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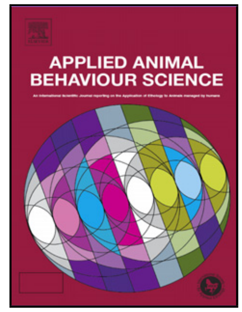

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Qualitative Behavioural Assessment of *Bos indicus* cattle after surgical castration.

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Highlights:

The application of Qualitative Behavioural Assessment (QBA) can aid in the improvement of animal welfare, allowing comparison between husbandry treatments and therefore informing pain management choices.

- Significant differences were recorded between treatment groups for *Bos indicus* calves in response to surgical castration and the administration of analgesia. Significant treatment x day interaction effects were apparent in the four experimental comparisons: C vs NC (in paddock and yard) and C vs CLM (in paddock and yard) contexts.

- Behavioural differences were more obscure in the feed yard than in paddock, however there were treatment x day interaction effects evident. Despite observers being blinded to the experimental treatments, they were able to detect differences in cattle expression between treatments which may reflect the animals' affective state and therefore presumably their experience of pain.

- Results suggested that analgesia (systemic meloxicam) may have provided relief after surgery and encouraged cattle to return to normal survival behaviour such as foraging and seeking food.

Abstract

There are various methods to perform castration of cattle, but surgical castration is the most common. Although it is well documented that surgical procedures inflict pain, analgesic use is usually omitted for surgical castration of production animals in Australia. This study compares the behavioural responses of castrated cattle (C) with non-castrated (NC) controls, and C with those castrated and given lignocaine and meloxicam (CLM) for analgesia. Brahman bull calves (n=8 for each treatment) were filmed in the morning pre- (day -1) and post- (day +1) castration in the paddock and feed yard ('context' of observation). Over four sessions, volunteer observers viewed the video footage for Qualitative Behaviour Analysis (QBA) using the Free Choice Profiling methodology comparing C and NC cattle, and C and CLM cattle under both contexts. The QBA consensus profiles explained 37.4% (C *vs.* NC) and 40.6% (C *vs.* CLM) of variation among observers for paddock sessions and 34.7% (C *vs.* NC) and 38.7% (C *vs.* CLM) for feed yard sessions. Significant *treatment x day* interaction effects were recorded in the paddock (P = 0.007 and P <0.001) and yard (P = 0.004 and P = 0.025) contexts for comparisons between NC vs C, and C vs CLM respectively. Compared to NC, post-castration C cattle were described as more 'bored'/'lethargic' and 'alone' (paddock) and were more 'calm'/'relaxed' and 'relaxed'/'lonely' (yard). Similarly, compared to CLM, post-castration C cattle were described as more 'docile'/'chilled' and 'curious'/'aware' (paddock) and were less 'hungry'/'alert' (yard). There was only one correlation between qualitative and quantitative behaviour scoring; ruminating showed significant correlation with one dimension in each context, that reflected a positive emotion ('calm, relaxed'). The comparison between C *vs.* NC suggest that C were less active and less engaged with their environment compared to the NC group following castration. The C *vs.* CLM comparison suggests a more subtle response whereby analgesia was associated with more positive valence (higher scores on 'calm/comfortable', and 'hungry/alert' dimensions) following castration. The interpretation of findings requires careful consideration of the emotional responses given these calves were unhabituated and reactive to their surroundings. These results suggest the body language of *Bos indicus* cattle may reveal indicators of pain, and that the administration of analgesia may be beneficial at the time of castration. The study highlights the complexities and challenges of identifying pain responses in Brahman cattle.

Keywords: Brahman cattle, Castration, Analgesia, Pain, Qualitative Behavioural Analysis (QBA)

Introduction

Castration is a routine husbandry procedure that is commonly performed to produce docile cattle, to reduce unwanted breeding, to modify carcass quality, and it is considered necessary for economic, safety and management reasons (Stafford and Mellor, 2005; (Lomax and Windsor, 2013). There are three common methods of castration (Phillips, 2010): surgical removal of testes; applying a rubber ring around the scrotum to cut off the blood supply to the testes; and crushing the spermatic cord with a Burdizzo instrument (Phillips, 2010). Of these methods, surgical castration is the most common method, with one study estimating its prevalence at 60% and 57% of all castration procedures, in Australia and USA, respectively (Coetzee et al., 2010a).

In companion animals, successful pain management is widespread; however, the same does not apply to farm animals undergoing painful husbandry procedures, including dehorning and castration (Landa, 2012). It has been well-documented that castration is painful (Molony et al., 1995; Fisher et al., 1996; Petherick, 2006; Coetzee, 2013), and without analgesia, can result in protracted pain lasting several days, or even weeks (Molony et al., 1995; Hay et al., 2003). Nevertheless, some producers believe that young animals generally do not require analgesia for routine elective surgeries such as castration or dehorning (Hewson et al., 2007a; b), and according to the Primary Industries Standing Committee: Model Code of Practice for the Welfare of Animals, Cattle (PISC, 2004), there is no requirement to provide analgesia for castration of cattle under 6 months of age, or under 12 months where castration is performed at the first muster. This approach is also consistent with Meat and Livestock Australia's *Best Practice Guidelines for Routine Husbandry Procedures in Beef Cattle* (Newman, 2007).

Pain is an important aspect of how animals protect their body and maintain their health. It is a signal that tissue damage might occur, is occurring, or has occurred, thereby eliciting immediate escape, withdrawal or other behaviour (Mellor et al., 2000). Pain can also substantially reduce animal wellbeing and prolong the time needed for recovery (Hellyer, 1998; Muir and Woolf, 2001). The use of analgesic drugs for livestock undergoing painful procedures is therefore beneficial not only for their welfare but is also likely to improve safety for personnel performing the procedure (Hewson et al., 2007b).

With the increasing public interest in animal welfare in production systems, refinement of such a common procedure as castration is warranted. In Australia, analgesic agents registered for use cattle are mainly non-steroidal anti-inflammatory analgesic drugs (NSAIDs) such as flunixin, meloxicam, and tolfenamic acid. Local anaesthetic drugs are also used, the most common being lignocaine (Huxley and Whay, 2006). A major issue with testing the efficacy of analgesic drugs is that many livestock species do not show obvious expressions of pain and, consequently, the degree to which animals suffer pain is difficult to describe and 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 assess the expressive qualities of animal demeanour, using descriptors such as ‘content’, ‘relaxed’ or ‘anxious’ (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001). Qualitative behavioural assessment has been applied to livestock under a range of housing, transport and experimental conditions, and QBA scores show significant correlations with meaningful physiological and behavioural measures (reviewed by Fleming et al., 2016). In Northern Australia, cattle are typically of *Bos indicus* breeds, which have resistance to heat stress and tropical diseases (Phillips, 2018). It is also well known that *Bos indicus* are less docile than *Bos taurus* (Hearnshaw et al., 1979; Elder et al., 1980; Fordyce et al., 1982; Burrow 1997).

The aim of this study was to use QBA to determine if *Bos indicus* cattle exhibit behaviour indicative of pain when undergoing surgical castration with and without analgesia. Bull calves were filmed in a paddock and a feed yard pre- (Day -1) and post-castration (Day +1). The behavioural expression (using QBA) of castrated calves with and without analgesia were compared with non -castrate controls to determine any treatment and/or time effects. We hypothesized that castrated calves which received analgesia will display similar behaviour to that of non -castrated calves on Day +1. It was expected that castrated calves which did not receive analgesia will display more painful behaviour in comparison to those receiving analgesia.

Methods

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

Animals and experimental treatments

Twenty-four 6–8 - month-old *Bos indicus* (Brahman) bull calves with a mean weight of 166 ±18kg were sourced from an extensive cattle station in the Pilbara region, north-western Australia. Calves were transported to the Murdoch University campus farm in winter. They were identified by numerical ID tags placed in their right pinna and corresponding numbers spray painted on their rumps to facilitate identification from a distance. The calves were held in the same paddock for 8 days for acclimation post-arrival, during which time they were moved through a race and then held individually in a crush for habituation and sampling of physiological baseline measures (the calves had little to no contact with humans prior to this). This study formed part of a larger project where other measurements were taken, including bodyweight, pedometry, blood cortisol and nociceptive threshold testing (Laurence et al., 2016; Musk et al., 2016). Access to oaten hay and water was allowed *ad libitum* and a complete mixed ration (EasyBeef pellets, Milne AgriGroup Pty Ltd, Perth, Western Australia) was fed daily at ~3% of bodyweight.

Each calf was randomly allocated to one of three treatment groups:

(i) Non-castrated (*NC*) calves were held in the crush for the same duration (5 minutes) as that taken for surgical castration (n=8).

(ii) Castrated (*C*) calves were surgically castrated while standing in the crush (n=8).

(iii) Castrated with analgesia (*CLM*) calves were administered Lignocaine (2 mg/kg, Lignocaine 20, 20 mg/mL, Ilium, Troy Laboratories, Glendenning, NSW, Australia) into each testicular parenchyma 5 minutes prior to castration, surgically castrated, and then administered meloxicam subcutaneously (0.5 mg/kg, Meloxicam 20, 20 mg/mL, Ilium, Troy Laboratories, Glendenning, NSW, Australia) immediately after castration (as described in Laurence et al., 2016).

Behavioural assessments

Quantitative Analysis

Animals were observed and filmed daily in two contexts the day before (Day -1) and after (Day +1) castration. Using binoculars to identify each animal, one observer recorded quantitative behaviour scoring pre-and post-castration in the paddock standing up to 80m away (between 07:00–08:00 h when they were undisturbed), and again at morning feeding in a small yard at approximately 1m away (between 11:00–13:00 h). Individuals were scored for 2 minutes each across eight behavioural categories (Table 1).

Qualitative Behavioural Assessment (QBA)

Concurrent with behavioural scoring, individuals were video recorded for 2 minutes using a handheld digital Panasonic SDR-H250 camcorder (Belrose, NSW, Australia). Footage was edited into 40–50 sec duration clips (Adobe Premier Pro CS3 and Adobe After Effects CS3, Chatswood, NSW, Australia) and presented in randomised order for each viewing session.

Volunteer observers were recruited via poster advertisements on campus and through Murdoch University social media pages. Four viewing sessions were held. Each viewing session compared footage of cattle before and after castration for paired treatments. Twenty observers were recruited for two sessions to assess footage of cattle in the paddock (*C vs. NC*, and *C vs. CLM*) and 30 observers were recruited for two sessions to assess footage of animals in the yard (*C vs. NC*, and *C vs. CLM*). All 20 observers who completed the paddock sessions also participated in the yard sessions. Observers included university staff, students, primary producers and the public. All observers were blinded to the experimental treatments or the context in which the animals had been filmed.

Free Choice Profiling (FCP) methodology was used for scoring video clips , which relies on observers generating their own unique set of descriptive terms (Wemelsfelder et al., 2001). Each observer therefore attended a term generation session where they were asked to list descriptive terms that they believed described cattle in a series of clips that showed a range of behavioural expressions. This session was followed by the scoring sessions where observers scored cattle in the experimental clips according to their own descriptive terms using a visual analogue scale. Observers were 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. Data were recorded in Excel files (Microsoft Excel 2013, North Ryde, NSW, Australia) for each observer.

Data were analysed by Generalised Procrustes Analysis (GPA) using a specialised software edition (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001; GenStat, 2008). Generalised Procrustes Analysis develops a consensus profile of all observers' scores by transforming the data and identifying complex patterns. A Procrustes Statistic was calculated, quantifying the percentage of variation between observers that was explained by the consensus. The Procrustes Statistic was compared with the result of this randomisation test (Dijksterhuis and Heiser, 1995) by a one-way *t*-test. Where the observer consensus profile falls significantly outside the distribution of the randomised profiles, this indicates that the consensus was a significant feature of the data set and not simply an artefact of the Procrustean calculation procedures (Fleming et al., 2013).

Subsequently, the numbers of GPA dimensions of the consensus profile are reduced through Principle Component Analysis. For descriptive understanding, the terms used by each observer to score cattle behaviour were correlated with each GPA dimension. Terms that show the strongest correlations with the GPA dimension scores (>75% of the highest absolute correlation coefficient values) (Mardia et al., 1979) were used to describe each dimension.

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.

Except where indicated, data are presented as mean ± standard deviation.

Results

The 20 participants in the paddock sessions generated 97 unique descriptive terms: 14 ± 5 (range 7–23) terms per observer. The 30 participants in the yard sessions generated 137 unique terms: 15 ± 5 (range 7–28) terms per observer. Procrustes Statistics (Table 2) indicated that the GPA consensus profile was significantly different from the mean randomised profile for all four viewing sessions.

The list of descriptive terms associated with each of the GPA axes is shown in Table 2. Significant *day x treatment interaction* terms are shown in bold. Ruminating was the only behaviour correlated significantly with any GPA dimension scores (described below).

In the paddock (C *vs.* NC), there was a significant day effect for GPA dimension 1, where cattle were described as 'relaxed'/ 'calm' vs. 'curious'/ 'alert'). Cattle were scored as more 'relaxed'/ 'calm' on Day -1 and scored more 'curious'/ 'alert' on day +1 (Figure 1a). There was no significant *treatment* effect or *treatment x day interaction*. There were significant *day,* and *treatment x day interaction* effects for both GPA 2 ('happy'/ 'contented' vs. 'bored'/ 'lethargic') and GPA 3 ('curious'/ 'lonely' vs 'alone'). Castrated cattle (C) became more 'bored'/ 'lethargic' (GPA2) and more 'alone' (GPA3) after castration when compared to NC cattle (Figure 1: GPA 2 & 3).

In the paddock (C *vs.* CLM), there was a significant day effect but no significant *treatment x day* interaction for GPA dimension 1 (‘uncomfortable’/ ‘sleepy’ vs. ‘happy’/ ‘relaxed’). Both treatment groups were more ‘uncomfortable’/ ‘sleepy’ the day after castration (Figure 2a). There were significant *treatment* and *treatment x day interaction* effects for GPA 2 (‘curious’/ ‘inquisitive’ vs. ‘docile’) and GPA 3 (‘calm’/ ‘comfortable’ vs. ‘curious’/ ‘aware’). Castrated cattle (no analgesia) were scored as more ‘docile’ and ‘curious’/aware’ the day after castration (compared with the day before), while CLM cattle were scored as more ‘curious’/ ‘inquisitive’ and ‘calm’/’comfortable’ the day after (Figure 2b, c). Ruminating was negatively correlated with GPA 3 scores ($r_{s\ 32} = -0.50$, $P < 0.05$) with animals scored as more ‘calm’/’comfortable’ having higher ruminating scores than those that were scored as more ‘curious’/ ‘aware’.

In the yard (C *vs.* NC), there were both *treatment* and *treatment x day* interaction effects for GPA dimension 1 (‘agitated’/ ‘anxious’ vs. ‘calm’/ ‘relaxed’). Castrated cattle were scored as more ‘calm’/ ‘relaxed’ the day after castration, while there was no significant change for NC. All three effects (Day, Treatment and Treatment x Day) were significant for GPA 2 (‘relaxed’/ ‘confused’ vs. ‘watchful’/ ‘frightened’). Non-castrated cattle became more ‘watchful’/ ‘frightened’ the day after castration compared with C. Ruminating was negatively correlated with GPA dimension 2 scores ($r_{s\ 32} = -0.38$, $P < 0.05$), with animals scored as more ‘relaxed’/ ‘confused’ having higher ruminating scores than those that were scored as more ‘watchful’/ ‘frightened’. There were significant *day* and *treatment* effects (but not their interaction) for GPA 3 (‘angry’/ ‘annoyed’ vs. ‘affectionate’/ ‘comfortable’). Castrated cattle were scored as more ‘affectionate’/ ‘comfortable’ than NC (scored as more ‘angry’/ ‘annoyed’) and both groups were more ‘affectionate’/ ‘comfortable’ the day after castration.

In the yard (C *vs.* CLM), there was a significant *day* effect for GPA dimension 1 ('alert'/ 'tense' vs. 'calm'/ 'relaxed') although there were no significant post-hoc results (Figure 4a). All three effects (Day, Treatment and Treatment x Day) were significant for GPA dimension 2 ('tired'/ 'sad' vs. 'hungry'/ 'alert'); CLM cattle were scored as more 'hungry'/ 'alert' the day after castration. There were significant *day* and *treatment* effects (but not their interaction) for GPA 3 ('startled'/ 'unsure' vs. 'free/ 'comfortable') with both treatment groups becoming more 'startled'/ 'unsure' the day after castration (Figure 4b, c).

Discussion

Significant differences were recorded between treatment groups for *Bos indicus* calves in response to surgical castration and administration of analgesia. Significant *treatment x day* interaction effects were apparent in the four experimental sessions: C vs NC and C vs CLM paddock and yard contexts. Compared to NC, C cattle were more 'bored'/'lethargic', and 'alone' (paddock; GPA 2, and 3) and were more 'calm'/'relaxed' and 'relaxed'/'lonely' (yard; GPA 1 and 2) post-castration (Day +1). This result suggested that C animals showed less activity and engagement with their environment and tended to remain alone, compared to the NC group. Similarly, compared to CLM, the C cattle were more were more 'docile'/'chilled' and 'curious'/'aware' (yard; GPA 2 and 3) and were less 'hungry'/'alert' (yard; GPA 2) post-castration (Day +1). This result suggested that analgesia may have provided some relief after surgery (CLM showed less passive, and more typical behaviour) and encouraged cattle to return to normal survival behaviour such as foraging and seeking food. After the handling and procedure, it would be expected that the cattle would display signs of hunger, yet those in pain may not.

Cattle behaviour is complex, and the display of pain can be masked by other signs. Cattle that displayed less active behaviour such as liveliness and play, may have higher blood lactate and glucose levels, meaning they are fearful and distressed (Camerlink et al., 2016). Displaying less active movement could be due to discomfort from the surgical procedure and a subsequent reluctance to move normally. Both de Oliveira et al. (2014) and Mellor et al. (2000) stated that when pain is evident, standing still, lying still and moving around/eating less may reduce pain. When animals in pain did move around, they did so with restriction and/or with short steps or hunched backs (de Oliveira et al., 2014). This type of 'less active' behaviour may be described as being 'calm' or 'happy' to observers blinded to the experimental treatments. Applying QBA in conjunction with physiological measures, such as blood cortisol or pedometer scores, might aid in clarifying the valence of behaviour. Pedometer and blood cortisol results from Laurence et al. (2016) showed that *Bos indicus* calves would benefit from the administration of analgesia. Calves that received lignocaine before castration had significantly lower cortisol concentrations and when peri-operative meloxicam was provided, calves were more active compared to other treatment groups (Laurence et al., 2016).

Calves in group CLM became more active on Day +1, as they were described as more 'hungry'/'alert' compared to calves in group C; this may be reflect the fact that CLM calves were less painful and moving more to seek food. Pain relief, provided as local anaesthesia and a NSAID, administered before castration and/or dehorning, has been shown by others to markedly reduce the indicators of acute pain (Sutherland et al., 2013; Thür et al., 2007). Although behavioural differences were more obscure when calves were filmed in the feed yard, there were still *treatment x day* interaction effects evident. Even though observers were not aware of the experimental treatments and their descriptive terms did not focus on pain behaviour, they were still able to detect differences in behavioural expression between treatment groups that could be related to their affective state and therefore presumably their experience of pain.

The provision of analgesia resulted in the manifestation of behaviour that was viewed more positively by the observers, evidenced by the behaviour shown in group CLM. Various studies have demonstrated that indicators of pain, such as behavioural and physiological responses, are significantly reduced if NSAIDs 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. Behavioural differences between *Bos indicus* and *Bos taurus* is not known however, from this experiment there were evidence that providing some form of analgesia (meloxicam) after surgery does benefit the animal's well-being.

The context in which animals are observed can influence QBA scoring patterns (Fleming et al., 2014) and detecting pain associated with castration may therefore require consideration of the context in which the animals are filmed. When the calves were filmed from a distance in the paddock, *treatment x day* interaction effects were evident for GPA dimensions 2 and 3 (but not GPA 1). When the calves were filmed in the feed yard, at closer proximity, the degree of activity and arousal was elevated for all animals; significant *treatment x day* interaction effects were evident for GPA 1 and 2, although they were less obvious than in the paddock. Given the first GPA dimension captures the most obvious behavioural differences and the majority of variance in scoring, it is likely the amount of activity among the cattle would have a greater influence on the QBA scores (Fleming et al., 2016). Similar findings of arousal have been reported in pigs (Temple et al., 2011b, Clarke et al., 2016) and sheep (Phythian et al., 2013). Data from the present study suggests that stockpersons are more likely to be able to identify individuals experiencing pain if they observe animals under quiet conditions, such as while they are in the paddock, further away from humans, rather than when they are aroused (e.g. while being handled or fed). Interestingly that the one activity, ruminating showed a correlation to one GPA dimension in both the yard and the paddock. Both these dimensions suggested positive valence; where an increasing number of cattle ruminating was associated with cattle being described as more 'calm' and 'relaxed'. This suggests that cattle ruminating could be useful indicator of positive emotional state.

Conclusion

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; QBA is able 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). QBA can provide an assessment of the animal's whole response to its environment and what is happening to the animal. Therefore QBA 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). Qualitative behaviour assessment may therefore reveal important aspects of how animals interact with their environment and their affective states including pain responses.

Traditionally, some stockpersons have under-rated the pain caused by castration (Byrne et al., 2001) with the attitude that cattle are unable to experience negative emotions. Hence pain relief has not always been provided. However, there is ample evidence that castration causes pain (Stafford and Mellor, 2005). The future of animal welfare requires educating both professionals and general public on the importance of pain assessment. Application of behavioural assessment tools can aid in the pursuit of better animal welfare for livestock species, allowing comparison between experimental husbandry treatments and therefore informing pain management choices.

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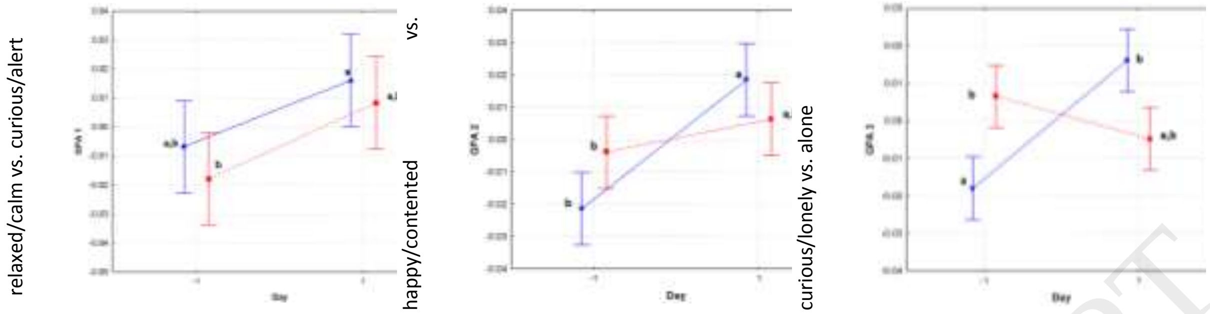

Figure 1: QBA Sessions on pre and post castration (in paddock), comparing C vs. NC cattle for GPA dimensions 1, 2 and 3.

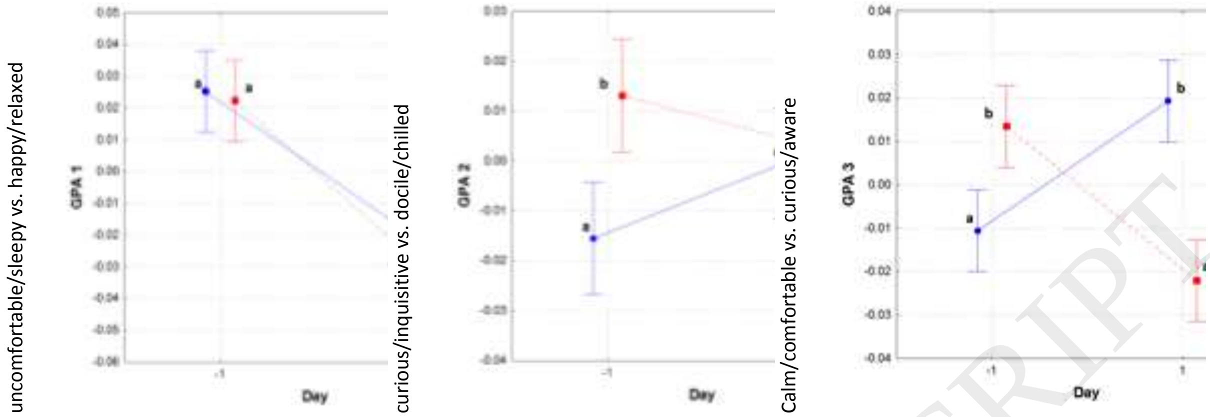

Figure 2: QBA Sessions on pre and post castration (in paddock), comparing C vs. CLM cattle for GPA dimensions 1, 2 and 3.

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

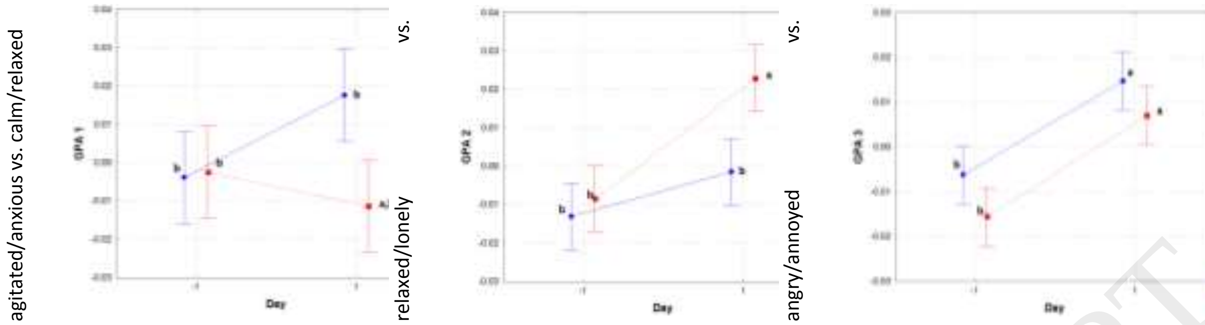

Figure 3: QBA Sessions on pre and post castration (in yard), comparing C vs. NC cattle for GPA dimensions 1, 2 and 3.

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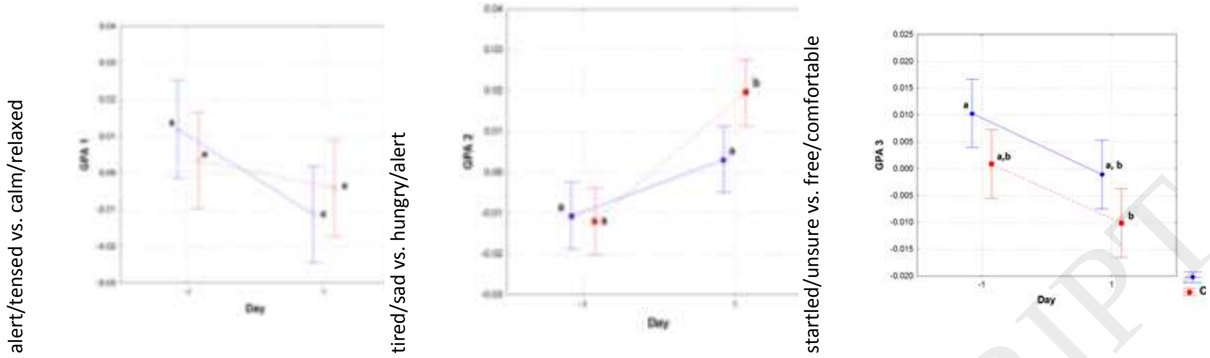

Figure 4: QBA Sessions on pre and post castration (in yard), comparing C vs. CLM cattle for GPA dimensions 1, 2 and 3.

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

Table 1 Behavioural scoring (2 minutes of observation per animal)

	Score
Position in group	0 = isolated (alone by itself, more than two body lengths away from others) 1 = semi-isolated (with one other animal within two body lengths away but not in a big group) 2 = together (with more than one other animal within two body lengths)
Grazing	0 = Nil (not eating) 1 = Intermittent (eating but not continuous) 2 = Constant (eating the entire 2 minutes)
Ruminating	0 = Nil (no movement in mouth region) 1 = Intermittent (ruminating but not continuous) 2 = Cud chew (constantly chewing / non-stop the entire 2 minutes)
Social Behaviour (grooming, sniffing, licking)	0 = Nil (no evidence of social behaviour) 1 = Intermittent (evidence of social behaviour but not continuous to itself or another) 2 = Constant (non-stop display of social behaviour to itself or another)
Weight shifting	0 = >3x/min 1 = 1-2x/min 2 = Nil (standing still)
Hindleg stamping	0 = >3x/min 1 = 1-2x/min 2 = Nil (standing still)
Scrotal area grooming	0 = >3x/min 1 = 1-2x/min 2 = Nil (no attempt)
Tail swishing	0 = >3x/min 1 = 1-2x/min 2 = Nil (no movement in tail region)

Table 2: Terms used by observers to describe behavioural expression of calves pre-and post-castration filmed in the paddock or in the feed yard the day before (Day -1) and the day after (Day +1) surgical castration.

		Terms correlated with the GPA dimension axis ‡			
Treatment Groups	**GPA Dimensions**	**Low values**	**High values**	**Treatment (MANOVA) §**	**Effect**
Footage from the paddock (Day -1 and +1)					
Session A1: *Castrated vs. Non-castrated (C vs. NC) Procrustes Statistic 34.6% (t_{99} = 32.07; P <0.001)*	1 (36.95%) †	Relaxed (5), Calm (5), Laid-back, Chilled, Contented, Quiet, Apathetic, Satisfied	Curious (3), Alert (3), Agitated (3), Stressed (2), Restless (2), Anxious, Disquieted, Distressed, Timid, Frightened, Cautious, Inquisitive, Nervous, Weary, Scared, Unsettled, Defensive, Lost, Uncomfortable	Day Treatment Treatment X Day	$F_{1,19}$ = 20.87, P < 0.001 $F_{1,19}$ = 2.87, P = 0.107 $F_{1,19}$ = 0.055, P = 0.818
	2 (18.8%) †	Happy (2), Content, Relaxed, Active	Bored (3), Lethargic (2), Uncomfortable (2), Exhausted (2), Tired (2), Disinterested, Sleepy, Sore	Day Treatment **Treatment X Day**	$F_{1,19}$ = 22.12, P < 0.001 $F_{1,19}$ = 0.17, P =0.680 **$F_{1,19}$ = 9.01, P = 0.007**
	3 (9.9%) †	Curious (2), Lonely (2), Confused, Agitated, Afraid, Trapped, Hesitant, Weary, Impatient, Bored, Cautious	Alone	Day Treatment **Treatment X Day**	$F_{1,19}$ = 10.60, P = 0.004 $F_{1,19}$ = 0.63, P = 0.438 **$F_{1,19}$ = 33.45, P < 0.001**

Treatment Groups	GPA Dimensions	Terms correlated with the GPA dimension axis ‡ Low values	High values	Treatment Effect (MANOVA) §
Session A2: *Castrated vs. Castrated with analgesia (C vs. CLM) Procrustes Statistic 38.7% (t_{99} = 45.12; P < 0.001)*	1 (26.8%) †	Uncomfortable (4), Sleepy (2), Nervous, Sad, Stressed, Unsettled, Depressed, Timid, Tired, Unsure, Disinterested, In_pain	Happy (4), Relaxed (4), Contented (3), Calm, Excited, Aware	**Day $F_{1,19}$ = 44.02, P < 0.001** Treatment $F_{1,19}$ = 1.36, P = 0.259 Treatment X Day $F_{1,19}$ = 1.50, P = 0.235
	2 (17.9%) †	Curious (6), Inquisitive (2), Alert (2), Weary, Frustrated, Aware, Distressed, Excited, Unsure, Bored	Docile, Chilled	Day F1,19 = 0.36, P = 0.556 Treatment $F_{1,19}$ = 11.82, P = 0.003 **Treatment X Day $F_{1,19}$ = 4.69, P = 0.043**
	3 (13.3%) †	Calm, Comfortable, Not afraid, Satisfied, Uncomfortable, Tired, Uncertain, Tender, Friendly *Ruminating	Curious, Aware, Dominant	Day $F_{1,19}$ = 0.82, P = 0.375 Treatment $F_{1,19}$ = 6.23, P = 0.022 **Treatment X Day $F_{1,19}$ = 26.91, P < 0.001**
Footage from the feed yard (Day -1 and +1)				
Session B1: *Castrated vs. Non-castrated (C vs. NC) Procrustes Statistic*	1 (30.7%) †	Anxious (4), Agitated (4), Excited (2), Nervous (2), Frightened (2), Restless (2), Edgy, Tensed, Disturbed, Annoyed, Scared, Irritated, Alert, Stressed, Energetic, Startled	Calm (5), Relaxed (3) Comfortable (2), Contented, Chilled, Settled, Quiet, Happy, Patient, Aimless	Day $F_{1,19}$ = 1.655, P = 0.208 Treatment $F_{1,29}$ = 9.13, P = 0.005 **Treatment X Day $F_{1,29}$ = 10.01, P = 0.004**

Treatment Groups	GPA Dimensions	Terms correlated with the GPA dimension axis ‡ — Low values	High values	Treatment Effect (MANOVA) §
40.56% (t_{99} = 32.07; P < 0.001)	2 (16.3%) †	Relaxed (2), Lonely, Calm, Tired, Confused *Ruminating	Watchful, Frightened, Amused, Sociable, Unsure, Angry	Day $F_{1,29}$ = 20.07, P = 0.001 Treatment $F_{1,29}$ = 26.23, P < 0.001 **Treatment X Day $F_{1,29}$ = 5.57, P = 0.025**
	3 (8.8%) †	Angry (3), Annoyed (2), Stressed, Restless, Discontented, Confused, Aggressive, Unpleasant, Anxious, Uncomfortable, Inquisitive	Affectionate, Comfortable, Playful, Motivated, Excited	Day $F_{1,29}$ = 15.99, P < 0.001 Treatment $F_{1,29}$ = 21.10, P < 0.001 Treatment X Day $F_{1,29}$ = 0.07, P = 0.792
Session B2: *Castrated vs. Castrated with analgesia (C vs. CLM)*	1 (39%) †	Alert (3), Tense (2), Agitated (2), Edgy, Scared, Tensed, On edge, Irritated, Dominant, Uncomfortable, Anxious, Restless, Nervous, Stressed, Frightened, Excited	Calm (4), Relaxed (4), Contented (2), Settled, Chilled, Comfortable, Quiet, Enjoying	Day $F_{1,29}$ = 7.78, P = 0.009 Treatment $F_{1,29}$ = 0.04, P = 0.838 Treatment X Day $F_{1,29}$ = 3.41, P = 0.075
Procrustes Statistic 37.37% (t_{99} = 45.12; P < 0.001)	2 (15.1%) †	Tired (7), Sad (6), Lifeless, Sick, Disoriented, Deflated, Drowsy, Depressed, Lethargic, Restless, Lonely, Bored	Hungry (2), Alert, Excited	Day $F_{1,29}$ = 22.55, P < 0.001 Treatment $F_{1,29}$ = 8.08, P = 0.008 **Treatment X Day $F_{1,29}$ = 12.95, P = 0.001**

Treatment Groups	GPA Dimensions	Terms correlated with the GPA dimension axis ‡ Low values	High values	Treatment (MANOVA) §	Effect
	3 (9%) †	Unsure, Startled	Free, Comfortable Placid, Happy, Relaxed Agitated	Day Treatment Treatment X Day	$F_{1,29}$ = 14.77, P < 0.001 $F_{1,29}$ = 18.94, P < 0.001 $F_{1,29}$ = 0.01, P = 0.935

GPA = Generalised Procrustes Analysis

† The percentage of variation explained by each GPA dimension 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.

* 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**.**