final report

Project code: B.AWW.0008

Prepared by: J. Carol Petherick

The University of Queensland
Queensland Alliance for
Agriculture and Food Innovation

Date published: December 2012

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Welfare outcomes of calves of two ages castrated by rings

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

The current Code of Practice for the Welfare of Cattle states that rings should not be used for castrating calves older than 2 weeks age, although anecdotal reports suggests ring castration occurs in cattle up to 10 or more months of age in northern Australia. This study examined the welfare outcomes of 60, 3- and 6-month-old Belmont Red calves surgically, ring or sham castrated in central Queensland in late February 2012. There were few differences with age, but behavioural responses suggested greater pain in the surgical than ring castrates during the first few days post-castration. Thereafter, wound inflammation steadily declined and healing proceeded in the surgical castrates, but inflammation increased in the ring castrates to 2 weeks post-castration before declining at week 3. Behavioural responses of the ring castrates indicated that they were in pain during this latter period and there was evidence of delayed wound healing, particularly in the 6-month-old calves. Responses observed here and reported in the literature indicate that castration is painful. Therefore to improve calf welfare, pain relief should be provided for castration and, given the timing of inflammatory pain, this is more likely to be most practical and cost effective, with less handling stress, for surgical castration, although efficacy requires testing.

Executive summary

With increasing scrutiny of livestock management practices, particularly those procedures that are invasive and likely to cause pain, the beef cattle industry needs objective data to defend or modify practices. There are anecdotal reports that rings are increasingly being used for calf castration and, particularly in northern Australia, they appear to be used for cattle of up to, at least, 1-year-old. The current Code of Practice for the Welfare of Cattle states that rings should not be used for the castration of calves older than 2 weeks of age. It seems likely, however, that this stipulation was made at a time when ring castration of calves was conducted using lamb rings, which were deemed, as a consequence of size and thickness, unsuitable for use on calves older and larger than those 2 weeks of age. There are now available rings that are larger and thicker than lamb rings and which are marketed and sold as being suitable for calves of more than 6 months of age. Unfortunately, the term "bands" adds to the confusion about rings because it can be used to mean rings or the lengths of latex that are usually used in conjunction with a specific applicator that allows tensioning of the band. There is some evidence that rings and bands produce different welfare outcomes for cattle and so they should be considered to be different castration methods.

Few studies have examined the welfare outcomes of calves castrated by different methods and at different ages and those that have been performed have mostly been conducted on *Bos taurus* calves of a few weeks or months of age and in temperate climates. The current cattle welfare code is undergoing revision and any decisions made about the use of rings should be informed by science that is relevant to the Australian beef cattle industry. Thus, this project examined the welfare outcomes for calves that were about 3- and 6-months-old and surgically or ring castrated. Welfare outcomes were assessed from measures of behaviour, selected blood parameters that are indicative of pain and stress, rates of wound healing and liveweight changes.

The experiment was conducted in central Queensland at a time when calves in this region would often be castrated (end of February). Sixty, Belmont Red calves were used, with 30 in each of two age groups: the 6-month-old group contained calves ranging in age from 5 to 7 months (mean liveweight 163.3 kg) and the 3-month-old group contained calves ranging in age from 2.5 to 4 months (mean liveweight 93.7 kg). Within age group there were three treatments: ring castration (n = 10), surgical castration (using a scalpel) (n = 10) and sham castration (calves were restrained and handled the same as the other calves, but not castrated) (n = 10). The calves were unweaned and were not dehorned until the experiment had finished. Blood samples for measurements of packed cell volume, plasma total protein, cortisol, creatine kinase and haptoglobin were taken by jugular venipuncture immediately prior to castration and at 30 minutes, 2, 7, 24, 48 and 72 hours, and 1, 2, 3 and 4 weeks post-castration. Behavioural recordings were made by direct observation on the day of castration when calves were apart from their mothers and days 1 to 3 post-castration when calves were with their mothers, and by continuous remote logging to 2 weeks post-castration. Scrotal circumferences were measured, and wounds photographed and scored for healing at day 3 and weeks 1 to 5 post-castration. Liveweights were recorded weekly to 45 days post-castration.

Behavioural and cortisol responses on the day of castration appeared to be confounded by stress and probable conflicting motivations induced by the separation of the calves from their mothers, but it was evident from behavioural responses that ring castration caused less pain and stress compared to surgical castration during the procedure. All calves, including the controls (sham castrates) experienced muscle damage from the repeated restraint and tipping in the calf-cradle, and blood sampling. Three calves required treatment for injuries apparently sustained during excessive struggling in the calf-cradle; these calves were in the

highest 10% for flight speed indicating that poor temperament may have contributed to them sustaining injuries. The youngest calf in the experiment died between the 2nd and 3rd day post-castration; post-mortem examination suggested that neither castration method (ring) nor blood sampling *per se* caused the death which was probably a consequence of the calf failing to cope with the overall stressful conditions.

There was no evidence of excessive blood loss in the surgical castrates, but there was some behavioural evidence that the surgical castrates, regardless of age, experienced more pain and stress than ring castrates during the first 3 days post-castration which coincided with a non-significant elevation in haptoglobin concentrations, indicating tissue inflammation. Thereafter, haptoglobin concentrations declined steadily over time in the surgical castrates, but increased significantly in the ring castrates to peak at 2 weeks post-castration in both age groups before declining at 3 weeks post-castration. There was evidence from behavioural patterns that the ring castrated calves experienced pain during this period. This pain was a likely consequence of inflammation of wounds, as ring castrates were scored more highly than surgical castrates as having evidence of sepsis and delayed wound healing during weeks 2 and 3 post-castration, particularly in the 6-month age group. Wounds of all calves proceeded to heal with no sign of infection from week 4 post-castration, at which time all but one of the ring castrates had lost their scrotal sacs. Liveweight gains were significantly lower in the castrated compared to control calves, but there was no effect of castration method on liveweight gains. The 3-month-old age group, however, showed superior gains to the 6-month-old group, which probably reflected a higher nutritional plane, as a consequence of their mothers being at peak lactation. All of these findings are generally supported by the limited scientific literature on ring and surgical castration from experiments conducted in temperate climates on calves of about 1 week to 4 or 5 months of age.

Our findings demonstrate that both ring and surgical castration cause pain and stress post-castration and there is little difference between 3- and 6-month-old calves in their responses to castration. Thus, in order to improve calf welfare, providing pain relief is the preferred option for castration by both methods and for both ages. As the pain and stress associated with surgical castration is immediate and comparatively short-lived (about 1 to 3 days post-castration), administration of an analgesic would be relatively straightforward, as it could be administered either just before or at the time of castration. In contrast, the onset of pain and stress from ring castration is associated with longer-term wound inflammation and, so, analgesics would need to be repeatedly administered to cover a period of, at least, 2 weeks post-castration. This would evidently involve repeated mustering and handling of calves which would be costly and possibly detrimental to their welfare, in addition to the cost of multiple administrations of the analgesic. Testing the efficacy of analgesia is, however, required explicitly for calves that are typical of the northern beef cattle industry i.e. a minimum of 3 to 6 months of age and unaccustomed to handling and restraint.

Findings from this work do not address the issue of welfare outcomes from castration of calves of a few weeks of age and specifically the use of rings being limited to calves of less than 2 weeks of age. There is some evidence in the literature for the duration of the cortisol response to castration to be reduced in young animals with, in one study, no detectable response in calves castrated using rings within 1 week of birth. This study, however, investigated cortisol responses only to 6 hours post-castration and would, therefore, have not detected any later onset of inflammatory pain from rings, as has been identified in the current and other work. Thus, studies on young calves should involve measures of cortisol and haptoglobin over a period of several weeks. Caution is needed, however, with repeated restraint and blood sampling of young calves and alternative methods for serial blood sampling should be explored e.g. in-dwelling catheters. The issues of stress and effects on behaviour due to dam-calf separation would also require addressing in order to optimise the acquisition of unambiguous data.

Contents

1 Background

Castration of beef cattle is one of the most common husbandry procedure conducted in northern Australia. Almost all males that will not be used for breeding are castrated because of perceived difficulties controlling and managing bulls, although the difficulties may be minimal in bulls up to 2 or 3 years of age[1]. Data collected in a 2009 survey of producers by MLA[2] reveal that, across Australia, 60% of producers surgically castrate (by knife or scalpel) and about 40% use "rubber rings". Proportions vary between states and territories in northern Australia, with about 80% castrating by surgery and 20% by rubber rings in Queensland and the Northern Territory (NT) and 15% by surgery and 85% by rubber rings in Western Australia (WA, the entire state and not just the northern regions). Surgical and rubber ring castration appear to be used across all ages of calves from less than 1 month of age to more than 12 months of age; most producers in Queensland and WA castrate when calves are 2 to 6 months old, and in the NT when calves are 3 to 12 months old.

Castration using rings is not to be confused with castration using bands; although the terms tend to be used interchangeably, there are differences. To add to the confusion, the devices used to apply rings are frequently marketed and sold as 'banders'. Further, there are two broad types of application devices, those that allow rings or bands to be tightened (tension-banders) and those that do not. Rings are closed circles of latex that are applied using a device that stretches the latex, usually by means of prongs over which the ring is placed, to increase the size of the opening (e.g. see (http://www.thecattleshop.com.au/category17_1.htm and http://horsleywholesale.com.au/products/Jumbo_Marking_Castration_Bander_Delivery-487-113.html). In contrast, bands are either lengths of latex with an aluminium clip at one end (e.g. the bands for the California Bander (InoSol Co. LLC, El Centro, CA, USA); http://www.inosol.com/thecaliforniabander.html) or lengths of latex that are joined by an aluminium clip to form a loop (e.g. the Callicrate Bander (No-Bull Enterprises Inc., St. Francis, KS, USA) loops; http://www.probeef.com.au/). Further confusion arises because of the use of the term 'elastrator ring'. Elastrator is a registered product (Elastrator Ltd, Bibra Lake, WA) and the term Elastrator® ring appears to specifically refer to the small, green rings (external diameter, 13.5 mm; internal diameter, 6.5 mm) advertised for castrating lambs and calves (http://www.heiniger.com/index.php?id=360&type=6).

According to the Model Code of Practice for the Welfare of Cattle (the Cattle Code)[3], rings should only be used on calves up to 2 weeks of age, although this Code is currently under review. It is probable that this age limit was established because, at the time the Cattle Code was prepared, only Elastrator® rings were available. There are available now, however, larger, thicker rings than the Elastrator® rings and which are promoted for castrating calves of more than 2 weeks of age. For example, the Tri-Bander (Wadsworth Manufacturing, St. Ignatius, MT, USA) is promoted for calves 3 to 4 months old and up to 350 lbs (160 kg) and it is stated that "tests show that banding causes less stress than any other method of castration" (http://www.thecattleshop.com.au/category17_1.htm). Another device from the same manufacturer as the Tri-Bander, is the XL Bander, which also uses rings. This device encourages producers to delay castration until 5 to 8 months of age (or 600 to 700 lbs; approximately 270 to 320 kg). Again, the method is stated as causing "minimal discomfort and stress to the animal". A similar device, the Jumbo Marking Castration Bander (Leader Products Pty. Ltd., Craigieburn, Victoria, Australia, e.g. http://horsleywholesale.com.au/products/Jumbo_Marking_Castration_Bander_Delivery-487-113.html) is promoted as being "ideal for castrating calves over 6 months", with the statement "to achieve more muscular and heavier calves it is recommended to delay

[1] Kilgour & Campin (1973) NZ Proc Anim Prod 33, 125-138
[2] MLA (pers. comm.)
[3] Primary Industries Standing Committee (2004) Cattle Welfare Code 2nd Edn.

castrating". In contrast, best practice states that castration at a young age is preferable for welfare, productivity and occupational health and safety reasons[4]. According to some researchers, however, "there is no convincing evidence that age affects [pain-induced] distress"[5] and it is presumption that younger animals will experience reduced stress and pain compared to older animals.

In previous work, we compared castration by tension-banding or by surgery (using a scalpel) in both weaner and mature bulls[6]. Tension-banding is perceived to be better than rings at preventing blood flow to the scrotum, leading to relatively rapid necrosis and dehiscence of the scrotum, compared to rings. Failure to achieve an effective seal between the scrotum and the living tissue proximal to the band or ring allows tissue fluids and contaminants (e.g. bacteria, pathogens, toxins) to move between the dying scrotal and living abdominal skin tissues causing inflammation and sepsis[7]. Our research indicated that welfare outcomes were inferior with tension-banding compared to surgical castration in both age groups of bulls, and tension-banding did not provide any productivity benefits compared to surgical castration. These findings would suggest that ring castration would also be inferior from a welfare perspective compared to surgical castration, but there was evidently a need to ascertain or refute this hypothesis given the large (and increasing, according to anecdotal reports) use of rings. Furthermore, with the revision of the Cattle Code ongoing, it was important to establish whether there is an effect of calf age on the welfare outcomes from ring castration, particularly in light of the data indicating the use of rings to castrate cattle up to 12 months of age (although it is acknowledged that those responding to the survey to collect these data may have confused rings and bands).

Pain is a subjective experience that cannot be measured directly. It is, however, accepted that the presence or absence of pain can be indicated by behavioural and physiological response, although caution in the interpretation of the responses is required[8]. Additionally, as a prey species, cattle may conceal symptoms of pain, and this may be particularly true of rangeland-reared cattle that are unaccustomed to humans[9]. For this experiment, in addition to various measures of pain-related behaviour, we measured plasma cortisol concentrations, which are commonly used in studies of pain induced by husbandry procedures, and which allow the assessment of the overall unpleasantness or noxiousness of procedures[10]. Haptoglobin is reported to be one of the most sensitive acute-phase proteins in cattle and is indicative of systemic inflammation induced by infection or tissue damage[11]. Although not yet determined in cattle, social and psychological stressors can also elevate haptoglobin in some species[12], but to a much lesser degree than does inflammation. An assessment of total protein (TP) and packed cell volume (PCV) was used to evaluate acute fluid and electrolyte changes, with blood loss generally resulting in a decrease in both PCV and TP concentration[13]. Damage to muscle tissue due to mechanical trauma or high muscular activity can be determined by measuring plasma concentrations of creatine kinase (CK)[14]. In addition, we scored the castration wounds to assess healing and liveweight changes to determine whether there were impacts on growth as a consequence of stress responses.

[4] Newman (2007) Best practice guide for branding, branding and dehorning. MLA
[5] Mellor & Stafford (1999) In Practice, Sept. 436-446
[6] Petherick (2011) Pain Management in Castrated Beef Cattle. MLA
[7] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48
[8] Stafford & Mellor (2010) In: Improving Animal Welfare: A Practical Approach (Grandin, ed.)
[9] ibid.
[10] Mellor et al. (2000) In: The Biology of Animal Stress (Moberg & Mench, eds.)
[11] Horadagoda et al. (1999) Vet Rec 114, 437-441
[12] Maes et al. (1997) Psychoneuroendocrinol 22,397-409
[13] Carlson (1997) In: Clinical Biochemistry of Domestic Animals (Kaneko et al., eds.)
[14] Radostits et al. (2007) Veterinary Medicine 10th Edn.

1.1 Project objective

- To document the welfare outcomes, via measures of blood parameters, behaviour, productivity, and morbidity and mortality rates, of two ages of calves (approximately 3 and 6 months of age) castrated by rubber rings or by surgery under northern Australian conditions.

2 Method

2.1 Research team

University of Queensland	CSIRO	DAFF Qld
Carol Petherick	Alison Small	Debra Corbet
Richard Holroyd (casual employee)	Drewe Ferguson	David Reid
	Ian Colditz	
	Rob Young	
	Jim Lea	
	Dom Niemeyer	
	Warren Sim	
	Phil Orchard	

2.2 Location and weather

The experiment was conducted at Belmont Research Station, approximately 26 km north of Rockhampton (150° 22' 57'' E, 23° 13' 26'' S) between 26 February and 13 April 2012. Temperatures and rainfall during the experiment are given in Table 1.

Table 1. Temperatures (°C) and rainfall during the experimental period of 26 February to 13 April 2012

	Mean max. temp.	Max. temp. range	Mean min. temp.	Min. temp. range	Rainfall (mm)	Mean RH (%) at 9.00 h	Range RH (%) at 9.00 h	No. wet days
Feb 26-29	29.7	28.9-30.6	23.1	22.7-24.0	19.6	85	77-91	3
March	30.0	25.4-34.7	21.3	16.2-24.4	198.4	70	41-90	11
Apr 1-13	29.5	27.2-33.3	18.3	15.1-20.6	0	63	37-75	0

2.3 Animals and treatments

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

Belmont Red calves that were born on Belmont Research Station between 30 August and 20 December 2011 were used for the experiment. The calves were ear-tagged within 24 hours of birth and branded in early January 2012, but were not dehorned. 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 by liveweight and flight speed as measured on 11 January 2012. Flight speed was measured according to a validated method[15] 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[16], 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[17] [18].

[15] Burrow et al. (1988) Proc Aust Soc Anim Prod 17, 154-157
[16] Petherick (2009a) Appl Anim Behav 120, 18-27
[17] Petherick et al. (2002) Aust J Expt Agric 42, 389-398
[18] Petherick et al. (2009b) Appl Anim Behav Sci 120, 28-38

There were six treatment combinations of age of calf and castration method, which were:

- 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)
- 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 birth dates for the 6-month-old calves ranged from 30 August to 3 October 2011 and for the 3-month-old calves it was 1 November to 15 December 2011. Thus the 6-month-old calves ranged in age from 5 to 7 months and the 3 month-old calves ranged from 2½ 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.

Plates 1 & 2 Calves in races illustrating size differences in age groups

Due to time and daylight constraints, 30 calves were castrated on 2 successive days (27th and 28th February). Calves were allocated to 10 blocks, each containing one animal for 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 hours, respectively on both days.

2.4 Procedures

On the day before the experiment started (26 February 2012) the calves and their mothers were mustered from their paddock, walked to the yards and cows and calves drafted into the two groups (Batch A and B). Calves were weighed and returned to their dams. Batch B were returned to their home paddock and Batch A held in a small paddock adjacent to the yard complex.

On the day of castration, cows and calves were walked into the yards and calves drafted from their mothers and into the five block groupings. The cows were retained in the yard complex, thus their calves could hear 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 20 G needles into vacutainers. Scrotal circumference was measured[19] and an IceTag3D™ motion sensor device (data logger) was fitted to the right hind leg in accordance with the manufacturer recommendations (IceRobotics, Roslin, Midlothian, Scotland). The calves were then castrated by the pre-assigned method. All castrations were conducted by the same, highly-skilled operator.

Plate 3 Cows and calves in yard

[19] Entwistle & Fordyce (2003) Evaluating and reporting bull fertility, AACV

Plate 4 Measuring scrotal circumference

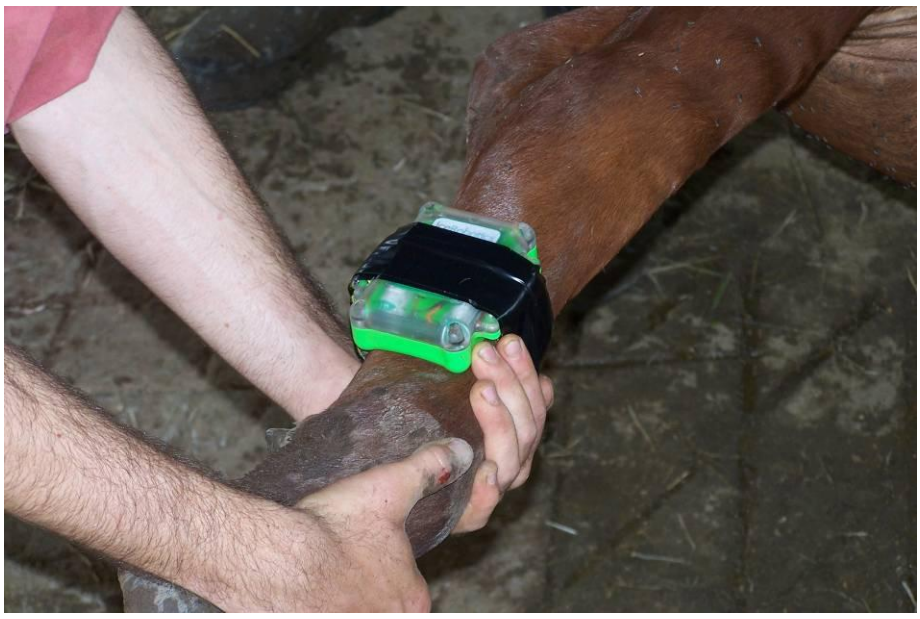

Plate 5 IceTag fitted to hind leg of calf

2.4.1 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 the MLA Guide[20], 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 cut 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

[20] Newman (2007) Best practice guide for branding, branding and dehorning, MLA

immediately righted and released to a grassed yard, with the entire procedure (from the start to end of restraint) taking approximately 1 min.

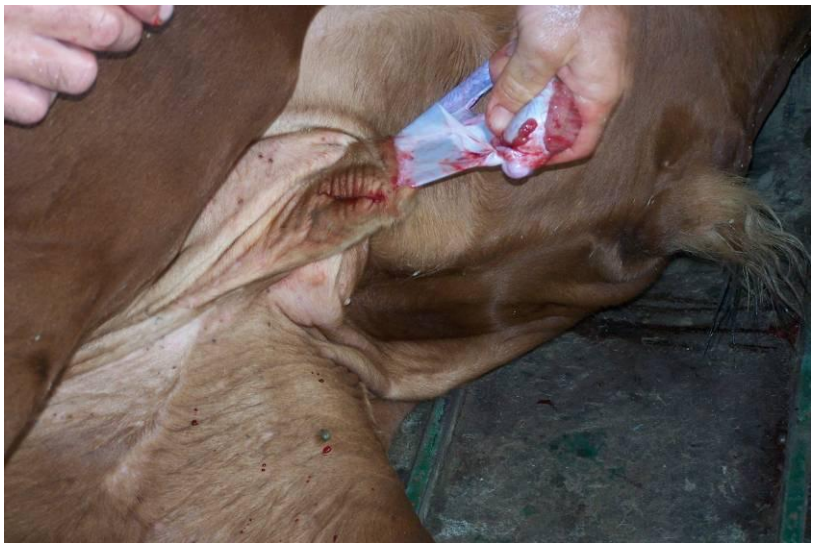

Plate 6 Surgical castration

2.4.2 Ring castration

Calves were restrained in the calf cradle as described in 2.4.1 and the operator conducted the castration according to the MLA Guide[21], 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.). 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 righted and released to a grassed yard. The entire process (from the start to end of restraint) took approximately 1 min.

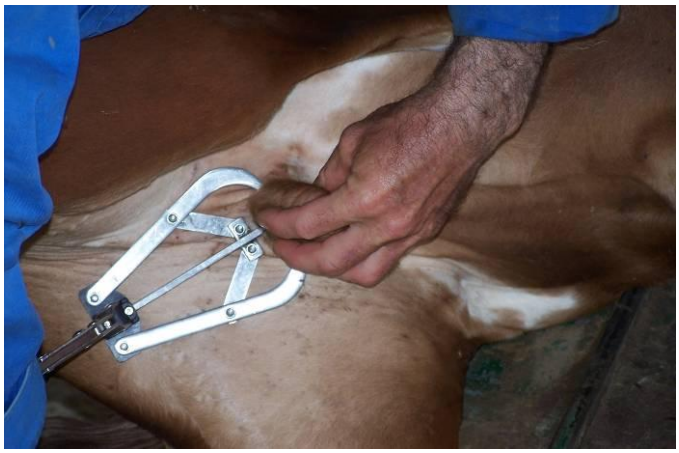

Plate 7 Applying ring

[21] ibid.

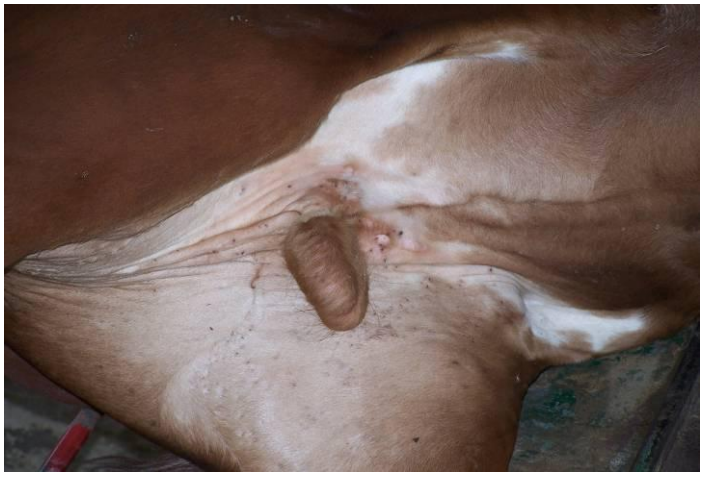

Plate 8 Ring in place

2.4.3 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.

2.4.4 Post-castration management

When all six calves had been treated, the group was either moved into the race to return to the calf cradle for another sample, or moved to a "home" yard (approximately 50-70 m^2) with shade, and Lucerne hay and water available *ad libitum,* until a few minutes before the next blood sample was due. For the second blood sample (at 30-min post-treatment) the animals were kept in the order in which they had been castrated, but for subsequent samples they were blood sampled in the order that they entered the crush. After blood-sampling they were returned to their home yard. Thus, each block of six 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 some time to pair-up and then released to a small paddock (approximately 1 ha) 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 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.

2.4.5 Blood sampling

Blood samples were taken on restraint (time 0) and at 30 min, 2 hours and 7 hours post-castration. Samples were collected into EDTA and sodium heparin vacutainers (Becton Dickinson, North Ryde, NSW, Australia) and kept refrigerated 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 up 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 the duplicate percentages read off the haematocrit scale (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).

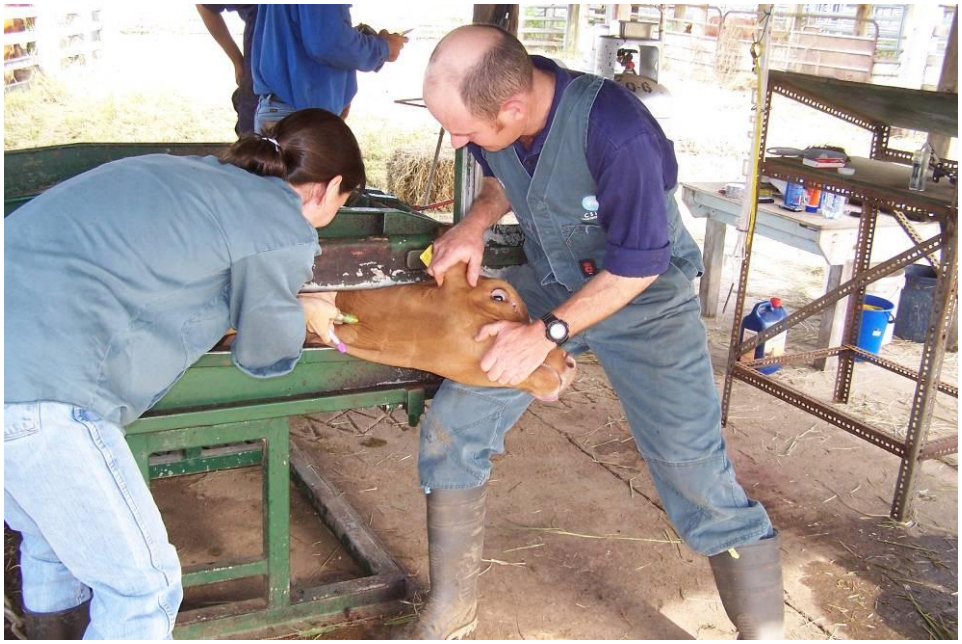

Plate 9 Taking blood sample

The sodium heparin vacutainers were centrifuged on the day of collection at 2500 rpm for 20 min and the plasma extracted and stored at -20 ºC 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 ovine plasma, as described previously[22]. Detection limit of the assay was 5.0 nmol/L. The intra-assay coefficients of variation (CV) for samples containing 33.3, 75.9 and 144.7 nmol/L cortisol were 7.6, 9.7 and 9.4%, respectively. The inter-assay CV for the same samples were 7.9, 11.4 and 9.9%, respectively.

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, no. 63 and 150, and one Surg6, no. 45) had previously sustained leg/shoulder injuries, apparently from vigorous struggling in the cradle. The injury to calf 150 was sufficiently severe that it was not restrained and sampled at weeks 3 and 4.

On all of 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

[22] Paull et al. (2007) Aust Vet J 85, 98-106

technically days 13, 20 and 27 for the batch B cattle, but for simplicity, these dates will be considered to be 2, 3 and 4 weeks post-castration for all animals.

2.4.6 Behavioural recording

Behaviour was recorded during castration by direct observation by a trained and experienced observer. Being restrained in a calf cradle, the calves were limited in what behavioural patterns they could show; counts were scored for:

- vocalisations;
- struggles (moving back and forth and/or side-to-side in the cradle with head and legs flailing);
- kicks (with one or both hind legs to the rear, even if manually restrained); and
- tail flicks (sideways movement of the tail from vertical and return to vertical).

On day 0 post-castration, blocks of animals were directly recorded by 5-min focal animal sampling by two highly-experienced observers. The behaviours recorded are given in Table 1 and were derived from the literature on behavioural responses to castration and in our previous work[23]. Those behaviours having a duration of 5 sec or more were categorised as States and other behaviours (lasting less than 5 sec) classified as Events. States were mutually exclusive and total durations (sec) were calculated for each state and the proportion of the total time (300 sec) spent in each state determined. Counts of all events were summed for each 5-min observation period. A number of behaviours were combined into behavioural 'categories', as indicated in Table 1 and some were not analysed due to too few data. 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 group were randomly allocated to each observer on each occasion, to minimise any biases 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. Further, 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 hours post-castration. Inspections of plots of the observations 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. With one exception (when recording on day 0 and day 1 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 hours on day 1, 12.45 and 17.00 hours on day 2, and 6.45 and 12.45 hours (with blood sampling between about 9.00 and 11.00 hours) on day 3, for both batches.

The percentage of time spent standing and lying, the number of steps taken and a "motion index" (indicating extent of activity) were automatically determined from the IceTag3D™ data. Some of the loggers (23) were removed at week 2 post-castration because lesions had developed under the straps (probably due to the wet weather). The remainder were removed at week 3 post-castration. Three loggers failed to record any data (two Sham3, calf

[23] Petherick (2011) Pain management in castrated beef cattle. MLA

numbers 464 and 506, and one Sham6, calf number 57) and two provided partial recordings (one Ring3, calf number 417, and one Sham3, calf number 462).

Table 2. Ethogram developed from observations conducted 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	
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	
Walk forward	Forward locomotion (mainly walk, but occasionally trot or gallop)	
Walk backwards	Backwards locomotion (walk)	
Eat	Ingesting hay, grazing, browsing	Feed
Suck	Sucking dam's teats	
Drink	Ingesting water	
Groom self	Licking, mouthing, nuzzling self or attempting to do so	Comfort
Scratch	Standing or lying and using the hind hoof to scratch part of the animal, or rubbing any part of the animal against another animal or an inanimate object	
Tail tuck	Standing or lying with tail held taught between the hind legs	
Urinate	Passing urine	
Events (counts)		
Stand alert	As for state	
Stand relaxed	As for state	
Stand head down	As for state	
Walk forward	As for state	
Walk backwards	As for state	
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	
Head shake	Head lateral movement to one side and the other	Head movement
Ear flick	Ear movement from relaxed position and return	
Leg lift	Raising of front or hind foot, may involve a "stamp"	Leg movement
Leg shake	Raising of front or hind foot and shaking the leg	
Kick	Rapid movement of one or both hind legs to the rear or the belly of the animal	
Stretch	Extending the body and limbs and tightening muscles whilst standing or lying	Comfort
Scratch	Standing or lying and using the hind hoof to scratch part of the animal, or rubbing any part of the animal against another animal or an inanimate object	
Groom self	Licking, mouthing, nuzzling self or attempting to do so	
Vocalisation	Any sound emitted (e.g. bellow, moo) from the open or closed mouth of a calf	
Agonistic	Head butting, pushing, charging, chasing, mounting or being mounted by an animal, and withdrawing/retreating from these actions by an animal	
Defaecate[†]	Passing faeces	

[†]This behaviour sometimes had a duration greater than 5 sec, but all incidences were scored as events

2.4.7 Recording liveweight and wound healing

Liveweights were recorded on days -1 (or -2 for Batch B calves) and 7, and at weeks 2 (day 14) to 5 (day 35) post-castration (these were a day less on each occasion for the batch B calves). An additional liveweight was obtained at day 45/46 prior to the calves being dehorned after the experiment.

Castration sites were checked on the same occasions, with an additional assessment on day 3, to week 5 post-castration to determine the extent of healing. On these occasions, for each animal, photographs of the scrotal area were taken and a verbal 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
6. Wound fully open, moist and pus present

Scrotal circumferences were measured (as an indicator of oedema and shrivelling) to week 4 post-castration, at which time the scrotums of all but one of the ring-castrated calves had dehisced and those of the surgically castrated calves, with one exception, had been scored as fully-healed (score 1). 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 give a score of 4, or a score of 6 if one wound was fully open with pus present.

2.5 Statistical methods

One calf died and two were injured (see 3.1 below), so all data for these animals were removed.

2.5.1 Behaviour

2.5.1.1 Direct observations 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.

2.5.1.2 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 hours following castration with times grouped into three periods: 0 to 40 min (0-1 hours), 68 to 230 min (1-4 hours) and 238 to 440 min (4-7 hours) post-castration.

Behavioural states of walking forward and standing alert within each time period were modelled with a GLM assuming a binomial error distribution, a logit link function and binomial totals of the time recorded in the sampling period (300 sec) 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), head movements (primarily ear flicking), leg movements (primarily leg lifting), vocalising, walking forward and walking backward within each time period, and agonistic events in the 4-7 hours 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 during the 0-1 hour period and three records (a leg movement and one behaviour each of walking backwards and agonistic) in the 4-7-hour period 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 hours was modelled as a GLM with Poisson error and log link function with the dispersion parameter estimated.

2.5.1.3 Direct observations on days 1 to 3 post-castration

Behavioural states of walking forward, standing (primarily standing alert), lying and feeding (primarily 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 time of sampling (900 sec) 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), head movements (primarily ear flicking), and walking forward, 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.

2.5.1.4 From IceTags

Examination of the data from the IceTag of a Surg6 calf (no. 39) revealed that it was an extreme outlier on all measures recorded (extremely large quantities of data compared to all other IceTag files) and, so, the data were not used in the analysis. After week 2 post-castration, there were small experimental numbers for some treatments, so analysis was conducted on only the data collected to week 2. IceTag data (motion index, percent 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 hours post-castration, in line with direct observations) and for 13 consecutive 24-hour periods post-castration, where the first period for each animal was the first 24 hours post-castration (i.e. 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 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-hour periods post-castration were analysed as repeated measures using REML and modelling the variance-covariance matrix with an unstructured correlation

structure. Motion index and number of steps were log-transformed and percent standing were arcsine-transformed prior to analysis.

2.5.2 Blood parameters

Blood parameters (PCV, TP, CK and cortisol) on the day of castration 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 (no. 24 (Ring6) at 120 min post-castration) and was treated as missing data. Haptoglobin concentrations at week 2 (day 14) for calf 49 and at week 4 (day 28) for calf 76 (both Sham6 treatment) were identified as outliers and so were treated as missing data.

2.5.3 Liveweight, scrotal circumference and wound healing

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 the castrated animals 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 (no. 516) died between days 2 and 3, after being detected as unwell on the morning of day 2. Post-mortem examination showed nothing abnormal, suggesting that it was unlikely that the treatment or the blood sampling *per se* were the cause of death.

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 4mL/100 kg liveweight, according to manufacturer recommendations). One Surg6 calf (no. 60) was treated at weeks 1 and 2 post-castration, and one each of Surg3 (no. 388) and Ring6 (no. 159) at week 3.

Two calves (one each of Ring6 (no. 63) and Surg6 (no. 45)) 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 week 1 post-castration. As indicated above, data for calf 63 were removed from analysis, as it lost a large amount of weight during the first week when it was lame, but calf 45 showed no sign of being an outlier. Another Ring6 calf (no. 150) was no longer restrained and sampled after week 2 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 sec.

3.2 Behaviour at castration

The proportion of animals vocalising during castration did not differ ($P > 0.10$) among castration treatments (29%) but did differ ($P < 0.05$) between age groups; more 3-month-old calves vocalised than the 6-month-old calves (41 ± 9% vs 14 ± 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 ($P = 0.056$) between age groups (2.1 ± 0.5 and 5.1 ± 1.6 vocalisations for the 3- and 6-month-old calves, respectively). More surgically castrated animals struggled than those from the sham and ring treatments ($P < 0.05$, 90 ± 7% compared with 20 ± 9% and 24 ± 10%, respectively) and, if they struggled, they performed more struggles (1.9 ± 0.2, 1.1 ± 0.3 and 1.1 ± 0.3 for surgical, sham and ring, respectively).

3.3 Behaviour post-castration

In the first hour post-castration, there was a significant castration method x age interaction ($P < 0.05$) in the number of leg movements for calves performing the behaviour. The 3-month-old surgically castrated calves (2.7 ± 1.3) and ring castrated calves (3.3 ± 1.4) performed less leg movements than the shams (6.0 ± 1.2), but 6-month-old surgically castrated (3.8 ± 1.1) and ring castrated calves (5.3 ± 1.5) performed more than the sham castrated calves (1.6 ± 1.8). The data from the IceTags revealed that castration method significantly affected the number of steps/hour ($P < 0.05$) and the percentage of time spent standing ($P < 0.01$). The ring castrated calves took more steps/hour than the surgically castrated calves, with the sham group intermediate (6.19 (back transformed 485), 5.83 (338) and 5.91 (366), respectively; LSD 0.29). Sham and surgically castrated calves spent about 100% of the time standing compared to the ring castrated calves that spent about 86% of the time standing (transformed means 1.57, 1.51 and 1.19, respectively; LSD 0.11). More ($P < 0.05$) transitions between behavioural states were performed by 3-month-old than 6-month-old calves (16.6 ± 1.3 vs. 12.6 ± 1.1), but 6-month-old calves tended ($P = 0.060$) to perform more walking backward events (2.3 ± 2.8) than 3-month-old calves (1.3 ± 2.5).

Overall, there were few differences between treatments in behavioural responses; the ring castrated calves were the most active and the surgically castrated calves were the least. The 3-month-old calves appeared more restless than the 6-month-old animals.

In the 1-4 hour period post-castration there were significant interactions between castration method and age for numbers of tail ($P < 0.05$) and head ($P < 0.01$) movements for those calves performing the behaviours (Table 3). Ring3 calves performed more tail movements than Surg3 calves, but there was no difference in castration method for the 6-month-old calves. Ring3 calves also performed more tail movements than Ring6 calves. Surg6 calves performed more head movements than Sham6 calves, but there were no differences for 3-month-old calves. There was also a tendency ($P = 0.068$) for a castration method x age interaction for the proportion of calves performing leg movements (Table 3), with Ring3, Surg3 and Surg6 performing more than Ring6 calves. The IceTag data revealed a significant ($P <0.05$) effect of castration method on the motion index with it being significantly higher in the sham than surgically castrated calves, with the ring castrated calves intermediate (6.74 (back-transformed 846), 6.31 (548) and 6.45 (629), respectively; LSD 0.32). There was a tendency ($P = 0.060$) for the sham castrated calves to spend a greater percentage of time standing compared with both the surgically and ring castrated calves (1.36 (back-transformed 95%), 1.11 (80%) and 1.14 (82%), respectively; LSD 0.22). For those calves performing walking backwards events, there was a tendency ($P = 0.066$) for 3-month-old calves to perform more than 6-month-old calves (3.7 ± 0.7 and 1.8 ± 0.6, respectively).

Table 3. Mean behaviours (± s.e.) performed in the 1-4 hour period post-treatment 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
Tail movement (no.)	22.7±6.5	36.7±8.2	21.3±6.3	30.6±7.5	48.7±10.8	19.6±6.8
Head movement (no.)	6.8±1.6	1.9±0.9	4.1±1.5	12.4±2.6	4.6±1.9	7.5±1.9
Leg movement (% calves)	54±14	54±14	69±13	64±13	77±12	21±11

Consistent behavioural responses were, again, difficult to discern in this period; there was some evidence from tail and leg movements that the Ring3 calves were feeling more pain than the other treatments. The IceTag data indicated that the surgically castrated calves were least active, regardless of age.

Table 4. Mean behaviours (± s.e.) performed in the 4-7 hour period post-treatment by calves sham, surgically or ring castrated

	sham	surgical	ring
Walk forward (% time)	11 ± 2.1	5 ± 1.5	11 ± 2.2
Head movement (no.)	5.5 ± 1.1	9.4 ± 1.5	9.3 ± 1.7
Walk backwards (% calves)	62 ± 6.7	42 ± 7.1	67 ± 6.7
Vocalisations (% calves)	84 ± 5.2	58 ± 7.1	63 ± 6.9
Vocalisations (no.)	9.5 ± 1.3	6.9 ± 1.3	8.7 ± 1.5

In the 4-7 hour period post-castration, castration method significantly ($P < 0.05$) affected the proportion of calves vocalising and performing walking backwards events, with less by the surgically castrated calves compared to the shams (Table 4). There was also a tendency ($P = 0.061$) for castration method to affect the percentage of time spent walking forwards, with the surgically castrated calves spending less time than both the sham and ring castrated calves (Table 4). Castration method also tended to affect the number of head movements ($P = 0.065$) and vocalisations ($P = 0.068$) for those calves performing these behaviours (Table 4), with the surgically castrated calves performing more head movements but less

vocalisations than the sham calves. Significantly (P < 0.05) more head movements were performed by 3-month-old than 6-month-old calves (9.7 ± 1.2 and 6.0 ± 1.0, respectively) and there was also a tendency (P = 0.071) for more 3-month-old than 6-month-old calves to perform leg movements (73 ± 5% and 59 ± 6%, respectively). There were no effects of treatment on the IceTag-recorded data in this period.

Overall, in this period the surgically castrated calves were the least active and tended to perform more pain-related behaviours compared with the ring and sham castrated calves. They were also the least vocal. The 3-month-old calves performed more pain-related behaviours than 6-month-old calves.

During days 1 to 3 post-castration, there were significant castration method x age interactions (P < 0.05) for both the percentage of time spent walking forward and the number of tail movements for those calves performing the behaviours (Table 5). The Sham3 calves spent more time walking forward than the Ring3 and Surg3 calves, but there were no differences between castration methods in the 6-month-old animals. There were also no castration method differences for tail movements in the 6-month-old calves, but Surg3 calves performed more than Ring3 and Sham3 calves.

Table 5. Mean behaviours (± s.e.) performed during days 1-3 post-treatment 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 ± 3.1	7 ± 2.5	4 ± 1.8	9 ± 2.6	11 ± 3.1	4 ± 2.0
Tail movement (no.)	69 ± 22	66 ± 22	235 ± 41	116 ± 29	47 ± 19	21 ± 33

There was a significant (*P* < 0.001) interaction between time periods (13, 24-hour periods) and castration method for motion index (Figure 1). In the first period (the first 24 hours post-castration), the surgically castrated calves had a low motion index compared with the ring and sham castrated calves, but then the index of the ring castrated calves decreased to a level similar to the surgically castrated calves, with both being less than the sham calves to period 7. Thereafter, the motion index of the surgically castrated calves increased to be similar to that of the sham castrated calves, whilst the index of the ring castrated calves remained lower. Ring castrated calves spent less (*P* < 0.05) time standing than sham or surgically castrated calves (53%, 55% and 55%, respectively) with no difference (*P* > 0.10) among castration methods in the number of steps taken. Three-month-old calves had a greater motion index (724 vs 663), spent less (*P* < 0.001) time standing (53% vs 56%) and tended (*P*=0.062) to take more steps (206 vs 193 steps/hour) than 6-month-old calves averaged over time periods and castration methods.

In summary, during the first 3 days post-castration, there was evidence that the surgically castrated calves, and the Surg3 calves in particular, were experiencing most pain. The surgically castrated calves were least active initially, suggesting they were in pain, but then the ring castrated calves reduced their activity, and both the ring and surgically castrated calves showed reduced activity until the 7th period (day 7). The surgically castrated calves then became more active, but the ring castrated calves continued showing reduced activity. The ring castrated calves also spent least time standing (more time lying) compared to the other methods, also perhaps indicating greater pain. All 3-month-old calves were more active than all 6-month-old calves during the 2 weeks post-castration.

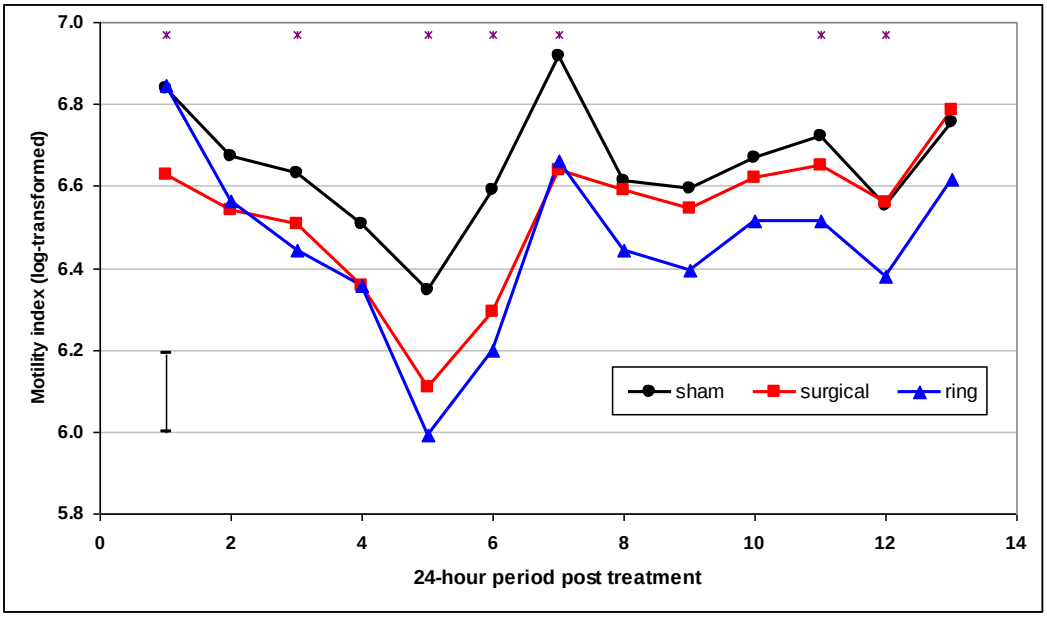

Figure 1. Mean motion index (the greater the number, the more active the animal) for calves sham, surgically or ring castrated during the 13, 24-hour periods post-castration. The vertical bar represents the average l.s.d. at P = 0.05 with significant differences between castration methods at time points indicated by *.

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 420 min post-treatment. Overall means were 35.55% ± 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 Figure. 2. There was a significant ($P < 0.05$) time x castration method x age interaction, as concentrations decreased most rapidly in the sham castrated calves, and the surgically castrated calves and the Ring3 showed similar patterns of cortisol decline, but the Ring6 calves failed to show a reduced cortisol response at 2 hours post-castration. By 7 hours post-castration, all treatment groups had similar concentrations.

Cortisol profiles on days 1 to 28 post-castration are given in Figure 3; 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 ($P < 0.01$) for 3-month-old than 6-month-old calves (27.2 ± 1.0 vs 23.4 ± 1.0 nmol/L, respectively).

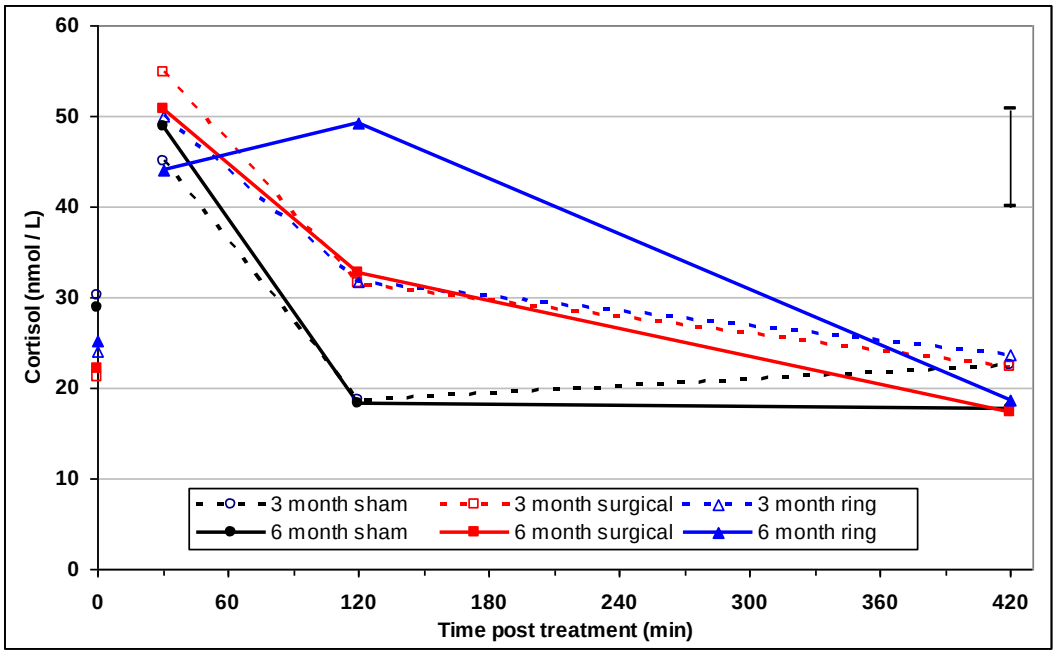

Figure 2. Profiles of predicted cortisol concentrations on the day of castration, adjusted for pre-castration cortisol concentrations (given at time 0), for 3- and 6-month-old calves sham, surgically or ring castrated. The vertical bar represents the average l.s.d. at P = 0.05.

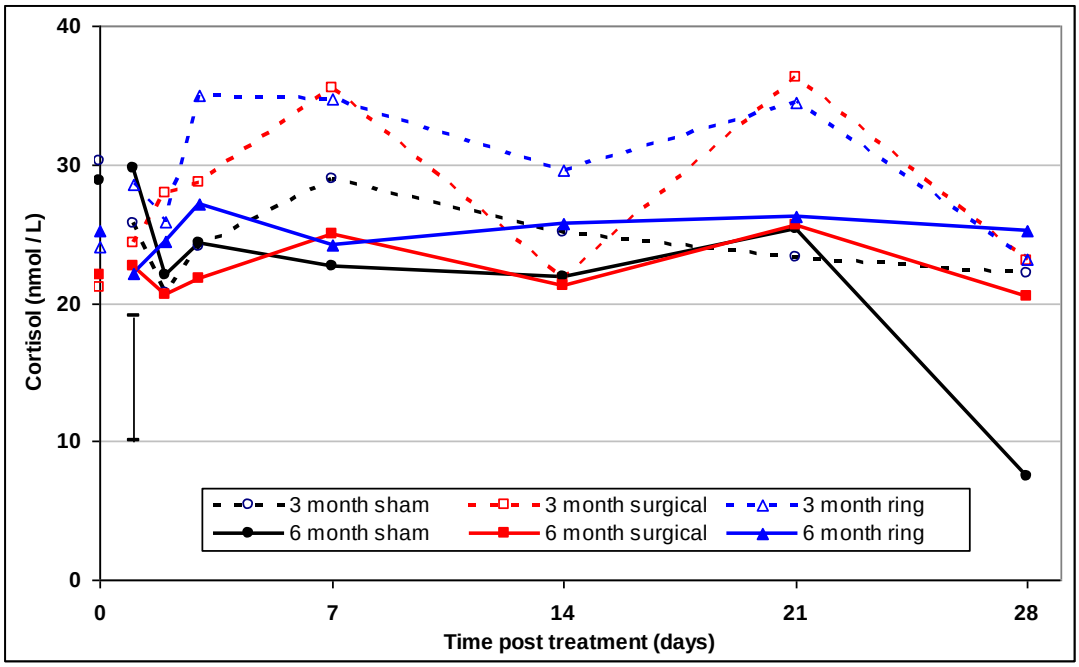

Figure 3. Profiles of predicted cortisol concentrations during days 1 to 28 post-castration, adjusted for pre-castration cortisol concentrations (given at time 0), for 3- and 6-month-old calves sham, surgically or ring castrated. The vertical bar represents the average l.s.d. at P = 0.05.

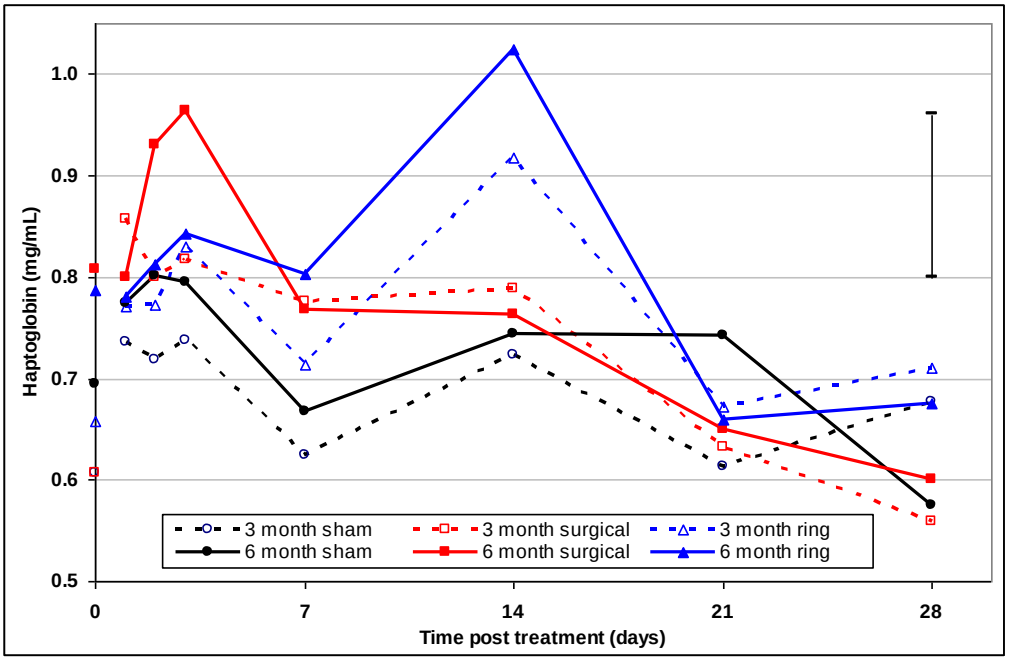

Figure 4. Profiles of predicted haptoglobin concentrations during days 1 to 28 post-castration, adjusted for pre-castration haptoglobin concentrations (given at time 0), for 3- and 6-month-old calves sham, surgically or ring castrated. The vertical bar represents the average l.s.d. at P = 0.05.

Haptoglobin profiles on days 1 to 28 post-castration are shown in Figure 4. There was a significant ($P < 0.01$) interaction between time and castration method; haptoglobin concentrations decreased slightly over time for the sham treatment group while levels increased slightly (but not statistically differently) for both castration methods over the first 3 days post-castration. Haptoglobin levels for the surgically castrated group then decreased steadily to levels similar to the sham group. Although the levels for the ring castration calves decreased on day 7, they increased significantly on day 14 before reducing to levels similar to the other groups by day 21 (Figure 5).

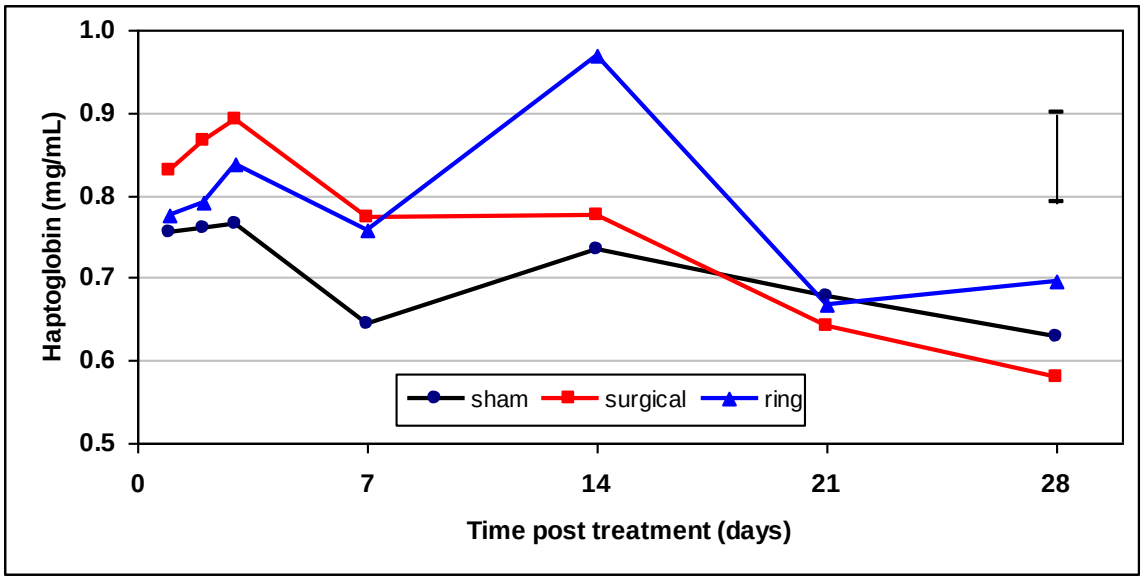

Figure 5. Predicted mean haptoglobin concentrations during days 1 to 28 post-castration, adjusted for pre-castration haptoglobin concentrations for calves sham, surgically or ring castrated. The vertical bar represents the average l.s.d. at P = 0.05.

3.4 Wounds

As anticipated, the number of scrotums present on the ring calves declined over time with all gone at week 5. All were present at day 3 and day 7, although at day 7 three were broken and the contents lost. At weeks 2, 3 and 4, 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) and weeks 4 and 5 (all in category 1) had insufficient variation to be analysed. There was no interaction between castration method and age group for wound score at weeks 1 and 3. Significantly ($P < 0.05$) more of the 3-month age group were in category 1 at week 1 than the 6-month age group, while more ($P = 0.062$) of the surgically castrated wounds were in category 1 at week 3 than the ring castrated wounds. Wound score at week 2 differed ($P < 0.05$) with both castration method and age group with fewer of the Ring6 calves in category 1 than the other treatment groups (Figure 6).

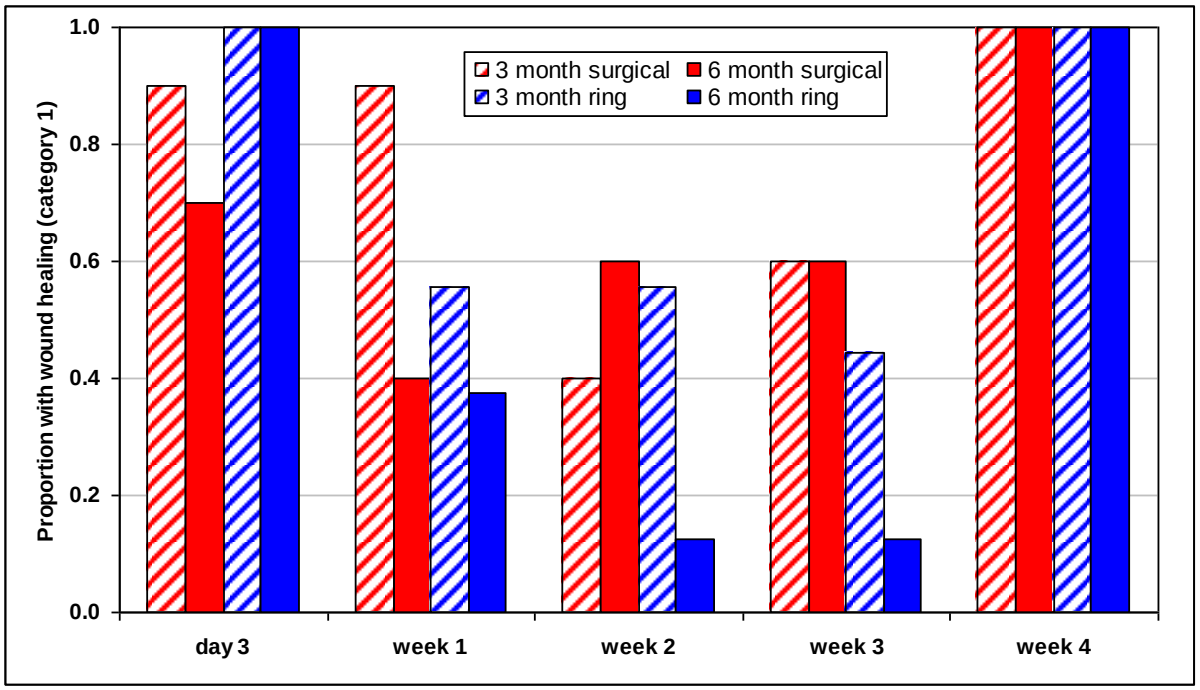

Figure 6. Proportion of wounds scored as healing (as opposed to delayed or abnormal healing) during a 4-week period post-castration for 3- and 6-month-old calves surgically or ring castrated.

The effect of castration treatment on scrotal circumference differed ($P < 0.001$) over time (Figure 7). Scrotal circumferences of sham castrated calves increased slightly (approx 0.5 cm) over the first 21 days post-castration which probably reflected normal growth of the calves. Circumferences of both the surgical and ring groups decreased by approximately 4 cm over this period, but the circumferences of the surgically castrated calves were about 4 cm greater than the ring castrated animals at all times. This size difference was probably a consequence of both the scrotal sac and contents drying and shrivelling in the ring castrated calves compared to removal of the testes, but retention of healthy, living scrotal sac tissue in the surgical castrates. By 28 days post-castration, all but one of the ring castrated calves had shed their scrotal sac.

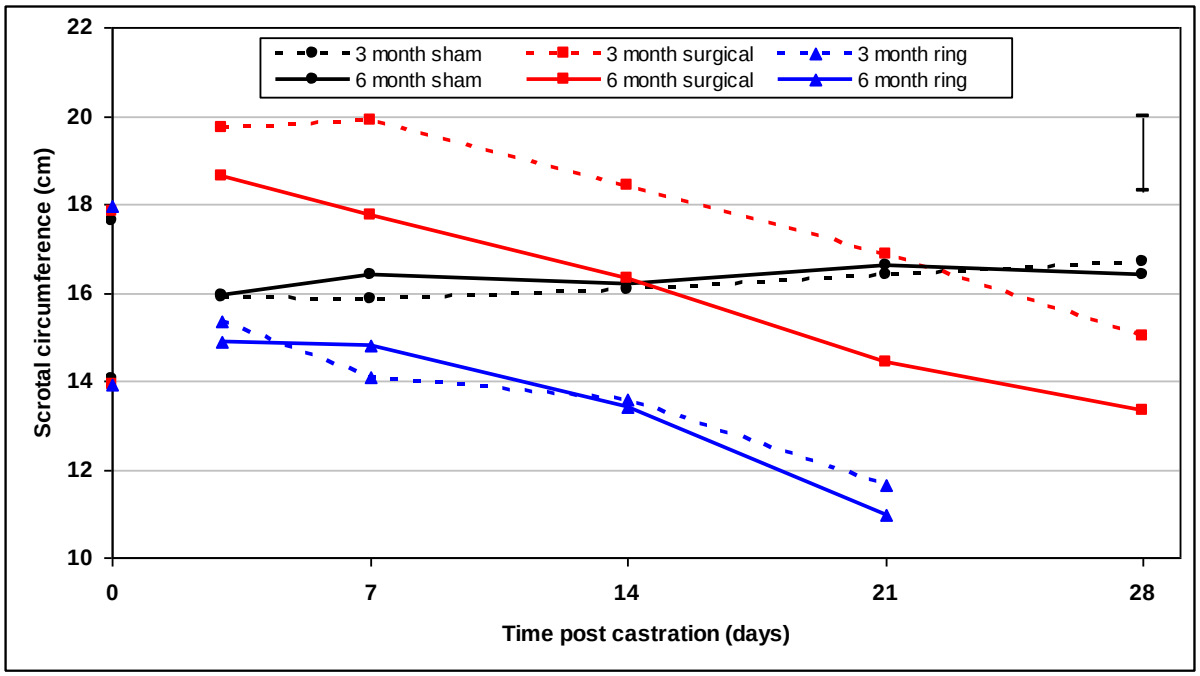

Figure 7. Predicted mean scrotal circumferences, adjusted for pre-treatment circumference (given at time 0), to day 28 post-castration for 3- and 6-month-old calves sham, surgically or ring castrated. The vertical bar represents the average l.s.d. at P = 0.05.

3.5 Liveweight gains

Across the 45 days post-castration, liveweight gain differed between age groups ($P < 0.05$), with greater gains in 3-month-old than in 6-month-old calves. Liveweight gain also differed between castration treatments ($P < 0.001$), with greater gains in shams, but no difference between ring and surgically castrated calves. The castration treatment by age interaction was not significant, nor were interactions between time (the weekly weighings) and method or age significant (Figure 8).

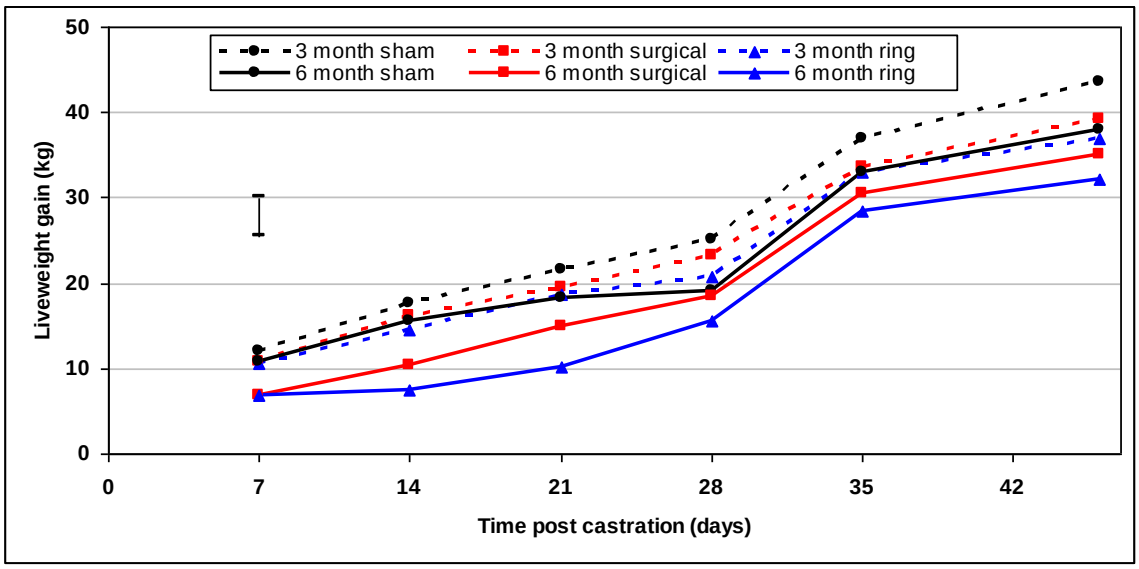

Figure 8. Predicted mean liveweight gains, adjusted for initial liveweight, to 46 days post-castration for 3- and 6-month-old calves sham, surgically or ring castrated. The vertical bar represents the average l.s.d. at P = 0.05.

4 Discussion

It was clear, from the extent of struggling that, during the actual procedures, surgical castration caused the most pain and discomfort whilst there was little difference between ring and sham castration, which agrees with the findings of others[24] [25]. Post-castration, however, there was little evidence of interactions between castration method and age on measures associated with welfare outcomes. Nevertheless, on the day of castration, only the Ring6 calves failed to show a decreased cortisol response at 2 hours post-castration, suggesting that they were experiencing pain and stress at this time. This finding contradicts other studies that have found higher cortisol plasma concentrations on the day of castration in surgically compared with ring castrated calves[26] [27] [28]. The calves in these studies were, however, much younger than the 6-month age group used in the present study, ranging from just 5 days to 11 weeks of age. Other studies comparing surgical castration with other methods, however, also report the greatest cortisol response in surgically castrated calves e.g. compared with chemical castration in 7 to 9-month-old calves, assessed by area under the cortisol curve[29]; compared with burdizzo castration in 5.5-month-old-calves, as assessed by peak cortisol[30]; and compared with burdizzo castration of 5.5-month-old-calves, but only at a single time-point (6 hours) post-castration[31]. In contrast, a ranking of the magnitude of the integrated cortisol response (the area under the curve above the pre-treatment value) during a period of 4.5 hours post-castration in 2 to 4-month-old calves indicated a greater response in ring castrated than surgically castrated calves, provided spermatic cords were cut in the surgical castrates. If the cords were pulled, then surgical castration produced a greater cortisol response compared with ring castration[32]. It is likely, therefore, that differences between studies in the cortisol response elicited by surgical castration may be a consequence of differences in the relative amounts of pulling and cutting of the spermatic cord tissue during the conduct of the procedure; procedures are usually insufficiently described to determine this.

In the current study, peak cortisol concentrations for both castration methods were mostly below the averages reported for ring (45 nmol/L) and surgical castration (129 nmol/L)[33], being about 47 nmol/L for the ring and 52 nmol/L for the surgical castrates at 30 minutes post-castration. Concentrations had returned to pre-treatment levels by 7 hours post-castration, which is in broad agreement with other research that has found a return to pre-castration concentrations of cortisol by: 12 to 24 hours post castration in 7 to 9-month-old surgically castrated calves[34]; 24 hours in surgically castrated 5-month-old calves[35]; 3.5 hours with ring castration, 5.5 hours with surgical castration and cutting of the spermatic cords, and 6.5 hours with surgical castration and pulling of the spermatic cords of calves of 2 to 4 months of age[36]; 6 hours in ring castrated calves of 3 to 4 weeks of age[37]; 4 hours in both ring and surgically castrated calves of 4 to 11 weeks of age[38]; and by 2.2 hours in both surgically and ring castrated calves of 5 to 7 days of age[39]. Thus, it appears that the duration

[24] Fell et al. (1986) Aust Vet J 63, 16-18
[25] Thüer et al. (2007) Vet J 173, 333-342
[26] Fell et al. (1986) Aust Vet J 63, 16-18
[27] Robertson et al. (1994) Res Vet Sci 56, 8-17
[28] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48
[29] Cohen et al. (1990) Can J Anim Sci 70, 1063-1072
[30] Fisher et al. (1996) J Anim Sci 74, 2336-2343
[31] King et al. (1991) Can J Anim Sci 71, 257-263
[32] Stafford et al. (2002) Res Vet Sci 73, 61-70
[33] Coetzee (2011) Appl Anim Behav Sci 135, 192-213
[34] Cohen et al. (1990) Can J Anim Sci 70, 1063-1072
[35] Carragher et al. (1997) Proc NZ Anim Prod 57, 100-104
[36] Stafford et al. (2002) Res Vet Sci 73, 61-70
[37] Thüer et al. (2007) Vet J 173, 333-342
[38] Fell et al. (1986) Aust Vet J 63, 16-18
[39] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48

of elevated cortisol is related to the age at castration, with longer times in older calves. Indeed, one study of calves ring castrated within a week of birth showed that there was no elevated cortisol response, suggesting that the calves experienced no pain and stress[40]. At the other end of the calf age spectrum, a study investigating the surgical castration of 5-month-old calves found elevated cortisol concentrations at days 3 and 7 post-castration compared with controls, even though there had been no difference between treatments at 1 day post-castration[41]. These high concentrations may have been related to inflammatory pain, as they coincided with elevated serum haptoglobin concentrations (see below for further discussion).

Few studies have specifically examined the effects of method of castration and age on welfare outcomes. One study used calves of similar ages to those in the present study (about 2.5 and 5.5. months of age), but compared surgical and burdizzo castration methods, and controls[42]. There were minimal differences between the castration treatments in the young calves; indeed, cortisol concentrations at 2 min post-treatment were greater in the controls than in the burdizzo castrates, with surgical castrates intermediate and not different to the other treatment groups. At 30 hours post-treatment the same patterns was seen, even though concentrations in the controls had been at levels similar to the other treatment groups at 3, 6, 12 and 24 hours post-treatment. In contrast, with the older calves, the cortisol response at 2 min post-treatment was significantly greater in the burdizzo compared with the surgical castrates, with no difference between these calves and the controls. At 3 hours post-castration both the burdizzo and surgical castrates had higher cortisol concentrations than the controls, but there was no difference between the castration methods. At 6 hours post-treatment, cortisol concentrations had decreased, but the surgical castrates were significantly higher than the burdizzo and control groups. Thereafter, there was no difference between treatments. The conclusions drawn from this work was that castration at 2.5 months of age caused minimal stress (maximum cortisol concentration was about 28 µg/L), but at 5.5 months of age the stress was significant (maximum cortisol response was about 44 µg/L) and that burdizzo castration caused less stress than surgical castration in the older calves. This conclusion would seem to be misleading, with few differences in the cortisol response of castrated and control calves in both age groups. Further, in the older calves, the cortisol response was greater in the surgical castrates compared to the burdizzo only at 6 hours post-castration (27.5 vs 15.3 µg/L, respectively), but burdizzo was greater than the surgical at 2 min post-castration (32.0 vs 18.6 µg/L, respectively) and at these times neither castration method was different to the controls.

One other study has examined the impacts of method of castration and age at castration on calf welfare, but the calves were castrated at 6, 21 or 42 days of age[43], so the relevance to the current study is questionable. Castration by rubber rings induced the greatest levels of pain-related behaviours in the 3 hours post-castration compared with burdizzo and surgical castration for all calf ages. In contrast, the cortisol response was greatest in the surgical castrates, with the highest concentrations in the 42-day-old calves and the lowest in the 21-day-old calves. Yet, based on these results, the authors concluded that the 6-day-old calves probably suffered least, that burdizzo castrated calves suffered least and the 42-day-old surgical castrates suffered most. This conclusion would seem to be dubious.

A review of castration studies concluded that the cortisol response was less in calves castrated at $\leq$ 6 months of age compared to those greater than 6 months of age, but made no comparison within the younger age group[44]. This same review also concluded that the

[40] Mellor et al. (1991) Res Vet Sci 51, 149-154
[41] Carragher et al. (1997) Proc NZ Anim Prod 57, 100-104
[42] King et al. (1991) Can J Anim Sci 71, 257-263
[43] Robertson et al. (1994) Res Vet Sci 56, 8-17
[44] Bretschneider (2005) Livest Prod Sci 97, 89-100

cortisol response (using the maximum concentration recorded) post-castration was significantly higher in surgically castrated calves compared to intact calves, with "banded" calves intermediate and no different from either the surgically castrated or the intact calves. The data, however, include studies using rings and tension-bands, which the author of the review appears to regard as equivalent. Rings and tension-banding, however, appear not to be equivalent in terms of the timing and magnitude of the cortisol response, behavioural responses and overall impacts on welfare[45] and, thus, the conclusion made from the cortisol response should be treated with caution.

In the current study, we found no differences between treatments in cortisol response on the days after castration, but behavioural responses suggested that the surgically castrated calves experienced more pain during the 24-hour period post-castration than the ring and sham castrated calves. From day 2 to 7 both the surgical and ring castrated calves appeared to be in pain. Thereafter, the surgically castrated calves behaved similarly to the sham castrates, but the ring castrated calves continued showing behavioural responses suggestive of pain. These behavioural changes may have been associated with inflammation of the castration wounds because, although haptoglobin concentrations declined steadily after day 3 post-castration in the surgically castrated calves, they were significantly elevated at 2 weeks post-castration in the ring castrated calves. This inflammatory response appeared related to the rate of wound healing as, at weeks 2 and 3, there were indications of infection and poor healing in the ring castrated calves and particularly in the 6-month-old calves. Our findings for surgical castrates are similar other studies; in one that used 5-month-old Friesian bulls haptoglobin concentrations were significantly elevated on days 1, 3 and 7 post-castration and cortisol was also elevated on days 3 and 7 in surgical castrates compared to controls[46]. In another recent experiment using 7-month-old Angus and Brangus calves there was evidence from both ceruloplasmin and haptoglobin concentrations of an early post-castration inflammatory response that did not differ from controls by day 9 post-castration[47]. Our findings relating to potential chronic pain in the ring castrates are also supported by other studies. One study investigated 1-week-old calves castrated by surgery, burdizzo, rings or a combination of burdizzo and rings[48]. Calves on which rings were used performed behaviours suggestive of pain at higher levels than calves castrated by other methods and the authors concluded that ring castration can result in chronic pain for at least 42 days post-castration. The performance of pain-related behaviours appeared related to inflammation, sepsis and the gradual healing of lesions; swelling, inflammation and infection started to develop after day 6 post-ring castration and peaked between 27 and 30 days. Another study used calves of 3 to 4 weeks of age and those castrated by rings showed higher scores for pain responses to palpation after day 10 and to week 8 post-castration[49]. Results from these studies are in broad agreement with our current findings; we found a peak in inflammation between 7 and 21 days post-ring castration, which also agrees with a comparative study of rings, burdizzo and surgical castration of 7-week-old calves[50]. It should be noted that the later peak of inflammation and sepsis in the aforementioned study on 1-week-old calves[51] may have been a consequence of the type of ring used; the authors stated they used lamb rings which may not have produced an effective seal, allowing the movement of fluids and bacteria between living and dying tissues. Our finding that wound healing was faster in the surgical compared to the ring castrates is also supported by other work with 7-week-old[52] and 2 to 4-month-old calves[53].

[45] Stafford et al. (2002) Res Vet Sci 73, 61-70
[46] Carragher et al. (1997) Proc NZ Anim Prod 57, 100-104
[47] Warnock et al. (2012) J Anim Sci 90, 2345-2352
[48] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48
[49] Thüer et al. (2007) Vet J 173, 333-342
[50] Fenton et al. (1958) Vet Rec 70, 101-102
[51] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48
[52] Fenton et al. (1958) Vet Rec 70, 101-102
[53] Stafford et al (2002) Res Vet Sci 73, 61-70

Our finding of greater inflammation and poorer wound healing in 6-month-old compared with 3-month-old calves may have been, in line with the suggestion above, 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[54] [55] [56] Other factors, however, are also likely to influence wound development and healing rate, 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 1 week post-castration, although these sacs appeared to be among the first to dry-out and dehisce.

Normal concentrations of haptoglobin are reported to be less than 0.35 mg/mL[57], but in both age groups and throughout the 4 weeks 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[58], it has been found to be elevated by social and psychological stressors in some species, although not yet determined in cattle[59]. It is possible, therefore, that elevated haptoglobin concentrations were a consequence of the stress experienced by the calves being separated from their mothers (see below for further discussion on this point).

Castration reduced liveweight gains compared with the sham castrates, but there was no difference between surgical and ring methods. This is in agreement with studies previously reviewed[60] and others not included in that review[61] [62] [63], although as noted before, the review confuses ring and tension-banding castration. 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 to 3 months of age compared to calves surgically castrated or left intact[64]. In the current study, 3-month-old calves had superior gains compared to the 6-month-old calves, which was expected for the castrated calves; previous work that analysed liveweight changes from a number of castration studies 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[65]. It is less apparent why higher weight gains were found for the 3-month-old compared to the 6-month-old sham castrated calves, but it was presumably 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[66]. 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 grown at a similar weight to the younger calves[67].

[54] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48
[55] Bretschneider (2005) Livest Prod Sci 97, 89-100
[56] Thüer et al. (2007) Vet J 173, 333-342
[57] Horadagoda et al. (1999) Vet Rec 114, 437-441
[58] ibid
[59] Maes et al. (1997) Psychoneuroendocrinol 22,397-409
[60] Bretschneider (2005) Livest Prod Sci 97, 89-100
[61] Fenton et al. (1958) Vet Rec 70, 101-102
[62] Fell et al. (1986) Aust Vet J 63, 16-18
[63] Warnock et al. (2012) J Anim Sci 90, 2345-2352
[64] Lents et al. (2001) Agric Expt Stn Oklahoma State Univ Rep P-986
[65] Bretschneider (2005) Livest Prod Sci 97, 89-100
[66] Grings et al. (2008) J Anim Sci 86, 768-779
[67] Tedeschi & Fox (2009) J Anim Sci 87, 3380-3391.

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 (Table 1), 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 extreme agitated response to being handled and restrained.

Despite using an ethogram developed from previous research on pain-related behaviours in cattle and which has revealed consistent behavioural responses in our previous research on castration[68], the behavioural data were inconsistent and equivocal in this experiment. A number of behaviours were influenced by calf age, in particular vocalisation and indicators of restlessness/activity, which were greater in 3-month-old than 6-month-old calves. Further, during the 4-7 hour period, by which time calves had been separated from their mothers for at least 4 or 5 hours, there were very high levels of vocalisation, particularly in the sham castrated calves (Table 4). Higher levels of vocalisation and restlessness in the younger calves on the day of castration may have been due to their greater attempts to establish contact with their mothers compared with the 6-month-old calves. According to a review of the effects of weaning in beef cattle[69], weaning is associated with persistent vocalising by calves, and increased activity and walking, behaviours which have been interpreted as a motivation to reunite with the dam. It is probable that this motivation would be greater in younger than older calves because of their greater dependency on the mother for their food supply[70]. Possibly, the sham castrated calves vocalised more during the 4-7 hour period compared to the surgically and ring castrated calves because they had experienced least pain and stress during the day. Behavioural responses may have also been further confounded because of competing motivational states in the calves and their attention being focussed on the absence of their mothers. Although not studied in cattle, competing motivational states and attentional shifts have been shown to reduce the performance of pain-related behaviours, resulting from a painful foot condition, in poultry[71]. Furthermore, separation of calves from cows is likely, in itself, to be stressful for the calves; certainly abrupt weaning results in elevated concentrations of blood cortisol in calves, although studies have involved permanent separation[72] [73] rather than for a few hours, as in the current experiment. We speculate that the reason we did not see clear behavioural and cortisol responses indicative of pain was a consequence of the calves experiencing stress due to their temporary separation from their mothers. Indeed, authors of another study on castration that involved temporary separation from dams suggested that as much or more immediate stress was caused by separation from the dam and restraint than by castration *per se*[74]. What is not clear, however, is why 3-month-old calves were also more active than 6-month-old calves in the days and weeks following castration when they were with their mothers and also had higher plasma cortisol concentrations. It is possible that these are simply developmental differences which may also be related to possible differences in the nutritional plane of the different ages of calves. Studies investigating cortisol concentrations of calves have focussed on the first few weeks or months of life; cortisol concentrations are

[68] Petherick (2011) Pain Management in Castrated Beef Cattle, MLA
[69] Enriquez et al. (2011) Acta Vet Scand 53, 28-35
[70] ibid.
[71] Gentle (2001) Anim Welf 10, S187-194
[72] Lay et al (1998) Appl Anim Behav Sci 56, 109-119
[73] Hickey et al. (2003) J Anim Sci 81, 2847-2855
[74] King et al. (1991) Can J Anim Sci 71, 257-263

high at birth[75] [76], but decline rapidly thereafter to achieve levels expected in adult cattle by 27 days of age[77].

Decreases in the concentrations of both TP and PCV can be indicative of blood loss[78]. Thus, we had anticipated a decrease in levels during the day of castration in the surgical castrates, as in our previous experiments on bull castration[79]. TP and PCV, however, increased during the day of castration, but as mean values were within normal ranges (PCV 24-46% and TP 57-81 g/L[80]), these increases are of little biological significance. Other work has found TP and PCV to be unaffected by surgical or chemical castration[81]. It was not surprising that concentrations of CK increased on all treatments during the course of the day of castration, as it is an indicator of muscle damage[82]. The mean value of 691 U/L greatly exceeded the upper limit of normal values (35-280 U/L for *Bos taurus* cattle[83]) and was likely due to the repeated movement of the calves through the yard complex, tipping and restraint in the calf cradle, and blood sampling.

[75] Knowles et al. (2000) Vet Rec 147, 593-598
[76] Burdick et al. (2009) J Anim Sci 87, 3202-3210
[77] Knowles et al. (2000) Vet Rec 147, 593-598
[78] Carlson (1997) In: Clinical Biochemistry of Domestic Animals (Kaneko et al., eds.)
[79] Petherick (2011) Pain Management in Castrated Beef Cattle, MLA
[80] Radostits et al. (2007) Veterinary Medicine 10th Edn.
[81] Cohen et al. (1990) Can J Anim Sci 70, 1063-1072
[82] Radostits et al. (2007) Veterinary Medicine 10th Edn.
[83] ibid.

5 Conclusions

Castration using rings caused less pain and discomfort during the procedure and during the first 1 to 3 days post-castration, compared with surgical castration. In contrast, inflammatory pain was associated with rings, which started to develop a few days post-castration and peaked at about 2 weeks post-castration. There were few differences in the responses of 3- and 6-month-old calves to castration by either method, apart from evidence of delayed healing of wounds in the 6-month-old ring castrated calves.

Our overall recommendation is that 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 there was little evidence for 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. The pain and stress associated with surgical castration is, however, shorter-lived than that associated with ring castration and onset differs with the two methods. This means that the administration of an analgesic will be easier and more cost-effective with surgical castration than with ring castration because it could be provided at the time of castration. In contrast, pain relief for ring castration would need to be repeatedly administered to cover a period of at least 2 weeks post-castration. This proposal does, however, require testing to ensure the efficacy of analgesia. Non-preferred procedures should only be used if there are compelling reasons; for example, currently it may be argued that analgesics may be difficult to obtain and administer because they are restricted to veterinarians.

6 Recommendations

We recommend that, as both ring and surgical castration cause pain and stress, the provision of pain relief is the preferred option for castration, but efficacy requires testing. The current study and the work of other researchers, indicate that the management of the pain associated with surgical castration is likely to be more practical and cost-effective than pain management from ring castration. Therefore, the efficacy of the administration of a non-steroidal anti-inflammatory drug (NSAID) to manage the pain associated with surgical castration of calves requires testing. As our past research has indicated that it takes time for a NSAID to take effect, it would be useful to compare pain relief achieved with the administration prior to (e.g. 30 to 40 minutes) versus at the time of castration. Administration at the time of castration would reduce handling and restraint which is preferable from a labour/time aspect and also from a welfare perspective, as handling and restraint are significant stressors for cattle unaccustomed to them.

As previously indicated, this work did not address pain from castration in very young animals and, specifically the use of rings in calves of less than 2 weeks of age. It is likely that some producers are able to access their calves within a week or 2 of birth and it would be useful to know if castration at this early age, compared to a few months of age, does improve welfare outcomes. In addition, the efficacy of a NSAID could be examined to determine if this further enhances welfare outcomes. The study should investigate pain over a period of several weeks, given that pain from ring castration appears to peak at 2 to 3 weeks post-castration. Conducting such a study would require careful conduct to minimise the stress induced by serial blood-sampling and from separation of calves from dams.

Another area relating to pain management that requires investigation is “attentional shifts” and the potential to distract calves from pain. This approach has been shown to be effective in poultry e.g. laying hens with an experimentally induced painful foot condition performed less pain related behaviours when their attention was shifted from the pain by a competing motivational state such as hunger, or nest seeking and building pre-laying. The behavioural and cortisol responses of the calves in the current study suggested that the calves may have been more focussed on the absence of their mothers than on the pain from castration. Whilst deliberate imposition of a significant stressor, such as separation from the dam for several hours, to act as a distraction from pain is dubious from a welfare perspective, it may be that other, less stressful manipulations could be imposed. For example, it may be possible to shift attention from pain by the provision of a highly palatable food, or by short-term separation and reunification of calves and group mates, or calves and mothers.

7 Acknowledgement

We thank Warren Sim and Phil Orchard at Belmont Research Station for cattle handling, management and care.

Bibliography

Bretschneider G (2005). Effects of age and method of castration on performance and stress responses of beef male cattle: a review. Livestock Production Science 97, 89-100.

Burdick NC, Banta JP, Neuendorff DA, White JC, Vann RC, Laurenz JC, Welsh TH Jr, Randel RD (2009). Interrelationships among growth, endocrine, immune, and temperament variables in neonatal Brahman calves. Journal of Animal Science, 87, 3202-3210.

Burrow HM, Seifert GW, Corbet NJ (1988). A new technique for measuring temperament in cattle. Proceedings of the Australian Society for Animal Production 17, 154-157.

Carlson GP (1997). Fluid, electrolyte, and acid-base balance. In: Kaneko JJ, Harvey JW, Bruss ML (eds). Clinical Biochemistry of Domestic Animals 5th Edn. Academic Press, San Diego. pp 485-516.

Carragher JF, Knight TW, Death AF, Fisher AD, Matthews LR (1997). Measures of stress and growth suppression in surgically castrated bulls. Proceedings of the New Zealand Society of Animal Production 57, 100-104.

Coetzee JF (2011). A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: practical implications for cattle production within the United States. Applied Animal Behaviour Science 135, 192-213.

Cohen RDH, King BD, Thomas LR, Janzen ED (1990). Efficacy and stress of chemical versus surgical castration of cattle. Canadian Journal of Animal Science 70, 1063-1072.

Enriquez D, Hötzel MJ, Ungerfeld R (2011). Minimising the stress of weaning of beef calves: a review. Acta Veterinaria Scandinavica 53, 28-35.

Entwistle K, Fordyce G (2003). Evaluating and Reporting Bull Fertility. Australian Association of Cattle Veterinarians, Brisbane. pp. 26-28.

Fell LR, Wells R, Shutt DA (1986). Stress in calves castrated surgically or by the application of rubber rings. Australian Veterinary Journal 63, 16-18.

Fenton BK, Elliot J, Campbell RC (1958). The effects of different castration methods on the growth and well-being of calves. The Veterinary Record 70(5), 101-102.

Fisher AD, Crowe MA, Alonso de la Varga ME, Enright WJ (1996). Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth and feed intake of bull calves. Journal of Animal Science 74, 2336-2343.

Gentle MJ (2001). Attentional shifts alter pain perception in the chicken. Animal Welfare 10, S187-194.

Grings EE, Roberts AJ, Geary TW, MacNeil MD (2008). Milk yield of primiparous beef cows from three calving systems and varied weaning ages. Journal of Animal Science 86, 768-779.

Hickey MC, Drennan M, Earley B (2003). The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocytes, acute-phase proteins and in vitro interferon-gamma production. Journal of Animal Science 81, 2847-2855.

Horadagoda NU, Knox KMG, Gibbs HA, Reid SWJ, Horadagoda A, Edwards SER, Eckersall PD (1999). Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Veterinary Record 144, 437-441.

http://www.heiniger.com/index.php?id=360&type=6, accessed 1/11/2012

http://horsleywholesale.com.au/products/Jumbo_Marking_Castration_Bander_Delivery-487-113.html, accessed 31/10/2012

http://www.inosol.com/thecaliforniabander.htm, accessed 31/10/2012

http://www.probeef.com.au, accessed 31/10/2012

http://www.thecattleshop.com.au/category17_1.htm, accessed 31/10/2012

Kilgour R, Campin DN (1973). The behaviour of entire bulls of different ages at pasture. Proceedings of the New Zealand Society for Animal Production 33, 125-138.

King BD, Cohen RDH, Guenther CL, Janzen ED (1991). The effect of age and method of castration on plasma cortisol in beef calves. Canadian Journal of Animal Science 71, 257-263.

Knowles TG, Edwards JE, Bazeley KJ, Brown SN, Butterworth A, Warriss PD (2000). Changes in the blood biochemical and haematological profile of neonatal calves with age. The Veterinary Record 147, 593-598.

Lay DC Jr, Friend TH, Randel RD, Bowers CL, Grissom KK, Neuendorff DA, Jenkins OC (1998). Effects of restricted nursing on physiological and behavioral reactions of Brahman calves to subsequent restraint and weaning. Applied Animal Behaviour Science 56, 109-119.

Lents CA, White FJ, Floyd LN, Wettemann RP, Gay DL (2001). Method and timing of castration influences performance of bull calves. Agricultural Experiment Station, Oklahoma State University, Animal Science Research Report P-986.

Maes M, Hendriks D, Van Gastel A, Demedts P, Wauters A, Neels H, Janca A, Scharpe S (1997). Effects of psychological stress on serum immunoglobulin, complement and acute phase protein concentrations in normal volunteers. Psychoneuroendocrinology 22, 397-409.

Martin P (1984). The meaning of weaning. Animal Behaviour 32, 1257-1259.

Mellor DJ, Stafford KJ (1999). Assessing and minimising the distress caused by painful husbandry procedures in ruminants. In Practice (September), 436-446.

Mellor DJ, Molony V, Robertson IS (1991). Effects of castration on behaviour and plasma cortisol concentrations in young lambs, kids and calves. Research in Veterinary Science 51, 149-154.

Mellor DJ, Cook CJ, Stafford KJ (2000). Quantifying some responses to pain as a stressor. In: Moberg GP, Mench JA (eds). The Biology of Animal Stress. Basic Principles and Implications for Animal Welfare. CABI, Wallingford. pp 171-198.

Molony V, Kent JE, Robertson IS (1995). Assessment of acute and chronic pain after different methods of castration of calves. Applied Animal Behaviour Science 46, 33-48.

Newman R (2007). A guide to best practice husbandry in beef cattle. Branding, castrating and dehorning. Meat & Livestock Australia Ltd., Sydney.

Paull DR, Lee C, Colditz IG, Atkinson SJ, Fisher AD (2007). The effect of topical anaesthetic formulation, systemic flunixin and carprofen, singly or in combination, on cortisol and behavioural responses of Merino lambs to mulesing. Australian Veterinary Journal 85, 98-106.

Petherick JC (2011). Pain management in castrated beef cattle. B.AWW.0206 Final Report, Meat & Livestock Australia Ltd., Sydney.

Petherick JC, Holroyd RG, Doogan VJ, Venus BK (2002). Productivity, carcass and meat quality of lot-fed Bos indicus cross steers grouped according to temperament. Australian Journal of Experimental Agriculture 42, 389-398.

Petherick JC, Doogan VJ, Holroyd RG, Olsson P, Venus BK (2009a). Quality of handling and holding yard environment, and beef cattle temperament: 1. Relationships with flight speed and fear of humans. Applied Animal Behaviour Science 120, 18-27.

Petherick JC, Doogan VJ, Venus BK, Holroyd RG, Olsson P (2009b). Quality of handling and holding yard environment, and beef cattle temperament: 2. Consequences for stress and productivity. Applied Animal Behaviour Science 120, 28-38.

Primary Industries Standing Committee (2004). Model Code of Practice for the Welfare of Animals. Cattle. 2nd Edn. CSIRO Publishing, Collingwood.

Radostits OM, Gay CG, Hinchcliff KW, Constable PD (2007). Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th Edn., Saunders, Philadelphia.

Robertson IS, Kent JE, Molony V (1994). Effect of different methods of castration on behaviour and plasma cortisol in calves of three ages. Research in Veterinary Science 56, 8-17.

Stafford KJ, Mellor DJ (2010) Painful husbandry procedures in livestock and poultry. In: Grandin, T (ed). Improving Animal Welfare: A Practical Approach. CABI, Wallingford. pp 88-124.

Stafford KJ, Mellor DJ, Todd SE, Bruce RA, Ward RN (2002). Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. Research in Veterinary Science 73, 61-70.

Tedeschi LO, Fox DG (2009). Predicting milk and forage intake of nursing calves. Journal of Animal Science 87, 3380-3391.

Thüer S, Mellema S, Doherr MG, Wechsler B, Nuss K, Steiner A (2007). Effect of local anaesthesia on short- and long-term pain induced by two bloodless castration methods in calves. The Veterinary Journal 173, 333-342.

Warnock TM, Thrift TA, Irsik M, Hersom MJ, Yelich JV, Maddock TD, Lamb GC, Arthington JD (2012). Effect of castration technique on beef calf performance, feed efficiency, and inflammatory response. Journal of Animal Science 90, 2354-2352.