castration in mature Brahman bulls, but was less effective in young weaners. Weary et al. (2006) indicated that NSAIDs were effective at reducing post-operative pain in the hours that followed the procedure. However, other NSAID studies have shown Ketoprofen, and oral Meloxicam, to be ineffective at mitigating pain after surgical castration (Repenning et al., 2013; Moya et al., 2014). While systemic analgesia (e.g. Ketoprofen) may be more effective than local anaesthesia during castration because it serves to alleviate the associated stress response (Earley and Crowe, 2002), local anaesthesia (e.g. Lidocaine) may provide effective pain-relief for two (2) to three (3) hours post-surgery (McMeekan et al., 1998a; McMeekan et
Indirect symptoms that can serve as indicators of pain in animals, include changes in both physiological and behavioural parameters (Molony and Kent, 1997). Clinical assessment of animal health and assessment of pain widely uses behaviour (Rutherford, 2002; Petherick et al., 2014). Dawkins (2004) added that behaviour has several major advantages in welfare studies as it is not only non-invasive, it is also in many cases non-intrusive. Morton and Griffiths (1985) proposed that the study of behavioural patterns should constitute a substantial part of pain assessment. Applying quantitative methods such as using composite behaviour scoring for pain assessment is another way of studying behavioural patterns as described in Chapter 3.

Qualitative Behavioural Assessment (QBA) uses observers to score qualitative aspects of behavioural expression (Fleming et al., 2016). The observers are asked to consider the whole animal and integrate many pieces of information, including incidental behavioural events, subtle details of movement and posture, and aspects of the context in which behaviour occurs (Wemelsfelder et al., 2001). QBA captures not so much what an animal does, but how it does so and the animal’s dynamic style of interaction with the environment (Wemelsfelder, 1997).

This study examined the behavioural expression of cattle allocated to six treatment groups, including a control group (no-castration), a castrated group (no analgesia), and four different analgesia protocols given to castrated groups. The bulls were filmed as they were being castrated and as they walked out of the crush immediately after castration. The footage was shown to volunteer observers for scoring using QBA, and a single observer for CBS analysis. The aims of this experiment were to:

1. Determine whether observers can distinguish between treatment groups using quantitative and qualitative behavioural assessment; and
2. Determine whether analgesia reduces the pain associated with castration.

4.3 Methods
This study was approved by Murdoch University Animal Ethics (Permit Number R2551/13) and Human Ethics (Permit Number 2008/021) committees.

4.3.1 Animals and treatment groups

Forty-eight (48) Brahman bull calves (mean weight 165 ± 17.5kg) from an extensive cattle station in the north-west region of Australia (Pilbara region) were transported to Murdoch University vet farm for the project duration. The calves were held in a communal paddock for eight (8) days for acclimation post-arrival, during which time they were passed through a race and crush for habituation to handling and measurement of baseline data for mechanical nociceptive study (Musk et al., 2016) and pedometer measurement (Laurence et al., 2016). They were randomly divided into six (6) treatment groups with eight (8) calves in each group. Treatment groups contained: castrated (C) without any analgesia, non-castrated (NC), castrated with Meloxicam post-castration and Lidocaine (CLM_post), castrated with Lidocaine (CLA), castrated with Meloxicam pre-castration (CM_pre) and castrated with Meloxicam post-castration (CM_post).

Calves were moved through a race individually into a crush for either sham treatment or surgical castration using the open technique. They were restrained in the crush using the head bail, and the scrotum was disinfected using 0.05% chlorhexidine solution. One testicle was held against the base of the scrotal skin and a quick firm incision with a scalpel blade was made allowing the testicle to be exteriorised. The vas deferens and tunica vaginalis were separated from the testicle. The testicle was then pulled away from the body such that vasculature tore and retracted back into the abdomen. The steps were repeated for second
testicle. Once complete surgical castration, they underwent routine blood sampling for another study (including NC calves) and released from the crush via the forward gate.

Each individual calf was filmed continuously using a fixed-position digital handheld camcorder (Panasonic SDR-H250) as it was held in the crush, and by a second hand-held camcorder as it was released from the crush and it walked into temporary holding yards before being released back into their communal paddock. The continuous footage in the crush was edited to isolate only footage at the time of the surgical incision for each animal.

### 4.4 Qualitative Behavioural Assessment (QBA)

Video footage was recorded and edited (Adobe Premier Pro CS3 and Adobe After Effects CS3, Chatswood, NSW, Australia) in to forty (40) to fifty (50) second clips. Observers were recruited on a volunteer basis. Each observer was shown the same clips of individual cattle, without being informed about their treatment groups. Free Choice Profiling (FCP) methodology was used for scoring cattle, which relies on observers generating their own unique descriptive terms to describe animals for a series of video clips shown to the observers as part of a term generation session (Wemelsfelder et al., 2001).

Two (2) qualitative viewing sessions were carried out, where (a) twenty (20) observers watched footage collected at the time of surgery, and (b) thirty (30) observers watched the footage as calves were released from the crush. During these sessions, each observer used the terms they had generated to score cattle behaviour on a visual analogue scale ranging from 0 to 100. The observers were asked to mark on the scale the intensity of the behavioural expression for each of their descriptive terms. These measurements were entered to individual Excel files (Microsoft Excel 2013, North Ryde, NSW, Australia) for each observer. The scores obtained for each clip were analysed by Generalised Procrustes Analysis (GPA)

GPA develops a consensus profile describing all observers’ scores collated within a viewing session by transforming all the data as well as identifying complex patterns. A Procrustes statistic is calculated, quantifying the percentage of variation between observers that is explained by the consensus. Whether this consensus is a significant feature of the data set, or, alternatively, an artefact of the Procrustean calculation procedures, is determined through a randomisation test (Dijksterhuis and Heiser, 1995). One-way t-test is used to determine whether the actual observer consensus profile falls significantly outside the distribution of randomised profiles (Fleming et al., 2015). Subsequently, the numbers of dimensions of the consensus profile is reduced through Principle Component Analysis (PCA). The terms used by each observer to score cattle behaviour are correlated with each GPA dimensions to identify those terms that show the strongest correlations (r) with each GPA dimension. These terms were identified by calculating >75% of the highest absolute correlation coefficient value for that dimension (Mardia et al., 1979). By using Mixed-model ANOVA, treatment groups comparisons were made for each of the three (3) GPA dimensions within each session.

### 4.5 Quantitative Analysis

A list of behaviour suitable for quantifying behaviour was collated based on published studies on cattle behaviour (Sylvester et al., 2004; Thüer et al., 2007; Napolitano et al., 2012; Sutherland et al., 2013; Petherick et al., 2014). A total of ninety-six (96) clips from both sessions (fourty-eight (48) video clips each) were observed for identification of nine behaviour categories: four mutually exclusive states (duration of more than five (5) seconds; measured
as proportion of time) and five (5) events (counts of behaviour with a duration of less than five (5) seconds) (Table 4.1). Spearman correlation was used to check for correlations against QBA results.

4.6 Results

4.6.1 Qualitative Behavioural Assessment (QBA)

A total of ninety-seven (97) descriptive terms were generated by twenty (20) observers for during surgery (in crush); with 14 ± 5 (range 7 - 23) terms per observer. After surgery (exit crush), thirty (30) observers generated one hundred thirty-seven (137) descriptive terms; with 15 ± 5 (range 7 – 28) terms per observer (Table 4.2). For each session, there were three GPA dimensions that explained 81.8% and 63.4% of the variation in the scoring pattern between cattle (percentage breakdown for each dimension are shown in Table 4.2). There were significant treatment group differences for all three (3) GPA dimensions for the footage collected during castration (Table 4.2a).

During castration (in crush), on GPA dimension 1, observers described calves in the C and CM\textsubscript{pre} as the most ‘agitated’, ‘restless’ and those in the NC and CLM\textsubscript{post} treatment groups as being the most ‘calm’, ‘relaxed’ (Figure 4.1a). GPA dimension 1 explained the majority (66.2%) of the variation in scoring between cattle, with terms such as ‘calm’, ‘relaxed’ correlated with the low end of the axis and ‘agitated’, ‘restless’ correlated with the high end of the axis. There were correlations with quantitative behavioural scores on this axis, with animals that were attributed high on GPA dimension 1 scores (more ‘agitated’, ‘restless’) scored as stand (struggling) ($r_{48} = 0.82, P < 0.001$), head shaking/struggling ($r_{48} = 0.84, P<0.001$), and head lowering ($r_{48} = 0.37, P < 0.05$) to the ground compared with cattle that were attributed low GPA dimension 1 scores (more ‘calm’, ‘relaxed’).
On GPA dimension 2, C and CLM\textsubscript{post} groups were described as more ‘playful’, ‘restless’, while the remaining treatment groups CLA, NC, CM\textsubscript{pre} and CM\textsubscript{post} were categorised as more ‘unsure’ by observers (Figure 4.1b). GPA dimension 2 (10.5% of variability) was described by terms such as ‘playful’, ‘uneasy’ correlated with low end of the axis and ‘unsure’ correlated with high end of the axis. Quantitative scores for stand (hunched) ($r_{48} = -0.57$, $P < 0.001$) and swallowing saliva ($r_{48} = 0.33$, $P < 0.05$) correlated with low and high GPA dimension 2 values respectively.

On GPA dimension 3, the CLM\textsubscript{post} group were described as most ‘sleepy’, ‘depressed’ and CLA and CM\textsubscript{post} the most ‘sore’, ‘frustrated’ (Figure 4.1c). GPA dimension 3 described only 5.1% of the variation between cattle, and was described by terms such as ‘sore’, ‘frustrated’ at the low end and ‘sleepy’, ‘depressed’ at the high end. Head – lowering ($r_{48} = 0.36$, $P < 0.05$) to ground was correlated with the high values on this dimension (more ‘sleepy’, ‘depressed’).

Quantitative behaviour that did not correlate ($P > 0.05$) to any of the GPA dimensions during castration are: stand (still), ears (flick), producing (saliva) and eyes (widening).

For animals filmed after castration (exit crush), there were significant treatment group differences for all three (3) GPA dimensions for the footage collected immediately after castration (Table 4.2b). On GPA dimension 1, observers described calves in the C and CM\textsubscript{post} as the most ‘calm’, ‘relaxed’ compared with the other treatment groups, which were described as more ‘agitated’, ‘anxious’ (Figure 4.1d). GPA dimension 1 explained 50.1% of the variation in scoring between cattle, with terms such as ‘agitated’, ‘anxious’ associated with the low end of the axis and ‘calm’, ‘relaxed’ associated with the high end of the axis. There were correlations with walking (backwards) ($r_{48} = -0.40$, $P < 0.05$) and running ($r_{48} = -0.57$, $P < 0.001$) on this GPA dimension, with ‘agitated’, ‘anxious’ at the low end and walking
(forward) \((r_{s.48} = 0.57, P < 0.001)\) at the high end with ‘calm’, ‘relaxed’. In GPA dimension 2, the CM_{pre} group were described as most ‘watchful’, ‘frightened’, while the other treatment groups were described as more ‘relaxed’, ‘confused’ (Figure 4.1e). GPA dimension 2 (7.9% of variability between cattle) was described by terms such as ‘relaxed’, ‘confused’ associated with the low end of the axis and ‘watchful’, ‘frightened’ associated with the high end of the axis. There were no correlations between qualitative and quantitative analysis in GPA dimension 2. In GPA dimension 3, CLA and CLM_{post} treatment groups were described as most ‘affectionate’, ‘comfortable’ while the C and NC groups were most ‘angry’, ‘annoyed’ (Figure 4.1f).

GPA 3 described only 5.4% of the variation between cattle, and was described by terms such as ‘angry’, ‘annoyed’ at the low end and ‘affectionate’, ‘comfortable’ at the high end. Running \((r_{s.48} = 0.38, P < 0.005)\) was correlated with more ‘affectionate’, ‘comfortable’ terms at the high end while weight-shifting \((r_{s.48} = -0.29, P < 0.05)\) and urinate/defecate \((r_{s.48} = -0.33, P < 0.05)\) were correlated with ‘angry’, ‘annoyed’ at the low end. Quantitative behaviour that did not correlate \((P > 0.05)\) to any of the GPA dimensions after castration are: stand, scrotal area grooming, hindleg stomping and tail swish.

The descriptive terms that showed the strongest correlation with each GPA dimension, for each session are shown in Table 4.2.

4.7 Discussion

This study used both quantification and qualification methods to determine treatment affects for cattle undergoing surgical castration and whether analgesia reduces pain associated with castration. Results showed that in GPA dimension 1, observers could distinguish NC and CLM_{post} from the rest of the group during castration. Cattle in those two (2) groups were
described as more ‘calm’ and ‘relaxed’ in GPA 1. This indicated that receiving both types of analgesia (Lignocaine at the site and Meloxicam post-castration) may have significantly reduced pain for CLM\textsubscript{post} group thus making their behaviour like the NC group. On GPA 1, observers described C and CM\textsubscript{post} cattle to be more ‘calm’ and ‘relaxed’ as they exited the crush, post-castration. This was surprising as we expected these cattle to be showing visible signs of pain, like aggression. These cattle were described as more relaxed and calmer perhaps due to their slower movement because of the pain, which also correlated with the walking-forward behaviour in the quantification behaviour list. NC, CLA, CLM\textsubscript{post}, CM\textsubscript{pre} and CM\textsubscript{post} groups were described as more ‘agitated’ and ‘anxious’ than the C group, which correlated with walking backwards and running behaviour. Thus, in both scenarios, observers could differentiate cattle that were experiencing the most pain from those that may have been in reduced, or no pain. This proved that analgesia (CLM\textsubscript{post}) can reduce pain and discomfort associated with castration, but the terms used to describe cattle behaviour may not always have a direct meaning and need to be carefully analysed.

QBA results obtained from this research indicate that there may be an alleviation of some pain and discomfort by providing a combined pain relief method. Being able to reduce some unpleasant stimuli to these prey animals is crucial as not only does it improve their welfare outcome, but also makes the stockperson’s job easier. Past studies have shown that local anaesthesia reminds effective for two (2) to three (3) hours post-administered (McMeekan et al., 1998a; McMeekan et al., 1998b) and NSAIDs for much longer (up to forty-eight (48) hours post-administration (Dumka and Srivastava, 2004)) following a procedure (Weary et al., 2006). By combining these two (2) types of pain relief, it would provide a more effective pain control method. Administering Meloxicam prior to castration, may benefit more than administering post-procedure. Stafford et al. (2002) conducted various castration methods
using similar pain relief protocols and found that when local anaesthetic and ketoprofen were given before castration, the cortisol response to all methods was virtually eliminated, being like that of control calves. Peak plasma cortisol concentration was thirty (30) minutes post-surgical castration according to Stafford et al. (2002) which was supported by Dumka and Srivastava (2004), in that the minimum therapeutic plasma concentration for Meloxicam is maintained from thirty (30) minutes to forty-eight (48) hours of being administered. Therefore, by giving an anti-inflammatory prior to castration may be more helpful with pain control. However, in this study, CMpre group (GPA dimension 1) post-castration were described as ‘agitated’, ‘anxious’, ‘restless’. Which were similar description to that of other treatment groups except for C group. C was the only group described as ‘calm’, ‘relaxed’, ‘comfortable’ that most likely indicated they were sore hence moving slowly.

During castration, calves that received the combined pain relief showed similar behaviour to that of NC group, being described as ‘calm’ and ‘relaxed’. However, after surgery, calves that received a pain relief protocol were displaying a more ‘active’ type of behaviour, than the C group. Pain and discomfort, especially in the groin region, can deter calves from moving too quickly therefore, those in the C group were less active. Being described as ‘calm’ and ‘relaxed’ may not necessarily mean that they were comfortable, but rather moving slower due to pain. Therefore, how we interpret these terms is important as one needs to understand the bigger picture rather than drawing direct conclusions. By performing quantitative analysis, it validated how observers described the cattle and validated those terms. For example, when calves were described as ‘agitated’ and ‘anxious’, there were also more walking (backwards) and running type of behaviour, which correlated with the terms used. Performing time budget analysis may help with clarifying QBA term use. For example, during castration, QBA terms such as ‘agitated’, ‘restless’ and ‘frightened’ correlated with
behaviour such as, stand (struggling), head shaking / struggling and head lowering to ground. QBA terms described the emotional state of the animal whereas time-budget analysis described the physical state of the animal at the very same moment. Therefore, the combined analysis made the scenario much clearer.

However, some results were unexpected and did not appear to support the use of pain relief. For example, the behavioural display of C calves were interpreted similarly to that of CLM post and NC calves in GPA dimension 2 and 3, during and post-castration. Due to their nature of being a prey specie, cattle behaviour can be hard to distinguish so it is crucial not to overlook any ambiguous behaviours but to carefully interpret them to determine why such behaviour was on display. Standing, lying and walking forward can be difficult to interpret regarding pain, but a reluctance to move is known to be indicative of pain (Molony et al., 1995; Stafford and Mellor, 2005a). Hence, most of the cattle displayed a natural fear response, and ran out of the crush upon being released, including those that had no surgery, but those that were in the most pain (C), masked that pain somewhat (were described as ‘calm’) and only walked out slowly.

Molony et al. (1995) reported that in surgically castrated calves, standing immobile is a pain-related response. Capturing animal behaviour both immediately after a painful procedure as well as later point will enable researchers to compare differences in their findings and be a better way to accomplish accurate results. Post-operative pain, occurring hours and days after the procedure, is less often treated (Walker et al., 2011) as the focus sometimes is immediately after the procedure. Due to the number of animals on a farm, it can also be difficult for stockmen to identify those in pain as they may be well hidden amongst their herd mates. On the other hand, abnormal behaviour can also be hard to identify as animals are
often restrained during surgery, making it difficult to see or interpret any behaviour responses (Johnson et al., 2005). There are various studies that monitored cattle for days following castration, especially with bloodless castration such as using Burdizzo or rubber ring techniques (Thüer et al., 2007), as these methods take much longer time for the spermatic cords to retract or atrophy. However, in surgical castration, even though the procedure is quick, the intensity of pain and discomfort and the time for wound healing are important and these animals still require monitoring post-procedure. Hence the importance of capturing their behaviour within the optimal time frame (example; seventy-two (72) hours of continuous monitoring) to be able to perform a detailed analysis. This will also enable researchers to differentiate between painful or fearful type of behaviour.

Cattle that are unhabituated to handling, restraint and physiological measurement for an extensive period in the crush might also exhibit fearful behaviour that make it hard to determine treatment differences. To avoid these issues, it would be beneficial to both animals and researchers to spend extra time habituating (more days spent on the property prior to castration with regular human contact) and restrict one physiological measurement at a time.

4.8 Conclusion
Using behaviour as a tool for assessing pain in Bos indicus cattle has been useful, but there were some limitations. QBA showed pain associated with surgical castration was present in the calves. However, it can be quite challenging for observers to differentiate between painful and normal cattle behaviour. As Rutherford (2002) mentioned, pain is a complex multidimensional phenomenon and the responses of animals to it are also complex. To properly assess behavioural changes induced by pain, an experienced observer may be the only method truly capable of capturing this complexity (Rutherford, 2002). However, in this
study, we found that inexperienced observers, those not expert in the field of animal sciences, could detect certain behaviours that are not specific to cattle assumed to be in pain, using terms such as ‘sore’, ‘uneasy’ or ‘in_pain’ to describe cattle. This was validated against quantitative measures. Using a combination of qualitative and quantitative methods to evaluate pain could produce a better outcome at understanding the underlying meaning of behaviour. Behaviour is likely to be the most practical tool for assessing pain in clinical situations (Hansen et al., 1997). It is the most convenient tool, because it is non-invasive, inexpensive and does not require special equipment. It can be used in conjunction with physiological measurements to obtain the most accurate results or on its own where physiological measurements are not available.
### Appendix

**Table 4.1** A list of behaviour developed for quantification (a) during castration and (b) post castration

(a).

<table>
<thead>
<tr>
<th>Category</th>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>States</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>States</td>
<td>Stand (still)</td>
<td>Stationary - torso upright position</td>
</tr>
<tr>
<td></td>
<td>Stand (hunched)</td>
<td>Stationary – shoulder slightly lowered</td>
</tr>
<tr>
<td></td>
<td>Stand (struggling)</td>
<td>Not stationary – torso moving vigorously</td>
</tr>
<tr>
<td>Events</td>
<td>Ears flick</td>
<td>Movement of one or both ears</td>
</tr>
<tr>
<td></td>
<td>Swallowing saliva</td>
<td>Visible swallowing reflex</td>
</tr>
<tr>
<td></td>
<td>Producing saliva</td>
<td>Producing clear oral secretion</td>
</tr>
<tr>
<td></td>
<td>Head (shaking/struggling)</td>
<td>Vigorously moving head</td>
</tr>
<tr>
<td></td>
<td>Eyes widening</td>
<td>Widening of eyelids / sclera visible</td>
</tr>
<tr>
<td></td>
<td>Head (lowering to ground)</td>
<td>Head below brisket, almost touching ground while standing</td>
</tr>
</tbody>
</table>

(b).

<table>
<thead>
<tr>
<th>Category</th>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>States</td>
<td>Walk (forward)</td>
<td>Forward locomotion</td>
</tr>
<tr>
<td></td>
<td>Walk (backwards)</td>
<td>Backwards locomotion</td>
</tr>
<tr>
<td></td>
<td>Stand</td>
<td>No movement of body, stationary</td>
</tr>
<tr>
<td></td>
<td>Run</td>
<td>Continuous motion of forward movement</td>
</tr>
<tr>
<td>Events</td>
<td>Scrotal area grooming</td>
<td>Licking scrotal area</td>
</tr>
<tr>
<td></td>
<td>Hind leg stomping</td>
<td>Stomping of either hind foot on ground</td>
</tr>
<tr>
<td></td>
<td>Weight shifting</td>
<td>Lifting either foot while maintaining balance</td>
</tr>
<tr>
<td></td>
<td>Tail swishing</td>
<td>Movement of tail from side to side</td>
</tr>
<tr>
<td></td>
<td>Urinate/Defecate</td>
<td>Excretion of bodily fluid / solid</td>
</tr>
</tbody>
</table>
Table 4.2 Terms used by observers to describe qualitative behaviour expression of calves during and immediately after surgical castration

<table>
<thead>
<tr>
<th>GPA Dimensions</th>
<th>Low values</th>
<th>High values</th>
<th>Treatment Effect (MANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) during castration</td>
<td><strong>1 (66.2%)</strong> Calm (8), Relaxed (5), Contented (3), Comfortable, Laid-back, Happy, Carefree, Docile</td>
<td>Agitated (7), Restless (5), Frightened (3), Frustrated (2), Unsettled (2), Anxious (2), Irritated (2), Stressed (2), Uncomfortable (2), In_pain, Hurt, Sore, Disquieted, Excited, Aware, Alert, Defensive, Bothered, Cautious, Nervous, Afraid, Scared</td>
<td>$F_{5,95} = 46.35, P &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td>*Stand (struggling)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*Head shaking / struggling</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*Head lowering to ground</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>2 (10.5%)</strong> Playful, Uneasy, Timid Trapped, Sad, In pain Tired</td>
<td>Unsure</td>
<td>$F_{5,95} = 5.98, P &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td>*Stand (hunched)</td>
<td>*Swallowing saliva</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>3 (5.1%)</strong> Sore, Frustrated, Anxious, Listening, Impatient</td>
<td>Sleepy (2), Depressed, Tired</td>
<td>$F_{5,95} = 14.90, P &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td>*Head lowering to ground</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) post-castration</td>
<td><strong>1 (50.1%)</strong> Agitated (4), Anxious (4), Restless (3), Nervous (2), Frightened (2), Excited (2), Startled, Energetic, Stressed Alert, Irritated, Anxious, Scared, Annoyed, Disturbed Tensed, Edgy</td>
<td>Calm (5), Relaxed (3), Comfortable (2), Contented, Chilled, Settled, Quiet, Happy, Patient, Aimless</td>
<td>$F_{5,145} = 21.91, P &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td>*Walk (backwards)</td>
<td>*Walk (forward)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*Running</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>2 (7.9%)</strong> Relaxed (2), Confused, Tired, Calm, Lonely</td>
<td>Watchful, Frightened Amused, Sociable Unsure, Angry</td>
<td>$F_{5,145} = 4.89, P &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td><strong>3 (5.4%)</strong> Angry (3), Annoyed (2), Inquisitive, Uncomfortable, Anxious, Unpleasant Aggressive, Confused Discontented, Restless, Stressed</td>
<td>Affectionate, Comfortable Playful, Motivated Excited</td>
<td>$F_{5,145} = 4.87, P &lt; 0.0001$</td>
</tr>
<tr>
<td>GPA Dimensions</td>
<td>Low values</td>
<td>High values</td>
<td>Treatment Effect (MANOVA)</td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td>-------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td></td>
<td>*Weight shifting</td>
<td>*Urinate/Defecate</td>
<td></td>
</tr>
</tbody>
</table>

GPA = Generalised Procrustes Analysis
†The percentage of variation explained by each GPA dimension is shown in brackets.
‡Terms that had 75% of the maximum absolute correlation value (Mardia et al., 1979) are shown for each end of the GPA dimension axis. Terms order is determined first by the number of observers to use each term (in brackets if >1), and second by weighing of each term. *indicates time budget categories behaviour that significantly correlated with the GPA dimension scores (*P < 0.05); shown on either left or right column.
Session (a) during castration

![Graph](Image)

Session (b) post-castration

![Graph](Image)

Figures 4.1 (a-f). Comparison of Qualitative Behavioural Expression during castration (a, b, c) and post castration (d, e, f).

NOTE: Letters link treatment groups that were not significantly different to each other.
5 Qualitative Behavioural Assessment to distinguish surgically castrated cattle from non-castrated and analgesia controls

5.1 Abstract
There are various ways to perform cattle castration, but surgical castration is adopted by most practitioners. Although it is well documented that surgical procedures inflict pain, analgesic use is routinely omitted in surgical castration in food animals. This study compares the behavioural responses of castrated cattle (C) and non-castrated (NC) controls with those castrated and given lignocaine and meloxicam (CLMpost) in the context of the home paddock and feeder yard. Eight (8) Brahman bull calves in each treatment group were filmed in the morning pre (day -1) and post (day +1) castration in the paddock and feeder yard. Video footage was shown to volunteer observers for analysis over four sessions using Qualitative Behaviour Analysis (QBA). Using Free Choice Profiling methodology, the consensus profile of the variation among observers for the analysis explained 37.4% and 40.6% for paddock sessions and 34.7% and 38.7% for feeder yard sessions. There were significant Treatment x Day interaction effects for GPA dimension 2 ($P = 0.007$) and 3 ($P < 0.001$) for the paddock; and GPA dimension 1 ($P = 0.004$) and 2 ($P = 0.025$) for feeder yard sessions. In the paddock, calves in C group on Day -1, GPA 2 were described as more ‘happy’, ‘contented’ and ‘relaxed’ than NC, and on Day +1 calves in C group were described as more ‘lethargic’, ‘disinterested’ and ‘bored’ than NC. In GPA 3, on Day -1 calves in C group were more ‘curious’, ‘itchy’ and ‘lonely’ than NC, but were more ‘alone’ on Day +1 than NC group. In the yard, calves in C group on Day -1, GPA 1 were described as more ‘agitated’, ‘anxious’ and ‘nervous’ than NC, and on Day +1 calves in C group were described as more ‘calm’, ‘relaxed’ and ‘comfortable’ than NC. In GPA 2, and on Day -1 calves in C group were described as more ‘relaxed’, ‘confused’ and ‘tired’
than NC but were less ‘watchful’, ‘frightened’ and ‘amused’ on Day +1 than NC. Quantitative behaviour measures were recorded in the paddock using Composite behaviour scoring (CBS). There was some correlation between qualitative and quantitative measures of behaviour; ruminating had significant correlation with GPA dimensions 3 (paddock) and GPA 2 (feeder). QBA showed there were significant differences between treatments; C were less active than NC and CLMpost. This study demonstrates that *Bos indicus* bulls are likely to benefit from the administration of analgesia at the time of castration. The study highlights the complexities and challenges of identifying pain responses in Brahman cattle.

**Keywords:** Cattle, Castration, Analgesia, Pain, Qualitative Behavioural Analysis (QBA), Generalised Procrustes Analysis (GPA)
5.2 Introduction

Castration of male calves is a routine husbandry procedure that is commonly performed without analgesia around the world (Lomax and Windsor, 2013). It is considered necessary for economic, safety and management reasons (Lomax and Windsor, 2013). There are three (3) common methods of castration (Phillips, 2010): 1) surgical removal of testes, 2) applying a rubber ring around the scrotum to cut off the blood supply to the testes, and 3) crushing the spermatic cord with a Burdizzo instrument (Phillips, 2010). Surgical castration is the most common method used in Australia and the USA, making up 60% and 57% of castration procedures, respectively (Coetzee et al., 2010).

It has been well-documented that castration is painful (Molony et al., 1995; Fisher et al., 1996; Petherick, 2006; Coetzee, 2013a). Nevertheless, there is no legal requirement for providing analgesia for castration of cattle under 6 months of age in or under 12 months if at first muster, according to the current Australian Code of Practice (PISC, 2004). In the USA, there no requirement for pain management for cattle castration, and there are no analgesic drugs approved for pain relief in cattle (FDA, 2015). With the increasing public interest in animal welfare and production, it is suggested that further refinement of such a common procedure is warranted.

Pain can substantially reduce animal well-being and prolong the time needed for recovery from the underlying condition (Hellyer, 1998; Muir and Woolf, 2001). Protracted pain results when analgesia is not used for surgical procedures, such as castration and dehorning (Molony et al., 1995; Stafford and Mellor, 2005a). Some veterinary practitioners who do not use analgesia believe that young animals generally do not require analgesia for routine elective surgeries such as castration or dehorning (Hewson et al., 2007a; b). However, studies have
shown that no matter which method was used these procedures still causes protracted pain lasting several days to weeks (Hay et al., 2003; Stafford and Mellor, 2005a).

Perioperative analgesics are regularly administered to companion animals such as cats and dogs, but not for livestock. Hewson et al. (2007a) surveyed livestock veterinary practitioners who mostly agreed that there is no long-acting, cost effective analgesic available for use in livestock and the long or unknown withdrawal periods of some drugs outweighed the benefits of using them. Analgesic agents available for livestock use are mainly non-steroidal anti-inflammatory analgesic drugs (NSAIDs) such as Flunixin, Meloxicam, and Ketoprofen. Procaine and Lignocaine are the most frequently used local anaesthetics (Huxley and Whay, 2006). It would be beneficial to use analgesia on livestock during painful husbandry procedures for their welfare, and it serves to keep the animal still, thus providing safety for the practitioners involved (Hewson et al., 2007b).

Pain warns an animal that tissue damage might occur, is occurring or has occurred, thereby eliciting or allowing immediate escape, withdrawal or other behaviour (Mellor et al., 2000). Pain is therefore an important aspect of how animals protect their body and maintain their health. However, there is also selection against the obvious expression of pain in prey species, which might single them out as vulnerable. Consequently, the presence of pain in cattle may be difficult to determine or quantify. Fitzpatrick et al. (2002) indicated that cattle practitioners would find it useful to have a formal method of assessing pain in practice, and several previous studies have used behavioural responses as an indicator of pain associated with castration (Thüer et al., 2007; Petherick et al., 2014).

One method of assessing animal behaviour is through Qualitative Behavioural Assessment (QBA), which uses a whole-animal methodology to assesses the expressive qualities of animal
demeanour, using descriptors such as “content”, “relaxed” or “anxious” (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001). QBA uses a Free-Choice Profiling (FCP) methodology that relies on human observers generating descriptors or a fixed list of terms provided to observers to score the animals (Wemelsfelder et al., 2001).

The aim of this study was to determine if *Bos indicus* cattle exhibit pain in behaviour when undergoing surgical castration, with and without analgesia. We compared the behavioural expression of cattle exposed to different analgesic regimes, using video footage of bulls collected in paddock and in the feeder-yard pre (Day -1) and post-castration (Day +1). Specifically, we compared:

(i) Cattle castrated without analgesia (*C*) with cattle that were not castrated (*NC*)

(ii) Cattle castrated without analgesia (*C*) with cattle that received local anaesthesia (LA - Lidocaine) and a non-steroidal anti-inflammatory drug (NSAID – Meloxicam) (*CLM post*)

Each treatment group contained eight (8) *Bos indicus* bull calves (*n* = 8). Video footage were shown to volunteer observers for scoring using QBA. We predicted that after surgery, the cattle castrated without any analgesia (*C*) would show more pain related behaviour than cattle not castrated (*NC*) and then cattle given analgesia (*CLM post*). In addition, we expected castrated cattle would show an increase in the frequency of pain related behaviours the day after castration when compared to the day before.

5.3 Methods

This study was approved by the Animal Ethics (Permit Number R2551/13) and Human Ethics (Permit Number 2008/021) committees, Murdoch University, Western Australia.
5.3.1 Animals and video recording

Forty-eight (48), six (6) to eight (8) month-old *Bos indicus* (Brahman) bull calves with a mean weight of 165.5 (±17.5) kg were sourced from an extensive cattle station in the north-west region of Australia (Pilbara region). They were not used to contact with humans and were transported to Murdoch University farm, South Street, Western Australia in winter 2013 for the duration of the project. The calves were held in the same paddock for eight (8) days for acclimation post-arrival, during which time they were moved through a race, and then held individually in a crush to be for habituation and sampling of other physiological baseline measures. These measures included bodyweight, pedometry, blood cortisol and nociceptive threshold testing and have been described elsewhere (Laurence et al., 2016; Musk et al., 2016). Eight (8) individuals were randomly allocated to each of the treatment groups:

- Non-castrated (NC) calves were held in the crush for the same duration (five (5) minutes) as that taken for surgical castration.
- Castrated (C) calves were surgically castrated while standing in the crush.
- Castrated with analgesia (CLMpost) calves were administered Lignocaine (2mg/kg, Lignocaine 20, Ilium, Troy Laboratories, Glendenning, NSW, Australia) into each testicle five minutes pre-castration, surgically castrated and then administered Meloxicam subcutaneously (0.5mg/kg, Meloxicam 20, Ilium, Troy Laboratories, Glendenning, NSW, Australia) immediately after castration as described in Laurence et al. (2016).

Calves were identified by numerical ID tags in their right ears and numerals spray painted on their rumps. Animals were filmed daily using a handheld digital Panasonic SDR-H250 camcorders in two contexts. Each was recorded for two (2) minutes firstly, in the paddock
(07:00 to 08:00) when they were undisturbed, and again at the time of morning feeding in a small yard, near the feeder (11:00 to 13:00) after they had been moved through the race for handling. Binoculars were used to allow ease of identification of animals in the paddock. One (1) observer recorded quantitative measures of behaviour pre-and post-castration in the paddock as described in chapter 3.

Table 5.1 Treatment groups and locations used in the study.

<table>
<thead>
<tr>
<th>Session series:</th>
<th>Treatment groups:</th>
<th>Location:</th>
<th>Number of observers:</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A).</td>
<td>• C vs NC</td>
<td>Paddock (Day -1 &amp; +1)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>• C vs CLM&lt;sub&gt;post&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B).</td>
<td>• C vs NC</td>
<td>Feeder (Day -1 &amp; +1)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>• C vs CLM&lt;sub&gt;post&lt;/sub&gt;</td>
<td></td>
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</tr>
</tbody>
</table>

5.4 Qualitative Behavioural Assessment (QBA)

5.4.1 Observers

Details regarding observers can be obtained from section 2.4.2 above.

Observers were recruited by advertisements on notice boards around the Murdoch University campus, and by using university social media; and included university students, staff, primary producers and the general public.

Video footage was recorded and edited (Adobe Premier Pro CS3 and Adobe After Effects CS3, Chatswood, NSW, Australia) into forty (40) to fifty (50) second clips. Two (2) series of viewing sessions were held in separate months; each series compared two (2) pairs of treatments in either the paddock or feeder yard location (Table 5.1). All observers were naïve to the experimental treatments or the context in which the animals had been filmed. Each observer attended three (3) viewing sessions, on campus; a term generation session (where the scoring process was explained, and terms generated) and two (2) video scoring sessions (where
observers scored cattle in the clips). In the first scoring session, the observers were shown clips of the sixteen (16) calves filmed on day -1 and +1 from the C and NC groups. On the second scoring session, observers were shown clips filmed on day -1 and +1 from the C and CLM_post groups. Free Choice Profiling (FCP) methodology was used for scoring the video clips (Fleming et al., 2016), which relies on observers generating their own unique set of descriptive terms (Wemelsfelder et al., 2001). Observers attended term generating session where they were shown a series of clips that showed cattle exhibiting a range of behavioural expressions and they were asked to list descriptive terms that they believed described the cattle. During subsequent scoring sessions, each observer used their own set of unique descriptive terms to score the behaviour of the cattle using a visual analogue scale set up on an Excel spreadsheet, with observers asked to mark on the scale the intensity of the behaviour expression for each of their descriptive terms ranging from 0=minimum to 100=maximum. Excel files (Microsoft Excel 2013, North Ryde, NSW, Australia) for each individual observer were then analysed by Generalised Procrustes Analysis (GPA) using a specialised software edition written for Francoise Wemelsfelder (GenStat, 2008). A detailed description of GPA procedures can be found in (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001).

GPA develops a consensus profile describing all observers’ score by transforming all the data and identifying complex patterns. A Procrustes Statistic is calculated, quantifying the percentage of variation between observers that is explained by the consensus. A randomisation test (Dijksterhuis and Heiser, 1995) is then used to determine whether this consensus is a significant feature of the data set, and not simply an artefact of the Procrustean calculation procedures. The Procrustes Statistic is compared with the result of this randomisation test by one-way t-test to determine whether the actual observer consensus profile falls significantly outside the distribution of the randomised profiles (Fleming et al.,
Subsequently, the numbers of dimensions of the consensus profile if reduced through Principle Component Analysis (PCA).

The terms used by each observer to score cattle behaviour were correlated with each GPA dimension to identify terms that show the strongest correlations (>75% of the highest absolute correlation coefficient values) for that dimension (Mardia et al., 1979). Mixed-model ANOVA was used to test for significant treatment differences in the GPA scores (StatSoft, 2007) with day and treatment as fixed factors and observer as a random factor.

5.4.2 Quantitative Analysis

Each morning (07:00-08:00h), behavioural scoring was carried out in the paddock by one observer for two (2) minutes per animal on pre- and post-castration days using the list of behaviour described in Table 3.1 (Chapter 3).

5.5 Results
5.5.1 Qualitative Behavioural Assessment (QBA)

Twenty (20) observers from Series A (paddock) generated ninety-seven (97) unique terms; 14 ± 5 terms per observer, (7 - 23). Thirty observers from Series B (feeder yard) generated one hundred thirty-seven (137) unique terms; 15 ± 5 terms per observer, (7 - 28). The Procrustes statistic indicated that the GPA consensus profile explained 34.65% (C vs NC) and 38.72% (C vs CLM<sub>post</sub>) of variation among observers for paddock analysis, which varied significantly from the mean randomised profile ($t_{99} = 32.07; P < 0.001$) for C vs NC and ($t_{99} = 45.12; P < 0.001$) for C vs CLM<sub>post</sub>.

For the feeder yard analysis, the Procrustes statistic indicated the consensus profile explained 40.56% (C vs NC) and 37.37% (C vs CLM<sub>post</sub>) of the variation among observers for feeder
analysis, which varied significantly from the mean randomised profile ($t_{99} = 32.07; P < 0.001$) for C vs NC and ($t_{99} = 45.12; P < 0.001$) for C vs CLM post.

5.5.2 Terms

The list of terms associated with each of the GPA axes is shown in Table 5.2 (paddock) and Table 5.3 (feeder yard). For the paddock context in C vs NC, the most frequently used terms for GPA dimension 1 were relaxed / calm on the low end and curious / alert on the high end of the axis. For GPA 2, happy / contented were the most common terms on the low end and bored / lethargic on the high end. For GPA 3, curious / itchy were on the low end and alone on the high end of the axis. In C vs CLM post, comparison, the most frequently used terms for GPA 1 were uncomfortable / sleepy on the low end and happy / relaxed on the high end of the axis. For GPA 2, curious / alert were on the low end and docile on the high end. In GPA 3, friendly / tender were associated with the low end and curious / aware on the high end of the axis.

For the feeder yard context in C vs NC, the most common used terms for GPA dimension 1 were, agitated / anxious on the low end and calm / relaxed on the high end of the axis. For GPA 2, relaxed / confused on the low end and watchful / frightened on the high end. For GPA 3, angry / annoyed on the low end and affectionate / comfortable on the high end. In C vs CLM post comparison, for GPA 1, alert / tensed was associated on the low end and calm / relaxed on high end. For GPA 2, tired / sad on the low end and hungry / alert on the high end. Finally, for GPA 3, startled / unsure was associated on the low end and freedom / comfortable on the high end.
5.5.3 Treatment effects

The paddock analysis showed significant treatment by day effect for C vs NC in GPA 2 ($F_{1,19} = 9.01, P = 0.007$) and 3 ($F_{1,19} = 33.45, P < 0.0001$), and for C vs CLM post in GPA 2 ($F_{1,19} = 4.69, P = 0.043$) and GPA 3 ($F_{1,19} = 26.91, P < 0.0001$). For the feeder analysis, treatment by day interaction effects were shown for GPA 1 ($F_{1,29} = 10.01, P = 0.004$) and 2 ($F_{1,29} = 5.57, P = 0.025$) for C vs NC and only in GPA 2 ($F_{1,29} = 12.95, P = 0.001$) for C vs CLM post comparison.

Castrated cattle (C) in the paddock scored significantly higher for GPA dimension 2 on day -1 than day +1; cattle were scored as more ‘happy’ and ‘contented’ on day -1 and scored more ‘bored’ and ‘lethargic’ on day +1 compared with NC cattle (Figure 5.1A). For GPA dimension 3, C cattle were scored more ‘curious’ on day -1 and more ‘alone’ on day +1 compared to NC cattle, which scored more ‘curious’ on day +1.

In the paddock, C cattle in GPA dimension 2 were described as more ‘bored’ and ‘unsure’ on day -1 and more ‘docile’ on day +1. This compared to CLM post cattle that described as more ‘docile’ on day -1 and more ‘bored’ and ‘unsure’ on day +1 (Figure 5.2B). In GPA dimension 3, C were scored more ‘friendly’ and ‘tender’ on day -1 and more ‘curious’ and ‘aware’ on day +1 compared to CLM post described as more ‘curious’ and ‘aware’ on day -1, but more ‘friendly’ and ‘tender’ on day +1 (Figure 5.2C).

In the feeder yard analysis, for GPA dimension 1, C cattle were scored as more ‘calm’ and ‘relaxed’ on day -1 and more ‘agitated’ and ‘anxious’ on day +1 compared to NC, that were scored as more ‘anxious’ and ‘agitated’ on day +1 compared to NC that were scored as more ‘startled’ and ‘agitated’ on day -1 but more ‘comfortable’ and ‘contented’ on day +1. In GPA dimension 2, both C and NC were described as ‘confused’ and ‘relaxed’ on day -1 and ‘watchful’ and ‘frightened’ on day +1 (Figure 5.3B).
In the feeder yard, for GPA dimension 2, C and CLM\textsubscript{post} cattle were scored as more ‘tired’ and ‘sad’ on day -1 but on day +1, CLM\textsubscript{post} cattle were scored as more ‘hungry’ and ‘alert’ than C (Figure 5.4B).

5.5.4 Correlations between quantitative and qualitative behaviour

Ruminating was the only behaviour to significantly correlate with any GPA dimension score. Ruminating correlated with GPA dimension 3 \((r_{32} = -0.50, P < 0.05)\) in paddock (C vs CLM\textsubscript{post}) and GPA dimension 2 \((r_{32} = -0.38, P < 0.05)\) in feeder yard (C vs NC) (Table 5.3).

5.6 Discussion

Studies have shown that castration of cattle is painful, yet few studies have looked at the behavioural responses of unhandled \textit{Bos indicus} cattle post castration. In this study, C and NC cattle were described as ‘lethargic’ and ‘disinterested’ on the day after handling and surgery in the paddock, indicating there was no difference between their behaviour, despite one group undergoing castration. Both C and NC were in low mood post-surgery as compared to being described as ‘happy’ and ‘relaxed’ the day before. Being a prey species with little to no interaction with stockpersons, most likely meant the overall handling and surgery may have increased their pre-existing fear. C cattle in the feeder were described as more ‘comfortable’ on day +1 and hence less active than NC cattle. Less active movements can indicate cattle were experiencing some pain from the surgical procedure, or it might represent a fearful or ‘frozen’ state. When compared with C cattle, pain relief appeared to provide some comfort as CLM\textsubscript{post} cattle displayed a more sociable behaviour, described as ‘friendly’ on day +1. However, there were also terms such as ‘tender’ and ‘uncertain’ being used in the same GPA dimension 3 which is quite different from ‘friendly’. This variation in term use could be due to an individual observer having a different view on the observed cattle behaviour hence the
variation. In the feeder yard, on GPA 2, both C and CLM post groups were scored similarly on day -1, however on day +1, C were described as less ‘hungry’ and more ‘tired’ and ‘sad’ than CLM post.

This study showed that typical pain related behaviour were displayed from cattle across all three (3) treatment groups. This can be explained by the fact that domesticated cattle were descended from wild cattle, which were prone to attack by predators, and therefore have strong evolutionary pressure that allows them to mask any signs of pain as this may imply weakness (Phillips, 2002). Hence, caution is needed when interpreting behavioural responses in cattle, and it suggests that cattle described as ‘quiet’ or ‘calm’ may not necessarily be free from pain. This display of behaviour was not consistent throughout all three (3) GPA analyses as even the NC cattle expected to be ‘calm’ and ‘happy’ did not consistently show quiet and calm behaviour. This could be due to cattle not habituated and still very reactive to handling. Paddock-born calves were difficult to handle, spending greater time running, attempts to escape and behaving aggressively towards handlers compared to calves born indoors, which were much easier to handle as they had frequent exposure to humans (Petherick et al., 2009).

The calves’ interaction with their environment and humans will reflect in their behavioural expression and is one of the key aspects of studying animal behaviour.

Fleming et al. (2016) stated that QBA can provide an assessment of the animal’s whole response to its environment and what is happening to it. QBA therefore measures ‘outcomes’, and can contribute to welfare assessment because it can capture variation in how animals respond to and deal with their environment at that instant (Fleming et al., 2016). This study showed that QBA, is a flexible assessment tool as it can be applied in different contexts, such as the paddock, feeder yard and in crush.
Interestingly, not all behaviour displayed by the calves that had surgery was negative. There were various descriptive terms used by observers describing cattle as ‘excited’ and ‘chilled’ on either pre-or post-castration day. This display of positive aspects of animal behaviour could be explained by the fact that these calves were kept with the presence of their herd mates, which reduces their emotional response (Phillips, 2010). Being placed in the feeder yard with others with fresh food pellets daily may have provided a positive environmental distraction for them. Especially as most would be highly engaged in eating, find it pleasurable and some show signs of curiosity with the feeder itself. QBA is one of the few current measures that capture positive aspects of animal welfare, such as animals being positively engaged with their environment, including being active, or alert (Keeling et al., 2013).

Cattle subjected to painful procedures will always be a challenge for both clinicians and handlers as recognising the signs of pain in these stoical species is difficult (Fitzpatrick et al., 1999; Barrett, 2004). Rutherford (2002) stated the importance of developing methods, such as observations of behaviour and measurement of physiological responses, which can be used to assess pain objectively. QBA can be applied under a range of conditions and can identify subtle differences in qualitative behavioural expression (Fleming et al., 2016). Body language is dynamic, so it allows QBA allows to capture subtle changes in an animal’s body language that can be important for welfare assessment and may otherwise be overlooked when individual behaviours are isolated and quantified (Wemelsfelder, 1997; Wemelsfelder, 2007; Meagher, 2009; Whitham and Wielebnowski, 2009).

Terms that the observers used appeared to make sense. At the feeder yard, observers viewed calves in C group as more ‘tired’ and ‘sad’ but calves in CLM\textsubscript{post} as more ‘hungry’ and ‘excited’. At first glance and in relation to the descriptive terms, it may seem that the terms do not
clearly describe cattle behaviour appropriately. However, these terms were a generalised view of behavioural display of the animal in context with the environment they were placed in. Hence, the term ‘hungry’ was used to describe the body language displayed as calves moved around the feed trough. C calves that we expected to be in pain, could have been described as tired and sad as they showed less energy or desire to seek food pellets.

Upon analysis of these terms, they appear describe what the animal may be feeling when comparing the two treatment groups. Those in the castrated with analgesia group ($CLM_{post}$) were viewed on a more positive aspect of behaviour, for example various studies have demonstrated that indicators of pain, such as, behavioural and physiological responses, are significantly reduced if NSAID are administered as part of the treatment protocol for surgical castration (Earley and Crowe, 2002; Stafford et al., 2002; Ting et al., 2003a; Ting et al., 2003b).

Huxley and Whay (2006) revealed that administering Meloxicam to cattle during dehorning showed a reduction in pain and distress. Therefore, it is quite clear that by providing some form of analgesia, it does benefit the animal’s well-being, and in return, a safer working condition for the practitioners and handlers is provided. There are some constraints as to why analgesia is not routinely given to livestock, such as the cost and type of analgesia approved for cattle use.

It is important that we do not disregard any behavioural display that may be deemed as ‘unusual’ or ‘inappropriate’ for cattle after performing painful husbandry procedures. Cattle may be masking fear or pain behaviour from observers or other species and may have felt the predatory threat due to their instinctive nature of being a prey species. Any subtle behaviour display needs to be taken into consideration, and studied at the whole animal level to fully understand what the animal is displaying.
The lack of clear treatment effect may be due to small sample size of this study. However, there were evidence that analgesia possibly alleviated some of the discomfort of castration. This was only possible though the application of QBA and needs to further this study with larger number of animals to see the extent of how provision of analgesia may provide more positive experiences for cattle. Welfare is an important focus of livestock production, which means that mitigating pain from any routine procedure performed on these animals throughout their life span should be desired goal for all.

5.7 Conclusion

The behavioural display of cattle can be dependent on the condition they are experiencing, as well as influence of the strong ancestral roots of wild cattle. It takes careful observation and behavioural analysis to understand the significance of their body language. According to our observers, some cattle behaviour such as, being calm, happy and excitable may be described as somewhat similar even the day after they have gone through unpleasant procedures. Some stockmen underrate the pain caused by castration, because of the traditional discounting of pain in livestock (Byrne et al., 2001) and the attitude that they are ‘just animals’ and possibly unable to experience such negative emotions, therefore not much attention to pain relief has traditionally been was given.

QBA may hold the key in breaking down barriers between the assumption that animals do not feel pain and improving ways to better understand animal behaviour. QBA can be used on its own or in conjunction with other physiological measures, such as blood cortisol levels, and pedometer and has the potential to become a useful assessment tool. The future of animal welfare and educating both professionals and the general public on the assessment of pain will be enhanced using QBA methodology. In conclusion, the application of QBA in this study
have been valuable and have given us the opportunity to look into pain related behaviour in cattle.
### 5.8 Appendix

**Table 5.2** Terms used by observers to describe qualitative behaviour expression of calves in paddock pre-and post-castration (Session A).

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>GPA Dimensions</th>
<th>Low values</th>
<th>High values</th>
<th>Significant</th>
<th>Non-significant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Castrated vs Non-castrated (C vs NC)</strong></td>
<td>1 (36.95%) †</td>
<td>Relaxed (5), Calm (5), Laid-back Chilled, Contented, Quiet Apathetic, Satisfied</td>
<td>Curious (3), Alert (3), Agitated (3), Stressed (2), Restless (2), Anxious, Disquieted, Distressed, Timid, Frightened, Cautious, Inquisitive, Nervous, Weary, Scared, Unsettled, Defensive, Lost, Uncomfortable</td>
<td>Day $F_{1,19} = 20.87$, $P = 0.0002$</td>
<td>Treatment $F_{1,19} = 2.87$, $P = 0.1066$ Treatment X Day $F_{1,19} = 0.055$, $P = 0.818$</td>
</tr>
<tr>
<td></td>
<td>2 (18.8%) †</td>
<td>Happy (2), Content, Relaxed, Active</td>
<td>Bored (3), Lethargic (2), Exhausted (2), Uncomfortable (2), Disinterested, Sleepy Tired, Sore</td>
<td>Day $F_{1,19} = 22.12$, $P = 0.0001$ Treatment X Day interaction $F_{1,19} = 9.01$, $P = 0.007$</td>
<td>Treatment $F_{1,19} = 0.17$, $P = 0.68$</td>
</tr>
<tr>
<td></td>
<td>3 (9.9%) †</td>
<td>Curious (2), Itchy (2), Lonely (2), Anxious, Cautious, Bored, Impatient, Weary, Hesitant, Trapped, Afraid, Agitated Confused</td>
<td>Alone</td>
<td>Day $F_{1,19} = 10.60$, $P = 0.004$ Treatment X Day interaction $F_{1,19} = 33.45$, $P &lt; 0.0001$</td>
<td>Treatment $F_{1,19} = 0.63$, $P = 0.438$</td>
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<td><strong>Castrated vs Castrated with analgesia</strong></td>
<td>1 (26.8%) †</td>
<td>Uncomfortable (4), Sleepy (2), Agitated,</td>
<td>Happy (4), Relaxed (4), Contented (3), Calm, Excited, Aware</td>
<td>Day $F_{1,19} = 44.02$, $P &lt; 0.0001$</td>
<td>Treatment $F_{1,19} = 1.36$, $P = 0.259$ Treatment X Day</td>
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<tr>
<td>Treatment Groups</td>
<td>GPA Dimensions</td>
<td>Low values</td>
<td>High values</td>
<td>Significant</td>
<td>Non-significant</td>
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<td>(C \text{ vs CLM}_{post})</td>
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<td>Unsettled, Depressed, Timid, Tired, Unsure, Disinterested In_pain, Stressed, Sad Nervous</td>
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<td>(F_{1,19} = 1.50, P = 0.235)</td>
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<td>2 (17.9%) †</td>
<td>Curious (6), Alert (2), Inquisitive (2), Bored, Unsure, Excited, Distressed, Aware, Frustrated, Weary</td>
<td>Docile</td>
<td>Treatment (F_{1,19} = 11.82, P = 0.003) Treatment X Day interaction (F_{1,19} = 4.69, P = 0.043)</td>
<td>Day (F_{1,19} = 0.36, P = 0.556)</td>
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<td>3 (13.3%) †</td>
<td>Friendly, Tender, Uncertain, Tired, Uncomfortable Satisfied, Not afraid Comfortable, Calm</td>
<td>Curious, Aware, Dominant</td>
<td>Treatment (F_{1,19} = 6.23, P = 0.022) Treatment X Day interaction (F_{1,19} = 26.91, P &lt; 0.0001)</td>
<td>Day (F_{1,19} = 0.82, P = 0.375)</td>
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GPA = Generalised Procrustes Analysis
†The percentage of variation explained by each GPA dimension is shown in brackets.
‡Terms that had 75% of the maximum absolute correlation value (Mardia et al., 1979) are shown for each end of the GPA dimension axis. Terms order is determined first by the number of observers to use each term (in brackets if >1), and second by weighing of each term. Italic indicates time budget categories behaviour that significantly correlated with the GPA dimension scores (*\(P < 0.05\)); shown on the left-hand column as they were negative correlated with the axis.
§Summary of the mixed model analysis (MANOVA) for each GPA dimension showing significant / non-significant results for Day, Treatment groups or Day and Treatment groups interactions.
Table 5.3 Terms used by observers to describe qualitative behaviour expression of calves in feeder pre- and post-castration (Session B).

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>GPA Dimensions</th>
<th>Low values</th>
<th>High values</th>
<th>Significant</th>
<th>Non-significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrated vs Non-castrated (C vs NC)</td>
<td>1 (30.7%) †</td>
<td>Agitated (4), Anxious (4), Nervous (2), Restless (2), Frightened (2), Excited (2), Startled, Energetic, Stressed, Alert, Irritated, Scared, Annoyed, Disturbed, Tensed, Edgy</td>
<td>Calm (5), Relaxed (3), Comfortable (2), Contented, Chilled, Settled, Quiet, Happy, Patient, Aimless</td>
<td>Treatment $F_{1,29} = 9.13$, $P = 0.005$ Treatment X Day interaction $F_{1,29} = 10.01$, $P = 0.004$</td>
<td>Day $F_{1,19} = 1.655$, $P = 0.208$</td>
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<td>2 (16.3%) †</td>
<td>Relaxed (2), Confused, Tired, Calm, Lonely *Ruminating</td>
<td>Watchful, Frightened, Amused, Sociable, Unsure, Angry</td>
<td>Day $F_{1,29} = 20.07$, $P = 0.0001$ Treatment $F_{1,29} = 26.23$, $P &lt; 0.0001$ Treatment X Day interaction $F_{1,29} = 5.57$, $P = 0.025$</td>
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<td>3 (8.8%) †</td>
<td>Angry (3), Annoyed (2), Inquisitive, Uncomfortable, Anxious, Unpleasant, Aggressive, Confused, Discontented, Restless, Stressed</td>
<td>Affectionate, Comfortable, Playful, Motivated, Excited</td>
<td>Day $F_{1,29} = 15.99$, $P = 0.0004$ Treatment $F_{1,29} = 21.10$, $P &lt; 0.0001$</td>
<td>Treatment X Day $F_{1,29} = 0.07$, $P = 0.792$</td>
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<tr>
<td>Treatment Groups</td>
<td>GPA Dimensions</td>
<td>Low values</td>
<td>High values</td>
<td>Significant</td>
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<td>Castrated vs Castrated with analgesia (C vs CLM&lt;sub&gt;post&lt;/sub&gt;)</td>
<td>1 (39%) †</td>
<td>Alert (3), Tense (2), Agitated (2), Excited, Frightened Stressed, Nervous Restless, Anxious Uncomfortable Dominant, Irritated On_edge, Tensed Scared, Edgy</td>
<td>Calm (4), Relaxed (4), Contented (2), Settled, Chilled, Comfortable, Quiet, Enjoying</td>
<td>Day F&lt;sub&gt;1,29&lt;/sub&gt; = 7.78, P = 0.009</td>
<td>Treatment F&lt;sub&gt;1,29&lt;/sub&gt; = 0.04, P = 0.838 Treatment X Day F&lt;sub&gt;1,29&lt;/sub&gt; = 3.41, P = 0.075</td>
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<td>2 (15.1%) †</td>
<td>Tired (7), Sad (6), Bored, Lonely Restless, Lethargic Depressed, Drowsy Deflated, Disoriented Sick, Lifeless</td>
<td>Hungry (2), Alert, Excited</td>
<td>Day F&lt;sub&gt;1,29&lt;/sub&gt; = 22.55, P &lt; 0.0001 Treatment F&lt;sub&gt;1,29&lt;/sub&gt; = 8.08, P = 0.008 Treatment X Day interaction F&lt;sub&gt;1,29&lt;/sub&gt; = 12.95, P = 0.001</td>
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<td>3 (9%) †</td>
<td>Startled, Unsure</td>
<td>Freedom, Comfortable Placid, Happy, Relaxed Agitated</td>
<td>Day F&lt;sub&gt;1,29&lt;/sub&gt; = 14.77, P = 0.0006 Treatment F&lt;sub&gt;1,29&lt;/sub&gt; = 18.94, P = 0.0001</td>
<td>Treatment X Day F&lt;sub&gt;1,29&lt;/sub&gt; = 0.01, P = 0.935</td>
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</tbody>
</table>

GPA = Generalised Procrustes Analysis

†The percentage of variation explained by each GPA dimension is shown in brackets.

‡Terms that had 75% of the maximum absolute correlation value (Mardia et al., 1979) are shown for each end of the GPA dimension axis. Terms order is determined first by the number of observers to use each term (in brackets if >1), and second by weighing of each term.

Italics indicates time budget categories behaviour that significantly correlated with the GPA dimension scores (*P < 0.05); shown on the left-hand column as they were negative correlated with the axis.

§Summary of the mixed model analysis (MANOVA) for each GPA dimension showing significant / non-significant results for Day, Treatment groups or Day and Treatment groups interaction.
Figure 5.1 QBA Session A (paddock) – C vs NC cattle for each GPA dimensions

Figure 5.2 QBA Session A (paddock) – C vs CLM_post cattle for all GPA dimensions
NOTE: Letters link treatment groups that were not significantly different to each other
Figure 5.3 QBA Session A (feeder yard) – C vs NC cattle for all GPA dimensions

Figure 5.4 QBA Session B (feeder) – C vs CLM\textsubscript{post} cattle for all GPA dimensions

NOTE: Letters link treatment groups that were not significantly different to each other
6 General Discussion

The Australian livestock industry is one of the world’s largest and most efficient commercial livestock industries and Australia is a major exporter of red meat and livestock; with an estimated total of 26.3 million head of beef cattle (ABS, 2013-14). Outback cattle stations are prevalent, where cattle graze free range over extensive areas with minimal handling by stockmen. Usually, cattle only encounter stockmen once yearly in the mustering season. Hence, most cattle are neither monitored closely nor habituated to humans. Therefore, even for simple routine husbandry procedures such as vaccination and ear-tagging, cattle will be in a stressed and fearful state. *Bos indicus* cattle are more excitable than *Bos taurus* cattle (Hearnshaw and Morris, 1984; Fordyce et al., 1988) and cattle reared in extensive systems are often more aggressive compared with cattle reared in intensive conditions because of less frequent interaction with humans (Fordyce et al., 1985).

The calves in this study appeared to express fear when directly handled by humans, during castration and the taking of physiological measurements. Cattle with excitable temperaments may have reduced feed intake compared with cohorts with less excitable temperaments (Fox et al., 2004; Nkrumah et al., 2007) and heightened stress-related physiological responses compared with calmer cohorts (Burdick et al., 2011). In addition, these cattle have heightened inflammatory and acute-phase responses following a stress stimulus (Hulbert et al., 2009), which is not a good welfare outcome. Having experienced the pain of surgical castration and the stress of being restrained in the crush, their behaviour was likely to have been a demonstration of a fear response. Hence, certain descriptive terms did not align with the behaviour expected from their analgesic groups. For example, a bull described as calm could be keeping very still thus masking any pain behaviour just to cope with the fearful situation.
or environment. The terms used to describe cattle in this study had polar opposite meanings; from displaying positive type behaviours such as ‘calm’, ‘relaxed’ to negative type of behaviours such as ‘annoyed’, agitated’ could very well be their coping mechanism. Some situations made understanding the complexity of their behavioural display hard to decipher.

Quantifying cattle behaviour using CBS in the first part of the study (Chapter 3) did not reveal differences in what the calves were experiencing while in the home paddock or during surgery in the crush. Surprisingly, there was also no variance in treatment when QBA was applied during surgery (Chapter 4). Thus, we believe that the stress and fear of handling these previously unhandled cattle might have been an overwhelming factor for their emotional state. Observers were not able to distinguish between analgesic treatment groups while cattle were in the crush, even though we do believe that castration would have been painful.

In a study done by Barrett et al. (2016) on pain-related behaviour in cattle following liver biopsy, there were no significant differences in behaviour between any treatment groups and it was postulated that only mild pain existed, which was not detected. However, cattle might be masking pain due to their behavioural evolution as prey species, to avoid any displays of abnormal behaviour that would make them vulnerable to predators (Livingston, 2010).

As cattle exited the crush, immediately after castration, there were detectable treatment differences. This might be interpreted that there was a sense of immediate relief (no foreign object blocking their path) when the crush gate was opened, and with the reduced stress and fear, cattle could exhibit their expression of pain behaviour. In addition, observers might also be context sensitive as they might have viewed all cattle in the crush as in a ‘negative’ situation and being ‘restrained’ unlike cattle outside the crush that had possibility of ‘freedom’. By conducting a comparison of free-choice profiling vs. fixed list for QBA Clarke et
al. (2016b), could determine if observers rating was influenced by context. Performing inter- and intra-observer reliability testing and cross-validation with other measures are crucial, as they are for all animal-based health and welfare indicators (Tuyttens et al., 2014).

Animal behaviour is determined by both the physical and mental state of an animal. An animal’s emotional state can be highlighted by its behavioural display such as decreased rumination, which is regarded as a general indicator of pain in cattle (Stafford, 2013), or abnormal standing, which has been documented as a pain-related response (McMeekan et al., 1999; Sylvester et al., 2004; Almeida et al., 2008; Kolkman et al., 2010; Petherick et al., 2014). However, fear might be so dominating that cattle remain motionless, which means some subtle stress behaviours are missed. Some observers might interpret this ‘frozen with fear’ state as cattle in a calm state and not experiencing any pain. Reluctance to move is indicative of pain (Molony et al., 1995; Stafford and Mellor, 2005b), and standing immobile is also a pain-related response that was previously reported in surgically castrated calves (Molony et al., 1995).

Several factors may limit our ability to interpret pain behaviour; removing fear responses and increasing time points for monitoring may assist with better interpreting their behaviour. Our results after surgery, showed that the analgesia could mitigate pain associated with surgical castration. This was supported by results from a companion study conducted by Laurence et al. (2016) which showed a decrease in blood cortisol concentration and weight gain in cattle that received a form of analgesia. Cattle that received meloxicam either pre or post castration were more active with fewer rest bouts according to the pedometer results in Laurence et al. (2016), which were similar to our findings of cattle that received the combined form of analgesia were described as more ‘calm’ and ‘relaxed’ like that of the non-castrated (NC)
group. Nociceptive threshold testing (NTT) was performed on these cattle for another companion study (Musk et al., 2016), but the results were inconclusive. However, NTT decreased during the course of study in all groups (Musk et al., 2016). This showed that habituation for these cattle is crucial especially to the handling process. If cattle were exposed to routine handling longer before castration, for example having arrived at the university farm a month prior to surgery with regular exposure to people as well as being put through the race and crush, then there is a possibility that their behavioural response to surgery (pain related behaviour) could be much clearer to interpret rather than being overwhelmed by fear or stress. Musk et al. (2016)

It was postulated that pain and associated stress with the injection of lignocaine, followed by the anticipation of that painful event at each sampling occasion, was enough to mask the beneficial effects of meloxicam (Laurence et al., 2016). This was suggested by the ambiguous results seen when comparing the behavioural observations of cattle expected to show no pain (NC), mild pain (CLMpost) or maximum pain (C).

Perhaps, a comparison between two (2) herds of cattle exposed to different stimuli in a longitudinal study in their behavioural display might enable one to use the list of behaviour displayed as a guideline for future studies. For example, observing cattle that had numerous painful or unpleasant procedures while another group of cattle were handled, without any pain involved. Therefore, when pain relief is provided there might be a possibility to distinguish differences in the behaviour of cattle experiencing painful stimuli. Another important factor which could be corrected in future studies is to observe as much of the animal body rather than just the head or parts of the body, as this limit viewing angles for observers which could result in incorrect judgements. QBA is the study of the whole body and
by applying QBA only on the head region will not make use of its full potential as an assessment tool.

Using behaviour assessment to study pain is practical as it is cost effective and non-invasive, contrary to physiological measurements. Pain is subjective and can only be measured indirectly; yet using behaviour is a more sensitive indicator of pain than cortisol and other physiological measures (Anil et al., 2002; Heinrich et al., 2009). QBA was used to test for effects of analgesia and to validate its accuracy by comparing two control groups (C versus NC) while in paddock and feeder yard for Chapter 5. It highlighted that Bos indicus bulls were likely to benefit from the use of analgesia and proved that QBA methodology for pain assessment is useful. However, any behaviour studies on prey species such as unhandled cattle require several larger sample sizes to confirm accuracy of treatments prior to on-farm application.

The lack of a consistent significant effect between the Meloxicam, Lignocaine and control groups may be caused by lack of power. It is feasible that effective plasma levels of Meloxicam were not present at the start of surgery, though further pharmacokinetic information for Meloxicam use in Bos indicus cattle must be gathered to fully understand this. A relatively small sample size can make detecting significant differences difficult, particularly in studies using large animals as we have, as often resources are restricted by logistics, facilities and financial constraints.

6.1 General Conclusion

QBA is a relative measure that is capable of detecting subtle differences in the behavioural expressions of animals (Fleming et al., 2016) and has proven to be applicable in several other behavioural studies such as sow housing (Clarke et al., 2016a), sheep and cattle road
transportation (Stockman et al., 2011; Wickham et al., 2012), piglet weaning (Lau et al., 2014), and cattle temperament (Stockman et al., 2012). QBA can also be used to measure handlers’ behaviour (Ellingsen et al., 2014), which looked at dairy calves and their handlers. Therefore, it has the potential to be useful in assessing painful behaviour in Bos indicus cattle since results from our study as well as of Laurence et al. (2016) found possible benefit towards the welfare of these cattle when analgesia was provided.

Suggestions for improvement include having a larger sample size, longer periods of observations with more time-points when monitoring cattle behaviour, less number of treatment groups and longer time spent habituating the cattle on the premises prior to any procedure. Being habituated is crucial as Musk et al. (2016) found a decrease in NTT overtime and Laurence et al. (2016) reported there were evidence of reduced cortisol concentrations post-operatively, increased activity and favourable weight gain over time regardless of treatment group. Given plenty of time and resources, continuing to record and observe cattle behaviour well after their habituation period might have benefited the study. We would be able to draw a clearer conclusion on why they behave a certain way as well as a chance to compare before and after habituation behaviours. By making these adjustments, it is highly likely to obtain desirable results that can be translated into a useful on-farm behavioural assessment tool.
### Appendices

#### 7.1 Behaviour scoring in paddock (Composite Behavioural Scoring chart)

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<td><strong>Tail Swishing</strong></td>
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**TOTAL SCORE**

**COMMENTS**
7.1 Post-graduate Poster Day November 2013

**Behavioural Assessment of Brahman Calves**

**Post Surgical Castration**

**Thirza Vindisvogel, Teresa Collins, Tim Fleming**

School of Veterinary and Life Sciences, Murdoch University

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### Background
- Societal concern over painful husbandry procedures without the use of analgesia or anaesthesia exists e.g. castration, dehorning.
- In Northern Australia, beef cattle are routinely surgically castrated without anaesthesia (few drugs are licensed for producers to use on cattle).
- The impact of such practices on cattle welfare is unclear.
- Monitoring cattle and assessing pain can be a challenge, on extensive cattle stations with thousands of livestock.

### Aims & Hypothesis
- **Aims**
  - To compare various types of analgesia used during surgical castration on post-surgery behaviour of Brahman cattle.
  - To develop and test a quantitative behaviour scoring system (Composite Behavioural Scoring; CBS)
- **Hypothesis**
  - The post-surgery behaviour of animals will be improved by use of analgesia.

### Materials & Methods
- **Material**
  - 48 Brahman bull calves, n=8 in each treatment group.
- **Composite Behavioural Scoring (CBS) example scoring sheet**
- **Methods**
  - CBS recorded each morning:
    - Day 0 1
    - Prior to castration, immediately after castration and post-castration.
    - 2 groups of calves (n=24), Group A & B.
    - Data analysed using Statistical.
    - CBS results illustrated on graphs below.

### Composite Behavioural Score (CBS) Result
- Note: calves treated together as a group.
- Small difference in shifting patterns pre & post castration.

### Conclusion
- The use of analgesia showed very little difference in calves' behaviour post-castration when using CBS.
- However, CBS still has the potential to be useful in conjunction with other assessment tools.

### Significance of Study
- Pain assessment in cattle needs further study with Bos Indicus breed.
- Assist producers in improving their knowledge of pain management.
2013 Poster Day

Congratulations!

We would like to congratulate you on being awarded the

Centre for Animal Production
prize for the
Animal Production Research
poster category

This award is acknowledgment of your hard work and dedication at Murdoch University and represents an excellent achievement.

In order to redeem your prize please complete the slip below and return in the reply paid envelope enclosed or to the Vet Trust Office.

To ensure accurate processing of your prize payment we ask that details are provided by return slip only along with your thank you letter to the donor.

Thank you!
Title: Qualitative Behaviour Assessment of Brahman cattle post-surgical castration.

Castration is a painful procedure which is routinely performed on livestock. In this study, the behavioural expression of Brahman cattle pre and post-surgical castration were examined using Qualitative Behaviour Assessment (QBA). The objective was to compare the pain behaviour in cattle provided with different analgesic protocols using a non-steroidal anti-inflammatory drug and local anesthesia. Forty-eight Brahman bull calves were divided into six treatment groups ($n = 8$): 1) No castration (NC), 2) Castration with no analgesia (C), 3) Castration with Meloxicam administered prior to procedure (CMpre), 4) Castration with Meloxicam administered post procedure (CMpost), 5) Castration with Lidocaine (CLA) and 6) Castration with Lidocaine and Meloxicam post procedure (CLMpost). Video footage of cattle were recorded pre-castration (Day -1), immediately after castration and post-castration (Day +1) for analysis to determine any treatment effect. There were significant differences identified between (C) with the rest of the treatment groups ($p < 0.01$) among 30 observers in their assessment of behavioural expression immediately after castration. However, no significant differences between treatment groups were found on both pre (Day -1) and post (Day +1) castration days. There was significant inter-observer reliability, with 50.1% of the variation in their scoring explained by the consensus profile. (136/300)

Authors: A/Prof Trish Fleming, Dr Teresa Collins, Thinza Vindevoghel
LOOKING FOR SOMETHING TO DO AFTER EXAMS??
YOU CAN HELP DEVELOP A TOOL TO MONITOR OUR WELFARE!! :)

Just watch 2-3 video sessions of cattle & receive a gift-card upon completion. Everyone is welcome to participate!!

Please contact Thinza (t.vindevoghel@murdoch.edu.au) ASAP!!
Dear participants:

**Re: Development of Qualitative Behavioural Assessment (QBA) in Cattle**

My name is Thimza Vindevoghel and I am currently undertaking a Masters research project. This study involves the objective measurement and analysis of animal behaviour in order to improve and understand welfare standards in the agricultural industry. It is funded by Meat and Livestock Australia.

**Nature and purpose of the study**

There is a growing need to monitor and assess the welfare of domestic animals, and to develop methods that allow efficient and reliable measures of multiple facets of animal behaviour. This need has sparked interest from a number of production industries towards developing objective measures of animal welfare. Support for the concept has also been gained from animal welfare scientists across the world. Welfare measures need to be practical, convenient, cost-effective, reliable and replicable if they are to accurately reflect the true welfare state of the animal. The Qualitative Behavioural Assessment (QBA) that we are developing will enable comparative, hypothesis-driven evaluations of different practices. The approach we envisage will develop real, workable, and ‘uncontroversial’ welfare measures that have broad applicability to the livestock industry.

**What this study involves**

As part of this study you will be asked to complete questionnaires that ask about your demographics, experience with livestock and views on welfare of livestock in Australia. You will also be asked to view video footage of cattle and make a descriptive assessment of their behaviour in order to understand their experiences. There will be 4 viewing sessions and 5 forms to fill in. The very first session is called Term Generation Session where you provide terms that best describe the cattle behaviour and then followed by Scoring Sessions 1 to 3. You will be scoring the cattle on an excel spreadsheet based on the terms that you have provided.

**Voluntary participation and withdrawal from the study**

Your participation in this study is entirely voluntary. All information is treated as confidential and no names or other details that might identify you will be used in any publication arising from the research. You may withdraw without discrimination or prejudice anytime during the study prior to the results from the study being submitted to the funding body. If you withdraw, all information you have provided will be destroyed.

If you consent to take part in this research study, it is important that you understand the purpose of the study and the procedures you will be asked to undergo. Please make sure that you ask any questions you may have, and that all your questions have been answered to your satisfaction before you agree to participate. If you have any questions about this study, please feel free to contact myself, Thimza Vindevoghel (t.vindevoghel@murdoch.edu.au) or my supervisor Dr Teresa Collins (t.collins@murdoch.edu.au).

Thank you for your time and participation!

This study has been approved by the Murdoch University Human Research Ethics Committee (Approval No. 2001/021)
Dear participants:

**Please read the following before answering any surveys or watching the videos**

In the envelope you will find:
- Instruction sheet for Session 1
- A consent form
- An information letter
- Survey 1
- A term generation form
- A USB (containing the video clips)

Firstly, please read both the Information Letter and Consent Form, and sign the form if you agree to participate.

Next, please complete Survey 1. You will note at the top of the Survey 1, you are asked to generate a unique code so that during analysis you remain anonymous. This code is generated in the following way:

- the first 2 letters of your mother’s name
- the first 2 letters of your father’s name
- the date of the day you were born (e.g. if you were born on December 17th you would write 17, if you were born on February 4th you would write 04)

Your final code should look something like this – SIAL17

Please write this code on the top of all the surveys and the Term Generation Form.

Now that you are done with the survey and generating your code, please read the following to learn how to generate your terms before watching the video footage:

3 easy steps to generate your terms:

(i). Pay close attention to each animal that you are watching (each video clip is fairly short)
(ii). Watch their body language and focus consciously on how the animal is behaving / interacting
(iii.) As you watch what the animal is doing, think about how it might be feeling / experiencing
E.g., excited, frightened etc

It is easy!! © There are no right or wrong terms.

Now you can view the video clips and record your terms on the Term Generation Form according to the clip number.

Once done, please return all the documents including the USB (except the information letter) in the return envelope.

**Thank you!!**
Instruction Sheet
For Scoring Sessions

Dear participants:

**Please read the following before answering any surveys or watching the videos**

In the envelope you will find:
- Instruction sheet on how to score the sessions
- Survey No. 2
- A USB (containing the video clips & Excel scoring sheet 1)
- A return envelope (please return survey & USB)

Important instructions before scoring begins:

There will be an excel file (named accordingly) which is your scoring sheet and video footage in the USB provided. The excel file has been named according to your unique code which was generated in Term Generation Session. Please do not change the name of the excel file.

When you open the excel file, a macro box will appear. You must click ‘enable macros’. The file will not work without macros.

Note: If you have the new version of excel (Vista), when you open the excel file, directly above the worksheet will be a Security Warning that says ‘Macro’s have been disabled’, shown in the picture below:

Click on that box ‘enable this content’, then click ‘ok’.

If a macro box does not appear but you have a security warning instead, follow these commands:

In excel, go to the ‘Tools’ menu, click ‘Macro’ then ‘security’. Select the ‘medium’ option (you can change it back to your current setting after the study). Once you have clicked medium, close the excel file and reopen it. The macros box should now appear. Please click ‘enable macro’.

How to score your video clips:

Once you have enabled the macros, familiarise yourself with the scoring sheet. You will see that there are 48 yellow tabs that correspond to 48 video clips from Session 1. The other tabs are for analysis purposes and you do not need to use them.
Instruction Sheet
For Scoring Sessions

On each scoring sheet, you will see a list of terms which you have generated. You will be scoring each clip against these terms between Min and Max. Click on the line and 'X' will appear which indicate your score.

E.g., Min____________________X____________________Max

If you made a mistake, you can delete the 'X' and score again. Please score all the terms.

When you are ready to start viewing, have the scoring sheet open and ready so that after viewing each clip, you can score it instantly. Please make sure you are scoring the correct clip. E.g., Video clip 1 corresponds to yellow tab clip 1 on the Excel scoring sheet.

"Please make sure you save your scoring sheets after scoring a few clips and also at the end of the session on the USB to avoid losing your work"

Please keep this instruction sheet for reference when scoring Sessions 2 & 3.

Thank you once again for your time!
Your gift-card will be sent to you once all scoring sessions have been completed.
Survey 1

To keep your identity anonymous please create a unique code from the following:

First two letters of your mother’s first name

First two letters of your father’s first name

Day of the month you were born (e.g. 4th = 06, 17th = 17)

These four letters and two numbers make up your unique code (e.g. SIAL17)

Your Unique Code is: ________________________________

1. Age:
   - [ ] 19 or under
   - [ ] 20-29
   - [ ] 30-39
   - [ ] 40-49
   - [ ] 50-59
   - [ ] 60-69
   - [ ] 70+

2. Gender:
   - [ ] Male
   - [ ] Female

3. Country of birth: ________________________________

4. Nationality: ________________________________

5. Current position:
   - [ ] Undergraduate student
   - [ ] Postgraduate student
   - [ ] Staff
   - [ ] Other: ________________________________
6. Area of study/employment
   - □ Biological Science & Biotechnology □ Business
   - □ Chemical & Mathematical Science □ Chiropractic □
   - □ Engineering & Energy □ Environmental Science □
   - □ Education □ IT □ Law □ Media □ Nursing □ Pharmacy
   - □ Psychology □ Sustainability
   - □ Social Science & Humanities □ Animal Science
   - □ Veterinary science □ Biomedical Science □
   - Other __________________________________________

7. Do you currently live on: □ urban property/environment
   □ rural property/environment

8. Have you ever lived on a rural property/environment □ Yes □ No
   If yes, how long did you live there?
   - □ Less than 1 month
   - □ 1 – 6 months
   - □ 7 – 12 months
   - □ 1 – 2 years
   - □ More than 2 years
9. Have you ever visited a farm which rears animals? □ Yes □ No

If yes, what animals did they farm? (Please select all that apply)

☐ Sheep (wool)
☐ Sheep (meat)
☐ Beef cattle
☐ Dairy cattle
☐ Chickens [eggs]
☐ Chickens [meat]
☐ Pigs
☐ Other: ________________________________________________________

10. Have you witness or involved any of the following husbandry procedures?

☐ Vaccination
☐ Drenching
☐ Branding
☐ Dehorning
☐ Castration
☐ Others: ________________________________________________________
11. Currently, how often would you say you come into contact with cattle?
   - Daily
   - Once or twice a week
   - Once a fortnight
   - Once a month
   - Once a year
   - A few times a year
   - Never

12. How much time have you spent working with cattle in your lifetime?
   - Never
   - A few occasions
   - A few days
   - A few weeks
   - A month or more
   - A year or more

13. Do you currently have any of the following as pets? (Please select all that apply):
   - Dog(s)
   - Cat(s)
   - Bird(s)
   - Fish
   - Horse(s)
   - Livestock
   - Reptile(s)
   - Rodent(s)
   - Other............................................
14. How often do you eat the following:

<table>
<thead>
<tr>
<th></th>
<th>Poultry</th>
<th>Lamb</th>
<th>Beef</th>
<th>Fish</th>
<th>Eggs</th>
<th>Dairy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td></td>
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<tr>
<td>Occasionally (less than once a week)</td>
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<tr>
<td>Often (1-4 times a week)</td>
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<tr>
<td>Always (&gt;5 times a week)</td>
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</tbody>
</table>

15. What factors do you consider when eating the following:

<table>
<thead>
<tr>
<th></th>
<th>Poultry</th>
<th>Lamb</th>
<th>Beef</th>
<th>Fish</th>
<th>Eggs</th>
<th>Dairy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetarian</td>
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<td>Religion</td>
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<td>Dietary intolerance</td>
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<td>Dislike product</td>
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<td>Animal welfare concerns</td>
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<td>Food safety</td>
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<td>Cost</td>
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<tr>
<td>Other</td>
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</tbody>
</table>
16. Do you believe that by purchasing animal welfare friendly products, you can positively influence animal welfare?

No  Don't know  Yes

Any comments?

17. If you consume meat, dairy or eggs, are you personally responsible for the purchasing these products?

☐ Yes  ☐ No  ☐ Not applicable

Any comments?

18. Do you believe animal welfare should be improved in Australia?

☐ Yes  ☐ No  ☐ Not sure

Any comments?
19. What factors influence your assessment of animal welfare?

☐ Media
☐ Education
☐ Personal experience
☐ Family/social/peers
☐ Religion
☐ Significant person (e.g., vets or scientists)
☐ Other ......................................................................................

20. As a consumer, would you be willing to pay more for products coming from facilities that are enhancing welfare beyond current industry standards?

☐ Yes  ☐ No

Any comments? ................................................................................

21. How do you rate the welfare of cattle in Australia?

Poor    Excellent

________________________________________

22. How do you rate the welfare of cattle during road transport in Australia?

Poor    Excellent

________________________________________
23. How do you rate the welfare of cattle during live export by sea?

Poor            Excellent

24. How do you rate the welfare of Australian exported cattle at their foreign destination?

Poor            Excellent

25. How do you rate the husbandry procedures on beef cattle?

Poor            Excellent
7.8 QBA observer survey part 2

Survey 2

Your Unique Code is: ________________________

1. I can tell how a group of beef cattle are feeling by the way they behave

(please mark (X) on the line at the appropriate place)

Strongly disagree  Strongly agree

Any comments?:........................................................................................................

2. I believe animal welfare is important

Strongly disagree  Strongly agree

Any comments?:........................................................................................................

3. I am influenced by the media in my perceptions of animal welfare

Strongly disagree  Strongly agree

Any comments?:........................................................................................................
4. **Cattle show visible behavioural responses**

   Strongly disagree   Strongly agree

   Any comments?

5. **It doesn't matter what a person's background is, they will be able to tell how cattle are feeling**

   Strongly disagree   Strongly agree

   Any comments?

6. **People who don't eat meat are better able to interpret how cattle are behaving**

   Strongly disagree   Strongly agree

   Any comments?
7. People who have pets are better able to interpret how cattle are behaving

Strongly disagree    Strongly agree

Any comments?

8. People who have no preconceived ideas of cattle behaviour are better able to interpret how cattle are behaving

Strongly disagree    Strongly agree

Any comments?
7.9  QBA observer survey part 3A and 3B

Survey 3A

1. I can tell how cattle are feeling by the way they behave

(Please mark X on the line to indicate your answer)

Strongly disagree ........................................ Strongly agree ........................................

Any comments? ..............................................................................................................

2. I believe animal welfare is important

Strongly disagree ........................................ Strongly agree ........................................

Any comment? ..............................................................................................................

3. I am influenced by the media in my perceptions of animal welfare

Strongly disagree ........................................ Strongly agree ........................................

Any comments? ..............................................................................................................
4. Cattle show visible behavioural responses

Strongly disagree                  Strongly agree

Any comments?..........................

5. It doesn’t matter what a person’s background is, they will be able to tell how an animal is feeling

Strongly disagree                  Strongly agree

Any comments?..........................

6. People who don’t eat meat are better able to interpret how cattle are behaving

Strongly disagree                  Strongly agree

Any comments?..........................
7. People who have pets are better able to interpret how cattle are behaving

Strongly disagree                         Strongly agree

____________________________________________________________________

Any comments?..............................................................................................

8. People who have no preconceived ideas of cattle behaviour are better able to interpret how cattle are behaving

Strongly disagree                         Strongly agree

____________________________________________________________________

Any comments?..............................................................................................
Survey 3B

(Please mark (X) on the line to indicate your answer)

1. During each session did you find it easier or harder to score the cattle as you moved from the first clip to the last clip?

Harder  About the same  Easier

Any comments?

2. Did you find it easier or harder to score the animals as you moved through your list of terms?

Harder  About the same  Easier

Any comments?

3. Please rate the following on how easy or hard you thought it was to score the cattle under the following circumstances. (Please mark (X) on the line to indicate your answer)

Inactive cattle

Hard  Don’t know  Easy

Any comments?
Active cattle

<table>
<thead>
<tr>
<th>Hard</th>
<th>Don’t know</th>
<th>Easy</th>
</tr>
</thead>
</table>

Any comments?

4. Do you think it is possible to score the behavioural expression of:

   a. a group of cattle  [ ] Yes  [ ] No  [ ] Not sure

   Any comments?

   b. an individual cow  [ ] Yes  [ ] No  [ ] Not sure

   Any comments?

   c. neither groups or individuals  [ ] Yes  [ ] No  [ ] Not sure

   Any comments?

5. In this session what was your overall impression of the cattle?

   __________________________________________________

   __________________________________________________

   __________________________________________________
6. What were the main things you looked at when watching the cattle to determine their
behavioural expression? (Tick all that apply)

☐ Position of head
☐ Position of ears
☐ Movement of ears
☐ Eyes
☐ Movement of lips / tongue

☐ Chewing / ruminating
☐ Breathing / respiration
☐ Vocalization
☐ Body position
☐ Movement of legs
☐ Movement of tail
☐ Interaction with others
☐ Speed of movement of animal as it moves around the paddock
☐ Other...........................................................................................................

Thank you very much for taking part in this study. Your participation has been a vital part of
this research in determining whether behaviour can be used as a measure for cattle welfare.

Thank you once again for your time and commitment! 😊
8 References

Barrett, L.A., Beausoleil, N.J., Benschop, J., Stafford, K.J., 2016. Pain-related behavior was not observed in dairy cattle in the days after liver biopsy, regardless of whether NSAIDs were administered. Research in Veterinary Science 104, 195-199.


Hemsworth, P.H., Coleman, G.J., 2011. Human-livestock interactions: the stockperson and the productivity and welfare of intensively farmed animals. CABI, Cambridge, MA; Wallingford, UK.
Animal Production Science.


