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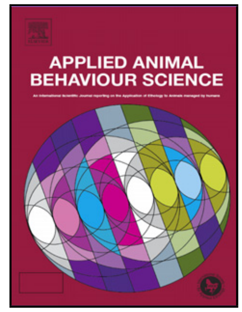

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A comparison of welfare outcomes for weaner and mature *Bos indicus* bulls surgically or tension band castrated with or without analgesia: 1. behavioural responses

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ABSTRACT

Tension-band castration of cattle is gaining favour because it is relatively simple to perform and is promoted by retailers of the devices as a humane castration method. Furthermore, retailers encourage delaying castration to exploit the superior growth rates of bulls compared with steers. Two experiments were conducted, under tropical conditions, comparing tension banding and surgical castration of weaner (7-10 months old) and mature (22-25 months old) *Bos indicus* bulls with and without pain management (ketoprofen or saline injected intramuscularly immediately prior to castration). Welfare outcomes were assessed using a wide range of measures; this paper reports on the behavioural responses of the bulls and an accompanying paper reports on other measures. Behavioural data were collected at intervals by direct observation and continuously via data loggers on the hind leg of the bulls to 4 weeks post-castration. Tension-banded bulls performed less movement in the crush/chute than the surgically castrated bulls during the procedures (weaner: 2.63 vs. 5.69, $P < 0.001$; mature: 1.00 vs. 5.94; $P < 0.001$ for tension-band and surgical castration respectively), indicating that tension banding was less painful then surgical castration during conduct. To 1.5 h post-castration, tension-banded bulls performed significantly (all $P < 0.05$) more active behavioural responses indicative of pain compared with surgical castrates e.g. percentage time walking forwards (weaner: 15.0% vs. 8.1%; mature: 22.3% vs. 15.1%), walking backwards (weaner: 4.3% vs. 1.4%; mature: 2.4% vs. 0.5%), numbers of tail movements (weaner: 21.9 vs. 1.4; mature: 51.5 vs. 39.4) and leg movements (weaner:12.9 vs. 0.9; mature: 8.5 vs. 1.5), respectively. In contrast, surgically castrated bulls performed more immobile behaviours compared with tension-banded bulls (e.g. standing in mature bulls was 56.6% vs. 34.4%, respectively, $P = 0.002$). Ketoprofen administration appeared effective in moderating pain-related behaviours in the mature bulls from 1.5 – 3 h, e.g. reducing abnormal standing (0.0% vs. 7.7%, $P = 0.009$) and increasing feeding (12.7% vs 0.0%, $P = 0.048$) in NSAID- and saline-treated bulls respectively. There were few behavioural differences subsequent to 24 h post-castration, but some limited evidence of chronic pain (3-4 weeks post-castration) with both methods. Interpretation, however, was difficult from behaviours alone. Thus, tension banding is less painful than surgical castration during conduct of the procedures and pain-related behavioural responses differ with castration method (active restlessness in response to tension banding and minimisation of movement in response to surgical castration). Ketoprofen administered immediately prior to castration was somewhat effective in reducing pain, particularly in the mature bulls.

1. Introduction

Castration is one of the most common husbandry procedures conducted on beef cattle, with data from the USA indicating between 7 million (Coetzee, 2011) and 15 million

(Coetzee et al., 2010) procedures per annum. There is ample evidence that castration causes pain (e.g. see review by Stafford and Mellor, 2005) but in Australia, there is no legal requirement for managing the pain for cattle under 6 months of age. For cattle over 6 months, the current code of practice does not mention pain management, but stipulates that castration should (or in some legislatures, must) be conducted by a veterinarian (Primary Industries Standing Committee (PISC), 2004). In the USA, not only is there no requirement for pain management for cattle castration, there are no analgesic drugs approved for pain relief in cattle (Coetzee, 2011).

In both Australia and the USA, the most common method is surgical castration (57% and 60% of cattle producers in the USA and Australia, respectively, Coetzee et al., 2010; Meat and Livestock Australia (MLA) pers. comm.). In the last 15 to 20 years, however, there appears to have been an increase in the use of banding as a castration method. In the USA, 22% of survey respondents reported using banders (Coetzee et al., 2010) and anecdotal reports suggest that banding has gained favour in some parts of Australia due to its perceived ease of application and superior outcomes in terms of reduced mortalities, particularly in older bulls.

Amongst beef cattle producers, there seems to be the perception that banding is less painful than surgical castration. This perception is reinforced by the manufacturers and retailers of the devices who promote them as being a humane method of removing the testicles stating, for example, that the process is “bloodless and avoids the stress, set backs and risk of infection often associated with surgical castration” http://www.farmerswarehouse.com.au/product.php?productid=16569. Further, the retailers appear to encourage producers to delay castration to exploit the superior liveweight gains achieved by intact males due to higher testosterone levels compared with castrated males (e.g. see http://www.nobull.net/bander/SBHumane.htm, http://www.probeef.com.au), although in Australia there is a recommendation that tension banding should be restricted to calves up to 6 months of age (http://www.probeef.com.au) although this contradicts the current code of practice which sets an upper age for ring castration of 2 weeks and does not distinguish between ring castration and tension banding (PISC, 2004).

Several welfare-related studies on tension banding have been conducted previously, but most of them have used a limited range of welfare-related measures and all were performed with *Bos taurus* cattle. Some studies have focussed on improving the understanding of the inflammatory response (Pang et al., 2006, 2008, 2009a, b, 2011), whilst only productivity measures were recorded (or reported) in another (Knight et al., 2000). No behavioural data were collected in the work of Stafford et al. (2002), whilst in other work, local anaesthetic and long-acting penicillin were administered to all bulls, potentially masking behavioural and wound healing differences between castration methods (Fisher et al., 2001).

The work of Gonzalez et al. (2010) was conducted in penned animals, used measures of production and stress, and collected limited behavioural data (feeding and lying only) but did not examine wounds and healing. Similarly, the work of Repenning et al. (2013) was conducted in a feedlot environment and focussed on production-related measures, although they did collected heart and respiration rate, and rectal temperature data as indicators of an inflammatory response. Furthermore, findings from research on *Bos taurus* cattle conducted in temperate climates are not perceived by beef cattle producers as being relevant to *Bos indicus* bulls in a tropical, rangeland environment, particularly since *Bos indicus* cattle are reported as being behaviourally and physiologically more reactive to handling than *Bos taurus* animals (Fordyce et al., 1988; Zavy et al., 1992).

Thus, the aim of this work was to assess the welfare outcomes, using a broad range of measures, for *Bos indicus* bulls castrated by tension banding in a tropical environment. As surgical castration is the method most commonly used in Australia and the USA, the welfare outcomes from tension banding were compared with those from surgical castration. Given the increasing attention on pain relief in livestock (Weary and Fraser, 2004), it seemed appropriate to determine whether effective analgesia could be achieved under commercial-type conditions and, with the apparent promotion of delayed castration, it was appropriate to examine the impacts on welfare for bulls of different ages. This paper describes the study methods and reports on the behavioural measures in response to castration, whilst a second paper (Petherick et al., submitted) reports on selected health, stress and production responses.

As there have been no previous studies on tension banding of *Bos indicus* cattle that have systematically recorded behaviour, it was unclear what the outcomes would be, so we predicted no difference between the methods in evoking pain-related behaviours. Ketoprofen is registered for use in cattle in Australia and has previously been shown to be effective in alleviating pain in surgically castrated (Earley and Crowe, 2002) and tension-banded calves (Stafford et al., 2002). Injection of it intramuscularly immediately prior to castration may be practicable in large commercial operations where hundreds of bull calves/weaners are to be castrated in a day. Thus, with this method of administration, we anticipated a time-lag of an hour or so before analgesia would be induced. We predicted, therefore, no differences in behaviours between the castration methods in this initial period, but that pain-related behaviours would be reduced for the following 12 or more hours. Further, we predicted that pain-related behaviours would be greater in older than younger bulls as a consequence of greater tissue damage, although this could not be examined statistically, as separate experiments were conducted for the two age groups of bulls.

2. Materials and methods

2.1. Animals and location

The use of the cattle in this experiment was approved by the CSIRO (Queensland) Animal Ethics Committee (approval A7/09). Two studies comparing surgical and tension-banding castration in weaner (7-10 months old) and mature (22 – 25 months old) bulls were conducted at Belmont Research Station, approximately 26 km north of Rockhampton, Queensland, Australia (150° 22' 57'' E, 23° 13' 26'' S). The weaner bull study was conducted during the 'dry' season (July to October) and the mature bull study in the early to mid-'wet' season (November to January). For the weaner bull study, the mean monthly maximum and minimum temperatures ranged between 24.7 – 27.6°C and 10.7 – 17.0°C, respectively, with 203.6 mm rain (21 wet days). For the mature bull study, the mean monthly maximum and minimum temperatures ranged between 27.5 – 31.2°C and 19.4 – 22.9°C, respectively, with 726.2 mm rain (34 wet days).

2.2. Weaner bull protocol

The cattle were purebred Brahmans that were born and raised on Belmont Research Station. Thirty-two bulls (mean liveweight ± s.e. at allocation, 217.8 ±2.93 kg) were assigned to four treatment combinations (n=8 per treatment group) according to liveweight and scrotal circumference (Entwistle and Fordyce, 2003; 16.7±0.18 cm) measured 3 weeks before the experiment, and an average (1.70±0.094 m/s) of three flight speeds, with two recorded at weaning (April) and another at 3 weeks before the experiment. Flight speed was measured according to a validated method (Burrow et al., 1988) using specially manufactured equipment (Ruddweigh-Gallagher Animal Management Systems, Campbellfield, Vic, Australia). It was considered important to take into account flight speed in the allocation of bulls to treatments, as previous work has found relationships between flight speed, stress responses and liveweight gains (Petherick et al., 2002, 2009). Bulls were allocated to eight blocks, each containing one animal for each treatment, from spatial groupings in the first two dimensions from a principal components analysis of liveweight, flight speed and scrotal circumference data. These two dimensions encompassed 86% of the total variation of the three variables.

The four treatment combinations formed a 2 x 2 factorial combination of castration method and pain management and were: tension-banding castration and an intramuscular injection of saline (Band+saline); tension-banding castration and an intramuscular injection of a non-steroidal anti-inflammatory drug (Band+NSAID); surgical castration and an intramuscular injection of saline (Surgical+saline); and surgical castration and an intramuscular injection of a non-steroidal anti-inflammatory drug (Surgical+NSAID).

The tension banding was conducted using the Callicrate Bander (No-Bull Enterprises, St. Francis, Kansas, USA) and the NSAID used was ketoprofen (Ilium Ketoprofen, Troy Laboratories Pty., NSW, Australia) injected intramuscularly into the anterior of the neck at a rate of 3 mg/100 kg liveweight, according to manufacturer recommendations. We wished to simulate likely commercial conditions with the administration of a NSAID which was why we injected it intramuscularly immediately prior to castration. We were cognisant that analgesia would take some time, so did not expect to see effects on behaviours during the conduct of castration or immediately post-castration. The saline solution (0.9% sodium chloride, Baxter Healthcare Pty. Ltd., Old Toongabbie, NSW, Australia) was injected in the same location as the NSAID at an equivalent volume. Also, as these bulls were valuable experimental animals, they were given tetanus antitoxin (Equivac TAT, Pfizer Australia Pty. Ltd., West Ryde, NSW, Australia; 1500 IU/mL) at the rate of 1,000 IU/head to ensure there were no deaths from tetanus although, due to routine vaccinations, they should have been protected.

Due to daylight constraints, castrations were conducted on 2 successive days (day 0) with four randomly selected blocks castrated on the first day (Batch A) and the remainder (Batch B) on the second. The procedures for the four blocks were started at approximately 7:00, 7:45, 8:40 and 9:55 h, respectively and the procedures were the same on both days.

2.2.1. Procedures

All bulls were individually identifiable from ear-tags that had been inserted within 12 h of birth. On the day before the experiment, the bulls were weighed. On the day of castration, the bulls were moved individually into a veterinary crush/chute and restrained by head-bailing and two blood samples (both approximately 8 mL) were taken, via a single jugular venipuncture using18 G needles, into vacutainers. Blood parameter data are presented elsewhere (Petherick et al., submitted). According to treatment, NSAID or saline and tetanus antitoxin were injected (using 18 G needles). An IceTag3D™ motion sensor device (IceTag data logger) was fitted to the left hind leg in accordance with the manufacturer recommendations (IceRobotics, Roslin, Midlothian, Scotland). These devices have been validated for determining when cattle are standing/lying (Trenél et al., 2009; Tolkamp et al., 2010). It has been determined that these devices have the potential for over-estimating the number of steps taken by cattle, due to leg-lifting occasionally being recorded as a step (Nielsen et al., 2010). That work used dairy cows, however, and the leg-lifting was observed when the cows were in cubicles; when walking, there was good correspondence between the IceTag data and recordings from video footage. Thus, we were confident that reliable data on number of steps taken would be generated in free-moving bulls.

The bulls were then castrated by the pre-assigned method. Animals were restrained in a head bail within the crush/chute during castration. All castrations were conducted by the same operator who was experienced and skilled in both techniques. Surgical castration was conducted according to an industry best practice guide (Newman, 2007) using a cut to the scrotum for each testicle made with a hand-held scalpel blade. After incision, the scrotum was pulled back to expose the testicle, and the spermatic fascia incised to expose the testis. Once the testis was exposed, the cremaster muscle and proper ligament of the testis were separated from the testis. The testis was then pulled away from animal's body to expose as much of the spermatic cord (incorporating the ductus deferens and the testicular artery and vein) as possible. The cord was severed with a 'scraping/sawing' action (in order to minimise bleeding) as close to the animal's body as possible and proximal to the testicle, away from where a high density of blood vessels were clearly obvious. Once both testes had been removed, the animal was immediately released to a clean yard, with the entire procedure (from the start to end of restraint) taking approximately 3.5 min.

Tension banding was conducted according to the manufacturer and supplier instructions (e.g. see http://www.nobull.net/bander/SBhowtouse.html). The band was inserted into the bander and the bull restrained in a head-bail, with a kick bar inserted behind the animal to hold it forward in the veterinary crush/chute to minimise the risk of injury to the operator, who worked at the rear of the animal. The operator inserted his hand through the band and grasped the testicles, then drew the testicles through the band. The ratchet was cranked to put a light tension on the band, ensuring that both of the testicles were held in the scrotum below the band. The band was checked and adjusted to ensure it was appropriately positioned just above testicles with the aluminium clip located at the centre-rear of the scrotum. The band was tightened, via the ratchet, to the correct tension (when the tension peg reached the rear of the slot). The crimping lever was then pushed down to hold the band tension via the aluminium clip, and the band cut close to the spool. The animal was then released to a clean yard, with the entire procedure (from the start to end of restraint) taking approximately 3.5 min.

When all four animals in the block had been castrated, groups were moved into small yards (approximately 50-70 m^2) with shade, and hay and water available ad libitum, where they remained until it was time for their next blood sample to be taken. Thus, each block of four was maintained as a group in a separate yard on the day of castration. At the end of day 0, the blocks were combined and the cattle walked to a small holding paddock (approximately 3400 m^2) adjacent to the yard complex, with pasture and water available ad libitum. The following day they were walked to the yard complex for their day 1 blood sample and then returned to the holding paddock. This process was repeated for days 2 and 3 for both batches of cattle, with Batch B cattle being held in a 3 ha holding paddock, with access to a yard (85 m x 40 m) containing hay and water ad libitum. After the day 3 blood sample for Batch B, the batches were combined and grazed a series of small (6 to 23 ha) paddocks to ensure that there was ample forage of good quality available at all times.

2.2.2. Behaviour recording

Behaviour was recorded during castration by direct observation by an experienced observer, with counts made of each of the following behaviours: push at the head-bail; pull back from the head-bail; jump (all hooves off the ground); jerk (sudden, small jump with overall body tension); struggle (moving back and forth in the head-bail with legs flailing); kick (with one or both hind legs to the rear); and stamp (raising and lowering any leg with a swift action). Due to the small numbers of these individual behaviours, the counts were combined into a total movement score. In addition, the number of vocalisations was scored and a note made of whether the bulls kneeled or lay down in the crush during castration.

The ethogram used for observations post-castration is given in Table 1 and was derived from the many published studies on purportedly painful husbandry procedures performed on cattle and the extensive experience of the person conducting the observations (JCP) with these classes of cattle. Behaviours having a duration of 5 s or more were categorised as ‘states’ and other behaviours (lasting less than 5 s) were classified as ‘events’. States were mutually exclusive and total durations (s) were calculated for each state and the percentage of the total time spent in each state determined. Counts of all events were summed for each sample period for each animal. A number of behaviours were also combined into behavioural ‘categories’, as indicated in Table 1.

On day 0 post-castration, blocks of animals were directly observed by 5-min focal animal sampling by the observer standing in the yard complex. This method was chosen as a compromise to allow data collection for both long duration (states) and, what may have been, infrequent behaviours (events) and with the constraints due to limited manpower and the movement of cattle for the blood sampling schedule. The observer was unaware of the treatments applied to the bulls, although in some instances, due to the presence of blood on the hind legs, it was apparent which bulls had been surgically castrated. The order in which animals in a block were observed was as they were individually identified by the observer, who was mindful that attention may be drawn to overt, active behaviours. There was no fixed schedule of observations for each block, rather blocks were observed opportunistically to fit with the blood sampling schedule and the movement of cattle through the yard system. Each block was, however, observed on four to six occasions from immediately post-castration to immediately after the final blood sample at 7 h post-castration. Inspections of graphs of the observation times showed there was no bias in the times post-castration that the observations were made. Behaviour was also recorded by 5-min focal animal sampling, from a utility vehicle using binoculars as necessary, on days 1, 2, and 3 post-castration when the cattle were in the two batches and on the single group of 32, on days 6, 13, 19 and 27 post-castration (which were days 5, 12, 18 and 26 post-castration for Batch B cattle). For simplicity, these observation days will be, hereafter, referred to as weeks 1, 2, 3 and 4, respectively. The order of recording was on the basis of locating individuals. Observations were started between 6:15 and 8:15 h and took 3 to 4 h to conduct depending on the ease of finding animals. The method of recording behaviour at these times was again selected due to the physical difficulty, safety issues and length of time required to capture data on cattle in 'commercial' conditions. The percentage of time spent standing and lying, and number of steps/h were automatically determined from the Icetag data, with the loggers removed at week 4 post-castration.

2.3. Mature bull protocol

Unless stated, the protocol was identical to that used for the weaner bulls. The bulls had previously been used in an experiment investigating indicators of fertility and had been subjected to Bull Breeding Soundness Evaluations (BBSE; Entwistle and Fordyce, 2003) on three occasions at approximately 12, 18 and 24 months of age. As part of the BBSE the animals had been moved through the yards, restrained and head-bailed in a veterinary crush/chute and electro-ejaculated for semen collection.

Thirty-two bulls were assigned to four treatments (n=8 per treatment) according to liveweight (401.6 ±5.80 kg), scrotal circumference (29.9±0.32 cm), as measured about 3 weeks before the experiment, and an average (2.05±0.080 m/s) of five flight speed measurements: two at the time of weaning and three when the BBSE had been conducted. Blocks (eight, each containing one bull for each treatment) were formed from spatial groupings in the first two dimensions from a principal components analysis of liveweight, flight speed and scrotal size data. The two dimensions encompassed 72% of the total variation of the three variables.

As these cattle had received vaccinations, they should have been protected against tetanus, but to be certain, tetanus antitoxin was given at the rate of 1,500 IU/head (as they were mature animals). The bulls were weighed on the day before the experiment and castrations were conducted on 2 successive days, with four randomly selected blocks castrated on the first day (Batch C) and the remainder (Batch D) on the second. The procedures for the four blocks were started at approximately 6:30, 7:30, 9:50 and 10:50 h, respectively and the procedures were the same on both days.

2.3.1. Procedures

Bulls were surgically castrated as described in 2.2.1 above, but a Hausmann emasculator (Aesculap, Germany) instrument was used to cut the cord as close to the animal's body as possible and proximal to the testicle. The clamped cord was held in the emasculator for about 30 s to close-off the blood vessels and minimise bleeding. Once both testicles had been removed, the animal was immediately released to a clean yard, with the entire procedure (from the start to end of restraint) taking approximately 3.5 min. The procedure for tension banding was as for the weaner bulls, but in some instances it was necessary to draw the testicles through the band one at a time. The entire procedure (from the start to end of restraint) took approximately 3.5 min.

After the day 3 sampling of Batch D, the two batches were combined into a 21 ha paddock with ample forage of good quality. At week 10, however, the cattle had to be moved due to flooding, and for the remaining 2 weeks were held in a 7 ha paddock with ample forage of good quality.

2.3.2. Behaviour recording

Observations were made as described in 2.2.2 above, but on days 6, 13, 20 and 27 post-castration for Batch C (a day earlier for Batch D) which, again, are referred to as weeks 1 to 4, respectively, hereafter. We were unable to obtain sufficient IceTag loggers for all animals, so only 30 bulls were fitted (one Band+NSAID and one Surgical+NSAID did not have an IceTag).

2.4 Statistical analyses

The time-series nature of the data was taken into account by repeated-measures analyses of variance (Rowell and Walters, 1976), using the AREPMEASURES procedure of GenStat (2014). This forms a split-plot analysis of variance (split for time). The Greenhouse-Geisser epsilon estimates the degree of temporal autocorrelation, and adjusts the probability levels for this. Behavioural states were analysed as the percentages in each defined category of the total time observed, and these distributions proved to be approximately Normal. Counts of observed events were discrete in nature and moderately skewed, so the sqrt(x+0.5) transformation was adopted to stabilise the variances. Significant differences between means were determined using protected least-significant-difference (LSD) testing at the $P = 0.05$ level.

For the analyses of observed behaviours, day 0 was divided into three time periods post-castration: 0 to 1.5 h (mean time of 0.45 h for the weaners and 0.48 h for the mature bulls); 1.5 to 3 h (mean time 2.31 h for the weaners and 2.19 h for the mature bulls); and 3+ h (mean time 5.89 h for the weaners and 5.13 h for the mature bulls). The IceTag (continuous) data were extracted and analysed using two different resolutions; a daily basis (where days were 24-h periods post-castration) for the 4 weeks that IceTags were on the cattle and an hourly basis for the initial 24 h post-castration.

Initial analyses were conducted on the factorial treatment structure (NSAID administration by castration method) over all times. The interaction between castration method and time was notably pronounced for most variables (behavioural and non-behavioural measures), being significant ($P<0.05$) in 67% of the individual tests, and these time-patterns form the main focus in the results. These initial analyses were, however, less conclusive for the effect of NSAID. Research findings (Landoni et al., 1995) and manufacturer recommendations on the frequency of administration of ketoprofen indicate that the analgesic effect would likely be present only during the first 12 to 24 h post-administration. Thus, re-analyses were conducted, firstly using data up to 24 h only (for the parameters that were measured during this period). These analyses showed that NSAID did have an effect during day 0 across all parameters, the NSAID terms (the main effect and its interactions with time and castration method treatment) were significant ($P<0.05$) in 16% of the individual tests across weaners and mature bulls, notably more than would be expected from random chance alone. Importantly, these analyses also showed that the NSAID effect had effectively dissipated at the 24 h measurements, as the number of detected significant differences at this point only approximately reflected random variation. The result of the NSAID effect was, thus, re-analysed and presented separately to 24 h post-castration; thereafter the two levels (NSAID and saline) were pooled as replicates for the castration method treatment (in secondary analyses, of the post-24 h period).

3. Results

As statistical comparisons between weaner bulls and mature bulls are not valid due to confounding of bull age with the time each experiment was performed, results for the two age cohorts are presented separately.

3.1 Weaner bulls

3.1.1 Behaviour in the crush/chute at castration

Both castration method ($F_{1,21}$ = 16.5; $P<0.001$) and NSAID administration ($F_{1,21}$ = 8.34; P = 0.009) affected the total amount of movement during castration; the Surgical group moved more then the Band group (mean± s.e., 5.69 ± 0.63 vs. 2.63 ± 0.43, respectively) and the NSAID group moved more than the Saline group (5.25 ± 0.61 vs. 3.06 ± 0.46, respectively). There was no difference between treatments in the numbers of bulls that knelt (P = 0.50 for castration method and 0.49 for NSAID administration) or lay down during castration (P = 0.58 for castration method and 0.57 for NSAID administration). The mean proportion (± s.e.) of bulls kneeling and lying were 0.094 ± 0.0442 and 0.156 ± 0.0547, respectively. There were insufficient vocalisations for analysis.

3.1.2 Behaviour post-castration by direct observation

Behaviours that were influenced by time only are neither presented nor discussed. NSAID administration had few effects on behaviour on day 0; there was a tendency for it to reduce the numbers of tail movements ($F_{1,21}$ = 4.08; P = 0.056; Fig. 1). Square-root-transformed means (with back-transformed numbers in parentheses) were 2.01 (3.54) and 2.56 (6.05) for NSAID and Saline respectively, LSD = 0.565. The NSAID x castration method interaction ($F_{1,21}$ = 11.18; P = 0.003; LSD = 9.55) indicated significantly more time spent feeding by the Surgical+NSAID weaners (20.9%) than the Surgical+saline weaners (1.8%), with the Band+NSAID (11.5%) and Band+saline (14.1%) weaners being intermediate.

On day 0, castration method affected percentage time spent ruminating ($F_{1,21}$ = 5.67; P = 0.027; LSD = 9.11) with the surgical castrates ruminating significantly more (11.3%) than the banded weaners (0.9%). There was also a tendency ($F_{1,21}$ = 3.88; P = 0.062; LSD 1.032) for surgical castrates to spend more time drinking (1.37%) than the banded weaners (0.39%). There were significant castration method x time interactions for percentage of time walking forwards ($F_{2,54}$ = 5.43; P = 0.013), walking backwards ($F_{2,54}$ = 6.28; P = 0.011) and the numbers of tail ($F_{2,54}$ = 21.01; P < 0.001) and leg movements ($F_{2,54}$ = 24.56; P < 0.001), with behaviours for the banded weaners at high levels in the initial 1. 5 h post-castration, but stabilising or declining to the 3+ h period (Table 2). Percentage time walking forwards was significantly greater in the banded than surgical castrates in the first period (0 – 1.5 h post-castration), and similarly for walking backwards where this trend also held in the second period. Leg and tail movements were significantly greater in the banded weaners compared with the surgical castrates for the first period (Table 2 and Fig. 1), and then stabilised over time. Additionally, a significant castration method x time interaction after day 0 ($F_{6,180}$ = 2.57; P = 0.033) showed a significantly greater number of tail movements at week 3 in the Band compared with Surgical weaners (Fig. 1).

3.1.3 Behaviour post-castration via IceTags

During the first 24 h post-castration, there was a significant effect of castration method on the percentage of time spent lying ($F_{1,27}$ = 9.09; P = 0.006; LSD = 5.55); the Surgical weaners spent less time lying (34.0%) than the Band weaners (42.2%).

3.2 Mature bulls

3.2.1 Behaviour in the crush/chute at castration

Both castration method ($F_{1,21}$ = 62.3; P<0.001) and NSAID administration ($F_{1,21}$ = 5.68; P = 0.017) affected the total amount of movement during castration; the Surgical group moved more then the Band group (mean± s.e., 5.94 ± 0.609 vs. 1.00 ± 0.248, respectively) and the NSAID group more than the Saline group (4.25 ± 0.515 vs. 2.69 ± 0.409, respectively). There was no difference between treatments in the numbers of bulls that knelt (P = 0.163 for castration method and 0.632 for NSAID administration) and only two bulls lay down in the crush/chute, meaning the data could not be sensibly analysed. The mean proportion (± s.e.) of bulls kneeling was 0.219 ± 0.065. Again, there were too few vocalisations for analysis.

3.2.2 Behaviour post-castration by direct observation

Behaviours influenced by time only are neither presented nor discussed. NSAID administration influenced more of the behaviours on day 0 of the mature than weaner bulls. There was a significant NSAID x castration method effect on percentage time spent ruminating ($F_{1,21}$ = 7.59; P = 0.012; LSD = 7.68), with ruminating occurring only in the Surgical+NSAID bulls (14.4%). There were significant NSAID x time interactions for percentage time spent feeding ($F_{2,52}$ = 3.41; P = 0.048), standing ($F_{2,52}$ = 4.28; P = 0.027) and abnormal standing ($F_{2,52}$ = 6.90; P = 0.009). Table 3 shows that all of these behaviours did not differ between NSAID and saline treated bulls during the first (0 – 1.5 h) and final (3+ h) periods post-castration, but time spent feeding was significantly higher in the NSAID- than saline-treated bulls in period 2 (1.5 – 3 h post-castration) and time standing and abnormal standing were higher in the saline- than NSAID-treated bulls in this same period.

Castration method affected the percentage time spent standing ($F_{1,21}$ = 12.68; P = 0.002; LSD = 12.95) and lying ($F_{1,21}$ = 5.31; P = 0.032; LSD = 14.13) on day 0; Surgical bulls spent more time standing (56.6%) and less time lying (19.0%) than Band bulls (34.4% and 34.7%, respectively). There were significant castration method x time interactions for percentage time spent abnormal lying ($F_{2,52}$ = 6.51; P = 0.013), walking forwards ($F_{2,52}$ = 7.53; P = 0.002) and walking backwards ($F_{2,52}$ = 7.17; P = 0.002, Table 4), and for numbers of leg movements $F_{2,52}$ = 12.94; P < 0.001), as well as a tendency for numbers of tail movements to be affected ($F_{2,52}$ = 2.99; P = 0.060 Table 4). Walking forwards and backwards were significantly greater in the Band than Surgical bulls in the first period with no difference thereafter, and abnormal lying was not seen in the Surgical bulls, but the difference between castration methods was significant only during period 2. Numbers of leg movements were greater in the Band than Surgical bulls in periods 1 and 2 (to 3 h post-castration), and tail movements tended to be greater in the Surgical than Band bulls in the 3+ h period.

After day 0 there was a castration method effect on percentage time spent ruminating ($F_{1,23}$ = 7.06; P = 0.014; LSD = 7.88), with the Band bulls overall spending twice the amount of time ruminating (20.1%) compared with the Surgical bulls (10.0%). This did not appear to be related to the percentage time spent feeding for which there was a castration x time interaction ($F_{6,180}$ = 2.42; P = 0.040), as values were largely similar between methods except for week 2 when the Band bulls spent about twice the amount of time feeding compared with the Surgical bulls (although this was not statistically significant), and week 3 when the Surgical bulls spent twice the time compared with the Band bulls (Fig. 2).

3.2.3 Behaviour post-castration via IceTags

During the first 24 h post-castration, there was a tendency for castration method to affect the number of steps/h ($F_{1, 24}$ = 3.93; P = 0.059; LSD = 39.29) and the percentage of time spent lying ($F_{1, 24}$ = 4.02; P = 0.056; LSD = 8.01); steps/h were less for Surgical (200.8) than Band (238.6) and percentage time lying was also less for Surgical (30.9%) than Band (38.7%).

4. Discussion

During the conduct of castration, tension banding was less painful than surgical castration for both weaner and mature bulls, as evidenced by the relative numbers of movements performed within the crush/chute, a finding supported by that of Repenning et al. (2013). One other study comparing tension-banding and surgical castration of bulls (360 kg) investigated behavioural responses during the procedure (Rust et al., 2007) and, in contrast to the current study in which we recorded few vocalisations, these workers found more vocalisation during surgical compared to tension-banding castration. Given that vocalisation is recorded during stressful and painful situations (e.g. see Watts and Stookey, 2000), this finding of Rust et al. (2007) suggests that surgical castration was more painful than tension banding, which agrees with our finding. We did not expect any analgesic effect from the ketoprofen during castration, but it was unexpected that the NSAID-treated bulls (weaner and mature) moved more than the saline-treated bulls and we can only speculate that ketoprofen sensitised the bulls, but can suggest no mechanism.

The behaviours shown by the bulls on the day of castration were consistent with the behavioural responses of cattle undergoing purportedly painful procedures. Abnormal lying is reported to be a pain-related response (Mellor et al., 1991; Robertson et al., 1994; Molony et al., 1995) and has previously been documented in cattle castrated with 'heavy rubber bands' (Stafford and Mellor, 2010) and calves castrated with rubber rings (Molony et al., 1995). Repetitive leg and tail movements are reported to be associated with pain from castration (Molony et al., 1995; Fisher et al., 2001) and walking backwards is associated with painful procedures, such as dehorning (Graf and Senn, 1999) and castration (Robertson et al., 1994). Abnormal standing has been documented as a pain-related response in surgically spayed cattle (Petherick et al., 2013) and rumination is suppressed by pain (McMeekan et al., 1999; Sylvester et al., 2004; Almeida et al., 2008; Kolkman et al., 2010). Standing, lying and walking forward can be difficult to interpret with regard to pain, but a reluctance to move is indicative of pain (Molony et al., 1995; Stafford and Mellor, 2005) and standing immobile is a pain-related response previously reported in surgically castrated calves (Molony et al., 1995). Restlessness, which may be reflected by increased locomotion, is also reported to indicate pain (Mellor et al., 1991), although Gonzalez et al. (2010) suggest standing is indicative of restlessness due to pain. Our findings, therefore, indicate that both surgical castration and tension banding were acutely painful for both the weaner and mature bulls, which is not an unexpected finding.

Of greater interest is the relative painfulness of the two procedures and whether ketoprofen alleviated the pain. The effect of the NSAID on behavioural responses provides evidence additional to the comparison with the findings of others, as described above, that the bulls were experiencing pain, as opposed to, say, general stress. Due to the timing and route of administration of the NSAID we did not expect analgesia in the initial time-period (0 – 1.5 h) post-castration, but expected analgesia for the remainder of day 0. Unexpectedly, for the weaners there was no indication of a time period effect on day 0 on the efficacy of the NSAID, although the number of behaviours influenced was limited; time spent feeding was highest in the Surgical+NSAID weaners indicating that this combination of treatments was the most effective at alleviating pain. The Surgical+saline weaners spent the least time feeding, suggesting that they were in the most pain of all the treatments. There was also no effect of time period on tail movements by the weaners on day 0, but rather the NSAID-treated weaners tended to show fewer tail movements than the saline-treated weaners suggesting some efficacy of ketoprofen in alleviating pain.

Judging solely from the behaviours affected (feeding, ruminating, standing and abnormal standing) ketoprofen was more effective in alleviating pain in the mature bulls than in the weaners. Rumination occurred only in the Surgical+NSAID mature bulls and, in contrast to the weaners, there were effects of the time periods, as we'd anticipated, on other behaviours. There was no indication of efficacy during the initial period (0 – 1.5 h post-castration), but during period 2 (1.5 – 3 h post-castration) the ketoprofen had beneficial effects i.e., reducing the percentage of time spent standing and abnormal standing and increasing the percentage of time spent feeding. A finding of no differences in these behaviours between the NSAID- and saline–treated bulls in the third period (3+ h post-castration) shows that all treatment groups were experiencing a similar degree of pain at this time. The limited effectiveness of the NSAID concurs with the findings of Moya et al (2011). In the band castrated animals, tissue perfusion is stopped, and it may be that this prevents delivery of the NSAID to the site in sufficient concentrations to be effective. In the surgically castrated animals, it may be that tissue acidosis does not reach a sufficient level to dislodge the NSAID from plasma proteins into the site of insult.

The surgically castrated weaners spent more time ruminating then the banded weaners, suggesting that banding caused more pain than surgical castration. The surgically castrated weaners also tended to spend more time drinking which may have been a consequence of blood loss and dehydration in the surgical castrates. The reason it was not seen in the mature bulls may have been a consequence of these larger bulls being able to better tolerate fluid loss than the lighter, weaner bulls. Indeed we found this same pattern of drinking on the day of procedures with heifer and mature cows subjected to flank spaying (Petherick et al., 2013). Alternatively, the use of an emasculator for surgical castration of the mature bulls may have minimised blood loss.

The castration method x time interactions revealed similar effects on the weaner and mature bulls with the banded bulls showing more time walking forwards and walking backwards and a greater number of tail and leg movements compared with the surgical castrates during the initial period post-castration. Abnormal lying was seen only with banding in the mature bulls and at a high level (22% of the time) in the 1.5 – 3 h period post-castration. The number of steps during the first 24 h post-castration recorded for the mature bulls via the IceTag loggers also revealed that the banded bulls walked more than the surgical castrates. All of these responses are consistent with banding being more painful than surgical castration during the 24 h post-castration and particularly during the initial two time periods.

Having said this, there was also evidence that surgical castration caused pain. In the mature bulls the direct observations showed that the surgically castrated bulls spent more time standing and less time lying compared with the banded bulls. The data from the IceTags supported this finding with the time spent lying in the initial 24 h post-castration being less (hence, more time spent standing) in the surgical than banded castrates for both the weaner and mature bulls. Given that percentage of time standing was reduced by the NSAID in the mature bulls and that others have reported standing immobile to be indicative of pain (Molony et al., 1995; Gonzalez et al., 2010), there is evidence of pain from surgical castration, but the behavioural responses differed to those induced by banding. The pain associated with surgical castration is reflected by standing relatively immobile in contrast to the active pain-related behaviours (walking, leg and tail movements) arising from banding castration, although abnormal lying was also seen in the banded bulls. It is not possible to determine the relative painfulness of the two castration methods based only on these behavioural responses, as such responses are likely to vary with the location and amount of tissue damage (Stafford and Mellor, 2005). The second paper of this pair (Petherick et al., submitted) provides additional (non-behavioural) data to better evaluate the relative painfulness of the castration methods.

These findings of active behaviours in response to tension banding and standing immobile in response to surgical castration contrast with those from Fisher et al. (2001) who reported no difference in lying and walking and more leg and tail movements in surgically castrated than in tension-banded, 14-month-old *Bos taurus* bulls. Fisher et al.'s findings are somewhat surprising given that the bulls castrated by both methods had local anaesthetic administered into the testes and subcutaneously to the sites that were cut or banded. It is unclear as to the exact timing of behavioural observations in relation to castration, but possibly the behaviours occurred when the anaesthesia wore-off. Studies on young unanaesthetised *Bos taurus* calves provide support for our findings, with more leg movements shown by 1-week-old calves castrated by rings than by those castrated surgically (Molony et al., 1995) and more tail, foot and head movements in calves of between 6 and 42 days of age castrated by rings compared with burdizzo and surgical methods (Robertson et al., 1994).

There were few effects of castration method after the day of procedures; on average for the 4 weeks, the mature banded bulls spent more time ruminating than the surgical castrates and more time feeding at 3 weeks post-castration. These findings suggest that the surgical castrates were in more pain than the banded bulls, although they contrast with the findings for the weaners when, at 3 weeks post-castration, significantly more tail movements were performed by the banded than surgical castrates. Again, these apparently conflicting findings demonstrate why additional data are needed to assist with the interpretation of behavioural responses and the results reported in the accompanying paper (Petherick et al., submitted) assist with clarification.

In summary, our prediction that both castration methods would result in similar pain appears substantiated for short-term pain, but the behavioural responses differed with the methods. There was some limited evidence of chronic pain with both methods, which we had not predicted. As anticipated, there was a time-lag for analgesia induction, but whilst ketoprofen provided some pain relief for the mature bulls, it seemed less effective for the weaner bulls, which was contrary to our expectations. Consideration of the relative numbers and percentages of time spent performing pain-related behaviours suggests that, as we had anticipated, pain was greater for the mature than weaner bulls, although this could not be examined statistically.

5. Conclusion

Tension banding causes less pain than surgical castration during the conduct of the procedures. During the ensuing 24 h, weaner and mature bulls experience pain with both methods, but behavioural responses differ between methods; tension banding evokes restless activity in contrast to movement minimisation after surgical castration. There is some limited behavioural evidence of chronic pain with both methods, but the responses may differ with age; non-behavioural data are required to aid interpretation of these longer-term behavioural responses. Ketoprofen administered by intramuscular injection immediately prior to castration (as a practical method of administration in large, rangeland enterprises) appears to be less effective in weaners than in mature bulls for managing pain, based on behavioural responses.

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Fig.1 Mean number of tail movements (square-root transformed) performed by weaner bulls post-castration by tension banding (Band) or surgery (Surgical), and with or without the administration of a non-steroidal anti-inflammatory drug (NSAID) immediately prior to castration

Fig. 2 Mean percentage of time spent feeding by mature bulls post-castration by tension banding (Band) or surgery (Surgical), and with or without the administration of a non-steroidal anti-inflammatory drug (NSAID) immediately prior to castration

Table 1. Ethogram developed for observations conducted on bulls post-castration

Behaviour	Description	Category
States (durations)		
Stand alert	Standing with muscles tense, head held high, ears pricked, apparently looking at something	Stand
Stand relaxed	Standing with muscles relaxed, head held relaxed, ears loose, apparently not focusing visually	
Stand head down	Standing with head below brisket, looking “depressed” e.g. ears drooped, little/no response to external stimuli	‘Abnormal’ standing
Stand shaking	Standing with muscle and body tremors	
Lie alert	Lying with muscles tense, head held high, ears pricked, apparently looking at something	Lie
Lie relaxed	Lying on sternum with muscles relaxed, head held relaxed, ears loose, apparently not focusing visually	
Stand ruminating	Standing with slow chewing movements and regurgitations	Ruminate
Lie ruminating	Lying on sternum with slow chewing movements and regurgitations	
Lateral lying	Lying recumbent on side	‘Abnormal’ lying
Lie neck extended	Lying on the sternum with head and neck extended on the ground	
Walk forward	Forward locomotion (mainly walk, but occasionally trot or gallop)	
Walk backwards	Backwards locomotion (walk)	
Feed	Ingestion (eating hay, grazing, browsing)	
Drink	Ingesting water	
Events (counts)		
Tail flick	Sideways movement of the tail from vertical and return to vertical	Tail movement
Tail tuck	Standing or lying, tail pulled tight between the hind legs and released	
Leg lift	Raising and lowering of front or hind foot, may involve a “stamp”	Leg movement
Kick	Rapid movement of one or both hind legs to the rear or the belly of the animal	

Table 2. Effects on behaviour of weaner bulls of castration by surgery (Surgical) or banding (Band) during three time periods on the day of castration

Behaviour		Time periods post-castration (h)			
		0-1.5	1.5-3	3+	LSD
Walking forwards (%)	Surgical	8.12^{a}	5.00	5.90	3.802
	Band	15.04^{b}	3.54	6.27	
Walking backwards (%)	Surgical	1.38^{a}	0.21	0.33	1.220
	Band	4.26^{b}	1.25	0.43	
No. tail movements*	Surgical	1.38^{a} (1.40)	1.86 (2.96)	2.24 (4.52)	0.965
	Band	4.73^{b} (21.87)	1.61 (2.09)	1.89 (3.07)	
No. leg movements*	Surgical	1.18^{a} (0.88)	0.89 (0.29)	1.01 (0.52)	0.632
	Band	3.66^{b} (12.92)	1.51 (1.77)	0.78 (0.11)	

*values sqrt (counts + 0.5) with back-transformed means in parentheses
Super-scripted letters indicate significant differences (P < 0.05) between the castration methods, within each variable and time period.

Table 3. Effects on behaviour of mature bulls of administration of a NSAID or saline immediately prior to castration by surgery or banding during three time periods on the day of castration

Behaviour		Time periods post-castration (h)			
		0 - 1.5 h	1.5 - 3 h	3+ h	LSD
Feeding (%)	NSAID	3.6	12.7[a]	3.2	11.15
	saline	9.8	0.0[b]	2.6	
Standing (%)	NSAID	55.1	22.6[a]	43.8	22.0
	saline	56.0	57.5[b]	37.8	
Abnormal standing (%)	NSAID	7.74	0.00[a]	1.61	6.41
	saline	2.22	7.68[b]	2.33	

Super-scripted letters indicate significant differences (P < 0.05) between the methods, within each variable and time period.

Table 4. Effects on behaviour of mature bulls of castration by surgery (Surgical) or banding (Band) during three time periods on the day of castration

Behaviour		Time periods post-castration (h)			
		0-1.5	1.5-3	3+	LSD
Abnormal lying (%)	Surgical	0.0	0.0[a]	0.0	14.77
	Band	4.3	22.1[b]	0.7	
Walking forwards (%)	Surgical	15.10[a]	4.39	5.68	6.073
	Band	22.29[b]	2.86	2.76	
Walking backwards (%)	Surgical	0.50[a]	0.92	0.40	1.513
	Band	2.43[b]	0.63	0.23	
No. tail movements*	Surgical	6.32 (39.4)	4.73 (21.9)	7.42 (54.6)	2.897
	Band	7.21 (51.5)	3.66 (12.9)	5.45 (29.2)	
No. leg movements*	Surgical	1.42[a] (1.52)	1.06 (0.63)	1.44 (1.58)	0.813
	Band	3.01[b] (8.53)	1.75 (2.57)	1.16 (0.84)	

*values sqrt (counts + 0.5) with back-transformed means in parentheses
Super-scripted letters indicate significant differences ($P < 0.05$) between the castration methods, within each variable and time period.