

Welfare outcomes for 3- and 6-month-old beef calves in a tropical environment castrated surgically or by applying rubber rings



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ABSTRACT

Castration of cattle using rubber rings is becoming increasingly popular due to the perceived ease of the procedure and greater operator safety when compared with surgical castration. Few comparative studies have investigated the effects of different castration methods and calf age on welfare outcomes, particularly in a tropical environment. Thirty Belmont Red (a tropically adapted breed), 3-month-old (liveweight 71–119 kg) and 30, 6-month-old (liveweight 141–189 kg) calves were assigned to a two age × three castration (surgical, ring and sham) treatment factorial study (Surg3, Surg6, Ring3, Ring6, Sham3 and Sham6, $n = 10$ for each treatment group). Welfare outcomes were assessed post-castration using: behaviour for 2 weeks; blood parameters (cortisol and haptoglobin concentrations) to 4 weeks; wound healing to 5 weeks; and liveweights to 6 weeks. More Surg calves struggled during castration compared with Sham and Ring ($P < 0.05$, $90 \pm 7\%$ vs. $20 \pm 9\%$ and $24 \pm 10\%$) and performed more struggles (1.9 ± 0.2 , 1.1 ± 0.3 and 1.1 ± 0.3 for Surg, Sham and Ring, respectively), suggesting that surgical castration caused most pain during performance of the procedure. A significant ($P < 0.05$) time × castration method × age interaction for plasma cortisol revealed that concentrations decreased most rapidly in Sham; the Ring6 calves failed to show reduced cortisol concentrations at 2 h post-castration, unlike other treatment groups. By 7 h post-castration, all treatment groups had similar concentrations. A significant ($P < 0.01$) interaction between time and castration method showed that haptoglobin concentrations increased slightly to 0.89 and 0.84 mg/mL for Surg and Ring, respectively over the first 3 days post-castration. Concentrations for Surg then decreased to levels similar to Sham by day 21 and, although concentrations for Ring decreased on day 7 to 0.76 mg/mL, they increased significantly on day 14 to 0.97 mg/mL before reducing to concentrations similar to the other groups (0.66 mg/mL) by day 21. Significantly ($P < 0.05$) more of the wounds of the 3-month calves scored as 'healed' at day 7 (74% vs. 39%), while more ($P = 0.062$) of the Surg than Ring scored as 'healed' at day 21 (60% vs. 29%). At day 14 there were significantly ($P < 0.05$) fewer healed wounds in Ring6 compared with other treatment groups (13% vs. 40–60%). Liveweight gain was significantly ($P < 0.05$) greater in 3-month (0.53 kg/day) than in 6-month calves (0.44 kg/day) and in Sham calves ($P < 0.001$, 0.54 kg/day), than in Ring (0.44 kg/day) and Surg (0.48 kg/day) calves. Overall, welfare outcomes were slightly better for Surg than Ring calves due to reduced inflammation and faster wound healing, with little difference between age groups.

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1. Introduction

Castration is one of the most common routine husbandry procedures conducted in beef production systems and some of

the largest of these systems (e.g. North and South America and Australia) rear calves on extensive rangelands where interactions with people are infrequent. In northern Australia, for example, calves are likely to be mustered (gathered) from paddocks to yards for handling once or twice a year. In addition, many of the large beef producing areas are located in the tropics and sub-tropics where highly seasonal rainfall can temporarily restrict or prevent access to cattle and interfere with the timing of routine management procedures, such as castration (Petherick, 2005). Also, rearing of

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cattle in tropical environments has resulted in the increased use of tropically adapted breeds, such as Zebu (*Bos indicus*) and Sanga (*Bos taurus africanus*) cattle, and their crosses. There is evidence of differences between cattle breeds in physiological (e.g. Ledger, 1959; Phillips et al., 1987; Arthington et al., 2004) and production (e.g. Hammond et al., 1996; Frisch and O'Neill, 1998) parameters, and behavioural responses (e.g. approachability, Murphey et al., 1980; temperament or reactivity, Hearnshaw and Morris, 1984; Fordyce et al., 1988). Thus, it is not unreasonable to suggest that breeds will differ in their responses to routine husbandry procedures.

The majority of studies on the welfare impacts of calf castration have been conducted in temperate climates using dairy calves and *Bos taurus* crossbreeds derived from British and European parent stock (e.g. see reviews by Bretschneider, 2005; Coetzee, 2011). Furthermore, from the review by Bretschneider (2005), most experiments on castration have used calves accustomed to confinement, people and handling. Such studies on British or Continental breeds of calf may not provide an appropriate model for investigating the impacts of castration on the welfare of commercial beef calves reared in tropical and sub-tropical environments. Thus, one aim of this experiment was to assess the welfare impacts of castration on calves that are representative of extensive beef cattle production systems located in the tropics and sub-tropics.

The application of rubber rings is perceived to be “a simple, inexpensive and effective method of castration” (Becker et al., 2012) and, as a consequence, the method is, at least in Australia, increasingly being used. Furthermore, manufacturers and retailers promote rings as being the least stressful method of castration (e.g. see <http://www.thecattleshop.com.au/category17.1.htm>) and also encourage beef producers to delay castration (to 5–8 months of age) to exploit the superior liveweight gains and musculature of bulls compared with castrates (e.g. see http://horsleywholesale.com.au/products/Jumbo_Marking_Castration_Bander_Delivery-487-113.html). Currently in some parts of Australia it is legally permissible to castrate cattle of any age without the use of anaesthetics or analgesics, although in some States and Territories it is illegal to castrate an animal older than 6 months of age unless it is undertaken by a veterinarian (Primary Industries Standing Committee (PISC), 2004). It is incongruous, therefore, that this same Welfare Code of Practice (PISC, 2004) stipulates that “castration with rubber rings is only recommended for calves up to 2 weeks of age.” Thus, the second aim of this study was to compare the welfare outcomes for different ages of calves castrated using rings and surgical castration, the latter being the most common method used in both the USA (Coetzee et al., 2010) and Australia (Meat and Livestock Australia, pers. comm.). Herd management practices in tropical Australia made it impractical to study calves less than 2 weeks of age. In this environment, however, some commercial enterprises castrate calves of 2–3 months of age without known adverse effects on welfare, although most enterprises routinely castrate calves at around 6 months of age. Therefore, the age comparison comprised 3 months and 6 months.

To assess welfare status we used a combination of measures in line with our previous research on cattle castration (Petherick et al., 2014a,b) and that of other authors (e.g., Robertson et al., 1994; Molony et al., 1995; Stafford et al., 2002). Behavioural responses to pain can be difficult to interpret in isolation due to their variability both between and within individuals (Mellor et al., 2000) and so are best supported by other measures. Although there can be difficulties with interpretation of pain vs. generalised stress (Mellor et al., 2000), plasma cortisol concentration and liveweight changes have been measured to assess the pain and stress associated with castration in many studies (e.g., see review by Bretschneider, 2005). Increases in plasma concentrations of creatine kinase (CK) are associated with muscle damage (Radostits et al., 2007) and changes in

total protein (TP) and pack cell volume (PCV) reflect dehydration and blood loss (Carlson, 1997). Thus, we included these measures anticipating that they would assist with assessing welfare status in animals subjected to a castration method that involves cutting tissue (surgical) and one that does not (application of rubber rings).

Our specific hypothesis was that welfare outcomes, as assessed by changes in behaviour, certain blood parameters, wound healing rates and liveweight, would be no different for 3-month-old or 6-month-old calves castrated surgically or using rings.

2. Method

The use of the cattle in this experiment was approved by the CSIRO (Queensland) Animal Ethics Committee (approval A1-2012).

2.1. Location and experimental design

The experiment was conducted at Belmont Research Station, approximately 26 km north of Rockhampton, Queensland, Australia (150° 22' 57" E, 23° 13' 26" S) during the late wet season to early dry season (26 February–13 April), a time at which commercial enterprises in the region would castrate calves. The range of mean minimum and maximum temperatures were 15.1–24.4 and 25.4–34.7°C respectively, with 218 mm of rainfall (14 wet days) during the experimental period.

Belmont Red (a stabilised African Sanga × *Bos taurus* hybrid) calves that were born on Belmont Research Station between 30 August and 20 December of the previous year were used for the experiment. The calves were ear-tagged within 24 h of birth and branded in early January, but were not dehorned to eliminate potential confounding due to experience of restraint and pain. Sixty calves were assigned to six treatment combinations ($n=10$ per treatment group) according to birth date (3-month or 6-month age group), and stratified within age group by liveweight and flight speed as measured at the time of branding. 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). Three flight speeds, taken in succession, were recorded but as has been found previously, the first speed was poorly correlated with the others (Petherick et al., 2009a), thus a mean of the second and third was used. It was considered important to take into account flight speed in the allocation of the calves to the treatments, as previous work has found relationships between flight speed and stress responses and liveweight gains (Petherick et al., 2002, 2009b).

There were six treatment combinations of age of calf (two levels) and castration method (three levels): sham castration of 3-month-old calves (Sham3); surgical castration of 3-month-old calves (Surg3); ring castration of 3-month-old calves (Ring3); sham castration of 6-month-old calves (Sham6); surgical castration of 6-month-old calves (Surg6) and ring castration of 6-month-old calves (Ring6). Due to limitations on calf numbers from which to select the experimental animals, there was a range of ages within the two age groups; the 6-month-old calves ranged in age from 5 to 7 months and the 3-month-old calves ranged from 2.5 to 4 months. Liveweights averaged 163.3 kg (range 141–189 kg) and 93.7 kg (range 71–119 kg) for the 6- and 3-month-old groups, respectively.

Due to time and daylight constraints, 30 calves were castrated on 2 successive days (day 0). Calves were allocated to 10 blocks, each containing one animal from each treatment. Five blocks (randomly selected) were treated on each day (batch A and B) with the procedures for the five blocks starting at approximately 7:00, 8:00, 8:45, 9:45 and 11:00 h, respectively on both days.

2.2. Procedures

On the day before the experiment started, the calves and their mothers were mustered from their paddock, walked to the yards and cows and calves sorted into the two batches. Calves were weighed and returned to their dams. Batch B cows and calves were returned to their home paddock and batch A held in a small paddock adjacent to the yard complex.

On the day of castration (day 0), cows and calves in the batch (A and B) were walked to the yards complex and calves separated from their mothers and sorted into the five block groupings. The cows were retained in the yard complex; the calves could hear, but not see them. Calves were moved individually into a calf cradle, tipped onto their left side and two blood samples (approximately 8 and 4 mL) were taken via a single jugular venipuncture via a 20 G needle into vacutainers. Scrotal circumference was measured (Entwistle and Fordyce, 2003) and an IceTag3D™ motion sensor device (data logger) was fitted to the right hind leg in accordance with the manufacturer's recommendations (IceRobotics, Roslin, Midlothian, Scotland). All calves were then castrated by the pre-assigned method by one operator.

2.2.1. Surgical castration

Calves were individually restrained in the calf cradle, with additional manual restraint by a person holding the right hind leg. Using a hand-held scalpel blade, the operator conducted the castration according to a beef industry 'best practice' guide (Newman, 2007), using a cut to the scrotum for each testicle. 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 roughly severed (to minimise blood loss) 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 righted and released to a grassed yard, with the entire procedure (from the start to end of restraint) taking approximately 1 min.

2.2.2. Ring castration

Calves were restrained in the calf cradle as described in Section 2.2.1 and the operator conducted the castration according to the best practice guide (Newman, 2007), although the rings used were ones marketed and sold specifically for calf castration (LG Superior Bander and LG bands, for cattle weighing 120–340 kg; Bainbridge Veterinary Instruments Pty Ltd., Murarrie, Qld., Australia). The ring was expanded using an applicator which was positioned near the distal end of the scrotum, with the prongs towards the calf's body. The scrotum was then gently pulled through the expanded ring, with gentle pressure used at the neck of the scrotum to push the testicles below the ring. The ring was then allowed to close around the scrotal neck, above the testicles, by releasing the pressure on the applicator handles. The prongs were then withdrawn leaving the ring around the scrotal neck. Once it was ensured that the ring was secure above the testicles, the calf was returned to a vertical position and released to a grassed yard. The entire process (from the start to end of restraint) took approximately 1 min.

2.2.3. Sham castration

Calves were restrained as for the other castration treatments and had their scrotums manipulated in a way similar to that required for castration and for the same length of time.

2.2.4. Post-castration management

When all six calves in a block had been treated, the group was either moved into a holding pen at the end of the race to return to the calf cradle for the second blood sample (at 30 min post-treatment), or moved to a "home" yard (approximately 50–70 m²) with shade, and lucerne hay and water available *ad libitum*, until a few min before the next blood sample was due. For the second blood sample the animals were kept in the order in which they had been castrated, but for subsequent blood samples they were bled in the order that they entered the crush. After blood-sampling they were returned to their home yard. Thus, each block of six calves was maintained as a group in a separate yard on the day of castration.

After their final blood sample on the treatment day, blocks of calves were returned to the cows being held in the yard complex. Once all calves were returned to their mothers they were given time to mother-up and then cows and calves were released to a small paddock (approximately 1 ha) adjacent to the yard complex, with pasture and water available *ad libitum*. The following day, cows and calves were walked to the yard complex and calves separated from their dams for their day 1 blood sample and then returned to their mothers and to the original home paddock. This process was repeated for days 2 and 3 for both batches of cattle, with the batch A being returned to their home paddock on each occasion and batch B being held in small paddocks (each approximately 2.5 ha) adjacent to the yards. After the day 3 blood sample for batch B, the batches were combined into a 6.85 ha paddock consisting of Rhodes Grass (*Chloris gayana* var. Callide) pasture. Dry matter availability was estimated to be above 2000 kg/ha at all times and exceeded 4000 kg/ha at first grazing. The cows and calves were rotated through four, similar (in terms of area, pasture-type and DM availability) contiguous paddocks during the period of the experiment.

2.2.5. Blood sampling

Blood samples were taken on restraint (time 0) and at 30 min, 2 h and 7 h post-castration. Samples were collected into EDTA and sodium heparin vacutainers (Becton Dickinson, North Ryde, NSW, Australia) and refrigerated (4 °C) until processed. Whole blood samples (those collected into the EDTA tubes) were measured on site, immediately post-collection, for packed cell volume (PCV). Blood was drawn into duplicate micro-haematocrit tubes (Clinilab, Herley, Denmark) and sealed with Seal-Ease (Becton Dickinson, North Ryde, NSW, Australia). The micro-haematocrit tubes were centrifuged (Hawksley, Sussex, UK) for 20 min and the average PCV concentrations calculated from duplicate samples (Hawksley, Sussex, UK). Total protein (TP) and creatine kinase (CK) concentrations were analysed using an automated biochemical analyser (Olympus Reply Biochemistry Analyser, Sydney, NSW, Australia). The sodium heparin vacutainers were centrifuged on the day of collection at 2500 rpm for 20 min and plasma extracted and stored at –20 °C until plasma cortisol and haptoglobin assays were performed. Haptoglobin concentrations were assayed in the same biochemical analyser indicated above using Tridelta haptoglobin kits (Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland). Plasma cortisol concentrations were determined using a commercial radioimmunoassay (Spectria Cortisol RIA, Orion Diagnostica, Espoo, Finland), adapted and validated for bovine plasma, as described previously (Paull et al., 2007). The detection limit of the assay was 5.0 nmol/L. The intra-assay coefficients of variation (CV) for samples containing 34.6, 80.4 and 149.8 nmol/L cortisol were 10.3%, 11.0% and 9.1%, respectively. The inter-assay CVs for the same samples were 12.5%, 10.8% and 10.8%.

Blood samples were also taken on days 1, 2, 3, 7, 14, 21 and 28 post-castration. With the exception of the day 28 sample, the calves were restrained in the calf cradle. For the final sampling occasion, the calves were restrained via the head-bail in a veterinary

crush. This change was made because of the difficulty in restraining some of the 6-month-old calves in the calf cradle, due to their size. Furthermore, three of these older calves (two Ring6 and one Surg6) had previously sustained leg/shoulder injuries, apparently from vigorous struggling in the cradle. The injury to one Ring6 calf was sufficiently severe that it was not restrained and sampled on days 21 and 28.

On these blood sampling occasions, a single sample was collected (into a sodium heparin vacutainer) and samples were handled and stored as described above, for plasma haptoglobin and cortisol assays. Although the two batches of cattle were mixed after day 3 they were blood-sampled on successive days for the day 7 sample. Thereafter, the cattle were treated as a single group and, thus, samples taken on days 14, 21 and 28 were technically days 13, 20 and 27 for the batch B calves, but for simplicity, these were considered to be 14, 21 and 28 days post-castration for all calves.

2.2.6. Behavioural recording

Behaviour was recorded during castration by direct observation. Being restrained in a calf cradle, the calves were limited in what behavioural patterns they could show. Counts were scored for individual vocalisations; struggles (a movement back and forth and/or side-to-side in the cradle with head and legs flailing; kicks (a movement of one or both hind legs to the rear, even if manually restrained); and tail flicks (a sideways movement of the tail from vertical and return to vertical).

Post-castration on Day 0, blocks of animals were directly recorded, in the yard complex, by 5-min focal animal sampling by two observers using an ethogram developed for studies on the castration of *Bos indicus* bulls (Petherick et al., 2014a; Table 1). Those behaviours having a duration of 5 s or more were categorised as states and other behaviours (lasting less than 5 s) classified as events. States were mutually exclusive and total durations (s) were calculated for each state and the proportion of the total time (300 s) spent in each state determined. Counts of all events were summed for each 5-min observation period. Additionally, the number of 'transitions' between states was scored by counting, for every animal observation, when there was a change of behavioural state.

Three calves within a block were randomly allocated to each observer on each occasion, to minimise any bias that may have occurred from the same observer always recording the same calves. The order in which individuals in a block were observed was as they were individually identified by the observers. 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. Furthermore, rainfall prevented some block data from being collected. 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 plots of the observation times made it clear that there was no bias in the times post-castration that the observations were made.

Behaviour was also recorded by 5-min focal animal sampling on days 1 to 3 post-castration when the cattle were in the two batches in paddocks. With one exception, when recording on day 0 for batch B and day 1 for batch A clashed, these observations were conducted by a single observer. The order in which the animals were recorded was on the basis of locating individuals. Observations were fitted-in with blood sampling and so some were conducted post-blood sampling. Observations were conducted between 7:00 and 10:45 h on day 1; 12:45 and 17:00 h on day 2 and 6:45 and 12:45 h (with blood sampling between about 9:00 and 11:00 h) on day 3, for both batches.

The percentage of time spent standing and lying and the number of steps taken were automatically determined from the IceTag3D™ data. Some of the loggers (23) were removed at day 14

post-castration because minor lesions had developed under the straps (probably due to the wet weather) and we did not wish to risk leg infections and lameness. The remainder were removed at day 21 post-castration. Three loggers failed to record any data (two Sham3 and one Sham6) and two provided partial recordings (one Ring3 and one Sham3).

2.2.7. Liveweight and wound healing recording

Liveweights were recorded on days –1, 7, 14 and 35 post-castration for batch A calves (these were a day less on each occasion for the batch B calves). An additional liveweight was obtained on day 45/46 prior to the calves being dehorned after the experiment.

Castration sites were checked on the same occasions to determine the extent of healing, with an additional assessment on day 3 post-castration. On these occasions, for each animal, photographs of the scrotal area were taken and a description of the wounds (and presence/absence of the scrotum for those animals ring castrated) recorded. For the ring-castrated calves, only the area above the ring was considered, as any infection above the ring would likely have an adverse effect on welfare. In contrast, below the ring the tissues would shrivel and die due to lack of blood flow, with little or no consequence for welfare. Based on the photographs and descriptions, the wounds were scored on the following scale: (1) Wound closed/scabbed, dry and no pus; (2) Wound part-closed, dry and no pus; (3) Wound part-closed, moist and no pus; (4) Wound part-closed, moist and pus present; (5) Wound fully open, moist and no pus and (6) Wound fully open, moist and pus present. As two cuts were made in the surgically castrated animals, calves were given a score corresponding to the state of the least-healed cut e.g. if one cut was part-closed and had pus present then the animal was given a score of 4, or a score of 6 if one wound was fully open with pus present.

Scrotal circumferences were measured (as an indicator of oedema and shrivelling) to day 28 post-castration, at which time the scrotums of all but one ring-castrated calf had dehisced and those of the surgically castrated calves, with one exception, had been scored as fully-healed (score 1).

2.3. Statistical analyses

One calf died and two became lame (see Section 3.1 below). The calf that died contributed few data; one lame calf was an outlier due to substantial weight loss during the first week post-castration; and the other lame calf was sufficiently injured that we did not collect data after day 14. All data for these three animals were removed.

2.3.1. Behaviour at castration

Counts of vocalisations and struggles during castration were analysed by a two-stage analysis of zero-inflated data. The presence/absence of the behaviour was modelled as a Generalised Linear Model (GLM) with binomial error and logit link function with dispersion fixed at unity. Counts of behaviours for animals exhibiting the behaviour (present) were then modelled as a GLM with Poisson error and log link function with the dispersion parameter estimated. Models included the effects of castration method and age group. As only eight and 11 animals were observed performing tail flicking and kicking, respectively, these behaviours were not analysed.

2.3.2. Behaviour by direct observations on the day of castration

The number of occurrences of behavioural events and the time spent in behavioural states during a sampling period was recorded at various times in the 7 h following castration with times grouped into three periods: 0–40 min (0–1 h), 68–230 min (1–4 h) and 238–440 min (4–7 h) post-castration.

Table 1
Ethogram developed for observations conducted on calves 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 and sucking dam’s teats)	
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	
Tail lift	Tail raised and lowered	
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	
Leg Shake	Rapid side-to-side and/or up-and-down motion of a single leg (usually hind)	
Vocalisation	Calls emitted by a calf	

Behavioural states of walking forward and standing within each time period were modelled with a GLM assuming a binomial error distribution, a logit link function and binomial totals of the recording time in the sampling period (300 s) with the dispersion parameter estimated. Other behavioural states did not occur on sufficient occasions to be analysed.

Behavioural events of tail movements (primarily tail flicking), leg movements (primarily leg lifting) and vocalising within each time period post-castration were analysed by a two-stage analysis of zero-inflated data. The presence/absence of the behaviour was modelled as a GLM with binomial error and logit link function with dispersion fixed at unity. Counts of the behaviour for animals exhibiting the behaviour (present) were then modelled as a GLM with Poisson error and log link function with the dispersion parameter estimated. Models included the effects of castration method and age group. Two tail movement records and a leg movement record were identified as extreme outliers and were excluded from analyses. Other behavioural events did not occur in sufficient numbers within time periods to be analysed.

The number of transitions between behavioural states in each of the time periods 0–1, 1–4 and 4–7 h was modelled as a GLM with Poisson error and log link function with the dispersion parameter estimated.

2.3.3. Behaviour by direct observations on days 1–3 post-castration

Behavioural states of walking forward, standing (primarily standing alert), lying and feeding were totalled for the 3 days and modelled as a GLM assuming a binomial error distribution, a logit link function and binomial totals of the total sampling time (900 s) with the dispersion parameter estimated. Other behavioural states did not occur on sufficient occasions to be analysed.

The behavioural event of tail movements (primarily tail flicking) and the number of transitions were totalled for 3 days and modelled as a GLM with Poisson error and log link function and the dispersion parameter estimated. Other behavioural events did not occur in sufficient numbers within time periods to be analysed.

2.3.4. Behaviour via IceTags

Examination of the data from the IceTag of a Surg6 calf revealed that it was an extreme outlier on all measures recorded (extremely large quantities of data compared with all others) and so the data

were not used in the analysis. After 14 days post-castration, there were small experimental numbers for some treatments because the IceTags had been removed from some calves, so analysis was conducted on only the data collected to day 14. IceTag data (percentage time standing, percentage time lying and number of steps) were exported on a per hour basis and further summarised as averages for three periods on the day of castration (0–1, 1–4 and 4–7 h post-castration, in line with direct observations) and for 13 consecutive 24-h periods post-castration, where the first period for each animal was the first 24 h post-castration (i.e. the first period included the data from the day of castration). Percentage time standing and lying are reciprocal data, so only percentage time standing data were analysed and are reported.

The three periods on the day of castration were analysed separately using restricted maximum likelihood (REML), with a model including the effects of castration method and age group. Data for the 13, 24-h periods post-castration were analysed as repeated measures using REML and modelling the variance-covariance matrix with an unstructured correlation structure. Numbers of steps were log-transformed and percent time standing was arcsine-transformed prior to analysis.

2.3.5. Blood parameters

Blood parameters on the day of castration (PCV, TP, CK and cortisol) were analysed as repeated measures using REML and modelling the variance-covariance matrix to account for the correlation structure induced by the repeated sampling. A general unstructured covariance matrix was used. Similarly, haptoglobin and cortisol concentrations on the days following castration were analysed as repeated measures using REML with a general unstructured covariance matrix. The corresponding initial blood concentration (time 0 sample) was included in the models as a covariate. Inspection of residual plots revealed that CK data were skewed, so were log-transformed prior to analysis. A cortisol concentration was identified as an outlier (Ring6 at 120 min post-castration) because the value was inconsistent with other values for that calf and also about 30 units less than the mean of the treatment group. It was, therefore, treated as missing data. Haptoglobin concentrations for one calf on day 14 and another on day 28 (both Sham6 treatment) were identified as outliers because the values were about twice and three times, respectively, greater than the group means for those

days and were inconsistent with the remaining pattern of values for those individuals. These were, also, treated as missing data.

2.3.6. Liveweight, scrotal circumference and wound scores

Liveweight gains from the initial liveweight were calculated for all weights following castration. Liveweight gains and scrotal circumferences post-treatment were analysed as repeated measures using REML and modelling the variance-covariance matrix to account for the correlation structure induced by the repeated sampling. An antedependence structure of order 1 was used to model the correlation structure for both liveweight gains and scrotal circumferences. Initial liveweight or scrotal circumference was included as a covariate.

Wound scores for Ring and Surg calves were summarised into two categories: 1 = normal wound healing (scores 1–3) and 2 = delayed wound healing/infection (scores 4–6). Data were then subjected to logistic regression using a GLM with binomial error and logit link function.

3. Results

3.1. Mortalities and morbidity

One Ring3 calf died between days 2 and 3, after being detected as unwell on the morning of day 2. Post-mortem examination showed nothing overtly abnormal, suggesting that it was unlikely that the treatment or the blood sampling *per se* were the cause of death.

Castration wound inflammation and infection was sufficiently severe for three calves to warrant treatment with penicillin (Norocillin L.A., Norbrook Laboratories Australia Pty Ltd., Tullamarine, VIC, Australia), injected intramuscularly into the neck at a rate of 4 mL/100 kg liveweight, according to manufacturer recommendations. One Surg6 calf was treated at days 7 and 14 post-castration, and one each of Surg3 and Ring6 at day 21.

Two calves (one each of Ring6 and Surg6) were treated for lameness with ketoprofen (Ilium Ketoprofen, Troy Laboratories Pty., NSW, Australia) injected into the anterior of the neck at a rate of 3 mg/100 kg liveweight, according to manufacturer recommendations at day 7 post-castration. As indicated above (Section 2.3), data for the Ring6 calf were removed from analysis, as it lost a large amount of weight during the first week when it was lame, but the Surg6 calf showed no sign of being an outlier. Another Ring6 calf was no longer restrained and sampled after day 14 because of an apparent back injury which caused lameness. These three calves had allocation flight times (time to cover 1.8 m) of between 0.65 and 0.73 s, which were in the fastest 10% of calves.

3.2. Behaviour at castration

The percentage of animals vocalising during castration (29%) did not differ ($P > 0.10$) among castration treatments, but did differ (Wald = 4.93, 1 df; $P < 0.05$) between age groups; more 3-month-old calves vocalised than the 6-month-old calves (data presented are mean \pm s.e. unless otherwise stated; $41 \pm 9\%$ vs. $14 \pm 7\%$). For those that did vocalise, there was no difference ($P > 0.10$) in the number of vocalisations among castration treatments (average of 2.8 vocalisations) while there was a weak difference (Wald = 4.49,

1 df; $P = 0.056$) between age groups (2.1 ± 0.5 and 5.1 ± 1.6 vocalisations for the 3- and 6-month-old calves, respectively). More Surg calves struggled than Sham and Ring (Wald = 16.98, 2 df; $P < 0.001$, $90 \pm 7\%$ compared with $20 \pm 9\%$ and $24 \pm 10\%$, respectively) and, if they struggled, they performed more struggles (Wald = 6.84, 2 df; $P = 0.051$; 1.9 ± 0.2 , 1.1 ± 0.3 and 1.1 ± 0.3 for Surg, Sham and Ring, respectively).

3.3. Behaviour post-castration

In the first hour post-castration, there was a significant castration method \times age interaction (Wald = 7.53, 2 df; $P < 0.05$) on the number of leg movements for calves that performed the behaviour. Surg3 (2.7 ± 1.3) and Ring3 (3.3 ± 1.4) calves performed less leg movements than the Sham3 (6.0 ± 1.2) calves, but Surg6 (3.8 ± 1.1) and Ring6 (5.3 ± 1.5) calves performed more than the Sham6 calves (1.6 ± 1.8). The data from the IceTags revealed that castration method significantly affected the number of steps/h ($F_{2,48} = 6.99$; $P < 0.05$) and the percentage of time spent standing ($F_{2,48} = 27.13$; $P < 0.001$). The Ring calves took more steps/h than the Surg calves, with the Sham intermediate (6.19 (back transformed 485), 5.83 (338) and 5.91 (366), respectively; l.s.d. = 0.29). Sham and Surg calves spent about 100% of the time standing compared with the Ring calves that spent about 86% of the time standing (transformed means 1.57, 1.51 and 1.19, respectively; l.s.d. = 0.11). More (Wald = 4.52, 1 df; $P < 0.05$) transitions between behavioural states were performed by 3-month-old than 6-month-old calves (16.3 ± 1.3 vs. 12.6 ± 1.1).

In the 1–4 h period post-castration there was a significant interaction between castration method and age for numbers of tail movements (Wald = 7.48, 2 df; $P < 0.05$) for those calves performing the behaviour (Table 2). Ring3 calves performed more tail movements (48.7 ± 10.8) than Surg3 (21.3 ± 6.3) and Sham3 (22.7 ± 6.5), but there was no difference in castration method for the 6-month-old calves (19.6 ± 6.8 , 30.6 ± 7.5 and 36.7 ± 8.2 for Ring, Surg and Sham, respectively). Ring3 calves also performed more tail movements than Ring6 calves. There was a tendency ($F_{2,49} = 2.99$; $P = 0.060$) for the Sham calves to spend a greater percentage of time standing (as determined from the IceTag data) compared with both the Surg and Ring calves (1.36 (back-transformed 95%), 1.11 (80%) and 1.14 (82%), respectively; l.s.d. = 0.22).

In the 4–7 h period post-castration, castration method significantly (Wald = 8.04, 2 df; $P < 0.05$) affected the percentage of calves vocalising, with less Surg ($58 \pm 7.1\%$) and Ring calves ($63 \pm 6.9\%$) compared with Sham ($84 \pm 5.2\%$). There was also a tendency (Wald = 5.71, 2 df; $P = 0.061$) for castration method to affect the percentage of time spent walking forwards, with less time spent by the Surg calves ($5 \pm 1.5\%$) than both the Sham ($11 \pm 2.1\%$) and Ring calves ($11 \pm 2.2\%$). There were no effects of treatment on the IceTag-recorded data in this period.

During days 1–3 post-castration, there were significant castration method \times age interactions ($P < 0.05$) for both the percentage of time spent walking forward (Wald = 6.48, 2 df) and the number of tail movements (Wald = 8.24, 2 df) for those calves performing the behaviours (Table 2). The Surg3 calves spent less time walking forward than the Ring3 and Sham3 calves, but there were no differences between castration methods in the 6-month-old animals. Similarly, Surg3 calves performed more tail movements than

Table 2
Mean behaviours (\pm s.e.) performed during days 1–3 post-castration by calves of two ages sham, surgically or ring castrated.

	Sham		Surgical		Ring	
	3 month	6 month	3 month	6 month	3 month	6 month
Time walking forward (% time)	13 \pm 3.1	7 \pm 2.5	4 \pm 1.8	9 \pm 2.6	11 \pm 3.1	4 \pm 2.0
Tail movement (no.)	69 \pm 22	66 \pm 22	235 \pm 41	116 \pm 29	47 \pm 19	121 \pm 33

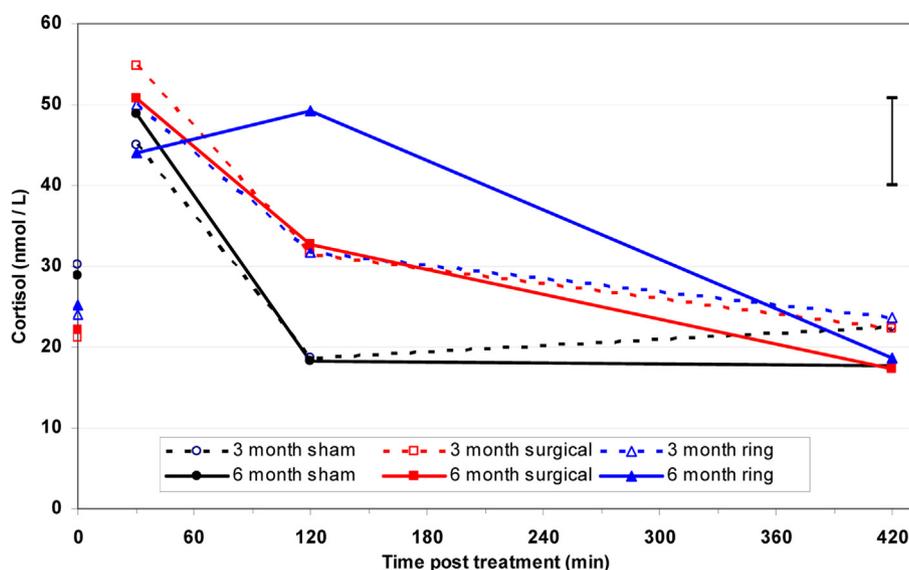


Fig. 1. Profiles of predicted cortisol concentrations on the day of castration, adjusted for pre-castration cortisol concentrations (given at time 0), for 3- and 6-month-old calves sham, surgically or ring castrated. The vertical bar represents the average l.s.d. at $P=0.05$.

the Ring3 and Sham3 calves with no differences among castration methods in the 6-month-old calves. The IceTag data revealed that Ring calves spent less ($F_{2,49}=4.72$; $P<0.05$) time standing than Sham or Surg calves (0.814 ± 0.007 (back-transformed 53%), 0.840 ± 0.007 (55%) and 0.838 ± 0.007 (55%), respectively) with no difference ($P>0.10$) among castration methods in the number of steps taken. Three-month-old calves spent less ($F_{1,49}=13.11$; $P<0.001$) time standing (0.817 ± 0.006 (back-transformed 53%) vs. 0.844 ± 0.006 (56%)) and tended ($F_{1,49}=3.66$; $P=0.062$) to take more steps (5.34 ± 0.03 (208 steps/h back-transformed) vs. 5.27 ± 0.03 (193 steps/h)) than 6-month-old calves averaged over time periods and castration methods.

3.4. Blood parameters

For PCV, TP and CK concentrations, there were no significant interactions between treatments (i.e. castration methods and age groups) and time, or between castration method and age group, and no differences between age groups or among castration methods. Each parameter, however, increased with time, being greatest at 7 h post-treatment. Overall means were $35.55\pm 0.25\%$, 70.20 ± 0.25 g/L and 6.54 ± 0.09 (691.3 U/L) for PCV, TP and CK, respectively.

Cortisol profiles on the day of castration are given in Fig. 1. There was a significant ($F_{4,59}=2.95$; $P<0.05$) time \times castration method \times age interaction; concentrations decreased most rapidly in the Sham calves, the Surg and Ring3 calves showed similar, but less rapid declines in cortisol concentrations, while the Ring6 calves failed to show a reduced cortisol response at 2 h post-castration. By 7 h post-castration, however, all treatment groups had similar concentrations (20.4 nmol/L). On days 1–28 post-castration there were no significant ($P>0.05$) interactions involving time, castration methods and age groups, or differences among castration methods (23.7 ± 1.2 , 25.6 ± 1.2 and 26.6 ± 1.3 nmol/L for sham, surgical and ring methods, respectively). Cortisol concentrations were greater ($F_{1,52}=8.09$; $P<0.01$) for 3-month-old than 6-month-old calves (27.2 ± 1.0 vs. 23.4 ± 1.0 nmol/L, respectively).

Haptoglobin profiles on days 1–28 post-castration are shown in Fig. 2. There was a significant ($F_{12,74}=2.47$; $P<0.01$) interaction between time and castration method; haptoglobin concentrations decreased slightly over time for the Sham calves while levels increased slightly (but not statistically) to 0.89 and 0.84 mg/mL for

Surg and Ring over the first 3 days post-castration. Haptoglobin levels for the Surg calves then decreased steadily to levels similar to the Sham calves by day 21. Although the levels for the Ring calves decreased on day 7 to 0.76 mg/mL, they increased significantly on day 14 to 0.97 mg/mL before reducing to levels similar to the other groups by day 21 (0.66 mg/mL).

3.5. Wounds

As anticipated, the number of scrotums present on the Ring calves declined over time with all gone by day 35. All were present at day 3 and day 7, although at day 7 three were broken and the contents lost. At days 14, 21 and 28, 6/19 (31.6%), 14/19 (73.7%) and 18/19 (94.7%) had dehisced, respectively.

Wound scores on day 3 (approximately 90% in category 1, indicative of normal healing) and on days 28 and 35 (all in category 1) had insufficient variation to be analysed. There was no interaction between castration method and age group for wound score at days 7 and 21. Significantly (Wald = 4.57, 1 df; $P<0.05$) more of the 3-month age group were in category 1 at day 7 than the 6-month age group ($74\pm 10\%$ vs. $39\pm 11\%$), while more (Wald = 3.48, 1 df; $P=0.062$) of the Surg calves had wounds in category 1 at day 21 than the Ring calves ($60\pm 11\%$ vs. $29\pm 11\%$). Wound score at day 14 differed (Wald = 3.66, 1 df; $P=0.056$) with both castration method and age group, with fewer of the Ring6 calves in category 1 than the other treatment groups (13% vs. 40–60%; Fig. 3).

The effect of castration treatment on scrotal circumference differed ($F_{7,89}=27.75$; $P<0.001$) over time (Fig. 4). Scrotal circumferences of Sham calves increased slightly (approx 0.5 cm) over the first 21 days post-castration. Circumferences of both the Surg and Ring calves decreased by approximately 4 cm over this period, but the circumferences of the Surg calves were about 4 cm greater than the Ring calves at all times.

3.6. Liveweight gains

Across the 45 days post-castration, liveweight gain differed ($F_{1,59}=6.53$; $P<0.05$) between age groups, with greater gains in 3-month-old (0.53 ± 0.02 kg/day) than in 6-month-old calves (0.44 ± 0.02 kg/day). Liveweight gain also differed ($F_{2,59}=11.62$; $P<0.001$) among castration treatments, with greater gains in Sham calves (0.54 ± 0.01 kg/day) than castrated calves, but with

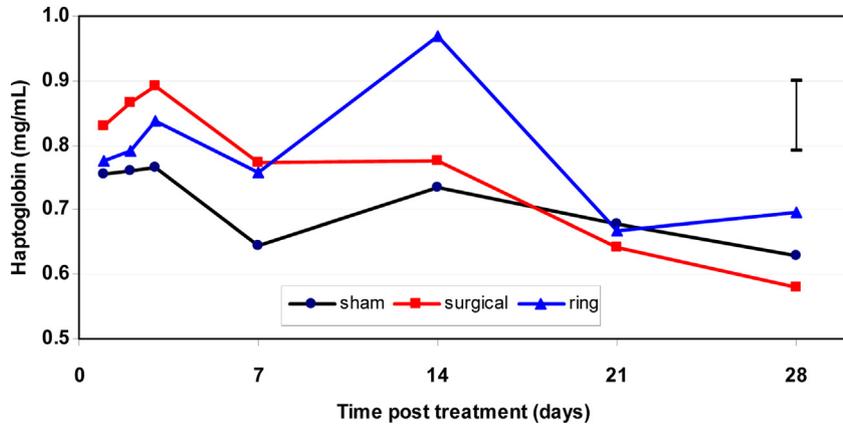


Fig. 2. Predicted mean haptoglobin concentrations during days 1–28 post-castration, adjusted for pre-castration haptoglobin concentrations, for calves sham, surgically or ring castrated. The vertical bar represents the average l.s.d. at $P=0.05$.

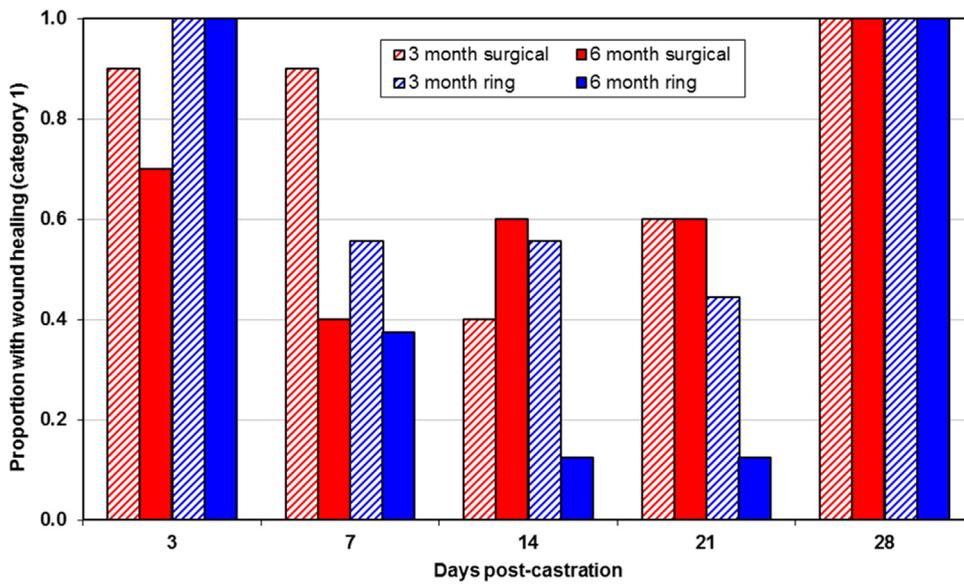


Fig. 3. Proportion of wounds scored as healing (as opposed to delayed or abnormal healing) during a 4-week period post-castration for 3- and 6-month-old calves surgically or ring castrated.

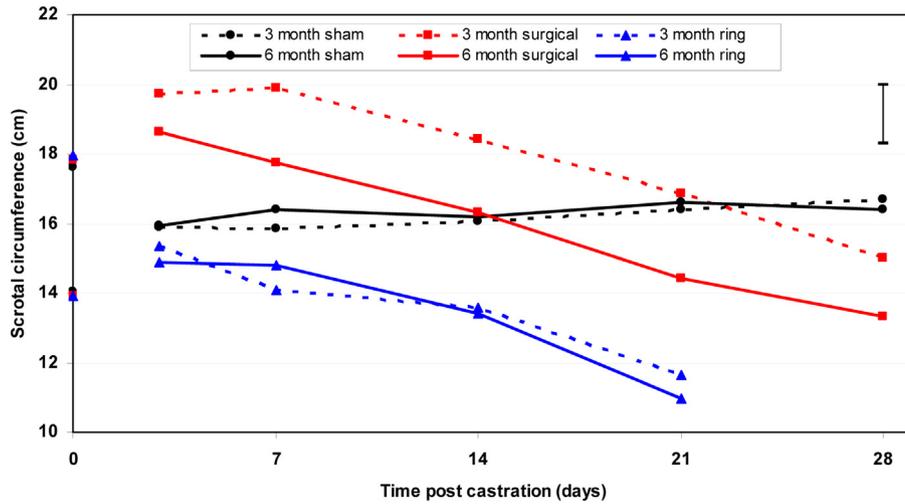


Fig. 4. Predicted mean scrotal circumferences, adjusted for pre-treatment circumference (given at time 0), to day 28 post-castration for 3- and 6-month-old calves sham, surgically or ring castrated. The vertical bar represents the average l.s.d. at $P=0.05$.

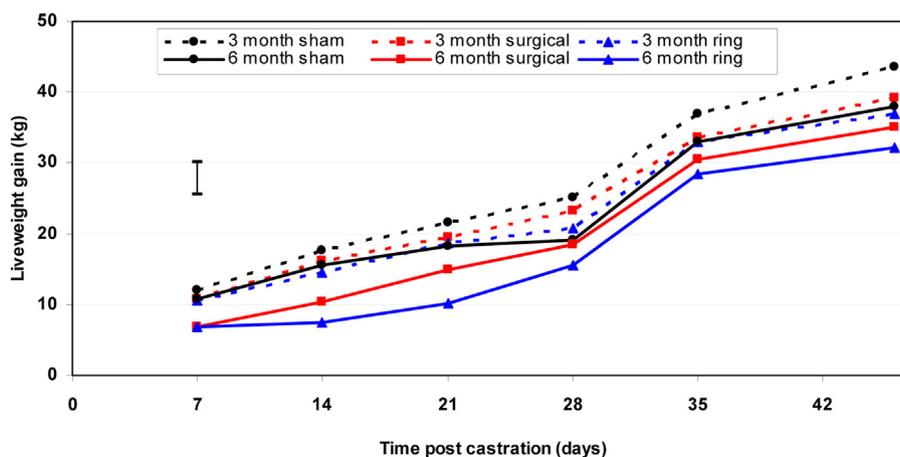


Fig. 5. Predicted mean liveweight gains, adjusted for initial liveweight, to 46 days post-castration for 3- and 6-month-old calves sham, surgically or ring castrated. The vertical bar represents the average I.s.d. at $P=0.05$.

no difference between Ring (0.44 ± 0.02 kg/day) and Surg calves (0.48 ± 0.01 kg/day). The castration treatment by age interaction was not significant, nor were interactions between time (the weekly weighings) and method or age (Fig. 5).

4. Discussion

Our findings indicated few differences in welfare outcomes between castrated 3-month-old and 6-month-old calves, but the castration method used did affect welfare. During the castration procedures it was clear, from the extent of struggling, that surgical castration caused more pain and discomfort than ring application, which is consistent with findings from other studies (Fell et al., 1986; Thüer et al., 2007) and our own work on tension-banding castration of beef cattle (Petherick et al., 2014a,b).

On the day of castration there was evidence from behavioural and cortisol responses that both ring and surgical castration caused pain. Active behavioural responses (e.g. walking and leg and tail movements) tended to be evoked with rings compared with stationary behaviours (e.g. standing) with surgical castration, which agree with our findings comparing surgical and tension-banding castration of beef cattle (Petherick et al., 2014a,b). Interestingly only the Ring6 calves showed increased cortisol concentrations at 2 h post-castration, although this did not coincide with any notable pain-related behavioural responses (both Ring6 and Surg6 showed more leg movement than Sham6 during the first h post-castration). This finding of an elevated cortisol response at 2 h post-castration in the 6-month ring castrates contradicts other studies that have found higher cortisol plasma concentrations on the day of castration in surgically compared with ring castrated calves (Fell et al., 1986; Robertson et al., 1994; Molony et al., 1995). The calves in these studies were, however, much younger (5 days to 11 weeks of age) than the 6-month age group used in the present study. Furthermore, the relative amounts of pulling and cutting of spermatic cord tissue during surgical castration are likely to contribute to differences observed in the cortisol response in different studies (Stafford et al., 2002). In the current study, peak cortisol concentrations for both castration methods were mostly similar to the averages reported for ring (45 nmol/L), or below those for surgical castration (129 nmol/L; Coetzee, 2011), being about 47 nmol/L for Ring and 52 nmol/L for Surg at 30 min post-castration. Concentrations had returned to pre-treatment levels by 7 h post-castration, which is in broad agreement with other research (Fell et al., 1986; Molony et al., 1995; Stafford et al., 2002; Thüer et al., 2007).

On the day of castration, due to the degree of tissue damage and blood loss, we had anticipated finding evidence of greater muscle

damage (CK, Radostits et al., 2007), and dehydration/blood loss (measures of TP and PCV, Carlson, 1997) in the surgically castrated calves compared with the ring castrates, but this was not the case. There were increasing CK concentrations during the day for all treatments groups and the mean value of 691 U/L greatly exceeded the upper limit of normal values (35–280 U/L for *Bos taurus* cattle, Radostits et al., 2007). It is likely that the high CK concentrations were due to the repeated movement of the calves through the yard complex, tipping and restraint in the calf cradle, and blood sampling. The increasing values of TP and PCV were unexpected, as there was no indication of decreased drinking during the day. As mean values were, however, within normal ranges (PCV 24–46% and TP 57–81 g/L; Radostits et al., 2007), these increases are of little biological significance. Other work has found TP and PCV to be unaffected by surgical or chemical castration (Cohen et al., 1990).

We found no differences between treatments in cortisol response on the days after castration, but behavioural responses suggested that the surgically castrated 3-month-old calves were in greater pain and discomfort than the Ring (and Sham) calves on days 1–3 post-castration. In contrast, there were no differences in the 6-month-old calves. Coincident with the behavioural responses in the surgical castrates was a rise in haptoglobin concentrations indicative of a systemic inflammatory response, although this occurred with both castration methods and regardless of age. In the longer-term, whilst haptoglobin concentrations steadily declined in the surgical castrates, they were significantly elevated at 14 days post-castration in the Ring calves of both age groups. This difference between methods in the pattern of the inflammatory response is supported by the findings of others (Fenton et al., 1958; Molony et al., 1995; Carragher et al., 1997; Warnock et al., 2012). The inflammatory response appeared related to the rate of wound healing as, at days 14 and 21, there were indications of infection and poor healing in the Ring calves and particularly in the 6-month-old calves. This finding that wound healing was faster in the surgical compared with the ring castrates is also supported by other work with 7-week-old (Fenton et al., 1958) and 2–4-month-old calves (Stafford et al., 2002). Our finding of greater inflammation and poorer wound healing in the Ring6 compared with Ring3 calves may have been a consequence of the rings exerting insufficient pressure on the scrotal neck to create an effective seal and cut off the blood supply in the older, larger calves, as has been suggested by others (Molony et al., 1995; Bretschneider, 2005; Thüer et al., 2007). Other factors, however, are also likely to influence wound development and healing rates, such as climatic conditions and the environment in which the cattle are kept post-castration, which could influence the propensity for contamination and infection

of wounds. The numbers of calves requiring treatment for wound inflammation and infection were too small to determine any relationship with calf age or castration treatment. We observed physical damage (punctures and tears) to some scrotums of the ring castrated calves at 7 days post-castration, although these sacs appeared to be among the first to dry-out and dehisce. The increase in the circumference of the scrotal sacs of the Sham calves probably reflected normal testicular growth in contrast to the decrease in size in both the Surg and Ring treatment groups. The size difference (of 4 cm) between these two groups was probably a consequence of both the scrotal sac and contents drying and shrivelling in the Ring calves compared with removal of the testes, but retention of healthy, living scrotal sac tissue in the Surg calves.

Normal concentrations of haptoglobin are reported to be less than 0.35 mg/mL (Horadagoda et al., 1999), but in both age groups and throughout the 28 days post-castration, concentrations were above this. Indeed, even the pre-treatment concentrations were elevated above normal. Although haptoglobin is reported to be a sensitive acute-phase protein in cattle indicative of systemic inflammation (Horadagoda et al., 1999), it has been found to be elevated by social and psychological stressors in some species, although not yet determined in cattle (Maes et al., 1997). The temporary separation of calves from their mothers, required for data collection in this study was likely, in itself, to be stressful for the calves (King et al., 1991; Enriquez et al., 2011). It is possible, therefore, that the elevated haptoglobin concentrations we found were a consequence of social stress.

Castration reduced liveweight gains compared with the sham castrates, but there was no difference between the surgical and ring methods. This is in agreement with studies previously reviewed (Bretschneider, 2005) and others not included in that review (Fenton et al., 1958; Fell et al., 1986; Warnock et al., 2012). Another study not included in that review reported significantly superior ADG (by about 0.3 kg/day) to weaning (timing not reported) in calves ring castrated at 2–3 months of age compared with calves surgically castrated or left intact (Lents et al., 2001). In the current study, 3-month-old calves had superior gains compared with the 6-month-old calves, which was expected for the castrated calves; Bretschneider (2005) analysed liveweight changes from a number of castration studies which indicates an expected liveweight loss of about 0.15 kg/day for 3-month-old calves and 0.3 kg/day loss for 6-month-old calves during the first month post-castration. It is less apparent why we found higher weight gains for the Sham3 compared with the Sham6 calves, but it was perhaps related to nutritional plane. A study on milk yield of grazing, primiparous beef cows indicates a decline in milk yield after about 100 days in milk (Grings et al., 2008). Thus, the 3-month-old calves would have been obtaining a greater proportion of their nutritional requirements from milk than the 6-month-old calves. It may have been expected, however, that the 6-month-old calves would have compensated for the reduced nutrient supply from milk by consuming more forage and would have grown at a similar rate to the younger calves (Tedeschi and Fox, 2009).

In the current study, one calf died and three others experienced injuries that required treatment, but all appeared unrelated to castration method *per se*. The experiment was conducted during hot, humid and wet conditions, although not necessarily atypical of the weather during which calves may be castrated in northern Australia. The calf that died was the youngest in the experiment, although was not the lightest. It is possible that the combination of the weather, castration (ring) and the repeated restraint and blood sampling were sufficiently stressful that the calf failed to cope. The three calves that were injured were amongst the fastest (flight speed) 10% of the calves, suggesting that their poor temperament may have contributed to their injuries, probably due to their extremely agitated response to being handled and restrained.

Some behavioural indicators of restlessness/activity were influenced by calf age, being greater in 3-month-old than 6-month-old calves. Further, during the 4–7 h period post-castration, by which time calves had been separated from their mothers for at least 4 or 5 h, there were higher levels of vocalisation by the Sham calves compared with the castrated calves. Higher levels of restlessness in the younger compared with older calves on the day of castration may have been due to higher motivation to establish contact with their mothers, as a consequence of their greater dependency on the mother for their food supply (Enriquez et al., 2011). The Sham calves possibly vocalised more during the 4–7 h period compared with the Surg and Ring calves as they were experiencing least pain and were, therefore, behaving the most 'normal' of the groups in trying to establish contact with their mothers. Throughout the day, pain-related behavioural responses may have been confounded by competing motivational states; although not studied in cattle, competing motivational states and attentional shifts have been shown to reduce the performance of pain-related behaviours in poultry (Gentle, 2001). Thus, temporary separation of calves from cows may have attenuated the castrated calves' pain-related behavioural responses because the calves were motivated to reunite with their dams and had their attention shifted from the pain.

5. Conclusion

Castration should be conducted by the procedure that has the least adverse impacts on cattle welfare. Both ring and surgical castration cause pain and stress post-castration and reduce liveweight gains, and there is little evidence of differences between 3- and 6-month-old calves. Thus, provision of pain relief is the preferred option for castration by both methods and for both ages. With ring castration there is evidence of systemic inflammation at about 2 weeks post-castration, but it is not clear if this is painful. In experimental situations where unweaned calves are temporarily separated from their mothers for data collection, pain-related behavioural responses may be attenuated due to calves being motivated to re-establish contact with their dams, which may switch their attention from pain. Competing motivational states and attentional shifts, thus, require consideration when interpreting pain-related behaviours.

Conflicts of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication and any financial support for this work has not influenced its outcome.

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