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Pain assessment and analgesia following surgical castration in bull calves

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Abstract

There is a necessity to develop practical, cost-effective, techniques to mitigate the welfare impost of surgical castration in pastoral cattle. Forty-eight six-month old bulls were randomly divided into six groups: no surgery control (NC); surgical castration (C) without analgesia; C and meloxicam (M) either pre or post-op; C and lignocaine (L); C, L and $M_{pre\text{-}op}$. Pain assessment included quantitative and qualitative behavioural analyses, body weight, serum cortisol, balk scores, crush scores, nociceptive threshold testing and pedometry. Behavioural scores showed only minor differences between animals post-surgery. Animals that received analgesia and/or local anaesthesia were more active, rested less and weighed more the day after surgery than those that did not. When comparing animals in the paddock the day after surgery, qualitative behavioural assessment (QBA) showed minor differences between treatments. QBA during the surgical procedure itself, showed animals that received analgesia or anaesthesia were perceived as more *calm* and *comfortable* than those that did not. Nociceptive threshold testing did not identify hyperalgesia in any treatment groups. While this experiment provides some evidence for the use of non-steroidal anti-inflammatory analgesia and local anaesthetic for the castration of bulls, results were not consistent and further work is needed to identify suitable techniques to improve animal welfare when castrating *Bos indicus* bull calves.

Executive summary

Cattle on pastoral lands are subjected to potentially painful husbandry techniques. This usually occurs once a year after the muster and these procedures are often performed on animals that are older than six months of age. It is seldom that any pain mitigating medications are employed at this time and there is increasing concern that this perceived impost on the animal's welfare will become more significant at both an economic and social level. There is a need to investigate the possibility of using simple, cost-effective, readily available medications, administered using relatively quick and easily taught techniques. Providing evidence that not only are these techniques effective in minimising pain and stress but that they are relatively inexpensive and practical will increase the chances of the industry adopting the use of these medications.

Investigation of the impact of analgesia and anaesthesia on the welfare of animals post-surgery has been extensive in the past but as yet pain assessment in older, *Bos indicus* cattle has not been adequately addressed. This research used castration as the test model because it is arguably one of the most common and most painful procedures endured by the animals.

"Station" cattle tend to be unhandled and unused to human contact, making the measuring of pain and post-surgical welfare particularly challenging. To best facilitate the detection of treatment differences, a host of parameters were used in this work. This included composite behavioural scores, qualitative behavioural analysis (QBA), periodic measures of live weight, blood cortisol concentrations, balk score, crush score, and nociceptive threshold testing. Additionally, pedometers were fitted to individuals in order to measure activity, number of periods of rest and the duration of those rest periods.

Qualitative behavioural analysis has during the last decade become a method to assess welfare as it provides a valid measure of an animal's emotional state. QBA was used to test if there was a difference in emotional states of animals undergoing surgical castration with and without analgesia. Animals with lower emotional states (described as more *agitated* and *restless*) were associated with castration without analgesia in the crush. Castrated bulls provided with and without analgesia were described as less *relaxed, happy* and *calm* in the paddock on the day immediately after experiencing surgery compared to the day before surgery. This provides

evidence that QBA can be used as a tool to assess the emotionality of cattle under field conditions, but suggests that it may not discriminate between fear and pain. The manner in which a prey animal experiences such negative emotional states is complex and may change instantaneously; hence it is likely that several tools will be needed to enable the accurate measurement of responses to handling and husbandry practices.

In general the results showed that the use of analgesia and or anaesthesia provided some welfare benefit to the castrated animals. Cortisol concentrations, level of activity and number and duration of rest bouts, live weight and observation of behaviour supported the hypothesis that pre-surgical local anaesthesia and/or analgesia helped to improve the wellbeing of the animals in the days after the procedure. However, the results were inconsistent. Where differences were detected between treatments that made biological sense, equally, where there should have been differences, none were detected. Qualitative behavioural analysis proved useful in discerning differences among treatments when the animals were actually enduring the procedure, but the technique was less sensitive when it came to observing the animals in the paddock in the days after surgery. It is postulated that the difficulties encountered in achieving consistent, measurable treatments in the various parameters was associated with the stoic nature of inherently anxious, unhandled, herd animals.

It was concluded that *Bos indicus* bull calves gained some short term benefit from the administration of both local anaesthesia prior to surgery and peri-operative meloxicam. The techniques used are readily adoptable by industry with respect to cost, efficiency and degree of technical skill required. However, it is suggested that showing longer term production and carcass benefits of these techniques would lend weight to the argument and better the chances of adoption.

Table of Contents

1. Background

There is growing societal concern over the treatment of livestock and in particular, performing routine surgical procedures, such as castration without pain relief. The provision of pain relief for surgical castration of calves may be required by some international standards and this aspect of cattle husbandry could affect market access into the future. Currently the ‘best practice’ recommendation for field castration of pastoral cattle up to six months of age in Australia does not include the administration of analgesia.[1] Furthermore, and the limiting factor in the decision making process for the administration of appropriate analgesia, there is no validated pain assessment tool for cattle. As a result it is difficult to assess the efficacy of analgesic drugs used in this species. The cattle industry requires a practical ‘best practice’ castration technique that may or may not include the use of local anaesthetic and/or systemic pain relief. This technique should be applicable in the field.

2. Project objectives

1. To investigate the efficacy of various analgesic techniques for surgical castration of calves.
2. To compare various tool for post-operative pain assessment in these calves.

3. Methodology

Brahman bull calves from an extensive cattle station were studied in two batches. The first batch (n=24) were *Bos indicus* animals with a mean weight of 186 (±18) kg (Photo 1) and the second batch (n=24) were *Bos indicus* crosses with a mean weight of 145 (±17) kg (Photo 2). The first batch of animals arrived in June 2013 and the second batch arrived in July 2013. Each batch was managed in the same way: transport from the property of origin occurred eight days prior to the surgery day to allow for acclimatisation to the University farm. The cattle were kept in a one hectare paddock. The cattle had not been handled by the pastoralist and were not familiar with humans. Access to hay and water was allowed *ad lib* and EasyBeef pellets were fed daily (Milne AgriGroup Pty Ltd, 103-105 Welshpool Rd, Welshpool, Western Australia, 6106) at approximately 3% of bodyweight. The day after arrival at the University farm 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 also fitted with a strap to the left hind leg just above the fetlock joint. The following day the cattle were once again brought into the race for prophylactic parasite treatment (Moxidectin 5g/L Cydectin Pour-on, Virbac Australia Pty Ltd, 361 Horsley Rd, Milperra, NSW, 2214). A blood sample was collected from the tail vein for antigen capture enzyme linked immunosorbent assay testing for Bovine Viral Diarrhoea Virus (Swans Veterinary Services, Lot 83 Sheldon Road, Esperance, Western Australia, 6450) and data collection commenced.

Photo 1: animals from batch 1

Photo 2: animals from batch 2

Analgesia

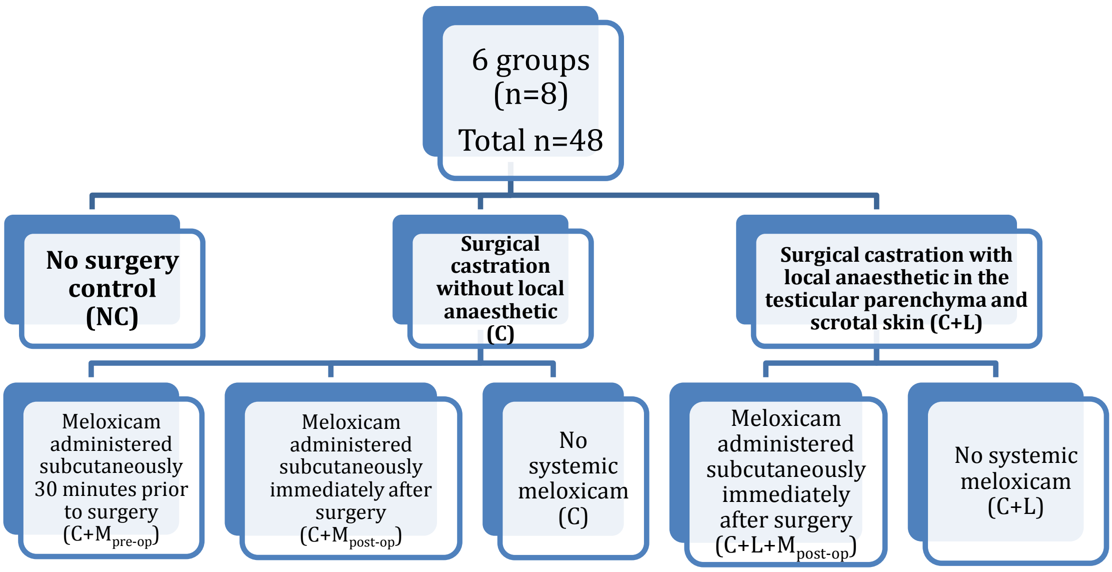
According to the study group lignocaine (L) (up to 2 mg/kg, Lignocaine 20, Ilium, Troy Laboratories, Glendenning, NSW, Australia) was injected into the testicle and subcutaneously at the incision site 5 minutes prior to surgery. The technique for injecting lignocaine was a *one-step* infiltration that involved insertion of a 1.5 inch needle into the testicular parenchyma. Injecting commenced as the needle was withdrawn in order to infuse lignocaine intra-testicle, into the subcutaneous tissue and the dermis. This technique was chosen as a technique that could be readily taught to producers. Meloxicam (M) (0.5 mg/kg, Meloxicam 20, Ilium, Troy Laboratories, Glendenning, NSW, Australia) was administered by subcutaneous injection. Meloxicam was administered either 30 minutes prior to surgery, or immediately after surgery.

Lignocaine is a local anaesthetic that will completely block the transmission of pain if applied to the nerves surrounding a surgical site. The onset time is approximately 5 minutes and the duration of action is usually about 60 minutes. Lignocaine is commonly used for desensitisation of tissues prior to surgery in conscious animals. Meloxicam is a non-steroidal anti-inflammatory drug which decreases pain associated with tissue inflammation. Both these drugs are licensed for use in cattle but require a prescription from a veterinary surgeon and can only be administered under veterinary supervision. The withholding periods for these drugs must also be considered when they are administered to food producing animals.

The current wholesale price for Meloxicam 20mg/mL, 100mL is $120 and for Lignocaine 2%, 100mL is $6.50. For these animals this equates to $4.20 to $6 for a dose of meloxicam and 91 c to $1.30 for a dose of lignocaine. The cost of syringes and needles must also be incorporated.

Study groups

Within each of the 2 batches of cattle (n=24 for each batch), the animals were randomly divided into six equal groups (n=4 for each group within each batch or n=8 for each group):



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

Pain assessment

Physiological and behavioural pain assessment strategies were employed at specific time points (Table 1). Physiological assessments included live weight changes and serum cortisol assays. Behavioural assessments included the balk score and crush score, composite behavioural score (CBS), nociceptive threshold testing (NTT), qualitative behavioural analysis (QBA) and daily pedometry.

Table 1: Sampling protocol per day

Day	QBA	CBS	Weight	Cortisol	Balk score	Crush score	NTT
-6		√	√	√	√	√	√
-1	√	√	√	√	√	√	√
0 (surgery)		√		√			
1	√	√	√	√	√	√	√
2		√	√	√	√	√	√
6	√	√	√	√	√	√	√
10		√	√	√	√	√	√
13			√	√	√	√	√

Surgical technique

Surgical castration was performed by two veterinarians with extensive experience in this 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 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.

Composite behavioural score

Behavioural scoring was done by one observer each morning in the paddock for a maximum of 2 minutes for each animal on pre and post castration days (Table 1). Cattle were sampled from left to right of the paddock. For the day of castration (Day 0), scoring was done in the afternoon. Eight common types of behaviour in cattle were chosen to be observed for scoring (Appendix 1: CBS sheet). The first four types of behaviour (*Position in Group, Grazing, Ruminating* and *Social Behaviour such as grooming each other, sniffing or licking*) were scored as follows: 0 = isolated/nil display of behaviour, 1 = semi-isolated/intermittent and 2 = together/constant display of behaviour. The remaining activities (*Weigh Shifting, Hind leg Stamping, Scrotal Area Grooming and Tail Swishing*) were scored as follows: 2 = nil display of behaviour, 1 = intermittent and 0 = constant. These were scored low (2) if behaviour was normal and high (0) if behaviour was abnormal. *Positioning in group, grazing, ruminating and social behaviour (SB)* was regarded as are normal behaviour for grazing animals. Data obtained was analysed using Statistica software performing

Repeated Measures Analysis of Variance (RM-ANOVA) to check for significant differences between treatment groups and day.

Qualitative behavioural analysis

Approximately three minutes of video footage was recorded for each calf in the paddock using a hand held Panasonic digital video camera at 7 am on pre and post-surgery days. Footage was edited using Adobe Premiere Pro CS3 and Adobe After Effects CS3 to produce 1-minute clip of each calf that was shown to observers for scoring.

Twenty observers were recruited from University staff, students and the public by advertising on email and flyers. Each observer was required to complete four sessions via correspondence or on campus. Before scoring cattle, observers were asked to complete a brief questionnaire regarding their demographic background, experience with cattle and their attitudes and opinions towards cattle behaviour and animal welfare. Observers were given detailed instructions on completing the four QBA sessions but were unaware of the treatment groups.

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 any words 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 the 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). Subsequent editing of the descriptive terms was carried out to remove terms that described actions, and terms that were in the negative form were transformed to the positive for ease of scoring (e.g. *unhappy* became *happy*). Each descriptive term was attached to a 100 mm visual analogue scale (minimum=0 to maximum=100). The list of terms was effectively randomly arranged, although ensuring that terms with a similar meaning were not listed together.

Quantification 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 (C and Mpost-op). In session 3, observers viewed 48 clips of individual cattle in the crush on day of surgery (Day 0) for all six treatment groups (C vs. NC vs. C and Mpre-op vs. C and Mpost-op vs. C and L vs.CL and Mpost-op).

Body weight

Body weight was measured on a scale in the race. Entering the scale was the first time the animal was isolated from its companions.

Cortisol

Blood samples were collected from the coccygeal vein as per Table 1. Samples were submitted to Vetpath Laboratory Services (Specialist Diagnostic Services, 39 Epsom Ave, Ascot, Western Australia, 6104). Measurement of serum cortisol concentrations was performed with an Immulite 2000 Cortisol competitive immunoassay.

Balk score

A balk score was attributed to each animal as it entered the scales for the first time (corresponding to the first occasion in which an animal was isolated from other animals in the race).

Balking ratings:

(1) Non-balker (No): enters voluntarily when the gate opens, or a light tap on the rump is required to induce the animal to enter the scale or the squeeze chute;
(2) Balker (Yes): a hard slap on the rump or tail twisting is required to induce the animal to enter the scale or squeeze.

Crush score

A crush score was determined from a 60 second period in the crush without the head restrained.

Temperament ratings:

(1) Calm, no movement
(2) Slightly restless
(3) Squirming, occasionally shaking the squeeze chute
(4) Continuous very vigorous movement and shaking of the squeeze chute
(5) Rearing, twisting of the body and struggling violently

Nociceptive threshold testing

A handheld manual pneumatic device (ProdPro, Topcat Metrology Ltd) with a 1 mm blunt pin was used to deliver a mechanical stimulus to a maximum of 27 Newtons (N) lateral to the sacrum. The operator stood on a raised platform next to a race which held six randomly selected animals at a time. The nociceptive threshold was recorded when a response to the stimulus was observed. Responses included stepping away from the stimulus, kicking, tail swishing or lifting the leg closest to the site of the stimulus. Each test was performed five times with at least five minutes between each test. The mean of the five tests were used for analyses.

Pedometry

Pedometers (afitag, Afimilk Ltd.Kibbutz Afikim,1514800, Israel) were strapped to the left hind leg of all bulls in the experiment two weeks before surgery. Each day, bulls were quietly mustered and run into a raceway. Daily data, including activity (number of steps taken), rest bouts (number of periods of recumbence), rest duration (length of each rest bout and rest time (total time resting), were recorded using a wand (afi2go, Afimilk Ltd.Kibbutz Afikim,1514800, Israel) by tapping the pedometer with the end of the wand. Data were collated using software (afifarm, Afimilk Ltd.Kibbutz Afikim,1514800, Israel). Pedometers were removed at the end of the data recording period.

Statistical analyses

For QBA: the distance from the start of the visual analogue scale to where the observer had made a mark was measured in millimeters and these measurements were entered into individual observer 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 (Genstat 2008, VSN International, Hemel Hempstead, Hertfordshire, UK;[2]). GPA calculates a consensus or 'best fit' profile between observer assessments through complex pattern matching. GPA provides a statistic (the Procrustes Statistic) which 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 is 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.

Nociceptive threshold testing data were analysed with a mixed effect linear model with the nociceptive threshold as the response variable and day and analgesic treatment as predictors. A step down model selection approach was then used to find the most parsimous model while maintaining the principle of marginality. Each term was removed in turn and an F-test used to test for a significant change in the fit of the model. A cut-off of P-value > 0.05 was used to remove a term.

A linear mixed model was fitted to the data for pedometers, weight and cortisol that was collected on each date in order to compare treatment effects over time. The fixed model included the effects of Day (4 df), Batch (1 df), Batch by Day, Treatment (5 df) and treatment by day. In addition the value for the appropriate measurement on day 0 was included as a covariate effect which was allowed to vary with Batch. The treatment effects were subdivided into five orthogonal contrasts each with one degree of freedom (df):

- Control vs Surgery treatments
- Pre-surgery analgesia vs surgery treatments with no pre-surgery analgesia
- No anaesthetic and no pre-surgery analgesia vs anaesthetic
- Post surgery analgesia vs no pre or post surgery analgesia
- Interaction between anaesthetic and post surgery analgesia

The random model included terms for animal and animal by day. An autoregressive model was used for correlation between days. Treatment effects have been assessed at the 5% level of significance and have been presented as deviations from the Control treatment to remove any influence of Day. Plots of residuals were used to check the assumptions underlying linear models were valid.

4. Results

All animals were negative for Bovine Viral Diarrhoea Virus.

Composite Behavioural Score

Results from the repeated measures analysis of variance (RM-ANOVA) did not show a significant difference (p = 0.866) between behaviour pre and post-surgery days (Day -6,-1, +1, +2, +3, +6 +10 +13) in all 6 treatment groups. Days -1 and +1 were selected to determine if there was a difference between the six treatment groups (Figure 1) Significant differences for five behaviours were seen; Positioning in Group, Grazing, Ruminating, Social Behaviour (SB) and Weight Shifting (p = 0.004).

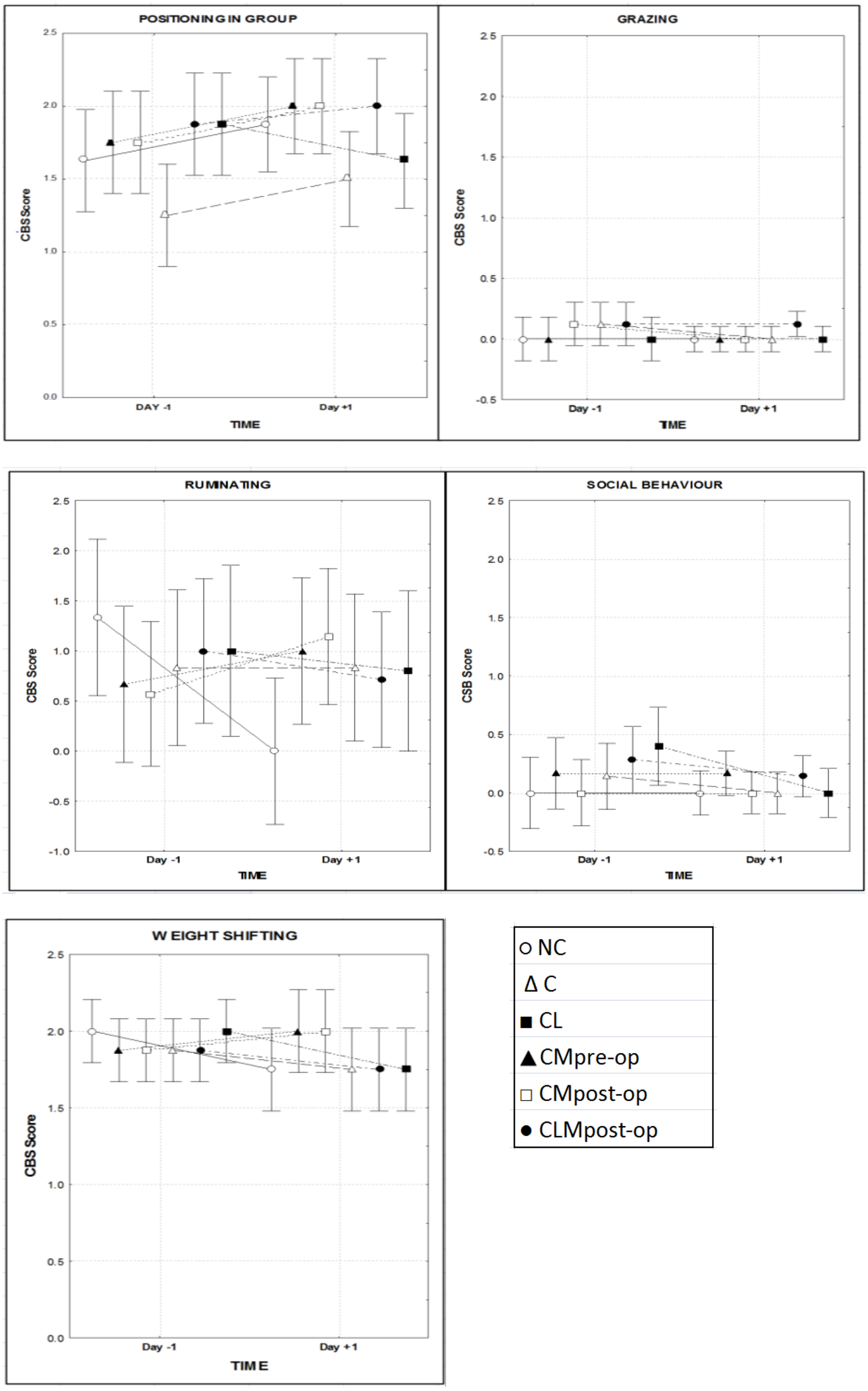


Figure 1: Analysis of behaviour in paddock pre and post surgery for all treatment groups.

Further analysis (RM-ANOVA) compared pre and post-surgery days (Day -1 vs. Day +1) for three pairs of treatment groups: Castrated (C) vs. Non-castrated (NC), Castrated (C) vs. Castrated with Local anaesthetic and post-surgery Meloxicam (CLMpost-op), and Castrated with pre-surgery Meloxicam (C Mpre-op) vs. Castrated with post-surgery Meloxicam (CMpost-op) (Figure 2, Figure 3, Figure 4).

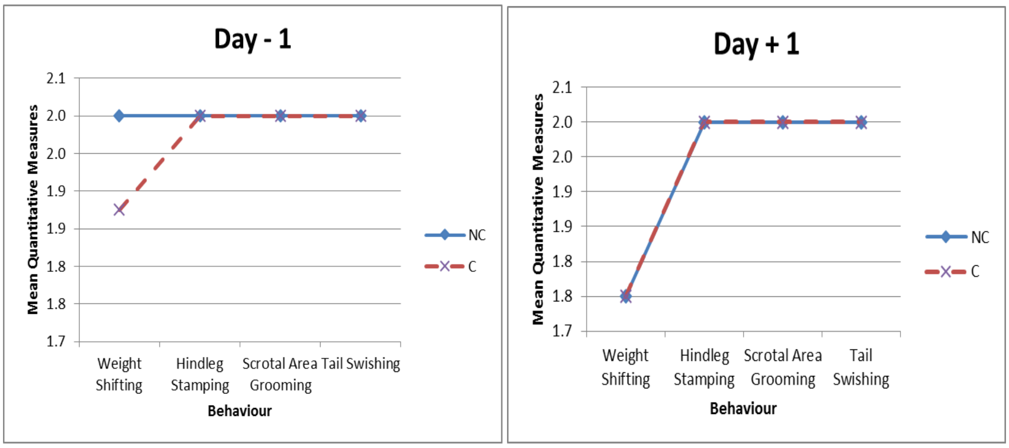


Figure 2: Behaviour in Castrated (C) vs. Non-castrated (NC) on pre and post-surgery (Day-1 vs. Day+1); *Weight Shifting, Hindleg Stamping, Scrotal Area Grooming* and *Tail Swishing*

Weight Shifting was detected in castrated calves on Day -1, and in castrated and non castrated calves on Day +1. There were no significant differences in any behaviour activity between the two treatment groups on Day +1 (p = 0.810).

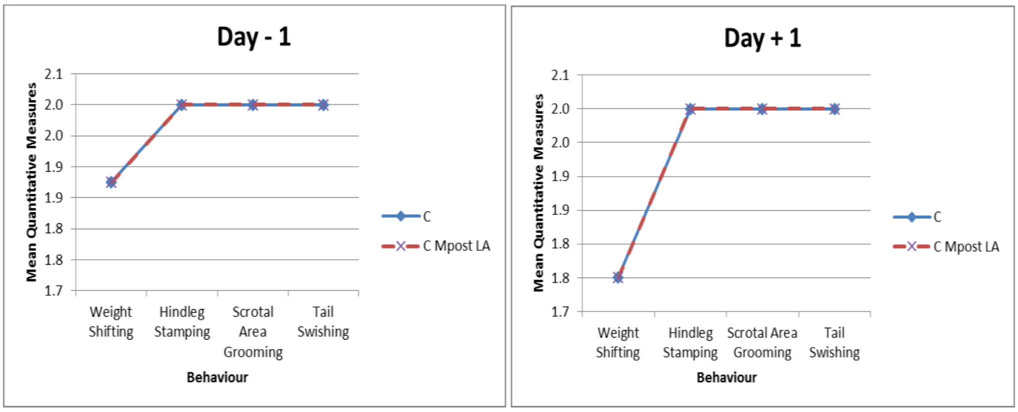


Figure 3: Behaviour in Castrated (C) vs. Castrated with Local anaesthetic and post-surgery Meloxicam (CLMpost-op) on pre and post-surgery (Day-1 vs. Day+1)

Four behaviours were analysed using RM-ANOVA for treatment groups Castrated (C) vs. Castrated with Local anaesthetic and post-surgery Meloxicam (CLMpost-op) on (Day -1 vs. Day +1). Both groups displayed intermittent weight shifting on both days but there were no significant differences (p= 0.810) between the groups.

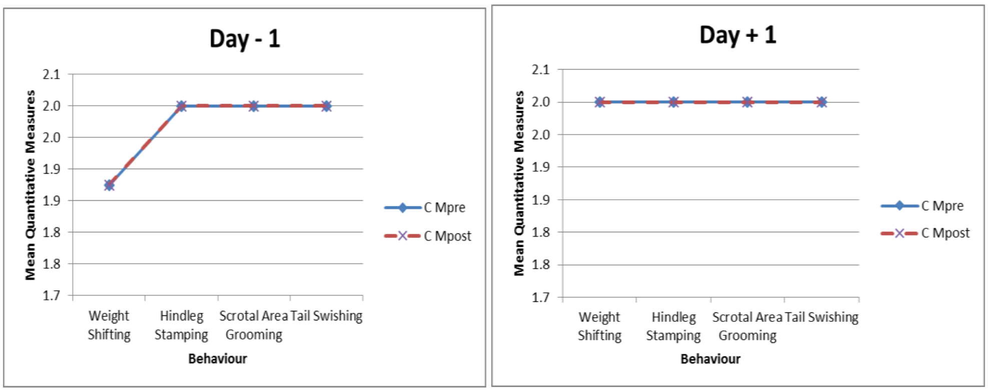


Figure 4: Behaviour of Castrated with pre-surgery Meloxicam (CMpre-op) vs. Castrated with post-surgery Meloxicam (CLMpost-op) on pre and post-surgery (Day-1 vs. Day+1)

Castration with pre-surgery Meloxicam (CMpre-op) vs. Castration with post-surgery Meloxicam (CLMpost-op) on (Day-1 vs. Day+1) was compared; there were no significant differences between treatment groups (p = 0.810).

Qualitative behavioural analysis

Twenty observers were recruited in this study; 95% were female, 50% were born in Australia and 50% either worked or studied in an animal related field (Table 2). The mean age of observers was 28 (range <19 to >60) years. Observers viewed three sessions of cattle footage and these sessions were analysed separately (Table 3). These sessions include:

a). Castrated (C) vs Non-castrated (NC) on pre and post-surgery day (Day-1 vs Day+1) in the paddock

b). Castrated (C) vs Castrated with Local anaesthetic and post-surgery Meloxicam (CL $M_{post-op}$) on pre and post-surgery days (Day-1 vs Day+1) in the paddock

c). All treatment groups on day of surgery; Castrated vs. Non-Castrated vs. Castrated with pre-surgery Meloxicam vs. Castrated with post-surgery Meloxicam vs. Castrated with Local anaesthetic vs. Castrated with Local anaesthetic and post-surgery Meloxicam (C vs. NC vs. C M_{pre-op} vs. C $M_{post-op}$ vs. CL vs. CL $M_{post-op}$) in the crush on the day of surgery (Day 0).

Observers generated a total of 96 unique terms to describe the cattle they were shown, with an average of 14 (min: 8, max: 23) terms per observer. An example of one observer's terms (Figure 5) mapped against GPA dimension1 and 2 for session 1: Castration (C) vs. Non-castration (NC), session 2: Castration (C) vs. Castration with Local anaesthetic and post-surgery Meloxicam (CL $M_{post-op}$) and session 3: all six treatment groups (C vs. NC vs. C M_{pre-op} vs. C $M_{post-op}$ vs. C L vs. CL $M_{post-op}$).

Table 2: Demographic description of observers

Attribute	Category: # of observers					
Sex	Female: 19	Male: 1				
Country of birth	Australia: 10	Other: 10				
Habitat	Urban: 18	Rural: 2				
Area of study/employment: animal-related	Yes: 10	No: 10				
Dietary preference: vegetarian	Yes: 1	No: 19				
Purchasing habit: purchases own meat/eggs/dairy	Yes: 14	No: 6				
Pet ownership	Yes: 18	No: 2				
Level of experience with cattle	Low: 11	Medium: 3	High: 6			
Age (yrs.) <19: 5	20-29: 9	30-39: 2	40-49: 2	50-59: 1	60-69: 1	>70: 0

Table 3: Summary of GPA results for Sessions 1, 2 and 3

Sessions	**Procrustes Statistic (%)**	**t-test**
1). C vs NC	**40.56%**	(t_{99}=37.02, P< 0.001)
2). C vs $CLM_{post\text{-}op}$	**37.37%**	(t_{99}=30.63, P< 0.001)
3). All six treatment groups (NC vs. C vs. $CM_{pre\text{-}op}$ vs. $CM_{post\text{-}op}$ vs. $CLM_{post\text{-}op}$ vs. CL)	**44.5%**	(t_{99}=30.63, P< 0.001)

Session 1: Castration (C) vs. Non-castration (NC) in paddock on Day -1 and +1

Terms with the strongest correlation with each of the GPA dimensions are shown in Table 4. For GPA dimension 1, low values were associated with terms such as *relaxed, calm and contented* and high values with terms such *alert, frightened and agitated*. For GPA dimension 2, low values were associated with terms such as *happy, relaxed and contented* and high values with terms such as *tired, bored and sleepy*. For GPA dimension 3, low values were associated with terms such as *curious, lonely and itchy* and high values with terms such as *sore, stressed and unsure*. Three main GPA dimensions made up a total of 65.6% of the variation between animals.

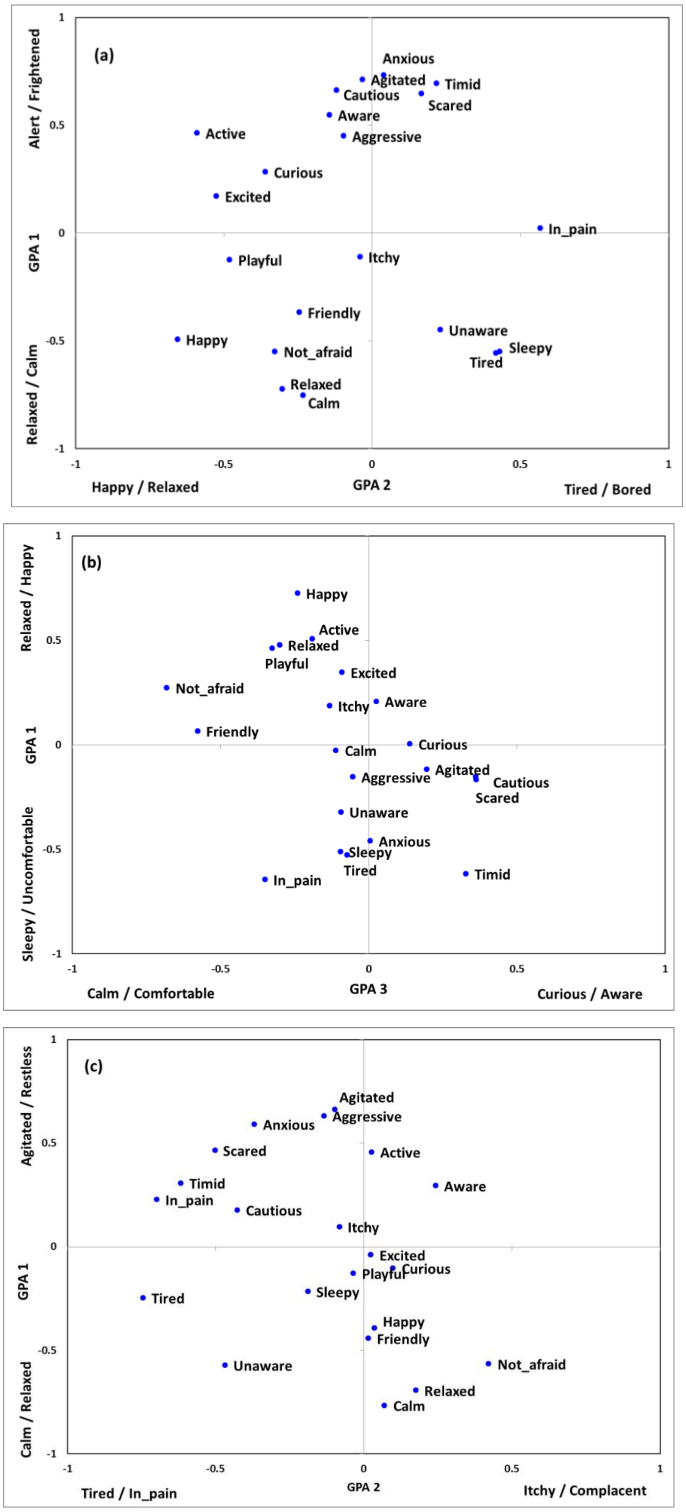


Figure 5: Word map of consensus profile for GPA dimensions of one observer viewing cattle (a) no castration (NC) vs. castration (C) on Day -1 and +1 in the paddock. (b) castration (C) vs. castration with local anaesthetic and meloxicam post-surgery (CLMpost-op) on Day -1 and +1 in the paddock. (c) all treatment groups (NC vs. C vs. CMpre-op vs. CMpost-op vs. CLMpost-op vs. CL) on day of surgery in the crush.

Table 4: Terms used by observers to describe cattle behavioural expression in the paddock and in the crush. Terms for all observers, showing the highest negative and positive correlation with GPA dimensions 1, 2 and 3 of the consensus profile are shown for three separate viewing sessions. Session 1: castrated without analgesia and non-castrated treatment groups (C vs. NC) in the paddock pre (Day -1) and post-surgery (Day +1), Session 2: castrated without analgesia and castrated with both local anaesthetic and post-surgery meloxicam treatment groups (C vs. $CLM_{post\text{-}op)}$ in the paddock pre (Day -1) and post-surgery (Day +1) and, Session 3: all six treatment groups, castrated vs. non-castrated vs. castrated with various combinations of analgesia (NC vs. C vs. $CM_{pre\text{-}op}$ vs. $CM_{post\text{-}op}$ vs. $CLM_{post\text{-}op}$, CL) on the day of surgery (Day 0), in the crush. Terms shown have a correlation of >0.5 (high values) and < -0.5 (low values) for GPA dimension 1 and 2 for session 1 (C vs NC), GPA dimension 1 and 3 for session 2 (C vs. CL $M_{post\text{-}op}$) and GPA dimension 2 for session 3 (NC vs. C vs. $CM_{pre\text{-}op}$ vs. $CM_{post\text{-}op}$ vs. $CLM_{post\text{-}op}$ vs. CL); a correlation of >0.4 (high values) and < -0.4 (low values) for GPA dimension 3 for session 1 (C vs. NC); a correlation of >0.6 (high values) and < -0.6 (low values) for GPA dimension 2 for session 2 (C vs. $CLM_{post\text{-}op}$) and GPA dimension 1 for session 3 (NC vs. C vs. $CM_{pre\text{-}op}$ vs. $CM_{post\text{-}op}$ vs. $CLM_{post\text{-}op}$ vs. CL). Order of terms is determined firstly by number of observers to use that term (in parentheses where >1) and secondly by weighting of each term

Treatment	GPA Dimension	Low Values	High Values	Treatment Effect
Session 1 **C vs NC**	**1 (36.9%)**	Relaxed (6), Calm (6), Contented (3) Laid_back , Chilled, Quiet, Apathetic, Satisfied, Comfortable, Carefree, Sleepy, Tired, Not_afraid, Sore, Bored	Alert (4), Frightened (3), Agitated (3), Curious (3), Unsure (2), Nervous (2), Unsettled (2), Stressed (2), Anxious (2), Restless (2), Lively, Aware, Tensed, Eager, Uncomfortable, Lost, Defensive, Scared, Weary, Inquisitive, Cautious, Timid, Distressed, Disquietened.	NS
	2 (18.8%)	Happy (4), Relaxed (2), Contented, Active, Energetic, Excited, Inquisitive.	Tired (3), Bored (3), Sleepy (2), Exhausted (2), Uncomfortable (2), Lethargic (2), Submissive, Sad, In_pain, Sore, Disinterested.	Day effect *F*=4.635, *P*=0.049
	3 (9.9%)	Curious (6), Lonely (2), Itchy (2), Confused (2), Bored (2), Relaxed (2), Agitated, Afraid, Trapped, Hesitant, Weary, Impatient, Cautious, Anxious, Observant, Friendly, Alert, Happy, Wary, Listening	Sore, Stressed, Unsure, Alone	NS
Session 2 **C vs**	1 (26.8%)	Sleepy (4), Uncomfortable (3), In_pain (2), Tired (2), Uncomfortable,	Relaxed (7), Happy (5), Calm (3), Contented (3), Excited (2), Content, Aware, Active	Day effect F=17.391, P<0.001

CLMpost-op		Nervous, Sad, Stressed, Disinterested, Unsure, Timid, Depressed, Unsettled, Agitated, Lost, Lethargic, Bored, Exhausted		
	2 (17.9%)	Curious (6), Inquisitive (2), Alert (2), Weary, Frustrated, Aware, Distressed, Excited, Bored, Unsure	Docile, Chilled, Unaware	NS
	3 (13.3%)	Calm (2), Comfortable (2), Not_afraid, Satisfied, Uncomfortable, Tired, Uncertain, Tender, Friendly, Unsettled, Depressed, Lethargic	Curious, Aware, Dominant,	Day x Treatment interaction F=4.673, P=0.048
Session 3 C vs NC vs CMPRE-OP vs CMPOST-OP vs CL vs CLMPOST-OP	1 (66.2%)	Calm (8), Relaxed (6), Contented (2), Docile, Carefree, Happy, Laid_back, Comfortable, Content, Chilled, Bored	Agitated (8), Restless (5), Frightened (4), Stressed (3), Unsettled (3), Uncomfortable (3), Alert (3), Anxious (2), Frustrated (2), Irritated (2), Sore (2), Nervous (2), Annoyed (2), Weary (2), In_pain, Hurt, Disquietened, Excited, Aware, Defensive, Bothered, Cautious, Afraid, Scared, Angry, Worried, Aggressive, Uneasy, Dominant	Treatment effect F=4.454, P=0.002
	2 (10.5%)	Tired, In_pain, Sad, Trapped, Timid, Uneasy, Playful, Weary, Aimless, Sleepy, Sore, Scared, Bored	Itchy, Complacent, Observant, Unsure, Bored	NS

There were no significant differences in treatment (F=1.792, p=0.202) or day (F=0.692, p=0.420) on GPA dimension 1. Similarly, there were no significant differences in treatment (F=0.002, p=0.962) or day (F=0.670, p=0.427) on GPA dimension 3. However, there was a significant day effect (F=4.635, p=0.049) on GPA dimension 2 as cattle in both treatment groups scored higher post-surgery (Day +1) than pre-surgery (Day -1). Cattle were described as more *alert, frightened and agitated* after surgery as shown in Figure 6.

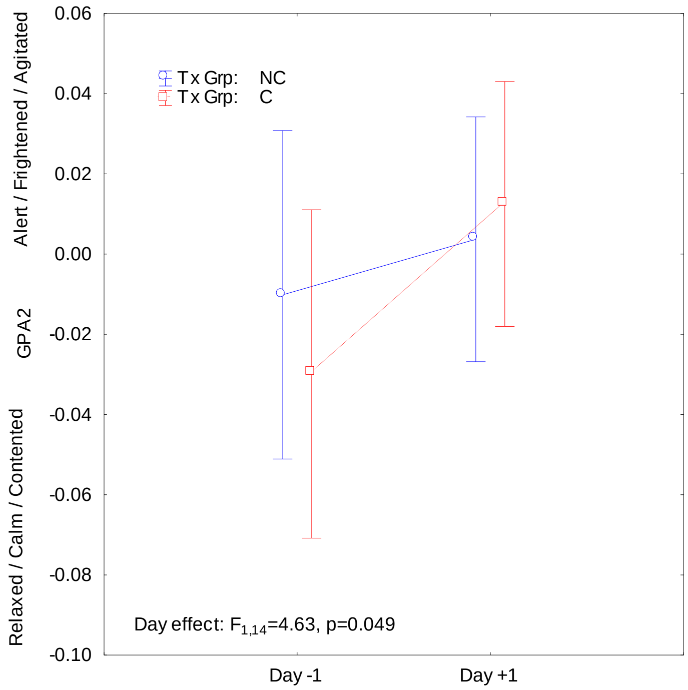


Figure 6: GPA dimension 2 showing day effect (F1,14=4.63, p=0.049). Both treatment groups were described as more *alert, frightened and agitated* post-surgery (Day +1).

Session 2: Castration (C) and Castrated with Local anaesthetic and post-surgery Meloxicam (CLMpost-op) in paddock on Day -1 and +1

Terms with the strongest correlation with each of the GPA dimensions are shown in Table 4. For GPA dimension 1, low values were associated with terms such as *sleepy, uncomfortable and in_pain* and high values with terms such as *relaxed, happy and calm*. For GPA dimension 2, low values were associated with terms such as *curious, inquisitive and alert* and high values with terms such as *docile, chilled and unaware*. For GPA dimension 3, low values were associated with terms such as *calm, contented, comfortable* and high values with terms such as *curious, aware and dominant.* Three main GPA dimensions made up a total of 58% of the variation between animals (Table 4).

Significant day differences on GPA dimension 1 (F=17.392, *p*<0.001) and day x treatment interaction on GPA dimension 3 (F=4.673, *p*=0.048) were found. On GPA dimension 1, cattle in both treatment groups scored lower post-surgery (Day +1) than pre-surgery (Day -1). Cattle were more *sleepy, uncomfortable and in pain* after surgery as shown in Figure 7. Day x treatment interaction on dimension 3 showed that castrated cattle were more *calm and comfortable* before surgery and were more *curious and aware* after surgery; however castrated cattle receiving Local anaesthetic and Meloxicam showed a different trend where they were described as more *calm and comfortable* after surgery as shown in Figure 8.

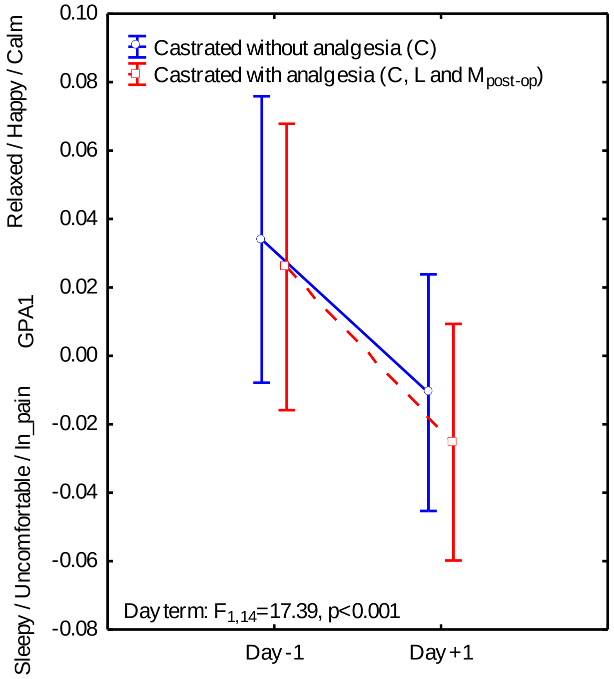


Figure 7: GPA dimension 1 showing day effect (F1,14=17.39, p<0.001). Both treatment groups were described as more *sleepy, uncomfortable and in_pain* post-surgery (Day +1).

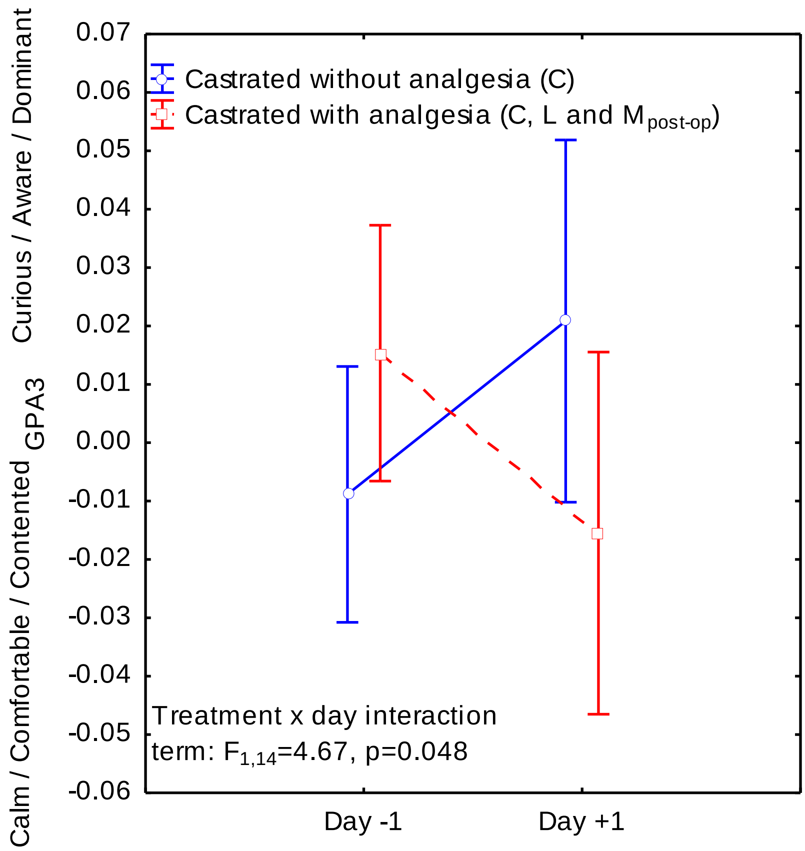


Figure 8: GPA dimension 3 showing day x treatment interaction ($F_{1,14}=4.673$, $p=0.048$). Castrated cattle (C) were less *calm* post-surgery (Day+1) and castrated cattle with local anaesthesia and meloxicam (CLMpost-op) were more *calm* post-surgery (Day +1).

Session 3: All treatment groups (NC vs. C vs. CMpre-op vs. CMpost-op vs. CLMpost-op vs CL) in crush on day of surgery (Day 0)

Terms with the strongest correlation with each of the GPA dimensions are shown in Table 4. For GPA dimension 1, low values were associated with terms such as *calm, relaxed and contented* and high values with terms such as *agitated, restless and frightened*. For GPA dimension 2, low values were associated with terms such as tired, in_pain and sad and and high values with terms such as itchy, complacent and observant. Two main GPA dimensions made up a total of 76.7% of the variation between animals (Table 4), as GPA dimension 1 accounted a large (66.2%) of total variation. Significant differences were found on GPA dimension 1 ANOVA (F=4.454, p=0.002) and by Tukey's HSD post-hoc analysis (Figure 9).

Qualitative Behaviour Assessment (QBA) ratings of calf body language in the crush at the time of surgery did not consistently reflect expected pain responses for each treatment group. No significant difference was shown in the responses of the castrated and non castrated cattle and there were no overriding treatment effects with the use of either Local anasethetic or Meloxicam. However, three pairs of treatment groups showed significant differences:

1) Castrated with pre-surgery Meloxicam (CMpre-op) vs. Non-castrated (NC); Calves that were not castrated (NC) were scored as more calm and relaxed than the CMpre-op calves on the GPA dimension 1 and the CMpre-op calves were scored as more agitated and restless
2) Castrated with pre-surgery Meloxicam (CMpre-op) vs. Castrated with anaesthetic and post-surgery Meloxicam (CLMpost-op): Castrated calves given Local anasethetic and post-surgery Meloxicam (CLMpost-op) were scored as more calm and relaxed during surgery compared to those castrated and given pre-surgery Meloxicam (CMpre-op).
3) Castrated (C) vs. Castrated with Local anaesthetic and post-surgery Meloxicam (CLMpost-op). Castrated calves were scored as more agitated and restless than CLMpost-op calves
The timing of the administration of Meloxicam did not appear to be important, as no significant difference was noted between castrated cattle given pre or post-surgery Meloxicam.FFIG

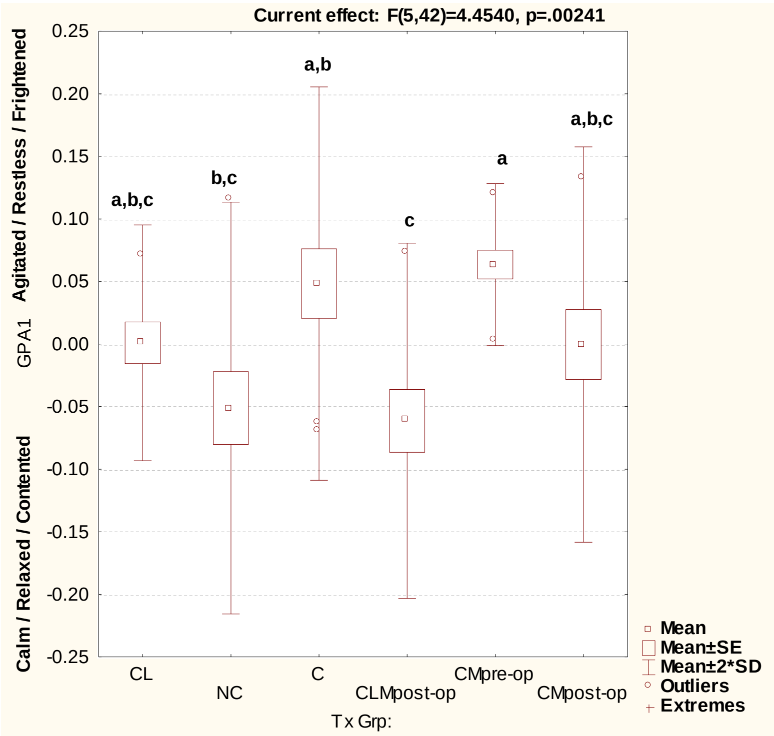


Figure 9: The effects of treatment on the day of surgery on GPA Dimension 1 using One-way ANOVA (F 5, 42=4.4540, p=0.002). Different letters[a,b,c] following the means define significant differences between treatment groups.

Body weight

Bulls that received post-surgery analgesia were consistently (at each day of measurement) heavier than those that didn't (**Error! Reference source not found.**).

Table 5: Levels of significance (P-values) for terms in model to analyse daily weight

Fixed term	Day 1	Day 2	Day 6	Day 10	Day 13
Experimental_Group	<0.001	<0.001	<0.001	<0.001	<0.001
cov_Weight	<0.001	<0.001	<0.001	<0.001	<0.001
Experimental_Group.cov_Weight	0.491	0.097	0.259	0.450	0.833
Control	0.250	0.530	0.035	0.042	0.102
Pre_surgery	0.544	0.507	0.294	0.217	0.162
Anaesthetic	0.437	0.056	0.116	0.772	0.687
Post_pain_relief	0.003	0.004	0.003	0.029	0.038
Anaesthetic.Post_pain_relief	0.888	0.774	0.427	0.384	0.465

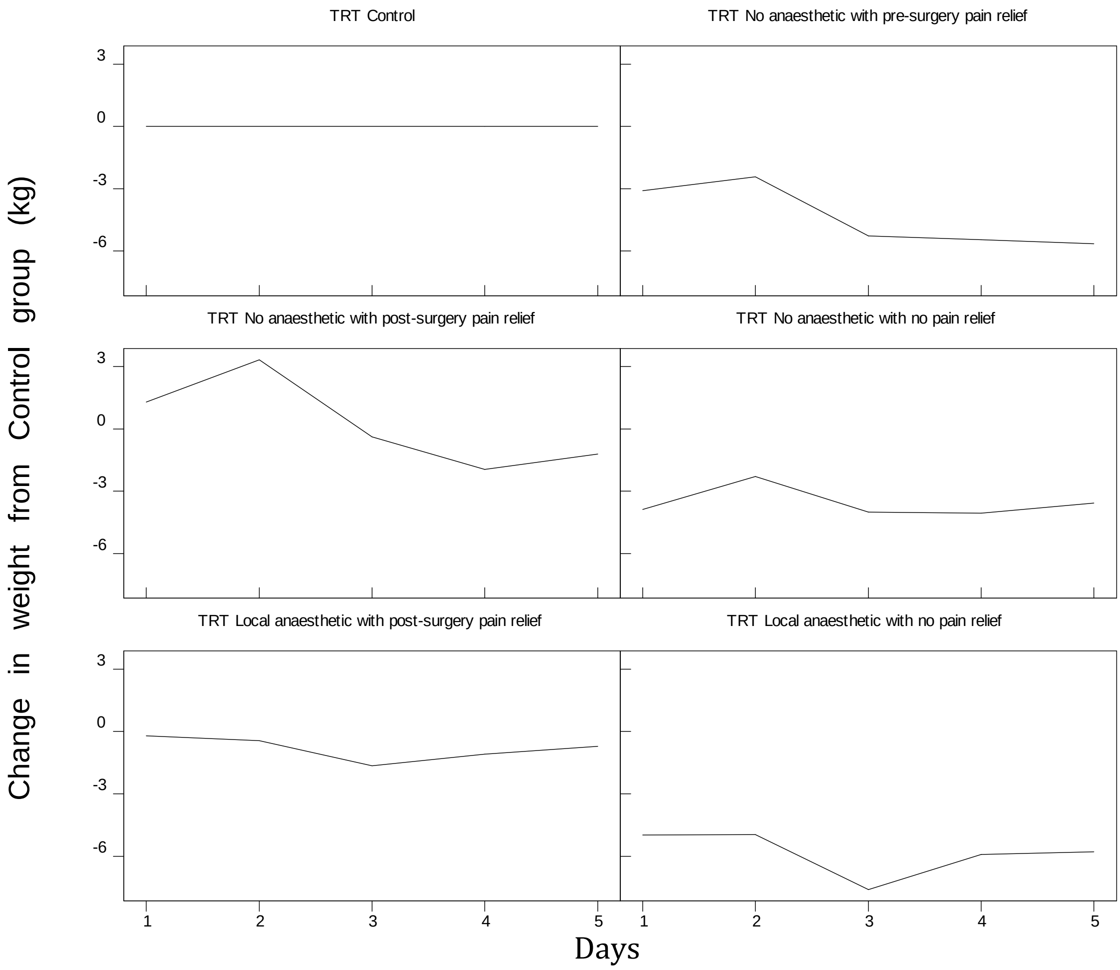


Figure 10: Differences in Daily mean weight from the control treatment for each of the treatment groups

Serum cortisol

On day 1, the administration of local anaesthetic reduces cortisol concentrations significantly when no post-surgery analgesia is given (P < 0.001, **Error! Reference source not found.**), ut not when it is compared to animals that had no analgesia or anaesthesia (compare treatment *local anaesthetic with no pain relief* to the average of treatments where post-surgery pain relief is given). Similarly on day 2 the administration of local anaesthetic reduced cortisol concentrations when no post-surgery analgesia was administered (P = 0,006, Figure 11.) (compare treatment *local anaesthetic with no pain relief* to the average of treatments where post-surgery pain relief is given).

Averaged across all days, post surgical analgesia is associated with lower plasma cortisol (P = 0.008).

Table 6: Levels of significance (P-values) for terms in model to analyse mean daily blood cortisol concentrations

Fixed term	Degrees of freedom	Day 1	Day 2	Day 6	Day 10	Day 13
Experimental_Group	1	0.448	0.480	0.092	0.034	0.137
cov_cortisol	1	<0.001	<0.001	<0.001	<0.001	0.024
Control	1	0.580	0.945	0.149	0.970	0.245
Pre_surgery	1	0.289	0.384	0.530	0.087	0.286
Anaesthetic	1	0.079	0.704	0.058	0.775	0.214
Post_pain_relief	1	0.370	0.712	0.029	0.117	0.877
Anaesthetic.Post_pain_relief	1	<0.001	0.006	0.428	0.483	0.984

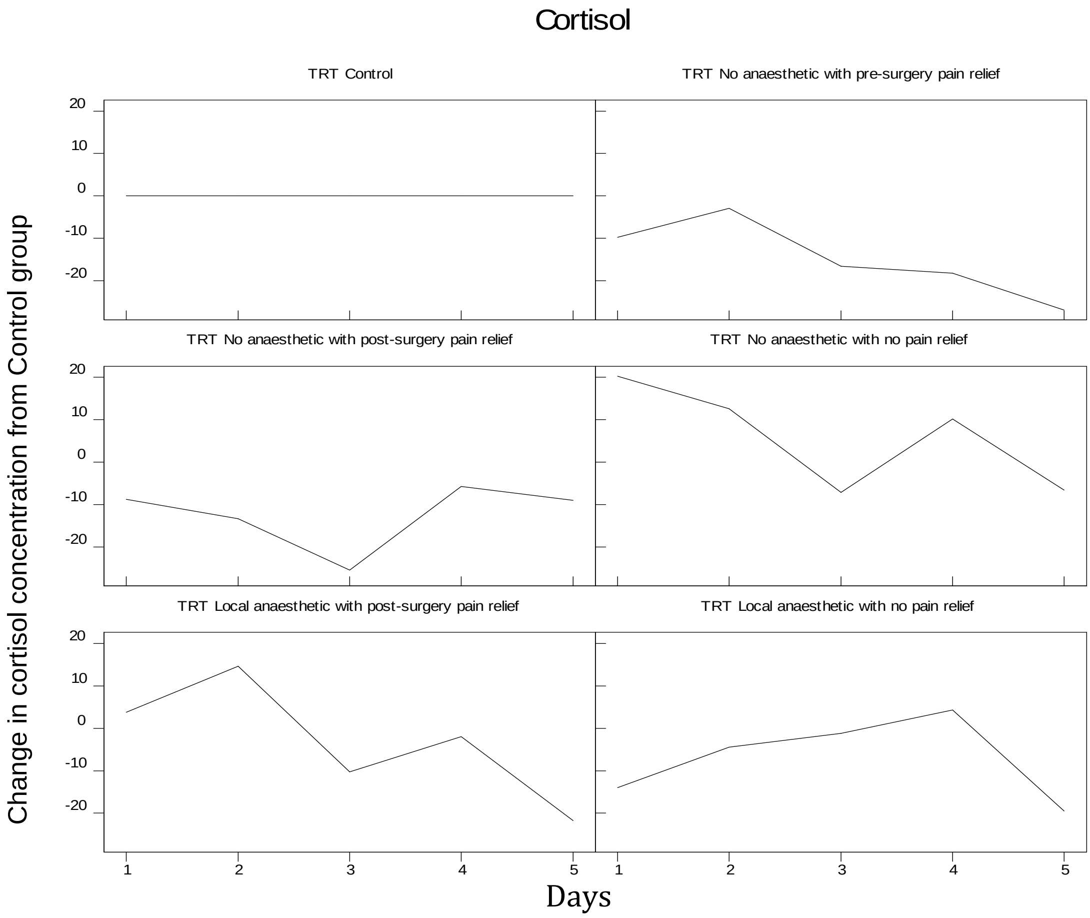


Figure 11: Differences in Daily mean cortisol concentration from the control treatment for each of the treatment groups

Balk score and crush score

Analyses of these measures revealed no differences between treatments (Tables 7 and 8).

Table 7: Balk score (number of animals that balked)

Study group	Day -6	Day -1	Day 1	Day 2	Day 6	Day 10	Day 13
NC	0	0	5	4	4	2	2
C	1	1	5	5	3	1	4
$C+M_{pre}$	0	1	4	5	4	4	2
$C+M_{post}$	0	3	1	6	2	4	5
$C+L+M_{post}$	0	1	6	5	4	2	4
C+L	1	2	4	4	2	2	2

Table 8: Crush score (average)

Study group	Day -6	Day -1	Day 1	Day 2	Day 6	Day 10	Day 13
NC	1.5	1.4	1.1	1.2	1.4	1.3	1.1
C	1.6	1.6	1.1	1.1	1.4	1.1	1.1
$C+M_{pre}$	1.6	1.3	1.1	1.2	1.3	1.1	1.2
$C+M_{post}$	1.8	1.3	1.2	1.3	1.4	1.1	1.0
$C+L+M_{post}$	1.6	1.4	1.3	1.4	1.1	1.1	1.1
C+L	1.4	1.5	1.1	1.6	1.4	1.1	1.1

Nociceptive threshold testing

An increase in the nociceptive threshold is suggestive of analgesic drug efficacy while a decrease in the nociceptive threshold indicates the development of hyperalgesia. For all groups, there was a trend towards decreasing nociceptive threshold over the study period but there were no significant differences between or within groups. Data is mean (SD) (Table 9).

Table 9: Nociceptive Threshold (Newtons) over treatment days. Data is mean(SD).

Study group	Day -6	Day -1	Day 1	Day 2	Day 6	Day 10	Day 13
NC	22 (3.7)	22.1 (4.2)	19.9 (4.5)	17.4 (5.1)	14.8 (5.3)	14.2 (3.7)	15.7 (4.8)
C	18.5 (3.9)	20.7 (4.5)	20.4 (5.4)	17.6 (5.9)	16.4 (5.8)	15.1 (3.9)	17.7 (5.4)
$C+M_{pre}$	14 (4.9)	21.8 (6.4)	20.1 (5.5)	17.2 (5.4)	15.7 (4.9)	13.3 (4.9)	14.5 (3.8)
$C+M_{post}$	18.9 (6.2)	22.6 (4.9)	17.5 (5.1)	14 (5.1)	13.5 (5.9)	14.4 (2.5)	15.3 (3.3)
$C+L+M_{post}$	18.8 (4.2)	21.8 (4.3)	21.4 (5.4)	17 (6.3)	15.6 (4.7)	15.3 (4.7)	15 (4.1)
C+L	21.9 (1.7)	20.3 (5.0)	16.9 (6.2)	16.7 (6.2)	15.9 (4.9)	16.2 (5.3)	14.7 (3.3)

There was no evidence of the development of hyperalgesia in these animals.

Pedometry

Table 10: Levels of significance (P-values) for terms in model to analyse daily pedometer data presents the levels of significance for the terms in the models used to analyse pedometer data each day. Table 11 presents this data but in a model that analyses the combined data over time. Figures 12-14 are the graphical representations of the treatment means, presented as degree of variation from the control treatment.

On day 1, bulls that received pre-surgical analgesia but no anaesthetic were more active than those that either received no anaesthetic or analgesia, or those that received anaesthetic and post-surgery analgesia (p = 0.038, **Error! Reference source not found.**).

Bulls that had no surgery had fewer rest bouts on day 1 than all bulls that had surgery (P = 0.013, Figure 13). Bulls that received post-surgery analgesia had fewer rest bouts on day 1 than those that received no analgesia/anaesthesia or those that received local anaesthesia but no analgesia (P = 0.001, Figure 13).

On day 4, bulls that received post-surgery analgesia had a shorter rest duration than those that received no analgesia/anaesthesia or those that received local anaesthesia but no analgesia (P = 0.002, Figure 14).

Table 10: Levels of significance (P-values) for terms in model to analyse daily pedometer data (Activity, Number of rest bouts and Duration of rest bouts). Shading indicates significance (P<0.05)

Fixed term	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
Activity													
Experimental_Group	<0.001	0.207	<0.001	0.130	0.430	<0.001	<0.001	<0.001	<0.001	<0.001	0.025	<0.001	0.004
cov_Activity	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
Control (1 vs 2-6)	0.137	0.908	0.828	0.500	0.846	0.581	0.876	0.325	0.782	0.896	0.382	0.550	0.487
Pre_surgery (2 vs 3-6)	0.038	0.806	0.231	0.528	0.907	0.870	0.968	0.791	0.542	0.119	0.405	0.229	0.647
Anaesthetic (3,4 vs 5,6)	0.479	0.849	0.409	0.398	0.384	0.716	0.064	0.199	0.357	0.208	0.501	0.980	0.378
Post_pain_relief (3,5 vs 4,6)	0.848	0.915	0.905	0.825	0.880	0.663	0.850	0.832	0.891	0.150	0.188	0.733	0.897
Anaesthetic.Post_pain_relief	0.625	0.750	0.400	0.933	0.805	0.349	0.535	0.759	0.581	0.867	0.633	0.919	0.951
Number of rest bouts													
Experimental_Group	0.011	<0.001	0.650	<0.001	<0.001	<0.001	0.812	<0.001	0.107	0.824	0.002	0.024	0.068
sqrt_cov_Rest_Bout	0.037	0.026	0.059	0.029	0.233	0.107	0.003	0.004	<0.001	0.004	0.002	0.001	0.001
Control (1 vs 2-6)	0.013	0.520	0.651	0.224	0.622	0.216	0.813	0.842	0.180	0.870	0.859	0.828	0.428
Pre_surgery (2 vs 3-6)	0.273	0.440	0.439	0.550	0.737	0.610	0.714	0.247	0.084	0.093	0.123	0.462	0.228
Anaesthetic (3,4 vs 5,6)	0.847	0.445	0.898	0.915	0.239	0.085	0.646	0.612	0.736	0.688	0.403	0.645	0.923
Post_pain_relief (3,5 vs 4,6)	<0.001	0.591	0.214	0.429	0.110	0.125	0.421	0.061	0.916	0.229	0.818	0.228	0.254
Anaesthetic.Post_pain_relief	0.472	0.437	0.950	0.849	0.971	0.366	0.935	0.729	0.955	0.855	0.585	0.551	0.017
Duration of rest bouts													
Experimental_Group	0.134	0.018	0.826	0.006	0.894	<0.001	0.413	0.147	0.002	0.002	0.005	0.793	<0.001
cov_Rest_Duration	0.803	0.041	0.931	0.095	0.075	0.009	0.023	0.033	0.071	0.684	0.057	0.762	0.140
Control	0.002	0.894	0.293	0.505	0.290	0.082	0.330	0.476	0.480	0.512	0.529	0.169	0.963
Pre_surgery	0.158	0.451	0.656	0.494	0.256	0.902	0.384	0.931	0.666	0.335	0.991	0.871	0.273
Anaesthetic	0.794	0.780	0.784	0.059	0.529	0.314	0.871	0.343	0.799	0.010	0.967	0.291	0.448
Post_pain_relief	0.923	0.101	0.258	0.002	0.378	0.550	0.469	0.333	0.519	0.241	0.843	0.977	0.528
Anaesthetic.Post_pain_relief	0.546	0.836	0.303	0.559	0.620	0.124	0.650	0.256	0.735	0.789	0.400	0.655	0.007

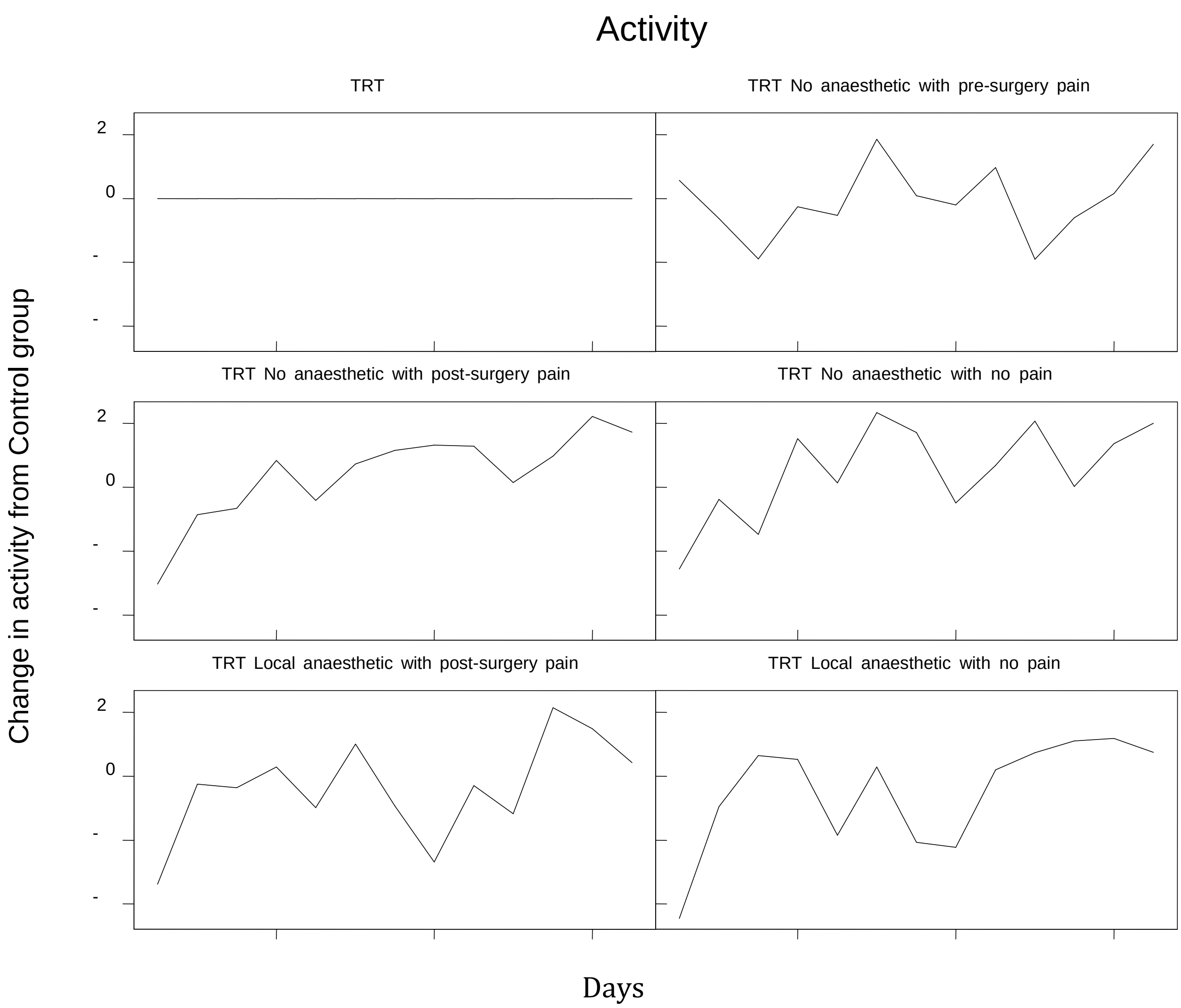


Figure 12: Differences in Daily mean activity (steps) from the control treatment for each of the treatment groups

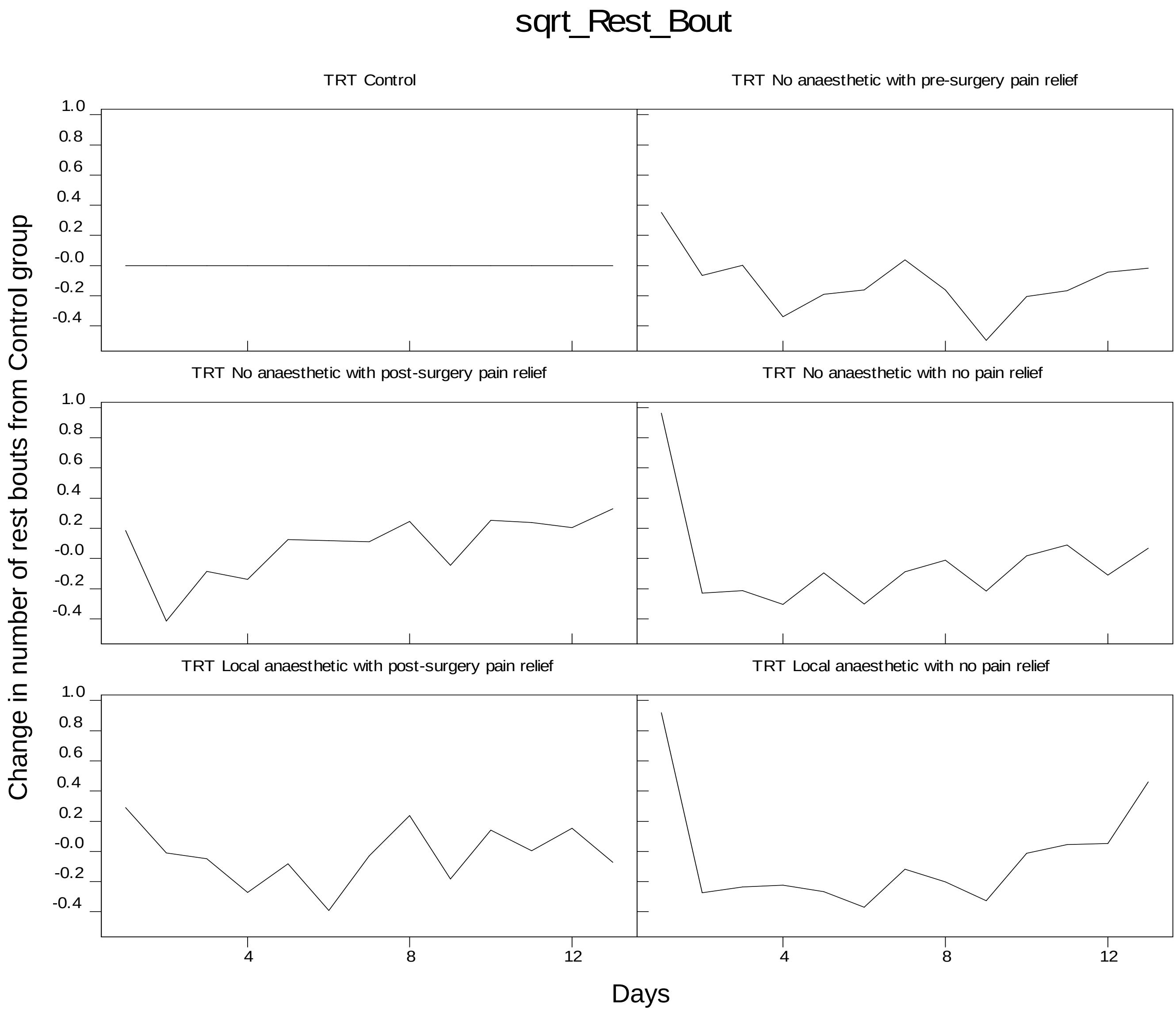


Figure 13: Differences in Daily mean rest bout (number of periods in recumbence) from the control treatment for each of the treatment groups

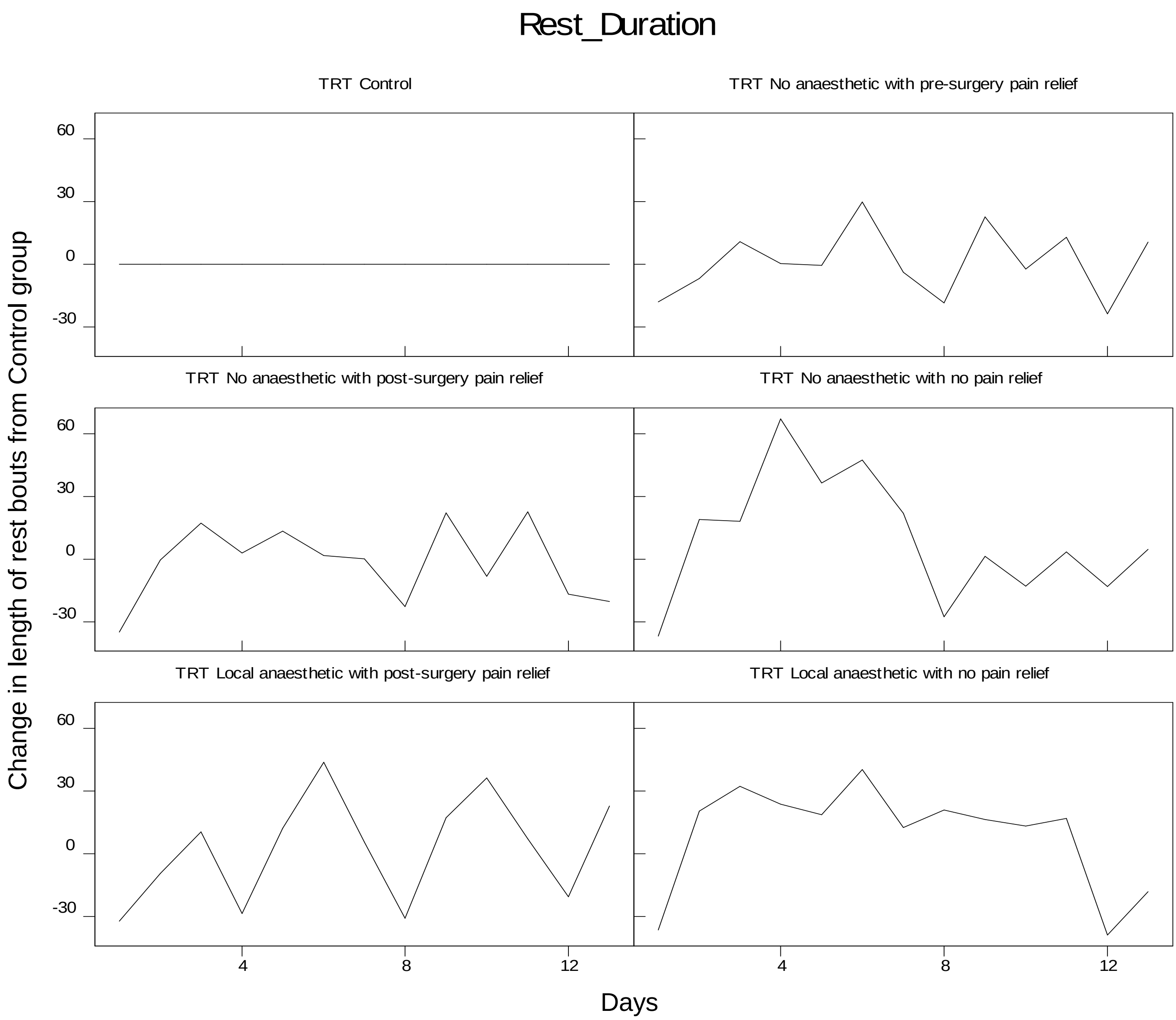


Figure 14: Differences in Daily mean rest duration (length of periods in recumbence) from the control treatment for each of the treatment groups

Table 11: Levels of significance (P-values) for terms in model to analyse combined pedometer data (Activity, Number of rest bouts, duration of rest bouts) over time. Shading indicates significance (P<0.05)

		Activity		**Number of rest bouts**		**Duration of rest bouts**	
Fixed term	Degrees of freedom	F statistic	F pr	F statistic	F pr	F statistic	F pr
Day	12	20.400	<0.001	21.760	<0.001	14.570	<0.001
Experimental_Group	1	65.240	<0.001	6.810	0.011	9.220	0.022
Day.Experimental_Group	12	63.960	<0.001	34.000	<0.001	10.330	<0.001
Covriate	1	73.070	<0.001	23.230	<0.001	10.130	0.018
Experimental_Group.covariate	1	0.260	0.614	2.850	0.097	0.350	0.574
Control	1	0.010	0.908	0.100	0.755	0.310	0.599
Pre_surgery	1	0.050	0.820	1.160	0.285	0.150	0.712
Anaesthetic	1	1.110	0.295	0.170	0.685	0.170	0.695
Post_pain_relief	1	0.000	0.976	0.000	0.976	1.330	0.292
Anaesthetic.Post_pain_relief	1	0.020	0.889	0.340	0.563	0.080	0.784
Day.Control	12	0.640	0.796	1.880	0.053	1.260	0.272
Day.Pre_surgery	12	1.530	0.145	0.930	0.527	0.530	0.880
Day.Anaesthetic	12	1.110	0.373	0.550	0.872	1.390	0.205
Day.Post_pain_relief	12	0.530	0.885	2.730	0.005	1.810	0.075
Day.Anaesthetic.Postpain_relief	12	0.410	0.955	0.930	0.522	1.300	0.250

5. Discussion/conclusion

Cattle production in northern Australia occurs on extensive pastoral land and is characterised by low-input, low-output enterprises [3]. The species that predominates is *Bos indicus* and *Bos indicus*-cross cattle. Reproduction occurs on a year-round basis with calving naturally spread over about nine months owing to feed availability and seasonal fertility of females [4].

Cattle are usually mustered once a year on cattle “stations”. This is the opportunity for producers to draft animals for sale, cull old or injured animals and perform necessary husbandry procedures. These procedures include vaccination and treatment with parasiticides. Additionally, there are surgical husbandry practices that are routine on most stations namely spaying female cattle, dehorning when required and castration of male animals [5]. Castration of bulls is considered vital to minimise danger to station staff and restrict the influence of unwanted genetics on the breeding herd. It has also been shown that while there is an impact on growth rate there are some beneficial carcass traits associated with castration [6,7]. Castration is probably the most common surgical husbandry procedure performed on station cattle.

Surgical husbandry procedures inevitably subject the animal to a degree of pain and stress[8,9]. The degree to which cattle suffer post-surgical pain, methodologies to categorise this pain and the methods to mitigate this pain are the subject of extensive research[10-38]. With the ever-increasing focus on animal production and its impact on animal welfare in Australia, comes the necessity to re-visit traditional husbandry practices, examine their impact and investigate ways to improve them. This research focussed on surgical castration.

This experiment is considered unique in terms of the context within which the research questions and aims were framed. The following is a list of the premises on which the experiment was based:

- that cattle on stations are usually subjected to husbandry procedures once a year and this often results in the castration of bulls when the animals are older than six months of age

- that surgical castration, without anaesthetic and/or analgesia, is a common method used to sterilise male bulls
- that cattle on stations are infrequently mustered and thus are not habituated to human handling or yard activities
- that any pharmacological intervention or modification to technique needs to be practical, affordable, efficient, demonstrably effective and different to current practice, while also being easy to teach to non-veterinarians to increase the chance of industry adoption.
- the experimental castration technique closely mirrored industry practice, namely a standing castration in a crush that employs a scalpel, manual tissue dissection and quick withdrawal of an un-ligated testicle and vessel [5]
- all experimental administration of drugs were readily applicable to a farm situation, namely standard subcutaneous injection of analgesia in the neck of the animal while standing in the race and a one-stage administration of anaesthetic that requires little knowledge of testicular anatomy, rather relies on the dispersion of drug through the testicle, subcutaneous tissue and skin as the drug is injected and on withdrawal of the needle.

Accepting the experimental framework defined here meant the aims became very specific. These were to measure the efficacy in reducing the welfare impost on surgically castrated animals of various combinations of drugs administered in a way that would mimic a high throughput system such as that found on a cattle station. Measuring efficacy then became the challenge and as a consequence a host of parameters were included in an attempt to detect treatment differences and possibly index the most useful measures.

In general terms it was shown that the administration of analgesia, anaesthesia or a combination of the two, improved the overall state of wellbeing of the animal evidenced by reduced cortisol concentrations post-surgery, increased levels of activity and favourable weight gain responses over time. In qualitative terms, during the surgical procedure calves that were castrated and administered either anaesthesia or a combination of analgesia and anaesthesia, were described as more *calm relaxed* and *contented* compared to calves with no analgesia. However, the results were not consistent when comparing treatments, and differences were not evident in some of the parameters measured, namely nociceptive threshold testing and behavioural scoring. It is concluded that this is more a consequence of the low sensitivity of certain parameters combined with the difficulty in measuring pain or distress in animals that are unfamiliar with handling and restraint. A recent castration study using *Bos indicus* bulls concluded that cattle unfamiliar with handling have a heightened cortisol stress response which may mask pain specific responses and any beneficial effects of analgesics (Petherick et al., 2014). In addition, there is likely to be significant variation in pain thresholds between individuals, especially young animals so larger numbers of animals per treatment may be required to see differences in group means.

Specifically, the reduced cortisol concentration associated with the administration of post-surgical analgesia is a finding consistent with other research [12,13,32,36,39,40]. However, it is unusual that there was an interaction with the administration of local anaesthetic on day one, in that the reduced cortisol concentrations associated with anaesthetic was not evident in combination with post surgical analgesia. It is postulated that owing to the relatively low number of animals in a treatment, a couple of excitable animals in the post-surgery analgesia group may have made detecting differences here difficult.

The use of pedometers to measure levels of activity and rest proved a useful tool in measuring the welfare impost of the different treatments. It is hypothesised that animals that are suffering acute or chronic pain take fewer steps, and rest more and for longer periods. This is supported by other literature [18,41,42]. The results in this experiment showed that bulls that received analgesia were more active and rested less than those that didn't. These differences were evident only on the day after surgery but arguably this is when the acute inflammatory response is at its most intense and painful, and the period when effective anti-inflammatories are likely to make the most difference.

Supporting the hypothesis that the use of analgesia reduces the welfare impost and increases production were the data that showed that calves administered analgesia were consistently heavier than those that didn't. Similar findings were found by Bretschneier *et al.* (2005) [12]. The difference in weight in the early post surgery days is arguably associated with the likelihood that treated animals are more active and willing to come to feed troughs and have a better appetite which in turn increases rumen fill. This effect was notably consistent over time in this experiment and is an argument that supports the message that better welfare increases production on farm.

Mechanical nociceptive threshold testing for assessment of analgesic efficacy in *Bos indicus* bull calves requires further refinement. The temperament of the animals, the proximity of the device operator to the animal, the site of stimulus application in relation to the surgery site and the type of stimulus may impact on the collection of meaningful data.

Nociceptive threshold testing involves the application of a potentially painful stimulus to an animal to elicit a specific response. This approach enables an objective assessment of hyperalgesia, hypoalgesia and analgesia as the threshold at which a response occurs can be measured and expressed as a number. The stimulus must be able to be terminated as soon as a response is observed, the response must be a natural behaviour of the animal, and tissue damage should not occur during the delivery of the stimulus [43,44]. Furthermore the stimulus may be thermal, mechanical, chemical or electrical [43] although contemporary literature most commonly refers to the use of mechanical or thermal stimuli in pain and analgesic efficacy studies [45,46]. For the cattle in this study we elected to use a mechanical stimulus and given the size of the animals and the location of the surgery we used a handheld device. Initial pilot tests focused on application of the mechanical stimulus to the scrotum, the perineum and the hock but these sites were too difficult to access safely and it became apparent that the soft tissues were not appropriate sites for testing as a force high enough to elicit a response could not be produced manually. The sacrum proved to be a suitable site to access safely and to cause a response. We settled upon application of the force to either side of the midline of the sacrum while leaning over the side of the race from a raised platform. The repertoire of responses should be small in this context so after initial pilot testing we accepted the following responses and terminated the stimulus accordingly: tail swish, leg lift or kick on the side of the stimulus and stepping away from the stimulus.

Although nociceptive threshold testing has been performed in a range of species including chickens, dairy cows, horses, pigs, cats, dogs and sheep; to our knowledge it has not been performed in *Bos indicus* cattle. Techniques that work in one species cannot always be transferred to another as access to the animal and the observed responses require an understanding of the normal behaviour of the animal in the study environment. These normal behaviours will vary within a species as the period of acclimatisation to the situation, other animals and personnel may impact upon the displayed behaviours. Ideally a stimulus would be delivered remotely and distractions such as noise, smell, companion animals and personnel would be eliminated but this is not always feasible. It is therefore necessary to develop a method which minimises distractions and maximises the display of normal behaviours while allowing the delivery of a stimulus and observation of the animal. We conclude from this study that nociceptive threshold testing requires further refinement in *Bos indicus* bull calves. The temperament of the animals, the proximity of the device operator to the animal, the site of stimulus application in relation to the surgery site and the type of stimulus require further investigation to develop a technique which can be used to investigate analgesic drug efficacy and acute pain.

Good measures of welfare should detect aspects of an animal's physical and psychological state and commonly include behavioural assessments. The EU-project Welfare Quality protocol states that welfare is related to animal's mental state and that welfare indicators should reflect this[47,48]. While there are several accepted physiological and behavioural measures of welfare, the use of qualitative descriptive measures of behaviour are relatively novel. QBA allows observers to describe the emotional state of an animal by looking at the 'whole-animal' and how it interprets changes in its environment. In this study we describe differences in behavioural responses of calves given different treatments using qualitative scoring that were not evident using quantitative behavioural scoring. Interestingly, when the ethograms of a repertoire of the bull calves behaviour (e.g. weight shifting, tail swishing) were compared, no differences between day or treatment were seen. This general assessment of cattle is typical of what stockmen routinely do on farm. While obvious deviation from normal behaviour can be detected (e.g. lying in lateral recumbency, not eating) other more subtle behavioural expressions that might better reflect animal demeanour or how the animal is feeling may go unnoticed. Hence, there is significant interest in the validity and reliability of using various observer judgements of animal behaviour and recent studies continue to support its use [49-51]

QBA allows assessors to focus carefully on one individual and how that individual interacts with its conspecifics and immediate environment. Qualitative assessments of cattle have been used on farm, during land transport and in lairage and have shown correlations with underlying stress physiology [52-54]. In this study, QBA of cattle in the paddock, revealed two findings. First, a strong day effect, where calves tested displayed less "settled" behaviour (less *happy* and *relaxed*) after the long day of handling, NTT testing and castration, compared to two days before. This change was detected regardless of the treatment administered and is likely to reflect a state of high arousal or fear especially given these were young *Bos Indicus* bulls that were not habituated to handling or yards. It is hypothesised that any pain experienced by surgery may be easily masked by the overwhelming response to a threatening environment. The second QBA finding was a day treatment interaction effect between the castrated group and those castrated with analgesia (given anaesthesia and meloxicam), in the paddock. The calves given analgesia were perceived as more *calm* and *comfortable*, indicating a more positive mood than calves without. Thirdly, observers were able to detect differences in body language of calves undergoing castration in the crush, and could differentiate between some but not all cattle given anaesthesia. We believe this reflects the subtle nature of the behavioural responses of Brahman calves to restraint and pain. It was evident that some calves were very traumatised by the restraint of the head bail alone and either struggled forcefully or dropped to the ground, irrespective of treatment. Importantly, this study reports the first application of QBA in *Bos Indicus* cattle which are generally regarded as less habituated to, and more fearful of humans. Further behavioural analysis of these cattle at different time points after surgery and in different contexts (yard versus paddock) may be useful as understanding the fear responses of such breeds to human handling is crucial for the industry to make optimal welfare cost-benefit decisions.

It is tempting to draw bold conclusions from this research because on the whole the findings support the premise that administration of analgesia and/or anaesthesia improved welfare in castrated bulls, but the investigators invite a more reserved interpretation of the results. While in general terms the above statement was true, there were inconsistencies in the results that raise questions. For instance, when there were significant differences evident between certain treatments, there were equally no differences where they would otherwise have been expected. Examples of this are that the behavioural scoring and QBA analysis did not show consistently broad differences between castrated and un-castrated animals. Additionally the effect of post-operative analgesia on cortisol was evident but the effect of pre-operative analgesia was absent. It is postulated that while the significant differences that were detected do indeed support the use of pain mitigation techniques, actually measuring pain and welfare impost remains a difficult challenge, particularly in older bull calves and those less accustomed to human contact and handling. It is hypothesised that the results that promote the use of strategic analgesia and anaesthesia in a manner that is readily adoptable by industry would be magnified in a large, more pointed study that refined the treatment design, increased animal numbers and used only the most promising and sensitive measures of pain identified in this experiment. Further, some inclusion of production and carcass parameters at the end of the finishing phase would lend weight to advice that improving welfare improves profitability, thus increasing the chance of industry adoption.

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Appendix 1 Combined Behavioural Score

Date: Start Time: Finish Time:

		Animal ID											
		13	14	15	16	17	18	19	20	21	22	23	24
Position in Group	2 Together												
	1 Semi												
	0 Isolated												
Grazing	2 Constant												
	1 Intermittent												
	0 Nil												
Ruminating	2 Cud Chew												
	1 Intermittent												
	0 Nil												
SB* - Grooming, Sniffing, Licking	2 Constant												
	1 Intermittent												
	0 Nil												
Weight Shifting	2 Nil												
	1 1-2x/min												
	0 >3x/min												
Hindleg Stamping	2 Nil												
	1 1-2x/min												
	0 >3x/min												
Scrotal Area Grooming	2 Nil												
	1 1-2x/min												
	0 >3x/min												
Tail Swishing	2 Nil												
	1 1-2x/min												
	0 >3x/min												
TOTAL SCORE													
COMMENTS													

* Social Behaviour: R = Receiving, I = Instigating ND = not detectable

NOTE: If Total Score < 2, report immediately