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Pain management in castrated beef cattle

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Abstract

Tension-bander castration is promoted as producing superior welfare and production outcomes compared to other castration methods, particularly for older bulls. The welfare outcomes for weaner and mature *Bos indicus* bulls castrated by tension-bander or surgery with and without analgesia (ketoprofen administered at castration) were investigated. Behavioural changes indicated that the banded bulls of both ages experienced greater pain than the surgical castrates immediately post-castration. Ketoprofen alleviated the pain, although it took time (about 1 hr) to take effect. Cortisol concentrations showed that both castration methods caused pain and stress and ketoprofen reduced cortisol only in the surgically castrated, mature bulls. Inflammation (scrotal swelling and haptoglobin) was initially greater in the surgical castrates, but it increased and remained high, to 4 weeks post-castration, in the banded bulls. Mature bulls had elevated cortisol concentrations at 2-4 weeks post-castration indicating inflammatory pain. Wounds were slower to heal in the banded than surgical castrates; all treatments had healed by 2 months post-castration. Liveweight changes were generally unaffected by treatment, although ketoprofen-treated, mature bulls had lower average liveweights over 3 months compared to those given saline. These results show that tension-banding castration produces inferior welfare outcomes to surgical castration for both weaner and mature bulls.

Executive summary

With increasing scrutiny of livestock management practices, particularly those that are invasive and likely to cause pain, the beef cattle industry needs objective data to defend or modify practices. The tension-bander, used for castrating bulls, is promoted as offering superior health, welfare and production outcomes compared to other castration methods, particularly for older bulls. There are anecdotal reports in the northern Australian industry that tension-banding is finding increasing favour in some regions. Further, although castration by rings is specifically mentioned in the current cattle welfare code of practice, with the recommendation that they should be used only on calves under 2 weeks of age, tension-banding is not mentioned. Any decisions made about the use of tension-banding should be on the basis of sound, scientific evidence that is relevant to the Australian beef cattle industry.

Few scientific examinations of the tension-bander have been conducted; none have been conducted under northern Australian conditions, few have focused on welfare outcomes by applying multiple measures (e.g. behaviour, physiology, health and productivity) and only one has used *Bos indicus* cattle. This project was, therefore, conducted to fill these gaps. It examined the welfare outcomes, assessed from measures of behaviour, certain blood and plasma parameters, wound healing and liveweight changes, of weaner (approximately 225 kg) and mature *Bos indicus* bulls (approximately 420 kg) castrated by the tension-bander (Callicrate™) with and without the administration of an analgesic (a non-steroidal anti-inflammatory drug (NSAID), namely, ketoprofen), and those castrated surgically with and without analgesia.

The weaner experiment was conducted during the dry season (July – October 2010) and the mature bull experiment in the wet season (November 2010 – January 2011). The mature bulls were accustomed to handling and restraint having been previously used in a study involving breeding soundness examinations and electro-ejaculation. The weaners had minimal experience of handling and restraint. For each cohort, 32 animals were used in a 2 x 2 factorial designed experiment with the treatment combinations being: tension-band castration with ketoprofen administered intramuscularly immediately prior to castration (Band+NSAID); tension-band castration with saline administered intramuscularly immediately prior to castration (Band+saline); surgical castration with ketoprofen administered intramuscularly immediately prior to castration (Surgical+NSAID); and surgical castration with saline administered intramuscularly immediately prior to castration (Surgical+saline). Blood samples for measurements of packed cell volume, plasma total protein, cortisol, creatine kinase and haptoglobin (all of which are indicators of pain and stress) were taken immediately before castration and at 30 min (weaners) or 40 min (mature) and 2, 7, 24, 48 and 72 hrs, and 1, 2, 3 and 4 weeks post-castration. Behavioural recordings were made on the day of castration and days 1, 2 and 3 and weeks 1 to 4 post-castration. Scrotal circumferences were measured and wounds checked, photographed and scored for healing at 1 to 6 weeks and 2 months post-castration and liveweights recorded at weeks 1 to 6, and 2 and 3 months post-castration.

Banding caused less pain and discomfort than surgical castration during the procedures, as evidenced by a significant difference in the amount the bulls moved in the crush. Changes in behaviour in the period to 1.5 hrs post-castration, however, indicated that the banded bulls of both cohorts experienced greater pain and stress than the surgically castrated bulls. Ketoprofen alleviated the pain, although it took time to take effect, with significant reductions in ‘abnormal’ behaviours (e.g. kicking, tail flicking, standing head down, walking backwards) and increases in ‘normal’ behaviours (e.g. feeding, ruminating, self-grooming) seen in the 1.5 to 3 hr period post-castration. There were rarely behavioural differences between the treatments after the day of castration.

Changes in plasma concentrations of total protein and packed cell volume indicated that the surgically castrated cattle lost more blood than the banded cattle, as would be expected. Creatine kinase concentrations rose for all treatments during the day of castration which was also as expected, due to the repeated handling, restraint and blood-sampling. The bulls given ketoprofen, however, had higher concentrations than those given saline. It is possible that this was a consequence of the effective analgesia which resulted in the ketoprofen-treated cattle being more active.

Plasma cortisol concentrations were significantly reduced post-castration in the surgically castrated but not the banded, mature bulls given ketoprofen, but there was no difference between any treatments in the weaners, all of which had high concentrations. Familiarity with handling and restraint was a difference between the cohorts, with the mature bulls being more accustomed than the weaners. Thus, it is likely that, in the weaners, the cortisol response to pain was masked by the cortisol response to handling and restraint. In contrast, the cortisol response in the mature bulls was reflecting pain. It would appear that the pain from tension-banding, unlike that from surgical castration, was not alleviated by the analgesic.

The mature, banded bulls showed significantly elevated cortisol concentrations at 2 to 4 weeks post-castration compared to the surgically castrated cattle. Haptoglobin concentrations were also significantly elevated in the banded cattle (both cohorts) during this period. Thus, the mature bulls were likely to have been experiencing chronic, inflammatory pain which was not apparent in the weaner cattle, perhaps due to their wound sizes being smaller. Wounds were significantly slower to heal in the banded than surgically castrated animals, although all were healed by 2 months post-castration.

Treatments generally did not affect liveweight changes, although the ketoprofen-treated mature bulls had lower average liveweights over 3 months compared to those given saline.

These results clearly demonstrate that tension-banding castration, using the Callicrate Bander™, did not provide superior welfare, health and productivity outcomes compared to surgical castration, in either weaner or mature bulls, as has been claimed by the manufacturer and retailers. Although tension-banding caused negligible pain and discomfort during application, it induced high levels of pain immediately post-castration, a potentially painful inflammatory response for, at least, 1 month post-castration, slow wound healing and no liveweight gain benefits compared to surgical castration. These findings are probably not unique to the Callicrate™ device, but are likely to apply to all tension-banding technologies. Thus, our overall conclusion is that tension-banding is an inferior option to surgery for the castration of cattle that are representative of the ages and genotype in the northern Australian beef cattle industry.

Administration of an analgesic provided some alleviation of pain, but for optimal effectiveness it would need to be given 20 to 30 min prior to castration, if administered intramuscularly. This requirement is likely to be difficult to accommodate with current cattle handling procedures, as it would necessitate either double-handling of the animals or a lengthy holding period in the race. Further, ketoprofen was associated with increased blood loss in the surgical castrates, which may be an additional risk for cattle with low haematocrit levels. Ketoprofen administration also had some unexpected consequences; average liveweights of the mature cattle were lower in the ketoprofen treated than saline-treated animals. This is paradoxical given that ketoprofen alleviated pain and has a short period of effect (12-24 hrs). Additionally, lateral lying was seen significantly more in the banded cattle given ketoprofen than other treatments and, at this time, we are unable to explain this finding.

Contents

The photographic appendices have been removed largely for reasons of size.

1 Background

Castration of male beef cattle is one of the most common husbandry procedures conducted in northern Australia. It is conducted on all males that will not be used for breeding because it is perceived that bulls are difficult to control and manage, although the difficulties probably rise with increasing bull age[1]. The most common technique is surgical castration in the USA (57% of producers[2]) and, although data are not available, this is probably true for northern Australia. Anecdotal reports suggest that castration using a tension-bander has gained favour in some parts of northern Australia due to its perceived ease of application and superior outcomes in terms of mortalities, particularly in older bulls. In the USA, 22% of survey respondents reported using banders (although there was no distinction between those devices with and without tension gauges), which was the second-lowest reported method (the lowest being burdizzo, at 21%)[3].

Castration using bands and rings are not to be confused; 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/).

According to the Model Code of Practice for the Welfare of Cattle (The Cattle Code)[4], rings should only be used on calves up to 2 weeks of age, although this Code is currently under review. Tension-banding *per se* is not mentioned in the current Code, but is perceived to be superior to rings for cattle of more than a few days or weeks of age, because it is reported to be better at preventing blood flow to the scrotum, leading to relatively rapid necrosis and dehiscence of the scrotum. 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[5]. There are, however, devices available in Australia that operate with thicker, stronger rings, compared to standard rings. 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".

[1] Kilgour & Campin (1973) NZ Proc Anim Prod 33, 125-138
[2] Coetzee et al. (2010) BMC Vet Res 6, 12-30
[3] ibid.
[4] Primary Industries Standing Committee (2004) Cattle Welfare Code 2nd Edn.
[5] Molony et al (1995) Appl Anim Behav Sci 46, 33-48

There seem to be three tension-bander devices that are available in Australia, but information about them is limited and appears to be available only from the manufacturer or retailer websites. The EZE Model T1 Castrator (http://www.castrator.com/shop/index.html, Out West Mfg., St. Ignatius, MT, USA) uses a ring of latex, a separate aluminium clip and involves a squeeze mechanism to tighten the ring. It appears to take some time and skill to load the device in preparation for use (http://www.castrator.com/eze_castrator_instructions.htm). The California Bander is a small, simple device, but it relies on the strength of the operator to tighten the latex band and, thus, may be less effective at occluding blood flow compared to the devices that use mechanised band tightening. The third device is the Callicrate Bander (No-Bull Enterprises Inc., St. Francis, KS, USA) which uses a ratchet mechanism to tension a loop of latex.

Since its introduction to Australia some 20 years ago, the Callicrate Bander has been a controversial method of castrating beef cattle (J. Griffin, pers. comm.) and tension-banding is not recommended by Meat and Livestock Australia (MLA) and the Cattle Council of Australia (CCA) because failure to completely occlude blood flow is reported to result in extreme pain and a potentially fatal swelling of the scrotum [6]. The basis for this assertion is, however, unclear. Further, the welfare consequences of tension-banding had not, until this current work, been evaluated under Australian conditions, although several welfare-related studies have been conducted in Ireland[7 8 9 10 11], New Zealand[12 13 14] and Canada[15], and productivity-related studies have been conducted in Canada[16] and the USA[17 18]. The USA studies also examined the effects of pain management for castration and one study[19] collected some limited behaviour-related data post-castration. The aim of the current project was, therefore, to evaluate the welfare of beef cattle castrated by tension-banding (using the Callicrate Bander) and to compare the welfare outcomes with those from surgical castration. In addition, because previous studies on tension-banding have been conducted with *Bos taurus* cattle (with one exception[20]), we used *Bos indicus* bulls, as these are the common genotype in northern Australia.

Tension-banding castration is also promoted for "delayed" castration to exploit the superior liveweight gains achieved by intact males due to higher testosterone levels compared to castrated males (e.g. see http://www.shoof.com.au/auscatalogue/page_20.pdf; http://www.probeef.com.au). Furthermore, there are anecdotal reports that stud breeders in northern Australia are increasingly delaying castration of bulls until they are mature, in order to better assess their reproductive potential. The Model T1 Castrator is promoted for use to castrate cattle "from 350 lbs on up to 1000 lbs +" (approximately 160 to 450+ kg) and, on USA websites, the Callicrate Bander is promoted for use on cattle ranging from 300 to 3000 lb (approximately 140 to 1400 kg), but in Australia there is a recommendation for use on calves up to 6 months of age (http://www.probeef.com.au) in line with the upper age limit for surgical castration without analgesia in the current Cattle Code. There appears to be no objective data on the impacts on welfare that tension-banding at different ages may produce.

[6] Newman (2007) Best practice guide for branding, branding and dehorning.
[7] Pang et al. (2006) J Anim Sci 84, 351-359
[8] Pang et al. (2008) Livest Sci 117, 79-87
[9] Pang et al. (2009a) BMC Vet Res 5, 36-42
[10] Pang et al. (2009b) J Anim Sci 87, 3187-3195
[11] Pang et al. (2011) Res Vet Sci 90, 127-132
[12] Knight et al. (2000) NZ J Agric Res 43, 187-192
[13] Fisher et al. (2001) Aust Vet J 79, 279-284
[14] Stafford et al. (2002) Res Vet Sci 73, 61-70
[15] Gonzalez et al. (2010) J Anim Sci 88, 802-810
[16] ZoBell et al. (1993) Can J Anim Sci 73, 967-970
[17] Rust et al. (2007) Bov Prac 41(2), 111-118
[18] Booker et al. (2009) Bov Prac 43(1), 1-11
[19] Rust et al. (2007) Bov Prac 41(2), 111-118
[20] Chase et al. (1995) J Anim Sci 73, 975-980

Also, as The Cattle Code indicates an upper age limit of 6 months on castration *per se* and is currently being reviewed, it would seem important to obtain objective data on age effects to inform this revision. Thus, we conducted the comparison between castration by the Callicrate bander and by the surgical method in weaner and mature bulls.

There is ample evidence that castration is a painful procedure (e.g. see review[21]), but the perception is that tension-banding is less painful than surgical castration. Indeed, the Callicrate Bander is promoted as being a humane method of removing the testicles (see http://www.nobull.net/bander/SBHumane.htm; http://www.shoof.com.au/auscatalogue/page_20.pdf) and causing "minimal stress to the animal" (quote from material provided with the Callicrate Bander by ProBeef Australia Pty Ltd; see also http://www.nobull.net/bander/SBHumane.htm). To determine whether there were differential effects from the use of an analgesic on the welfare of bulls castrated by surgery and tension-banding, we administered a non-steroidal anti-inflammatory drug (NSAID) to half of the bulls immediately prior to castration. The NSAID we used was ketoprofen, as it has been shown to be effective in alleviating pain in surgically castrated[22] and tension-banded calves[23].

1.1 Project objectives

- To document the behavioural, physiological and production responses of beef cattle in northern Australian conditions to castration by the tension-bander technique, with and without analgesia (NSAID) and to compare these responses with those to surgical castration with and without analgesia.
- To make recommendations on the use of the tension-bander and analgesia for castration, based on welfare outcomes.

[21] Stafford & Mellor (2005) NZ Vet J 53, 271-278
[22] Earley & Crowe (2002) J Anim Sci 80, 1044-1052
[23] Stafford et al. (2002) Res Vet Sci 73, 61-70

2 Method

2.1 Research team

DEEDI/QAAFI	CSIRO	Massey University	Private
Carol Petherick	Alison Small	Kevin Stafford	Ed Butterworth
David Mayer	Drewe Ferguson		
Debra Corbet	Ian Colditz		
	Rob Young		
	Nick Corbet		
	Jim Lea		
	Paul Williams		

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 22 July 2010 and 25 January 2011. The weaner bull data collection period ended on 14 October 2010 and the mature bulls were drafted into their experimental groups on 1 November 2010. Temperatures and rainfall during the experiment are given in Table 1.

Table 1. Temperatures (°C) and rainfall during the experimental period of 22 July 2010 to 25 January 2011

	Mean max. temp.	Max. temp. range	Mean min. temp.	Min. temp. range	Rainfall (mm)	Number of wet days
Jul 22-31	25.3	23.0-30.1	14.1	5.8-17.0	2.4	1
Aug	24.7	14.9-30.1	10.7	6.0-14.5	78.2	6
Sept	26.9	21.5-31.4	13.7	10.3-17.8	86.0	10
Oct	27.6	23.0-32.3	17.0	14.4-19.8	37.0	4
Nov	27.5	22.6-30.2	19.5	17.1-21.1	159.2	11
Dec	29.9	23.6-35.2	19.4	21.2-22.9	499.2	17
Jan 1-25	31.2	27.7-34.9	22.9	20.6-25.2	67.8	6

2.3 Animals and treatments

The use of the cattle in this experiment was approved by the CSIRO (Queensland) Animal Ethics Committee (approval A7/09).

The experimental protocol was conducted using two age groups of bulls: the weaner bulls were approximately 7-10 months old and the mature bulls 22-25 months old. The weaner bulls were castrated in July 2010 and the mature bulls in November 2010 and both age groups were monitored for a 3-month period post-castration.

2.3.1 Weaner bull protocol

The cattle were purebred Brahmans that were born (on Belmont Research Station) between 29 September 2009 and 8 December 2009 (designated as number 0 calves). The calves were branded and dehorned in February 2010 and worked through the yards in March 2010 before being weaned on 19/20 April 2010. Thirty-two animals (mean liveweight ± s.e., 217.8 ±2.93 kg) were assigned to four treatment combinations (n=8 per treatment group) according to liveweight and scrotal circumference[24] as measured on 1 July 2010 (16.7±0.18 cm) and an average (1.70±0.094) of three flight speeds (recorded on the day of weaning, 19/20 April 2010; 27 April 2010; and 1 July 2010). Flight speed was measured according to a validated

[24] Entwistle & Fordyce (2003) Evaluating and reporting bull fertility.

method[25] using specially manufactured equipment (Ruddweigh-Gallagher Animal Management Systems, Campbellfield, Vic, Australia). It was considered important to take into account flight speed in the allocation of bulls to treatments, as previous work has found relationships between flight speed and stress responses and liveweight gains[26 27]. To undertake the study, animals were allocated to eight blocks, each containing one animal for each treatment, from spatial groupings in the first two dimensions from a principal components analysis (PCA) of liveweight, flight speed and scrotal size data. These two dimensions encompassed 86% of the total variation of the three variables.

The four treatment combinations of castration method and pain management were:

- Tension-banding castration and an intramuscular injection of saline (Band+saline)
- Tension-banding castration and an intramuscular injection of a non-steroidal anti-inflammatory drug (Band+NSAID)
- Surgical castration and an intramuscular injection of saline (Surgical+saline)
- Surgical castration and an intramuscular injection of a non-steroidal anti-inflammatory drug (Surgical+NSAID)

The tension-banding was conducted using the Callicrate Bander (No-Bull Enterprises, St. Francis, Kansas, USA). On the advice of the veterinarians involved in the project and because it is registered for use in cattle and has been used in previous studies on cattle, the NSAID used was 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. The saline solution (0.9% sodium chloride, Baxter Healthcare Pty. Ltd., Old Toongabbie, NSW, Australia) was injected in the same location at an equivalent volume. Also, at the recommendation of veterinarians, all cattle were given tetanus anti-toxin (Equivac TAT, Pfizer Australia Pty. Ltd., West Ryde, NSW, Australia; 1500 IU/mL) at the rate of 1,000 IU/head, although the manufacturer of the Callicrate Bander states that tetanus toxoid (i.e. vaccine, not anti-toxin) must be used. These cattle should have been protected against tetanus, as they had received their vaccination for Clostridial diseases and booster at weaning but, as valuable experimental animals, we were not prepared to take risks.

Due to daylight-hour constraints, castrations were conducted on 2 successive days (22 and 23 July 2010, day 0) with four randomly selected blocks castrated on the first day (Batch A) and the remainder (Batch B) on the second. The procedures for the four blocks were started at approximately 7.00, 7.45, 8.40 and 9.55 hrs, respectively and the procedures were the same on both days.

2.3.1.1 Procedures

All bulls were individually identifiable from ear-tags that had been inserted within 12 hrs of birth. On the day before the experiment started (21 July 2010) the bulls were weighed. On the day of castration, the bulls were moved individually into a veterinary crush and restrained by head-bailing and two blood samples (both approximately 8 mL) were taken via a single jugular venipuncture via 18 G needles into vacutainers. According to treatment, NSAID or saline and tetanus-antitoxin were injected (using 18 G needles). An IceTag3D™ motion sensor device (data logger) was fitted to the left hind leg in accordance with the manufacturer recommendations (IceRobotics, Roslin, Midlothian, Scotland). The bulls were then castrated by the pre-assigned method. All castrations were conducted by the same operator who was experienced and skilled in both techniques.

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

Plate 1 Fitting IceTag

2.3.1.1.1 Surgical

Bulls were individually restrained in the head bail of a veterinary crush, with additional manual restraint by a person holding the animal against the crush side via the tail. The operator worked at the left side of the animal and using a hand-held scalpel blade conducted the castration according to the MLA Guide[28], using a cut to the scrotum for each testicle. Some slight variations to the method were made to cater for the fact that the animals were standing and to minimise risk of injury to the operator. 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 immediately released to a clean yard, with the entire procedure (from the start to end of restraint) taking approximately 3.5 min. The removed tissue was immediately weighed and the weight recorded.

2.3.1.1.2 Tension-bander

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

[28] Newman (2007) Best practice guide for branding, branding and dehorning.

Plates 2-4 Surgical castration

Plates 5-10 Tension-banding

2.3.1.1.3 Post-castration management

At the end of castration the animal was released and when all four animals in the block had been castrated they were moved into small yards (approximately 50-70 m^2) with shade, and hay and water available ad libitum, where they remained until it was time for their next blood sample to be taken. For the 30-min sample, 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 the same small yard. Thus, each block of four was maintained as a group in a separate yard on the day of castration.

At the end of day 0, the blocks were combined and the cattle walked to a small holding paddock (approximately 3400 m^2) adjacent to the yard complex, with pasture and water available ad libitum. The following day they were walked to the yard complex for their day 1 blood sample and then returned to the holding paddock. This process was repeated for days 2 and 3 for both batches of cattle, with the second batch of cattle being held in a 3 ha holding paddock, with access to a yard (85 m x 40 m) containing hay and water ad libitum. After the day 3 blood sample for the second batch of cattle, the batches were combined into a 6 ha paddock where the dominant grasses were Rhodes Grass and Bambatsi panic, between stands of Leucaena. The cattle were held here for 4 weeks; pasture yield at entry was estimated to be 2000 kg/ha and at exit was 1000 kg/ha. At entry it was estimated that the Leucaena had 25% leaf present and it was stripped at exit. For the following 4 weeks, the cattle were held in a 23 ha paddock of predominantly Rhodes Grass, with stands of Leucaena. At entry the pasture yield was estimated to be 1500 kg/ha with 25% leaf present on the Leucaena. At exit the Leucaena had been stripped and the pasture yield reduced to approximately 1000 kg/ha. For the remainder of the trial, the cattle were rotated through small paddocks (approximately 7 ha) at about 2-week intervals. The dominant pasture species was Rhodes Grass with some Siratro (approximately 10% of volume). At entry, the pasture yield was estimated to be 2000 kg/ha and the cattle were moved when this had reduced to 1000 kg/ha.

2.3.1.2 Blood samples

Blood samples were taken on restraint (time 0) and at 30 min, 2 hrs and 7 hrs post-castration. Samples were collected into EDTA and sodium heparin vacutainers (Becton Dickinson, North Ryde, NSW, Australia) and kept refrigerated until processed. The whole blood samples (those collected into the EDTA tubes) were transported chilled, to Brisbane where they were measured 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 (Clements Medical Equipment, North Sydney, NSW, Australia) for 20 min and the average PCV concentrations calculated from the duplicate percentages read off the haematocrit scale. The total protein concentrations were calculated from the mean of the duplicate micro-haematocrit tubes which was read from a refractometer (Bellingham and Stanley Ltd., Tunbridge Wells, Kent, UK). The creatine kinase (CK) concentrations were analysed using an automated biochemical analyser (Olympus Reply Biochemistry Analyser, Sydney, NSW, Australia).

The sodium heparin vacutainers were centrifuged on the day of collection at 2500 rpm for 20 min and 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 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. Briefly, human serum standards were used as provided or diluted in phosphate-buffered saline (PBS) and 20 µL of standard, control or unknown sample pipetted into anti-cortisol antibody-coated tubes. Five hundred µL of the provided ^{125}I-labelled cortisol tracer (diluted 1 in 2 in PBS) was then added before incubation for 2 hrs at 37^{o}C. Tubes were decanted, washed once with 1 mL of distilled water and counted for 1 min in a gamma counter.

Parallelism between the standard and unknown samples was demonstrated by serial dilutions of two ovine plasma samples: the calculated slopes of the binding vs. log cortisol concentration or dilution curves for the cortisol standards and the two samples were –0.172, -0.165 and –0.131, respectively.

The mean recovery of added cortisol to ovine plasma was 102% and the sensitivity of the assay was 10 nmol/L. The stated cross-reactivities of the anti-cortisol antibody with corticosterone, cortisone, dexamethasone, prednisolone and prednisone were 0.2, <0.1, <0.1, 45.3 and 0.3 %, respectively. The intra-assay coefficient of variation (CV) for samples containing (mean ± s.d.) 34.7 ± 2.0, 78.3 ± 4.8 and 157 ± 7.9 nmol/L cortisol respectively, were 6.5, 6.8, and 4.3%. The inter-assay CVs for the same samples were 5.7, 6.1 and 5.0% respectively.

Blood samples were also taken on days 1, 2, 3, 7, 14, 21 and 28 post-castration. On these occasions a single sample was collected (into a sodium heparin vacutainer) and samples were handled and stored as described above, for plasma haptoglobin and cortisol assays. Although the two batches of cattle were mixed after day 3 they were blood-sampled on successive days for the day 7 sample. Thereafter, the cattle were treated as a single group and, thus, samples taken on days 14, 21 and 28 were technically days 13, 20 and 27 for the batch B cattle, but for simplicity, these dates will be considered to be 2, 3 and 4 weeks post-castration for all animals.

2.3.1.3 Behaviour recording

Behaviour was recorded during castration by direct observation by a single observer, with counts made of each of the following behaviours:

- Push at the headbail
- Pull back from the headbail
- Jump (all hooves off the ground)
- Jerk (sudden, small jump with overall body tension)
- Struggle (moving back and forth in the headbail with legs flailing)
- Kick (with one or both hind legs to the rear)
- Stamp (raising and lowering any leg with a swift action)

Due to the small numbers of these individual behaviours, the counts were combined into a total movement score. In addition, the number of vocalisations was scored and a note made of whether the bulls kneeled or lay down in the crush during castration.

On day 0 post-castration, blocks of animals were directly observed by 5-min focal animal sampling by a single observer. The order in which animals in a block were observed was as they were individually identified by the observer. There was no fixed schedule of observations for each block; rather blocks were observed opportunistically to fit with the blood sampling schedule and the movement of cattle through the yard system. Each block was, however, observed on four to six occasions from immediately post-castration to immediately after the final blood sample at 7 hrs post-castration. Inspections of graphs 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, 2, and 3 post-castration when the cattle were in the two batches. The behaviours observed are given in Table 1 with those behaviours having a duration of 5 sec or more 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' and some were not analysed, as indicated in Table 1.

With one exception (when recording on day 0 and day 1 clashed), the observations were conducted by a single observer. With the exception of the final observation, which was made by another person, this same observer conducted 5-min focal animal sampling (on the single group of 32 head) on days 6, 13, 19 (scheduled for day 20, but heavy rain was predicted and, so the decision was made to conduct the observations a day early) and 27 post-castration (which were days 5, 12, 18 and 26 post-castration for batch B cattle). The order in which the animals were recorded was on the basis of locating individuals. Observations were started between 6.15 and 8.15 hrs and took 3-4 hrs depending on the ease of finding animals. On days 1-3 post-castration, observations were fitted-in with blood sampling, so some were done after sampling (but before 12.30 hrs). Also, on these days some observations were made with cattle in yards (85 m x 40 m) with hay and water available ad libitum, and others in small (approximately 3 ha) holding paddocks.

The percentage of time spent active, standing and lying was automatically determined from the IceTag3D™ data, with the loggers removed at week 4 post-castration.

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	
Stand shaking	Standing with muscle and body tremors	
Lie alert	Lying with muscles tense, head held high, ears pricked, apparently looking at something	Lie
Lie relaxed	Lying on sternum with muscles relaxed, head held relaxed, ears loose, apparently not focusing visually	
Stand ruminating	Standing with slow chewing movements and regurgitations	Ruminate
Lie ruminating	Lying on sternum with slow chewing movements and regurgitations	
Lateral lying	Lying recumbent on side	'Abnormal' lying

Lie neck extended	Lying on the sternum with head and neck extended on the ground	
Walk forward	Forward locomotion (mainly walk, but occasionally trot or gallop)	
Walk backwards	Backwards locomotion (walk)	
Feed	Ingestion (eating hay, grazing, browsing)	
Drink	Ingesting water	
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	
Groom other*	Licking, mouthing, nuzzling another animal or attempting to do so	
Mouth object*	Licking, mouthing, nuzzling an inanimate object	
Tail tuck	Standing or lying with tail held taught between the hind legs	
Urinate	Passing urine	
Events (counts)		
Tail flick	Sideways movement of the tail from vertical and return to vertical	Tail movement
Tail tuck	Standing or lying, tail pulled tight between the hind legs and released	
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
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	
Groom other*	Licking, mouthing, nuzzling another animal or attempting to do so	
Sniff other*	Standing or lying, animal places muzzle close to another animal (usually prepuce or scrotum) and inhales, sometimes followed by flehmen	
Mouth object*	Licking, mouthing, nuzzling an inanimate object	
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	

*These behaviours were recorded but are difficult to interpret in relation to pain/discomfort and were, therefore, not analysed

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

2.3.1.4 Liveweights and wound healing

Liveweights were recorded on days 7, 14, 21, 28 (1 month), 34 (5 weeks), 42 (6 weeks) 56 (2 months) and 84 (3 months) days post-castration (again, these were a day less on each occasion for the batch B cattle).

Castration sites were checked at these same times to 2 months post-castration to determine the extent of healing. On these occasions, for each animal, photographs of the scrotal area were taken, scrotal circumferences measured (as a measure of oedema and shrivelling), and a verbal description of the wounds (and presence/absence of the scrotum for those animals tension-banded) recorded. For the tension-banded animals only the area above the band was considered, as any infection above the band would likely have an adverse effect on welfare. In contrast, below the band 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 pus present
4. Wound fully open, moist and no pus present
5. Wound fully open, moist and pus present

As two cuts were made in the surgically castrated animals, the animal was 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 3, or a score of 5 if one wound was fully open with pus present.

2.3.2 Mature bull protocol

Unless stated, the protocol was identical to that used for the weaner bulls.

The cattle were purebred Brahmans that were born (on Belmont Research Station) between 17 September 2008 and 29 December 2008 (designated as number 9 calves). As calves these animals were branded and dehorned in February 2009 and worked through the yards in March 2009 before being weaned in May 2009. They were then used in an experiment investigating indicators of fertility and had been subjected to three Bull Breeding Soundness Evaluations (BBSE)[29] on three occasions, at approximately 12 (17 November 2009), 18 (6 April 2010) and 24 months (5 October 2010) of age. As part of the BBSE the animals had also been electro-ejaculated for semen collection.

Thirty-two animals (401.6 ±5.80 kg) were assigned to four treatments (n=8 per treatment) according to liveweight (measured on 21 October 2010) and scrotal circumference[30], as measured 5 October 2010 (29.9±0.32 cm), and an average (2.05±0.080 m/s) of five flight speed measurements: two around weaning (13 and 18 May 2009), and then at approximately12 (17 November 2009), 18 (6 April 2010) and 24 months (5 October 2010) of age. Blocks (eight, each containing one animal for each treatment) were formed from spatial groupings in the first two dimensions from a principal components analysis (PCA) of liveweight, flight speed and scrotal size data. The two dimensions encompassed 72% of the total variation of the three variables.

As these were mature cattle, tetanus anti-toxin was given at the rate of 1,500 IU/head. Again, these cattle should have been protected against tetanus having received annual boosters against Clostridial diseases post-weaning, but we were not prepared to take risks with them.

The bulls were weighed on the day before the experiment (1 November 2010) and castrations were conducted on 2 successive days (2 and 3 November 2010, day 0) with four randomly selected blocks castrated on the first day (Batch C) and the remainder (Batch D) on the second. The procedures for the four blocks were started at approximately 6.30, 7.30, 9.50 and 10.50 hrs, respectively and the procedures were the same on both days.

2.3.2.1 Procedures

2.3.2.1.1 Surgical

Bulls were individually restrained in the head bail of a veterinary crush, with additional manual restraint by a person holding the animal against the crush side via the tail. The

[29] Entwistle & Fordyce (2003) Evaluating and reporting bull fertility.
[30] Ibid.

operator worked at the left side of the animal and using a hand-held scalpel blade conducted the castration according to the MLA Guide[31], using a cut to the scrotum for each testicle. Some slight variations to the method were made to cater for the fact that the animals were standing and to minimise risk of injury to the operator. 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. Using a Hausmann emasculator (Aesculap, Germany) instrument, the cord was cut as close to the animal's body as possible and proximal to the testicle, away from where a high density blood vessels are clearly obvious. The clamped cord was held in the emasculator for about 30 sec to close off the blood vessels and minimise bleeding. Once both testicles had been removed, the animal was immediately released to a clean yard, with the entire procedure (from the start to end of restraint) taking approximately 3.5 min. The weight of the removed tissue was immediately recorded.

Plate 11 Application of emasculators during surgical castration of mature bull

2.3.2.1.2 Tension-bander

The procedure was as for the weaner bulls, but in some instances it was necessary to draw the testicles through the band one at a time. The entire procedure (from the start to end of restraint) took approximately 3.5 min.

At the end of day 0, the blocks were combined and the cattle walked to a small holding paddock (3400 m^2) adjacent to the yard complex, with pasture and water available ad libitum. The following day they were walked to the yard complex for their day 1 blood sample and then returned to the holding paddock. This process was repeated for days 2 and 3 for both batches of cattle (as for the weaner bulls) and then the batches were combined into a 21 ha paddock of predominantly Rhodes Grass, with Siratro present at about 30% by volume. The pasture yield was estimated to be 3000 kg/ha at entry. At week 10, the cattle had to be moved due to flooding, at which time the paddock yield was estimated to be 1000 kg/ha. For the remainder of the trial (2 weeks), the cattle were held in a 7 ha paddock of Rhodes Grass with 10% Siratro by volume, estimated to have a yield of 2500 kg/ha at entry and 1500 kg/ha at exit.

[31] Newman (2007) Best practice guide for branding, branding and dehorning.

2.3.2.2 Blood samples

Blood samples were taken on restraint and at 40 min, 2 hrs and 7 hrs post-castration. A longer time, compared to the weaner bulls, was allowed between the first and second samples, as we were uncertain as to whether we would have difficulties getting the bulls into the crush and restraining them (the head in particular) for blood-sampling. PCV assays were conducted on site immediately post-collection, in the same way as described for the weaner bulls, but using a different micro-haematocrit centrifuge and scale (Hawksley, Sussex, UK).

2.3.2.3 Behaviour recording

On day 0 post-castration, blocks of animals were directly observed by 5-min focal animal sampling by a single observer. The order in which animals in a block were observed was as they were individually identified by the observer. 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.
Observations were made on the group of 32 head on days 6, 13, 20 and 27 post-castration.

We were unable to obtain sufficient IceTag loggers for all animals, so only 30 bulls were fitted (one Band+NSAID and one Surgical+NSAID did not have an IceTag).

2.3.2.4 Liveweights and wound healing

Liveweights and wound assessments were recorded on the same days as for the weaner bulls, except that the 2-month recording was made on day 57.

2.3.3 Statistical methods for weaner and mature bulls

Generalised linear models[32] were used to analyse the data, in GenStat[33]. The Normal distribution was assumed for blood parameters, wound scores, scrotal circumferences and liveweights. Motion Index data (from the IceTags) data were skewed, so were ln(x+0.001) transformed prior to analysis. Behavioural states (being the proportions of total time observed in each defined category), and the proportions of cattle kneeling and lying during castration, all used the Binomial distribution with the logit link function. For a small number of proportion data sets the Binomial model would not fit, so the Normal approximation was used. Counts of observed events were discrete in nature and moderately skewed, so the Poisson distribution with a log link function was adopted. For the blood parameters and liveweights over days, the time-series nature was taken into account by an analysis of variance of repeated measures[34], via the AREPMEASURES procedure of GenStat. This forms an approximate split-plot analysis of variance (split for time). The Greenhouse-Geisser epsilon estimates the degree of temporal autocorrelation, and adjusts the probability levels for this.

For Cortisol, Total protein, PCV and CK analyses, the concentration in the sample taken at time 0 was used as a covariate. For liveweights, initial liveweight was used as a covariate.

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

[32] McCullagh & Nelder (1989) Generalized linear models.
[33] GenStat (2009) GenStat for Windows, release 11.1.
[34] Rowell & Walters (1976) J. Agric. Sci 87, 423-432

3 Results

Initial analyses were conducted on the factorial treatment structure (NSAID administration by castration method) over all times. The interaction between castration method and time was notably pronounced for most variables, being significant ($P<0.05$) in 62% (16 out of 26) of the individual tests (the random expectation for significance would be 5%), and these time-patterns form the main focus in the results. These analyses were, however, less conclusive for the effect of NSAID. Research findings[35] and manufacturer recommendations on the frequency of administration of ketoprofen indicate that the analgesic effect would likely to be present only during the first 12 to 24 hrs post-administration. Thus, re-analyses were conducted using data up to 24 hrs only (for the parameters that were measured during this period). These analyses showed that NSAID did have an effect during day 0, across all parameters, with the NSAID terms (the main effect and its interactions with time and castration method treatment) being significant ($P<0.05$) in 19% (28 out of 150) of the individual tests, considerably more than would be expected from random chance (which would be 7.5 from 150) alone. Importantly, these analyses also showed that the NSAID effect had effectively dissipated at the 24-hr measurements, as the number of detected significant differences at this point only approximately reflected random variation.

Hence, the result of the NSAID effect is graphically presented separately to 24 hrs; thereafter the two levels (NSAID and saline) are pooled as replicates for the castration method treatment, to better determine these patterns over times.

3.1 Weaner bulls

3.1.1 Behaviour at castration

Both castration method ($F_{1,21} = 16.54$; $P<0.001$) and NSAID administration ($F_{1,21} = 8.34$; $P = 0.009$) affected the total amount of movement during castration; the Surgical group moved more then the Band group (mean± s.e., 5.69 ± 0.63 vs. 2.63 ± 0.43, respectively) and the NSAID group moved more than the Saline group (5.25 ± 0.61 vs. 3.06 ± 0.46, respectively). The findings suggest that the Surgical and NSAID bulls experienced more discomfort during the castration procedure than the Band and Saline bulls.

There was no difference between treatments in the numbers of bulls that knelt ($P = 0.50$ for castration method and 0.49 for NSAID administration) or lay down during castration ($P = 0.58$ for castration method and 0.57 for NSAID administration). The mean proportion (± s.e.) of bulls kneeling and lying were 0.094 ± 0.0442 and 0.156 ± 0.0547, respectively.

3.1.2 Behaviour post-castration

3.1.2.1 Direct observations

There were too few recordings of the following states for meaningful analysis: time spent standing shaking, standing head down, tail tucking, drinking and urinating. Urinating was recorded only in the Band bulls. Time spent standing, lying and ruminating, and numbers of agonistic behaviours and defaecations were influenced only by time and are, therefore, not presented or discussed.

3.1.2.1.1 Effect of NSAID administration.

There was a significant NSAID administration x time interaction on the number of tail movements made ($P = 0.019$; Fig. 1). At all times on day 0, the amount of tail movement

[35] Landoni et al. (1995) Vet. Rec. 137, 428-431

was reduced in the cattle given the NSAID compared to those castrated by the same method and given saline, suggesting that the NSAID was effective, given the time delay to reach target tissues. In the period to 1.5 hrs post-castration, numbers of tail movements were greatest in the Band cattle regardless of NSAID administration, indicating discomfort in the Band animals that was not immediately alleviated by the NSAID. During 1.5 to 3 hrs post-castration, the level of tail movements reduced in the Band cattle. At weeks 2 and 3 tail movements increased in the Band cattle, with a significant difference to the Surgical cattle at week 3.

Fig. 1 Mean number of tail movements performed by weaner cattle

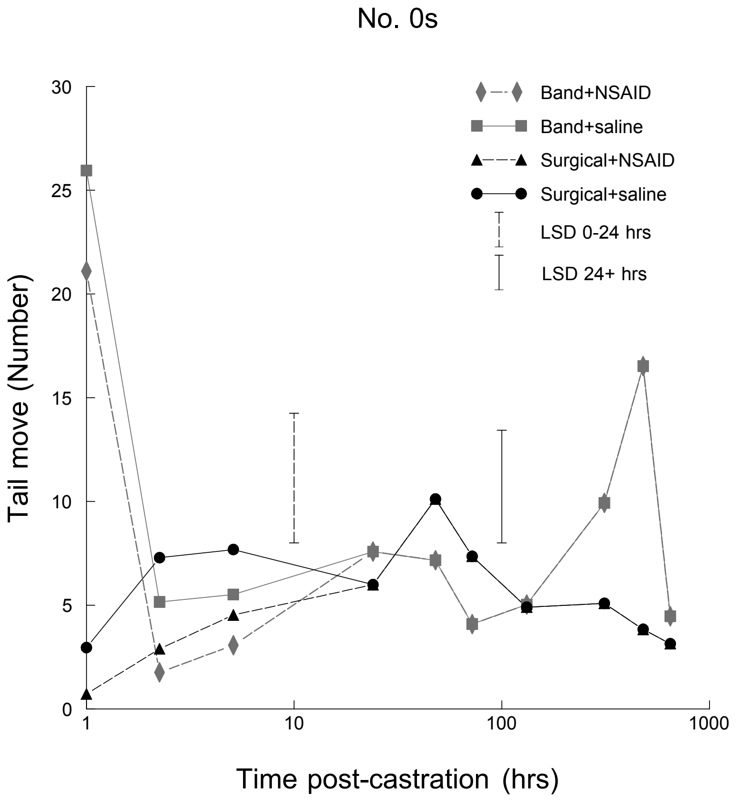
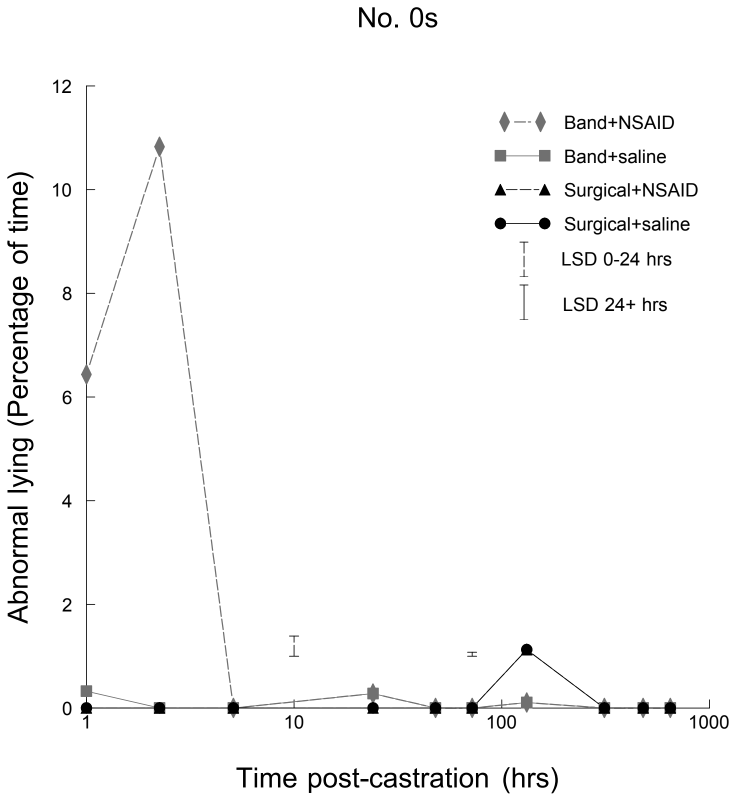


Plate 11 Lateral lying in banded and standing in surgically castrated weaners

There was a significant NSAID administration x castration method interaction on percentage of time spent in ‘abnormal’ lying ($P < 0.001$; Fig. 2) and feeding ($P = 0.006$; Fig. 3).

Abnormal lying was seen only in Band cattle on the day of treatment and mainly in the Band+NSAID group.

Fig. 2 Mean percentage of time spent in 'abnormal' lying by weaner cattle



On day 0, the amount of time spent feeding was greater in the NSAID treated cattle, again perhaps, indicating the effectiveness of the analgesic, with the Surgical showing more feeding time than the Band cattle. At the end of day 0 there was, however, a sharp increase in the time spent feeding by the Band+saline cattle, whilst feeding by the Surgical+saline cattle remained low, suggesting that the latter animals were still experiencing pain/discomfort at this time.

Plate 12 Stretching (comfort behaviour) by weaner post-castration

Fig.3 Mean percentage of time spent feeding by weaner cattle

No. 0s

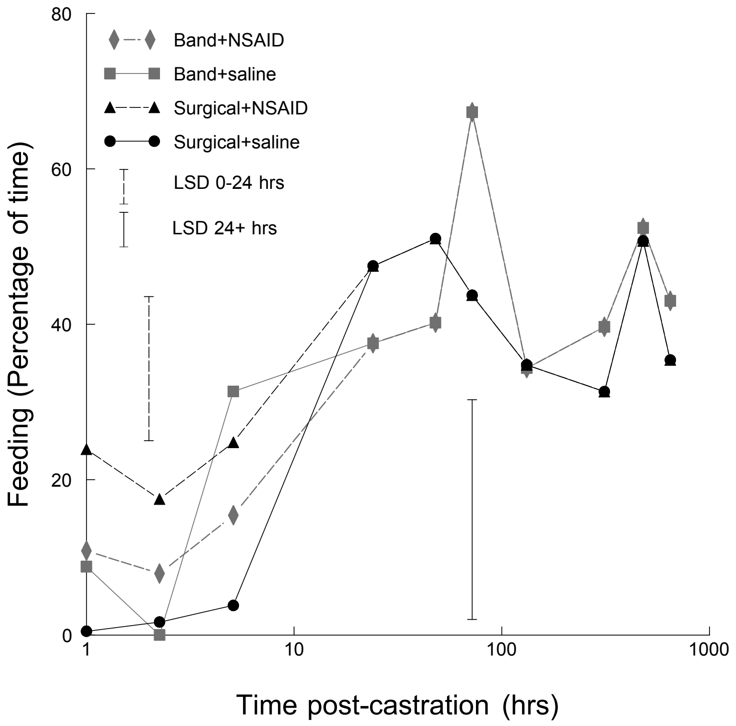
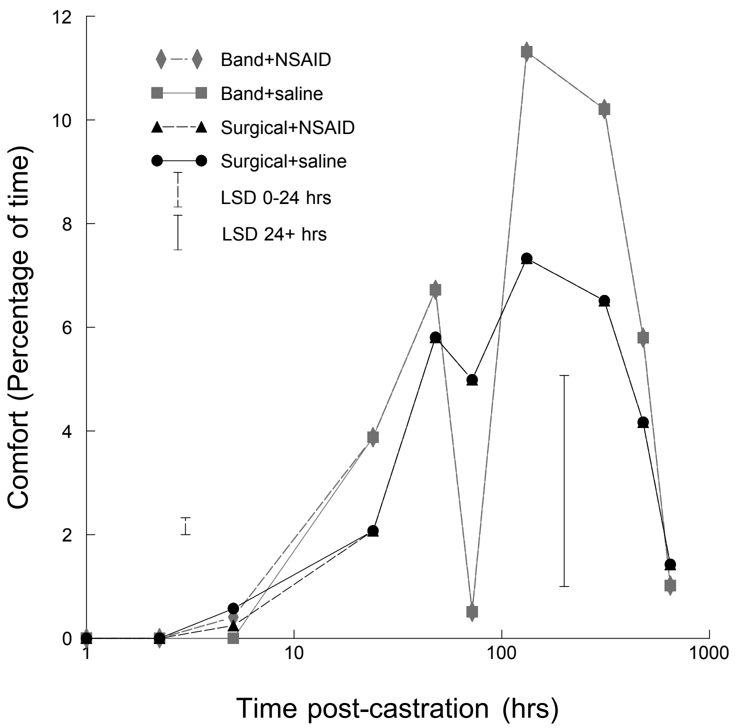


Fig. 4 Mean percentage of time spent in comfort behaviours

No. 0s

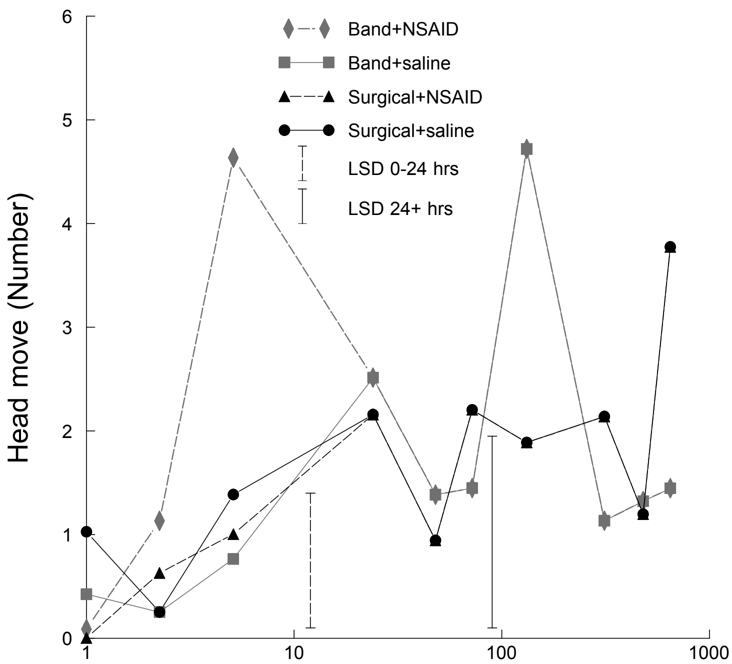


There was a significant NSAID administration x castration method x time interaction on the proportion of time spent in comfort behaviours (P<0.001; Fig. 4) and numbers of head movements made (P = 0.002; Fig. 5). The amount of time spent in comfort behaviours was negligible initially, but increased over time and was higher in the Band than Surgical animals at weeks 1 and 2 post-castration, perhaps indicating greater discomfort in the Band animals (although it is not clear why there was a marked drop at day 3). On day 0 at 3+ hrs, the Band+saline animals showed no comfort-related behaviours, yet the Surgical+saline showed the greatest amount, which is difficult to explain. Given that comfort behaviours were at very low levels initially and increased over time, it is probable that they are not shown in direct response to the pain and discomfort from castration but, like feeding behaviour, are suppressed when the animals are in pain.

Head movements showed a similar trend to comfort behaviours, generally increasing during the course of the trial. So, it is likely that these are also behavioural patterns that are suppressed by pain and discomfort. There is support for this hypothesis, with the initial decline in levels in the saline-treated animals, but a rise in the NSAID-treated ones, suggesting a beneficial effect of the analgesic.

Fig. 5 Mean number of head movements performed by weaner cattle

No. 0s



Time post-castration (hrs)

3.1.2.1.2 Effect of castration method

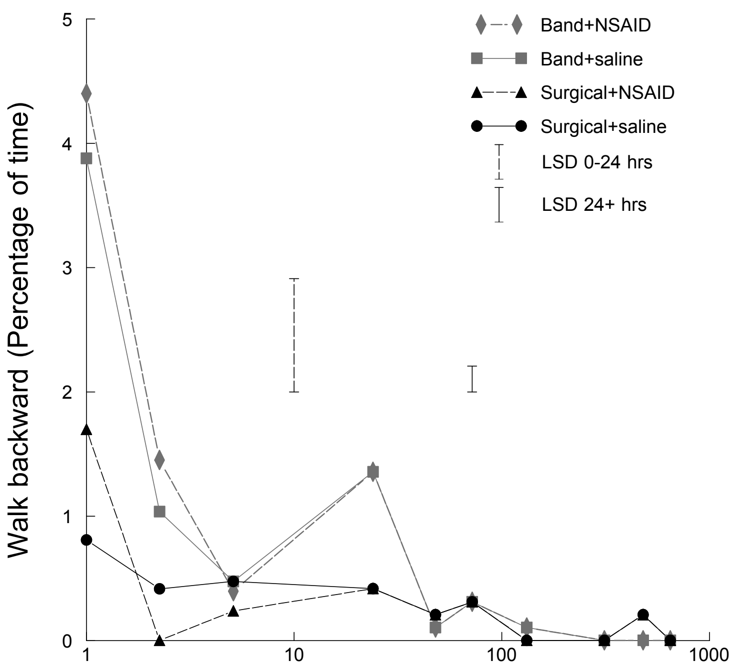
The interaction between castration method and time was significant ($F_{9,345}$ = 2.07; P = 0.032), with walking backwards initially being much greater in the Band than Surgical cattle post-castration, but then declining to levels similar to the Surgical cattle at 3+ hrs post-castration (Fig.6). This pattern indicates that the Band cattle experienced greater pain than the Surgical animals, with the NSAID having no effect on this behaviour. Levels were also greater in the Band cattle compared to the Surgical ones at 24 hrs post-castration, which suggest they were still in some pain/discomfort at this time.

Similar significant castration method x time interactions were also apparent for the numbers of tail movements (P < 0.001; Fig. 1), leg movements (P < 0.001; Fig. 7) and comfort behaviours (P = 0.003; Fig. 8). Leg and tail movements were greater in the Band than Surgical cattle immediately post-castration, but were at similar levels during the 1.5 to 3 hrs post-castration observation period and subsequently. As tail and leg movements are performed in response to pain, these results indicate that the Band cattle experienced more pain than the Surgical cattle post-castration. Unlike the amount of time spent in comfort behaviours, the number of comfort behaviours performed showed no obvious pattern over time and the NSAID did not have a consistent effect (reduction) on them. Thus, it is difficult to say whether an initial high level of these in the Band cattle is indicative of more or less pain than the Surgical animals.

The amount of time spent walking forward showed a similar pattern to the previous behaviours (Fig. 9). Initially there was a significant difference (P<0.05) between the Band and Surgical cattle, with similar levels reached at 1.5 to 3 hrs post-castration. Walking forwards can be difficult to interpret, but as the NSAID reduced the behaviour, it is likely that it was performed in response to pain. Therefore, these results support the other behavioural data indicating greater pain and discomfort in the Band compared to the Surgical cattle initially.

Fig. 6 Mean percentage of time spent walking backwards by weaner cattle

No. 0s



Time post-castration (hrs)

Plate 13 Kicking (leg movement) by weaner bull

Fig. 7 Mean number of leg movements by weaner cattle

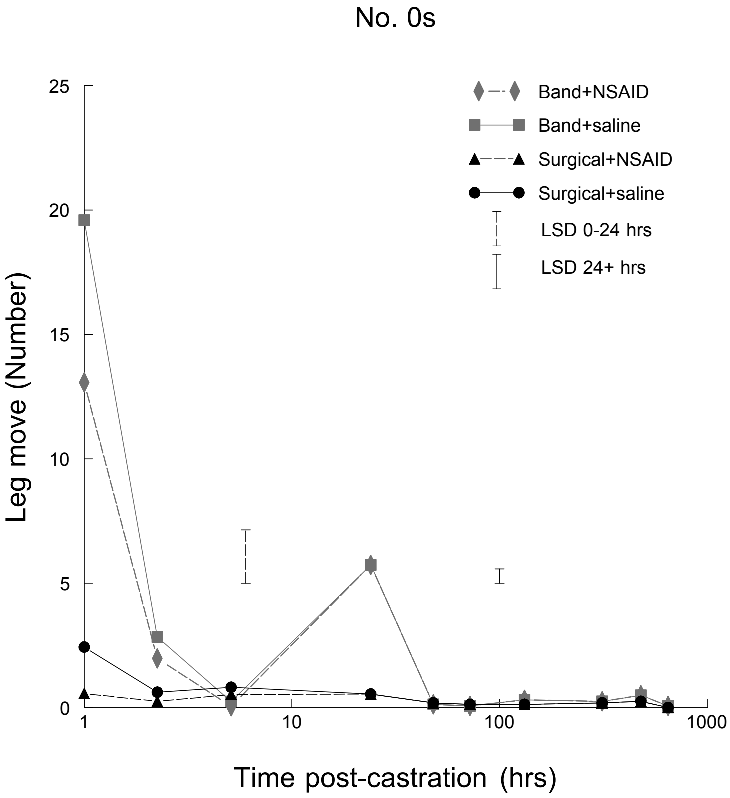


Fig. 8 Mean number of comfort behaviours performed by weaner cattle

No. 0s

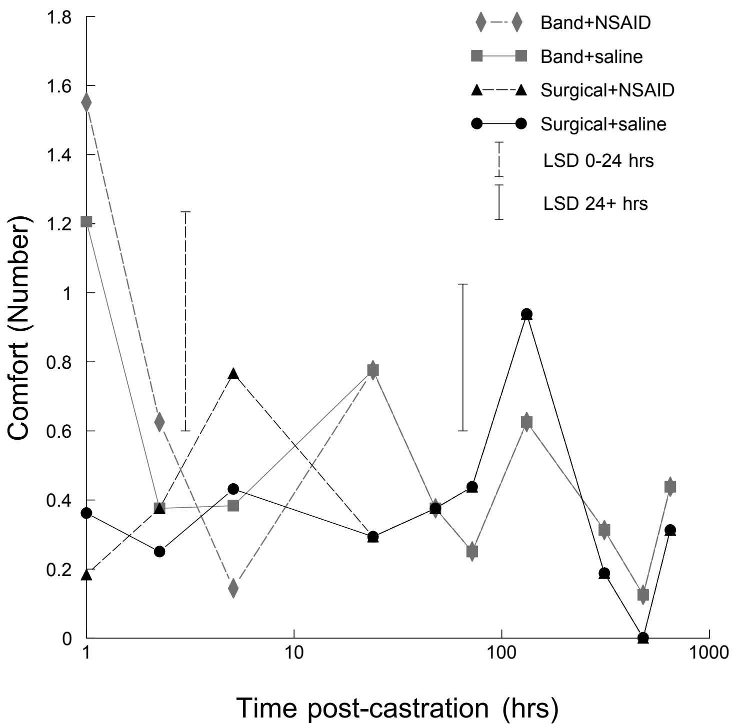
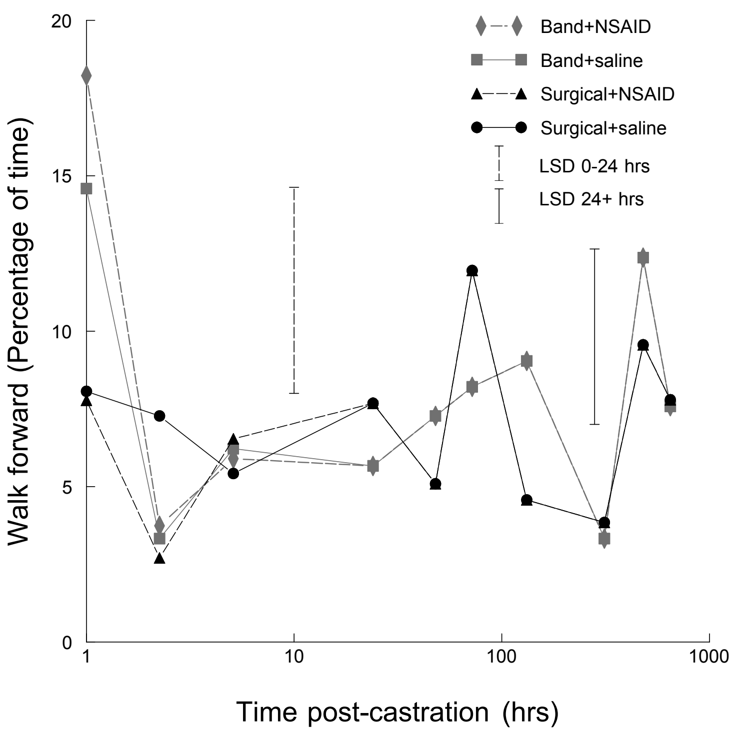


Fig. 9 Mean percentage of time spent walking forwards by weaner cattle

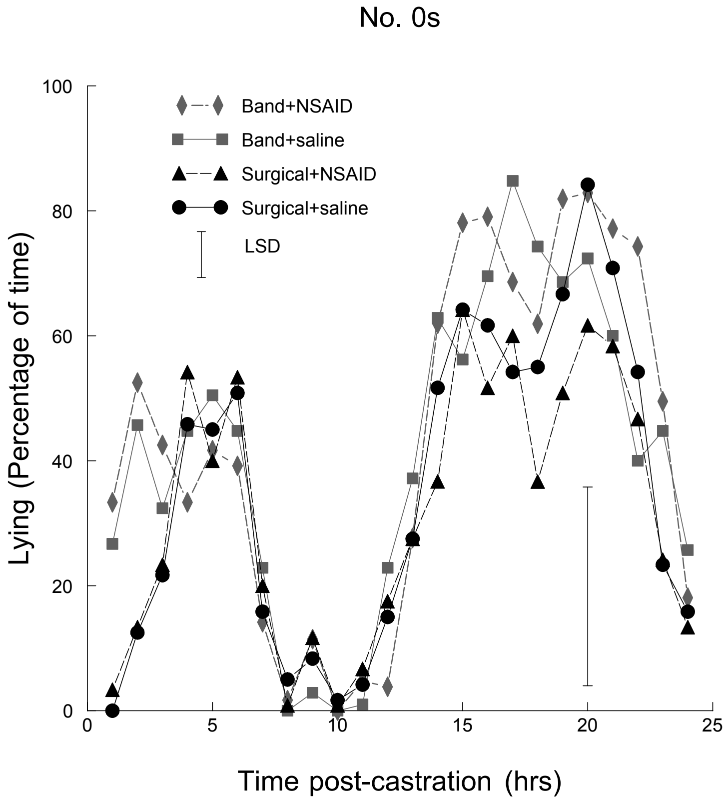
No. 0s



3.1.2.2 Via IceTags

During the first 24 hrs post-castration, there was a significant effect of castration method on the percentage of time spent lying ($F_{1,632}$ = 11.57; P < 0.001; Fig. 10), with the Surgical bulls spending less time lying overall than the Band bulls.

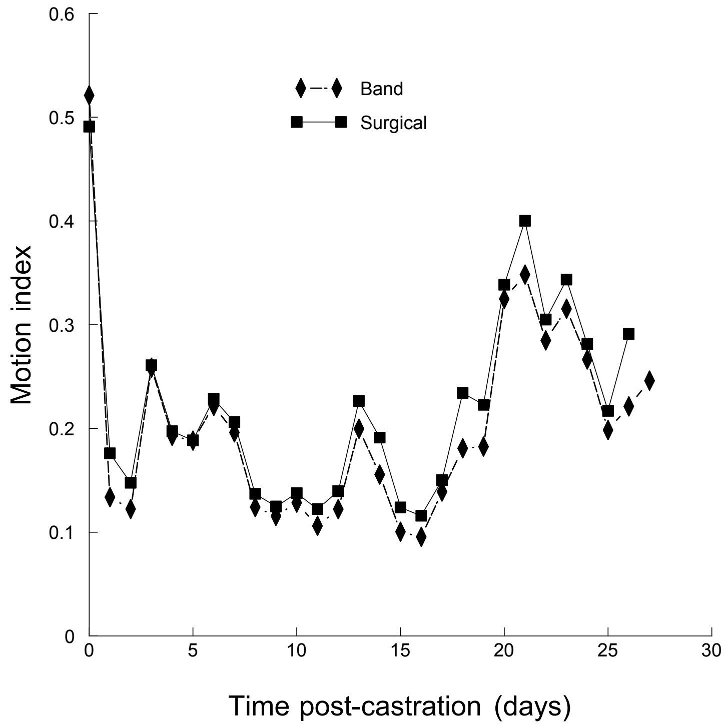
Fig. 10 Percentage of time spent lying in the 24-hr period post-castration, determined from IceTags on weaner cattle



In the 4 weeks post-castration, there was castration method effect ($F_{1,20}$ = 16.93; P < 0.001; Fig. 11) on the Motion Index measure, with ln(x+0.001) means and bias-corrected back-transformed values of -1.687 (0.184) and -1.564 (0.208) for the Band and Surgical bulls, respectively. This index is, according to the manufacturers of the IceTags, on a scale of 1 to 10 denoting increasing animal activity. Thus, overall, the Surgical cattle were more active than the Band cattle, although values were very small.

Fig. 11 Motion indices (back-transformed means) from IceTags on weaner cattle during the 4 weeks post-castration

No. 0s



3.1.3 Blood/plasma parameters

NSAID administration did not have a significant effect on any of the blood/plasma parameters.

3.1.3.1 Cortisol

There was a significant castration method x time interaction ($F_{9,252}$ = 2.76; P = 0.033; Fig. 12); at 2 hrs post-castration, cortisol concentrations were significantly higher in the Band compared to the Surgical animals, indicating greater pain and stress in the Banded bulls at this time. At 24 hrs post-castration, cortisol concentrations in the Surgical cattle were significantly greater compared to the Band cattle and, thereafter, there were no differences between the groups.

3.1.3.2 Haptoglobin

There was a significant ($F_{6,167}$) = 14.43; P = <0.001; Fig.13) castration method x time interaction on plasma haptoglobin concentrations. At days 2 and 3 and 1 week post-castration, Surgical bulls had significantly higher concentrations of haptoglobin than Band cattle. Haptoglobin concentrations in the Band cattle rose above those of the Surgical cattle at week 2, peaked at 3 weeks post-castration and remained significantly greater than those in the Surgical bulls at 4 weeks post-castration. This pattern indicates that inflammation and tissue damage were initially greater in the surgically castrated bulls, but after the first week it increased in the Band bulls and was still elevated 1 month post-castration.

Fig. 12 Mean plasma cortisol concentrations (nmol/L) of weaner cattle

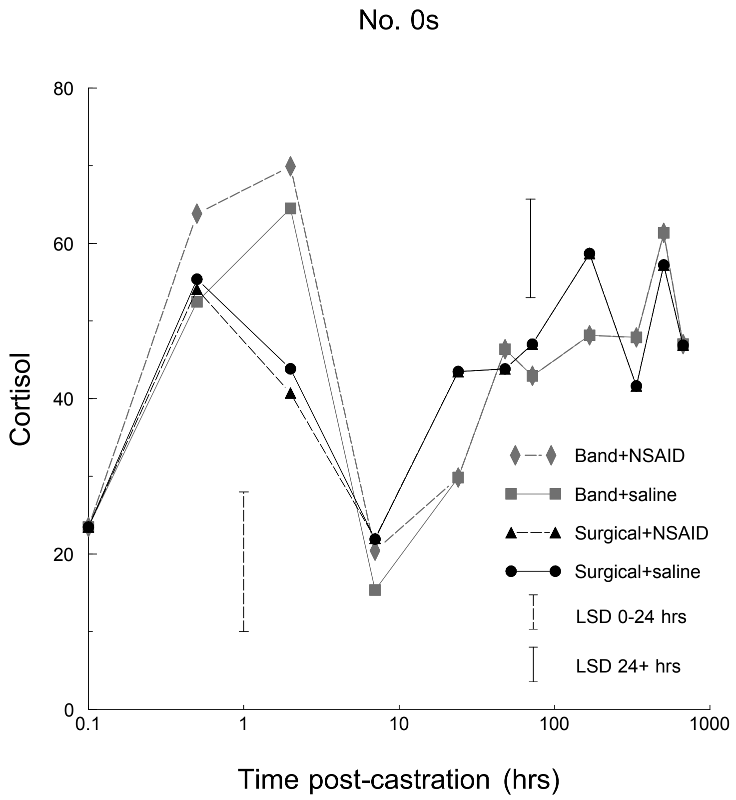
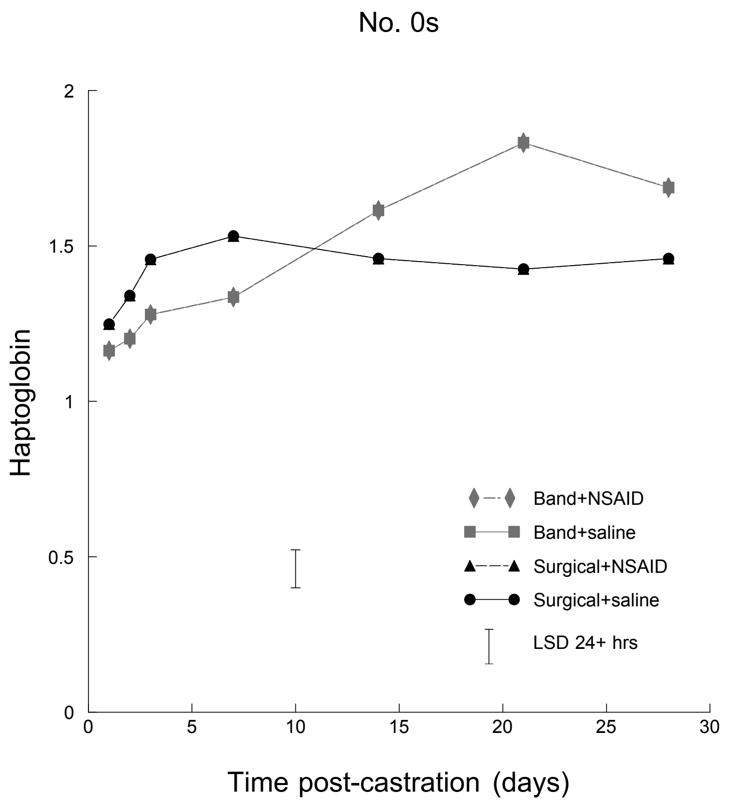


Fig. 13 Mean plasma haptoglobin concentrations (mg/mL) of weaner cattle



3.1.3.3 Total protein

Castration method ($F_{1,20}$ = 12.19; P = 0.002) and time ($F_{2,56}$ = 9.23; P = 0.001; Fig. 14) significantly affected total protein concentrations, with levels declining during the course of day 0, but with significantly higher concentrations at 2 and 7 hrs post-castration in the Band than Surgical bulls. The Surgical+NSAID animals had the lowest levels at the end of day 0.

3.1.3.4 Packed cell volume (PCV)

There was a significant castration method x time interaction ($F_{2,56}$ = 4.44; P = 0.027; Fig. 15) being the same order of magnitude as a marginally significant three-way interaction ($F_{2,56}$ = 3.15; P = 0.067) on PCV. Initially, there was no difference between the treatment groups, but at 2 hrs post-castration PCV was significantly greater in the Band+NSAID than Surgical+saline cattle, although there was no difference between Band+NSAID, Band+saline and Surgical+NSAID, or between Band+saline, Surgical+NSAID and Surgical+saline. At 7 hrs post-castration, the PCV in the Surgical+NSAID was significantly lower than the other three treatments, with no difference between these.

Fig. 14 Mean plasma total protein concentrations (g/L) of weaner cattle on the day of treatment

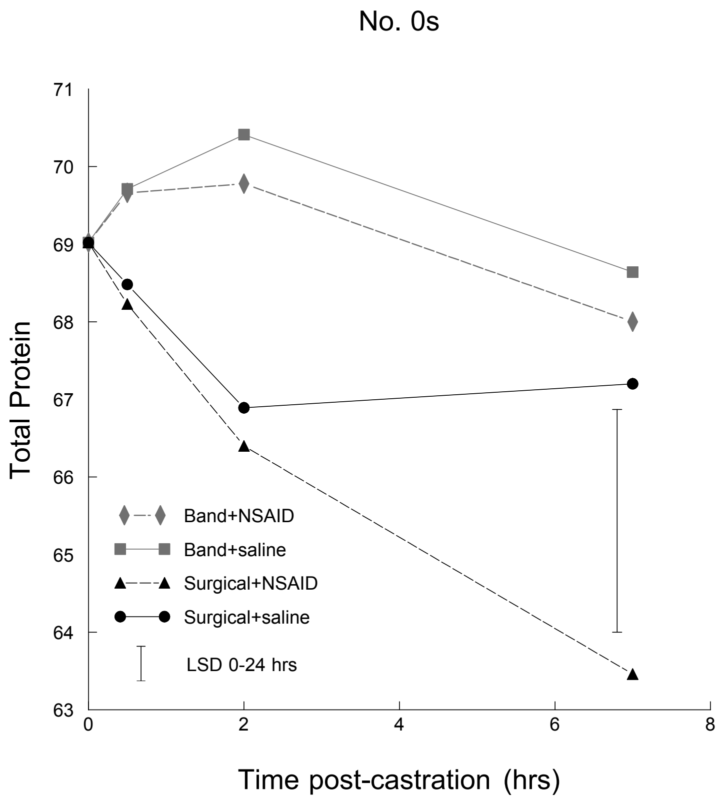


Fig. 15 Mean Packed cell volume (%) of weaner cattle on the day of treatment

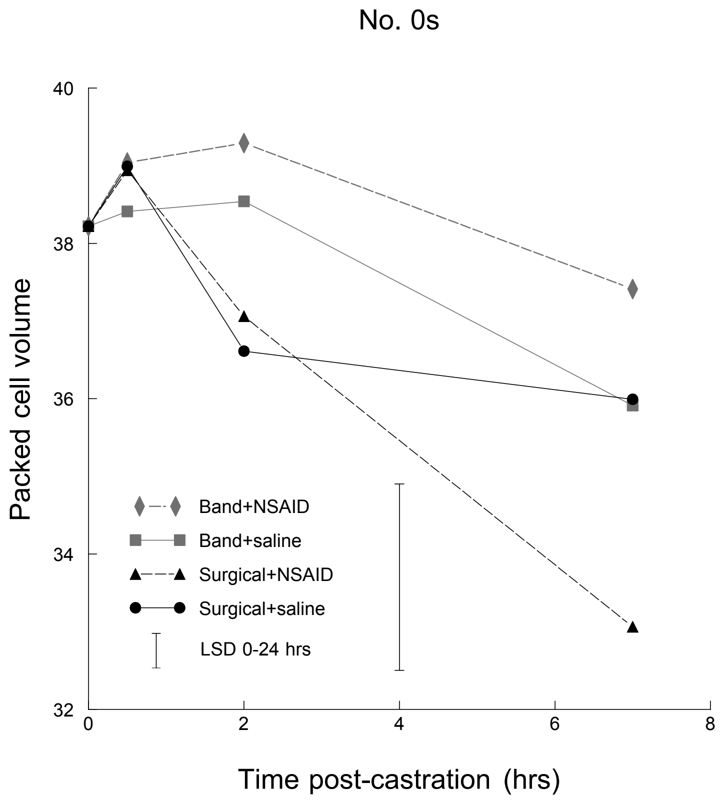
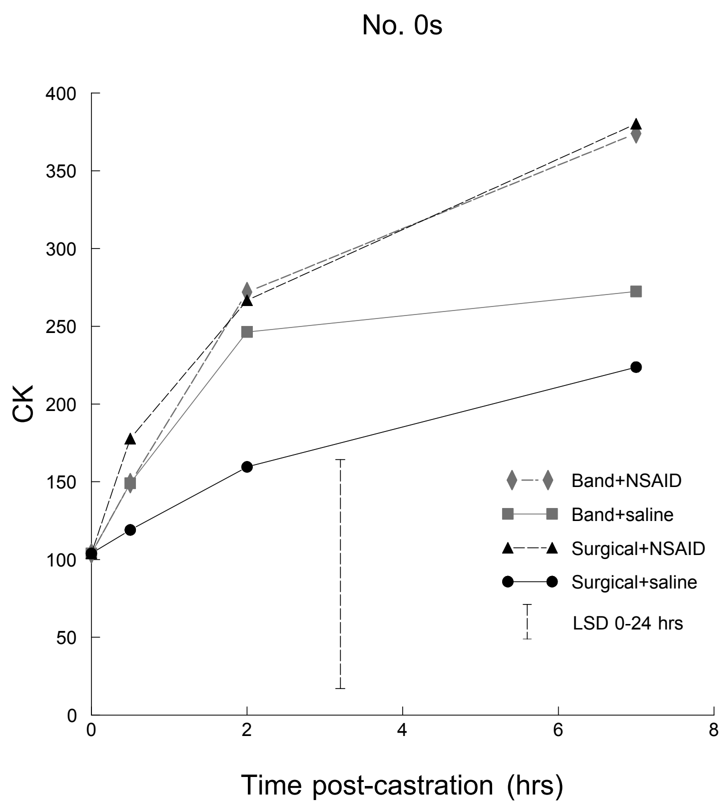


Fig. 16 Mean plasma creatine kinase (CK) concentrations (U/L) in weaner cattle on day of treatment



3.1.3.5 Creatine kinase (CK)

Time significantly affected concentrations of CK ($F_{2,56}$ = 24.31; P <0.001; Fig 16) with increases in all treatment combinations during the day of treatment. At 7 hrs post-castration both NSAID treatments had significantly higher levels of CK compared to the Surgical+saline treatment, with Band+saline being intermediate and not significantly different to any other.

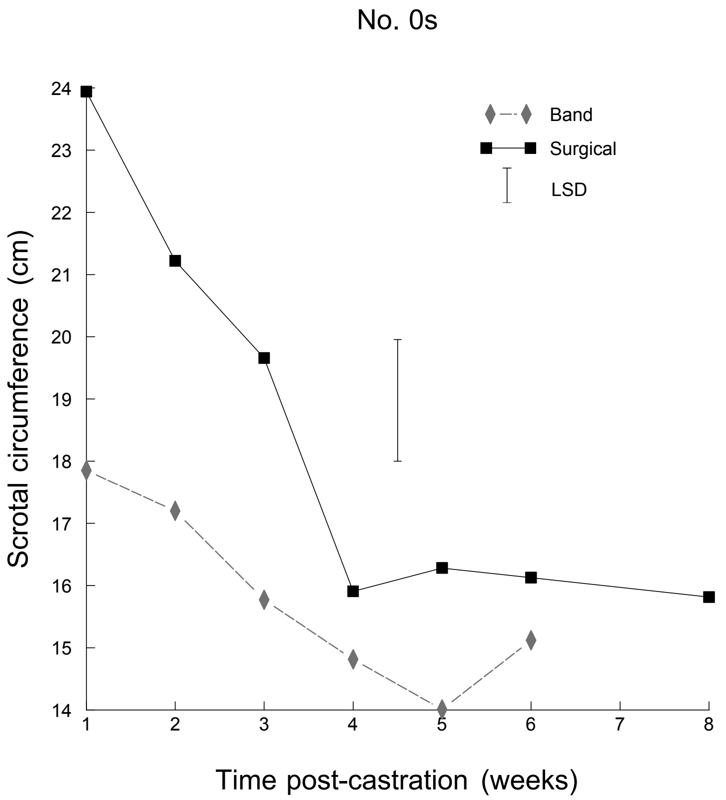
3.1.4 Wounds

There was a high correlation (r = 0.72; P<0.01) between the pre-experiment scrotal circumference and the weight of testicular tissue removed by surgical castration.

There was a significant time x castration method interaction on scrotal circumference ($F_{5,120}$ = 19.77; P<0.001; Fig. 17); as expected, the scrotums of the Band bulls gradually decreased in size over weeks, as they dried and shrivelled. In contrast, the scrotums of the Surgical bulls were larger compared to the pre-treatment value (of 16.7 cm) and compared to the Band bulls (P<0.05; LSD = 1.953) for the first 3 weeks post-treatment before stabilising at a mean of 16 cm for the remainder of the 8 weeks of measurement. This initial increase in scrotal size in the Surgical bulls probably reflected the amount of inflammation and oedema resulting from the castration procedure.

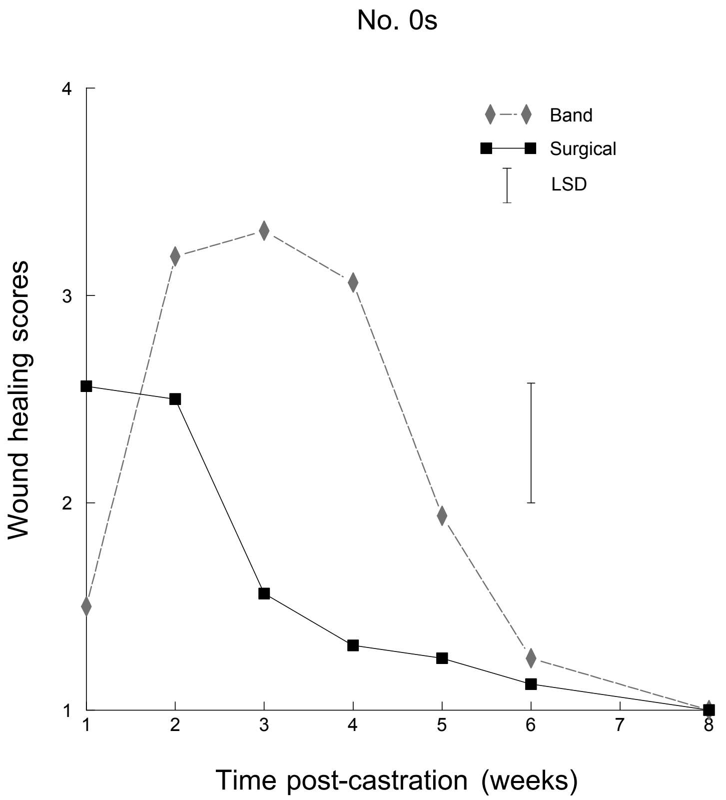
As anticipated, time affected the number of scrotums present on the Band bulls ($X^2_{6,91}$ = 17.12; P<0.001). The proportion of bulls with scrotums for weeks 1 to 6 were: 1.00 ± 0.000, 0.94 ± 0.043, 0.88 ± 0.060, 0.69 ± 0.078, 0.25 ± 0.080 and 0.13 ± 0.073 respectively, and all had dehisced by week 8.

Fig. 17 Scrotal circumference of weaner cattle during trial



There was also a significant time x castration method interaction for healing scores ($F_{6,168}$) = 17.11; P<0.001; Fig. 18). The Band bulls had a low score at week 1, which was significantly lower than the Surgical bulls (P<0.05; LSD = 0.5773). The score then increased at weeks 2 to 4 before decreasing, with the wounds fully healed at 8 weeks post-castration. At weeks 2 to 5 the score was significantly higher in the Band than Surgical bulls ($P<0.05$; LSD = 0.5773). In contrast, the Surgical bulls had high scores at weeks 1 and 2, then a significant decrease at week 3 ($P<0.05$; LSD = 0.5311) followed by a gradual, statistically non-significant decrease to all wounds being fully healed at week 8, although areas of bare, granulated skin remained in the banded cattle. Photographs of wounds are given in Appendix I.

Fig. 18 Healing scores of castration wounds of weaner cattle during trial
(1. Wound closed/scabbed, dry and no pus; 2. Wound part-closed, dry and no pus; 3. Wound part-closed, moist and pus present; 4. Wound fully open, moist and no pus present; and 5. Wound fully open, moist and pus) present)



There was a significant time by castration method interaction on the proportion of animals with scores of 3 to 5 ($X^2_{6,195}$ = 6.76; $P<0.001$) which are indicative of slow healing and/or the presence of infection (Table 3). Proportions were greater for the Band bulls compared to the Surgical bulls at weeks 2 to 5 post-castration.

Table 3. Mean proportion (± s.e.) of tension-banded and surgically castrated bulls with healing scores of 3 to 5* during 8 weeks post-castration

Week	Tension-banded	Surgical
1	0.25 ± 0.100	0.75 ± 0.097
2	0.81 ± 0.082	0.63 ± 0.102
3	0.94 ± 0.058	0.25 ± 0.099
4	0.81 ± 0.089	0.13 ± 0.078
5	0.38 ± 0.110	0.13 ± 0.078
6	0.06 ± 0.056	0.06 ± 0.056
8	0.00 ± 0.001	0.00 ± 0.001

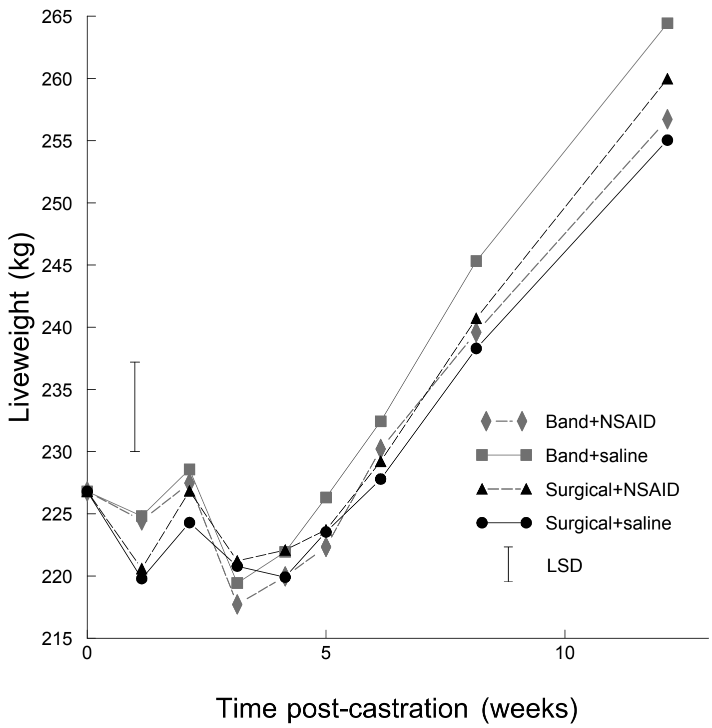
* 3. Wound part-closed, moist and pus present
4. Wound fully open, moist and no pus present
5. Wound fully open, moist and pus present

3.1.5 Liveweight and liveweight changes

There was no difference in liveweights between treatments at allocation (castration method P = 0.50; NSAID administration P = 0.42). There was no effect of castration method (P = 0.42), NSAID administration (P = 0.71) or an interaction (P = 0.08) on overall liveweight change (determined from average daily gains). Initial liveweight was a significant covariate for final liveweight (P< 0.001) and when adjusted for initial liveweights, final liveweights were unaffected by treatment (P = 0.40 for castration method and P = 0.70 for NSAID administration). As expected, there was a significant effect of time ($F_{7,196}$ = 305.13, P <0.001) on adjusted (for covariate) liveweights (Fig. 19).

Fig. 19 Liveweight changes of weaner cattle

No. 0s



3.2 Mature bulls

3.2.1 Behaviour at castration

Both castration method ($F_{1,21}$ = 62.32; P<0.001) and NSAID administration ($F_{1,21}$ = 5.68; P = 0.017) affected the total amount of movement during castration; the Surgical group moved more then the Band group (mean± s.e., 5.94 ± 0.609 vs. 1.00 ± 0.248, respectively) and the NSAID group more than the Saline group (4.25 ± 0.515 vs. 2.69 ± 0.409, respectively). The findings suggest that the Surgical and NSAID bulls experienced more pain and discomfort during the castration procedure than the Band and Saline bulls.

There was no difference between treatments in the numbers of bulls that knelt (P = 0.163 for castration method and 0.632 for NSAID administration) and only two bulls lay down, meaning the data could not be sensibly analysed. The mean proportion (± s.e.) of bulls kneeling was 0.219 ± 0.065.

3.2.2 Behaviour post-castration

3.2.2.1 Direct observations

There were too few recordings of the following states for meaningful analysis: time spent tail tucking, drinking and urinating. Standing shaking was not recorded at all. Time spent feeding, numbers of comfort behaviours, agonistic behaviours and defaecations were affected only by time, so are not presented or discussed.

3.2.2.1.1 Effect of NSAID administration

The NSAID effect (including its interactions with time and castration method) was significant (P < 0.05) in 7 out of 63 individual tests. As with the weaners, this was more than expected by random chance (random being 3.15 from 63), so we concluded that this was a real effect and focus on these patterns.

There was a significant effect of NSAID on the percentage of time spent in ‘abnormal’ lying (P <0.001); Fig. 20) and on the numbers of head movements (P = 0.009; Fig. 21). ‘Abnormal’ lying was seen only on the day of treatment, only in the Band cattle and mostly in the Band+NSAID group.

The numbers of head movements increased during day 0 for all treatments, with a significant difference between the Surgical+saline and Band+saline at 3+ hrs post-castration. The numbers increased at 1 day post-castration for the Band+saline animals and decreased for the Surgical+ saline animals. Thereafter, the patterns were similar between the treatments. In line with the weaner data, we suggest that the increase in these head movements did not result from the pain and discomfort of castration. The pattern suggests that all of the cattle were feeling less pain and discomfort as the trial continued, but with the most rapid easing of pain initially in the Surgical+NSAID cattle and, later on day 0, in the Surgical+saline and Band+NSAID animals.

Plate 14 Abnormal (lateral) lying by a banded mature bull

Fig. 20 Mean percentage of time spent in 'abnormal' lying by mature cattle

No. 9s

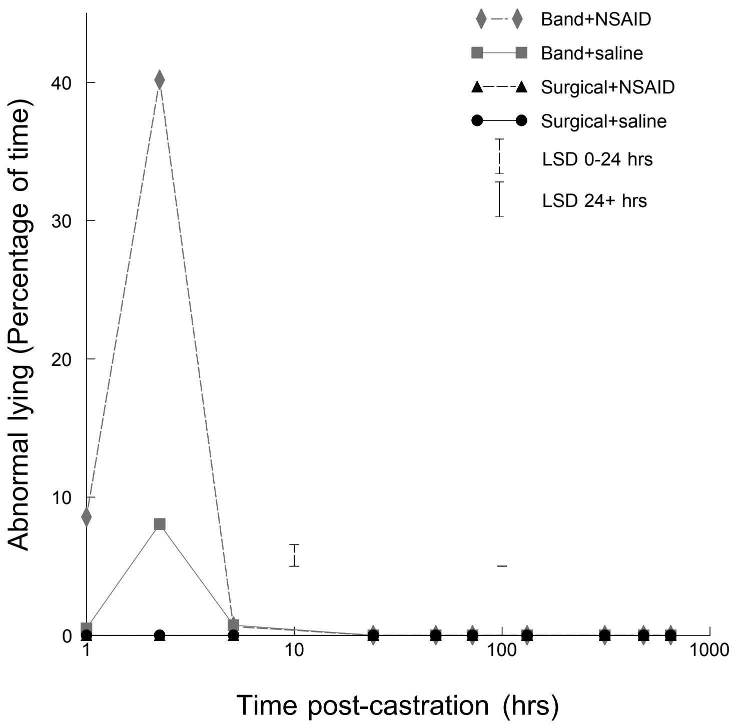


Fig. 21 Mean numbers of head movements by mature cattle

No. 9s

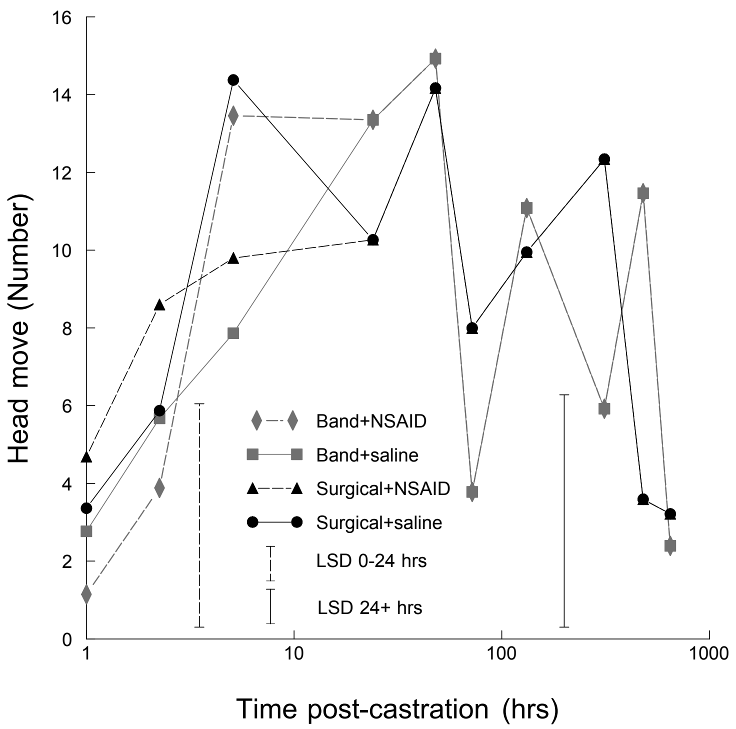
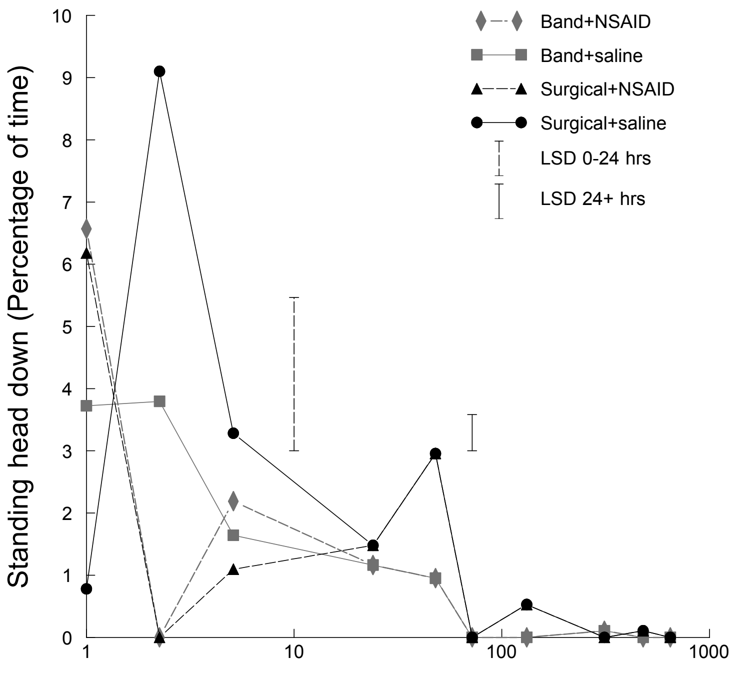


Plate 15 Standing head down (weaner, no photograph of mature bull available)

There was a significant NSAID x time interaction for the proportion of time standing with head down ($F_{3,177}$ = 11.0; P < 0.001; Fig. 22). Initially, the amount of time spent standing head down was low in the Sugical+saline cattle, high in the NSAID treatments, with Band+saline intermediate. At 1.5 to 3 hrs post-castration, the amount of time increased markedly in the Surgical+saline and reduced markedly for the NSAID treatments, suggesting that the NSAID was eventually effective at alleviating pain, and with greater pain experienced by the Surgical than the Band bulls. By the end of day 0, however, and subsequently, there was no difference between the treatments.

Fig. 22 Mean percentage of time spent standing head down by mature cattle

No. 9s



There was an NSAID x castration method x time interaction for the proportion of time spent in comfort behaviours ($F_{9,345}$ = 2.94; P = 0.002; Fig. 23) and ruminating ($F_{9,345}$ = 2.25; P = 0.019; Fig. 24). The amount of time in comfort behaviours was low for all treatments on day 0. At 1 day post-castration, however, the amount of time in comfort-related behaviours increased markedly for the Surgical cattle and to a lesser extent in the Band cattle. As described in relation to the weaner cattle, comfort behaviours appear not to be performed in direct response to the pain and discomfort associated with castration but, rather, are suppressed by pain. The greater amount of time spent by the Surgical cattle performing these behaviours on day 1 post-castration compared to the Band animals indicates less pain and discomfort in the Surgical than Band cattle.

The amount of time spent ruminating on day 0 was negligible for all treatments except Surgical+NSAID, indicating that these animals were in the least pain and discomfort. On days 1 and 2 post-castration, the Band cattle showed an increase in rumination time, perhaps as a “rebound” effect from the low levels on the treatment day. The Band cattle also spent significantly more time ruminating than the Surgical cattle at weeks 1 and 3 post-castration. The reason for this is unclear, particularly as levels were similar in the treatment groups at week 4.

Fig. 23 Mean percentage of time spent in comfort behaviours by mature cattle

No. 9s

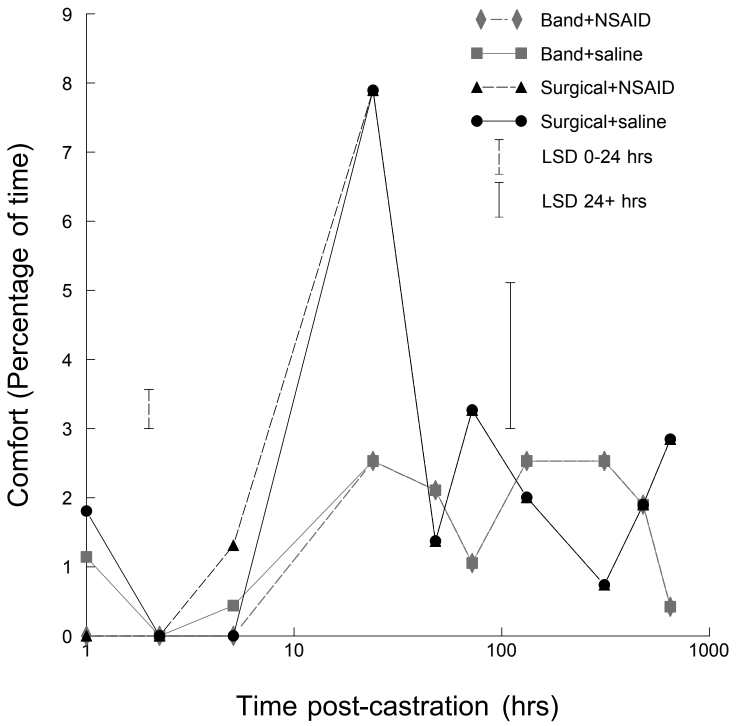
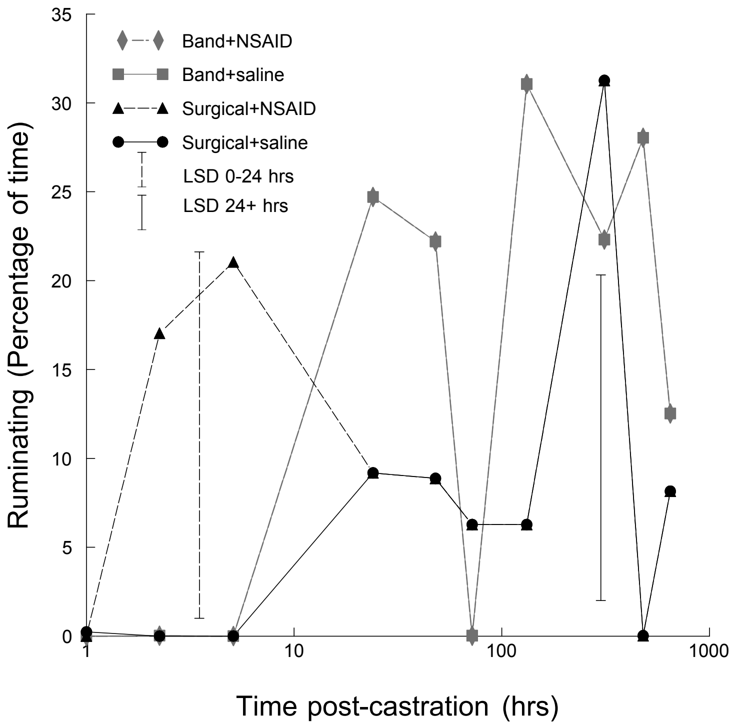


Fig. 24 Mean percentage of time spent in ruminating by mature cattle

No. 9s



3.2.2.1.2 Effect of castration method

Castration method had a significant effect on the amount of time in ‘abnormal’ lying ($F_{1,345}$ = 208; P < 0.001; Fig. 20), as previously described and also on the amount of time spent standing ($F_{1,345}$ = 7.31; P = 0.007; Fig. 25). Immediately post-castration, the Surgical cattle stood significantly more than the Band animals. The amount of time standing decreased in all treatments during day 0, with no difference between them at the 3+ hrs observation period. This suggests greater or a different type of discomfort in the Surgical than Band cattle initially. This is supported by a significant castration method x time interaction for the amount of time spent lying ($F_{1,345}$ = 2.4; P = 0.026; Fig. 26), with lying generally higher for the Band than Surgical cattle during day 0. At weeks 1 and 2 post-castration, the amount of time lying was non-significantly greater for the Surgical than Band cattle possibly indicating greater pain and discomfort in the Band than Surgical cattle at these times.

There were also significant castration method x time interactions on the numbers of tail movements ($F_{9,345}$ = 2.55; P = 0.007; Fig. 27), leg movements ($F_{9,345}$ = 6.10; P < 0.001; Fig. 28) and head movements ($F_{9,345}$ = 2.07; P = 0.032; Fig. 21, previously described above). Tail movements tended to decrease between the first and second observations, then rise at the third observation, particularly for the Surgical+saline group. At 1 day post-castration and subsequently, there was no difference between the treatments. The numbers of leg movements were significantly greater in the Band than Surgical cattle immediately post-castration, but decreased to be no different after 3 hrs post-castration. These patterns of behaviour indicate greater pain and discomfort in the Band than Surgical cattle immediately post-castration.

Fig. 25 Mean percentage of time spent standing by mature cattle

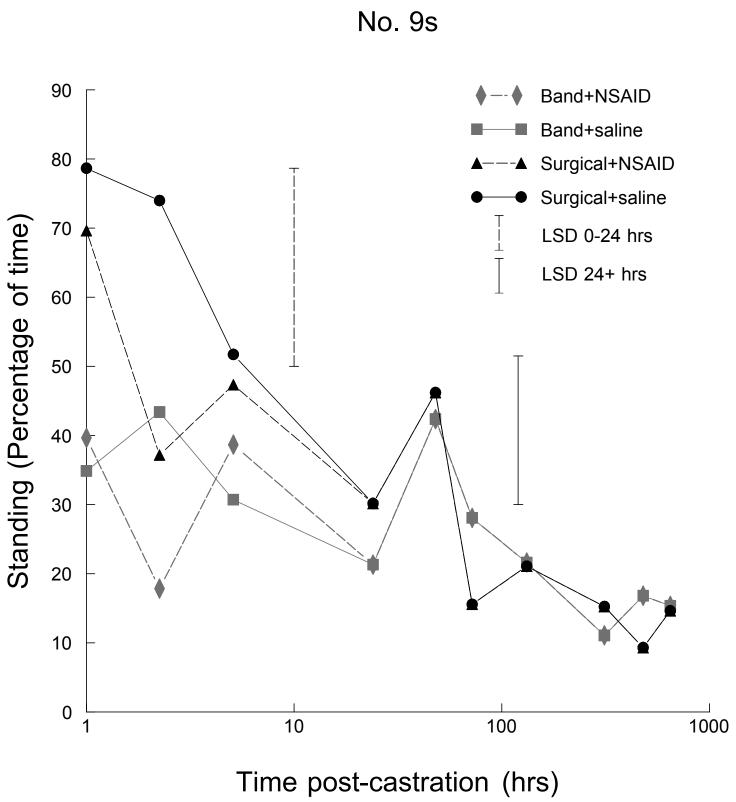


Fig. 26 Mean percentage of time spent lying by mature cattle

No. 9s

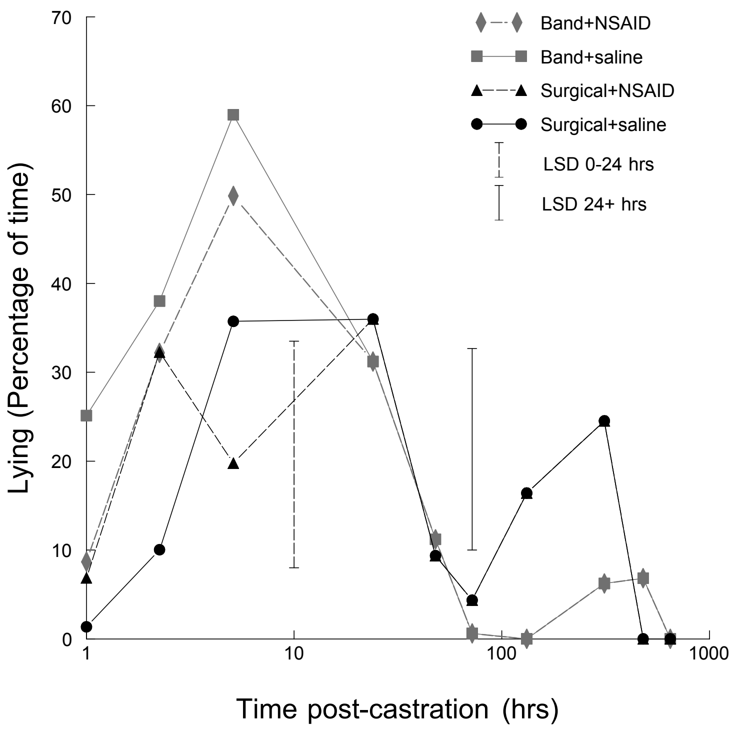


Fig. 27 Mean number of tail movements by mature cattle

No. 9s

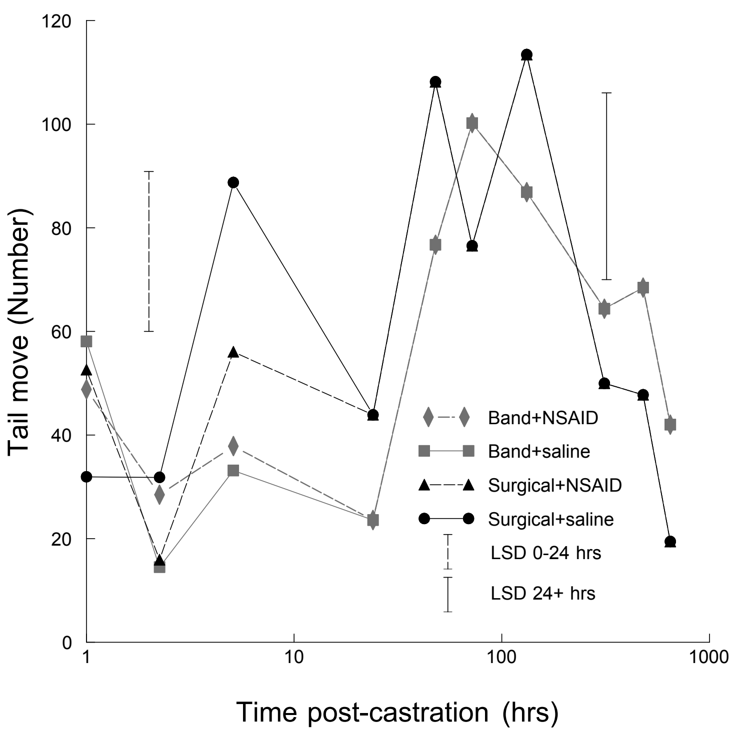
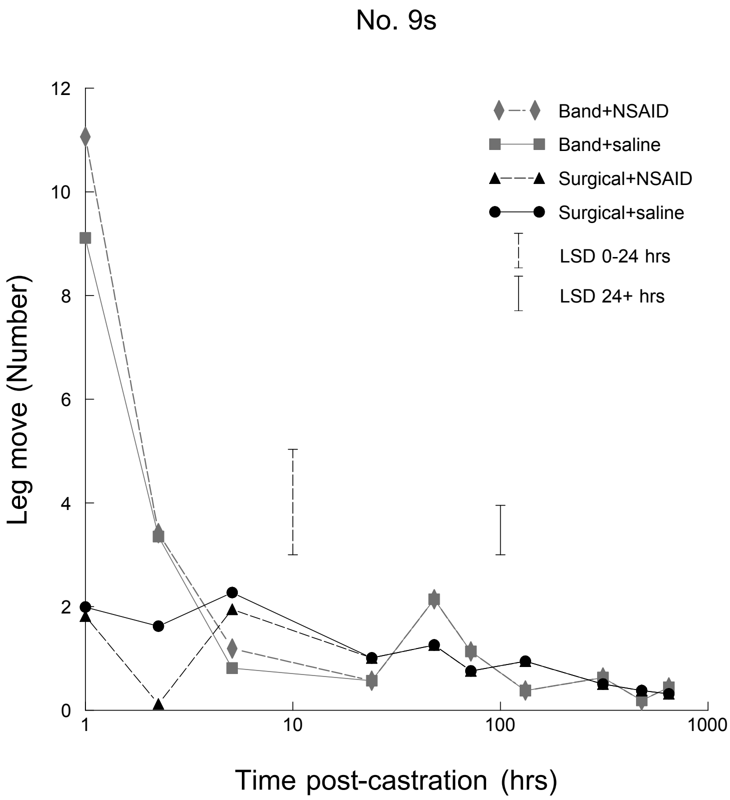


Plate 16 Mature bulls lying and performing tail flicking

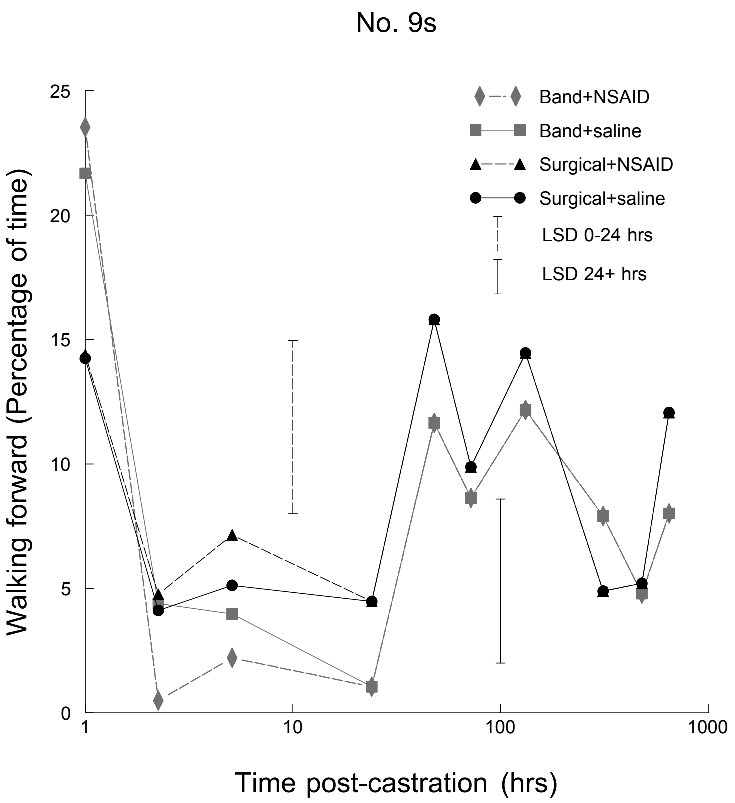
Fig. 28 Mean numbers of leg movements by mature cattle



There were significant castration method x time interactions for the amounts of time cattle spent walking forwards ($F_{9,345} = 11.45$; $P < 0.001$; Fig. 29) and walking backwards ($F_{9,345} = 2.82$; $P = 0.003$; Fig. 30). Immediately post-castration, the Band cattle spent significantly more time walking forwards than the Surgical bulls. Thereafter the patterns were very similar for the two treatment groups. The initial amounts of walking by the Band bulls are high compared to those seen during the remainder of the trial, suggesting that the "excessive" walking may have been a response to the pain and discomfort. The decline in walking in the NSAID-treated animals also suggests that walking was a response to pain which was alleviated by the NSAID.

Plate 17 Bull kicking

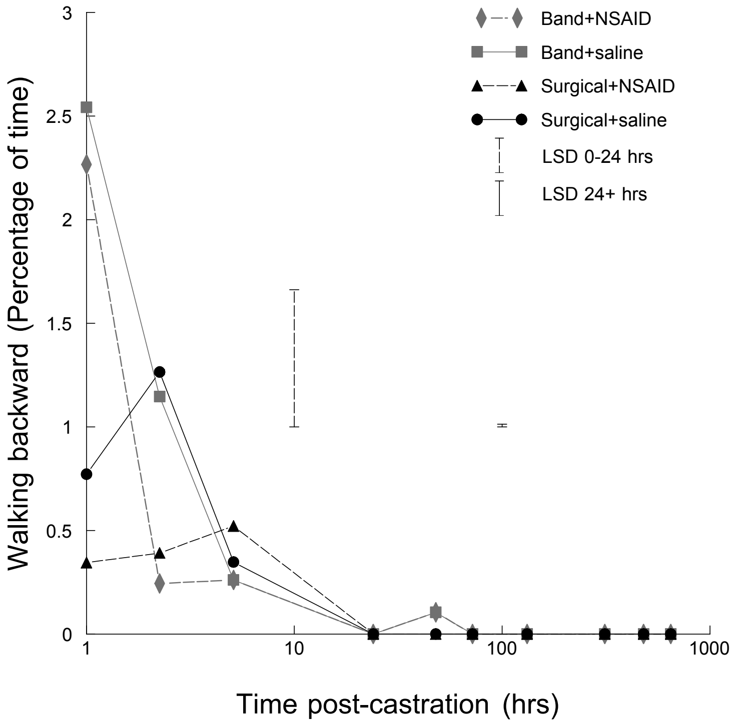
Fig. 29 Mean percentage of time spent walking forwards by mature cattle



Similarly, to 1.5 hrs post-castration, the Band cattle spent more time walking backwards than the Surgical cattle. At 1.5 to 3 hrs post-castration, however, the saline treated animals of both castration methods showed significantly more backwards walking compared to the NSAID treated cattle. Walking backwards is a behaviour that is seen in response to pain, such as post-dehorning. The fact that it was initially higher in the Band cattle indicates that they experienced greater pain and discomfort compared to the Surgical bulls. Furthermore, the fact that it was reduced in the NSAID treated cattle indicates that the ketoprofen was alleviating pain during the 1.5 to 3 hr post-castration period.

Fig. 30 Mean percentage of time spent walking backwards by mature cattle

No. 9s



3.2.2.2 Via IceTags

During the first 24 hrs post-castration, there was a significant effect of castration method on the number of steps/hr ($F_{1,576}$ = 7.17; P = 0.008; Fig. 31), with the Band bulls taking more steps than the Surgical bulls. There was a significant castration method x NSAID administration effect on the Motion Index (indicative of animal activity) ($F_{1,408}$ = 7.67; P = 0.006; Fig. 32) and proportion of time spent lying ($F_{1,\ 576}$ = 5.75; P = 0.017; Fig. 33). For Motion Index, the lowest value (least activity) was for the Surgical+saline cattle and there was no significant difference between the other treatment combinations. The ln(x+0.001) means and bias-corrected, back-transformed values were -2.854 (0.133), -2.374 (0.216), -2.316 (0.229) and -2.512 (0.188) for Surgical+saline, Sugical+NSAID, Band+saline and Band+NSAID, respectively. For lying, the proportions were lower for the Surgical castration compared to Band, although there was no difference between Surgical+saline and Band+saline. These results in combination indicate that the Surgical bulls were less active and stood stationary more than the Band bulls.

Fig. 31 Number of steps/hr taken by mature cattle in the 24 hrs post-castration

No. 9s

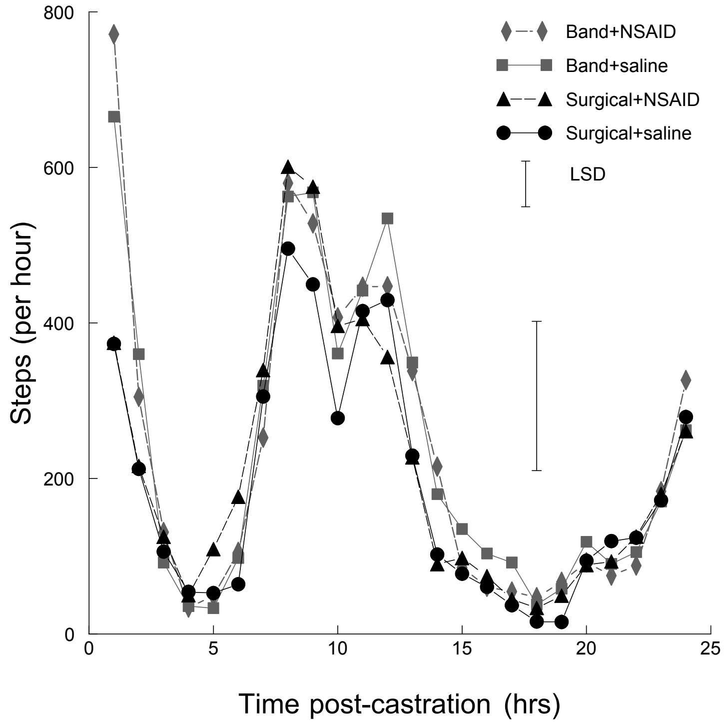


Fig. 32 Motion indices (back-transformed means) from IceTags on mature cattle in the 24 hrs post-castration

No. 9s

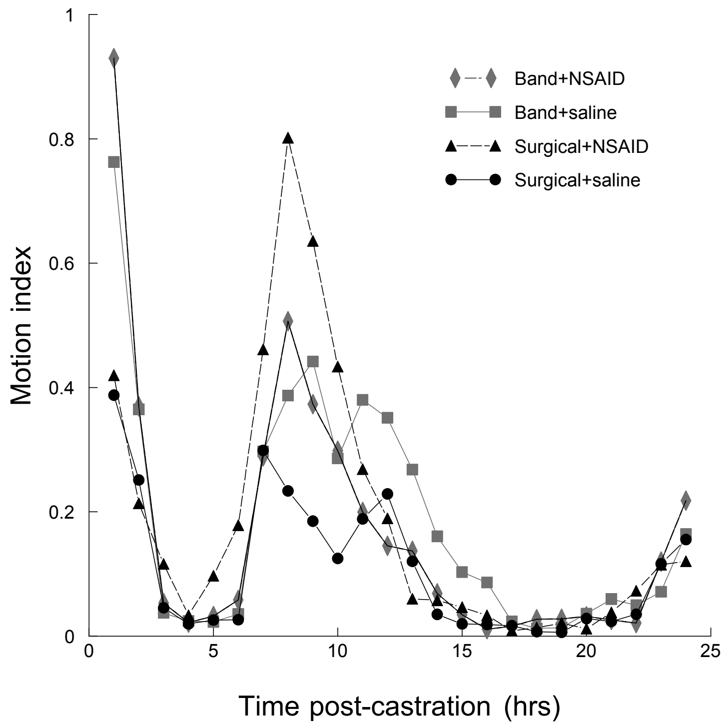


Fig. 33 Percentage of time spent lying in the 24 hrs post-castration determined from IceTags on mature cattle

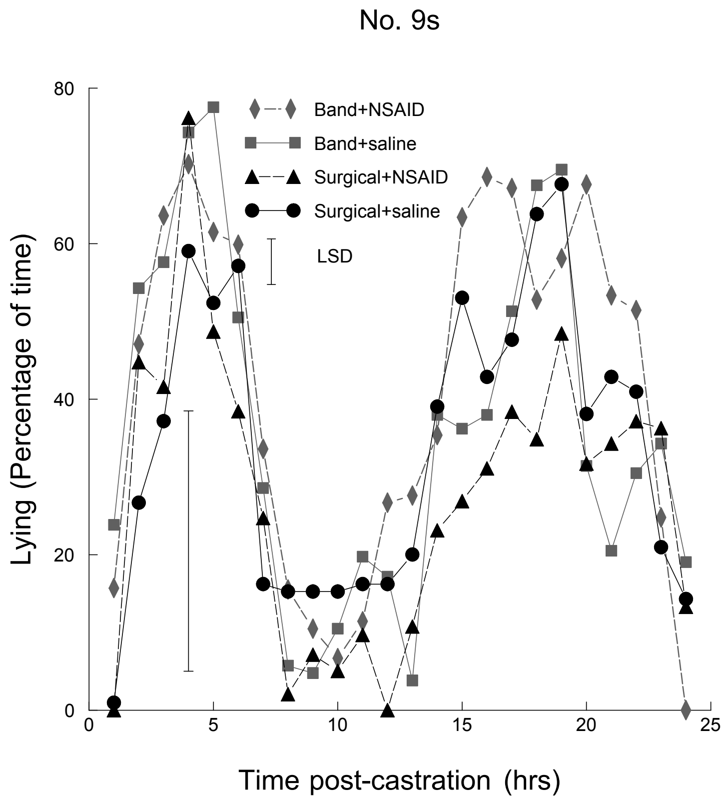
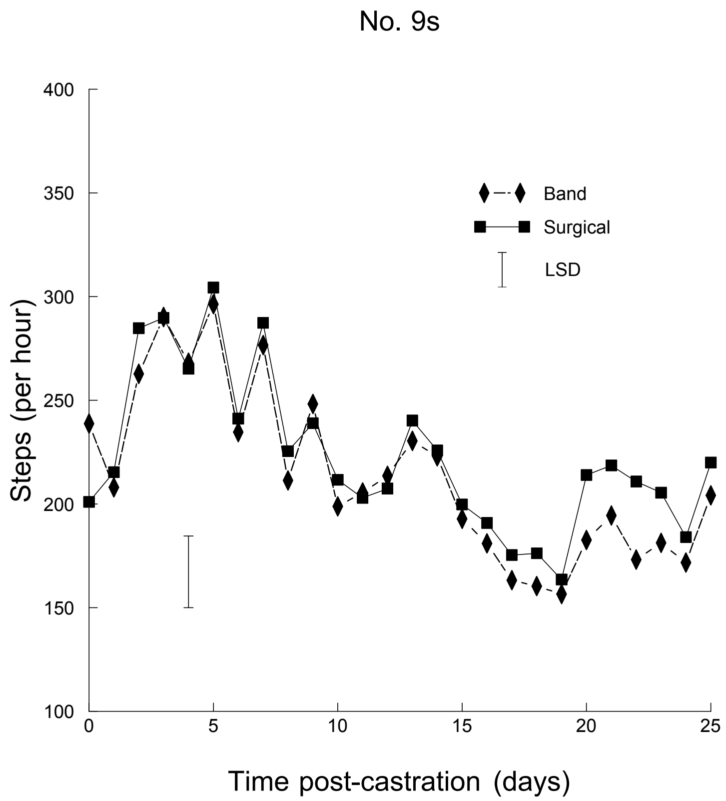


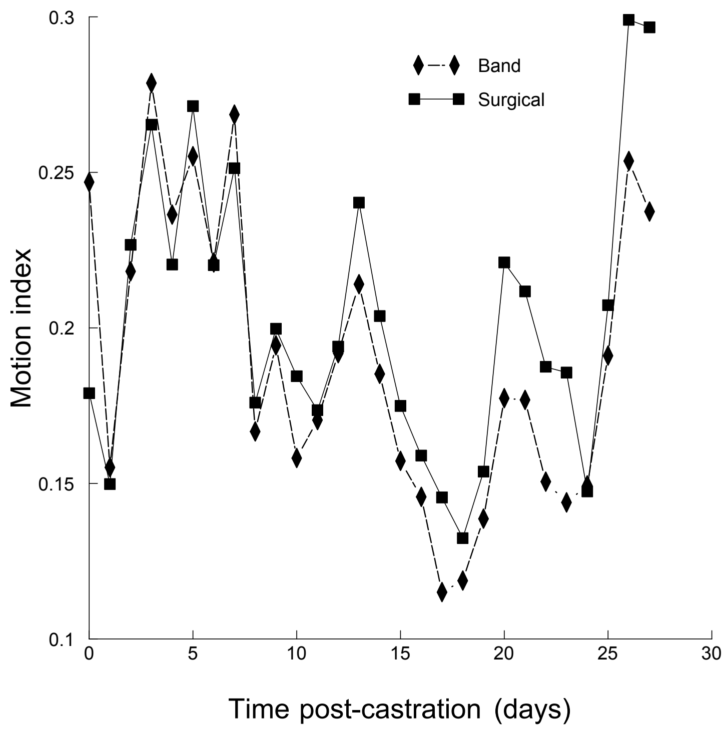
Fig. 34 Number of steps/hr taken by mature cattle in the 4 weeks post-castration



For the 4-week period that the IceTags were on the cattle, there was a significant effect of castration method on number of steps/hr ($F_{1,672}$ = 10.44; P = 0.001; Fig. 34) and the Motion Index ($F_{1,479}$ = 7.96; P = 0.005; Fig. 35). The Surgical bulls took more steps than the Band bulls overall and the Index was higher in the Surgical (ln(x+0.001) means and bias-corrected, back-transformed values: -1.622, 0.207) than Band bulls (ln(x+0.001) means and bias-corrected, back-transformed values: -1.692, 0.193). These results indicate more locomotion and activity in the Surgical compared to the Band bulls during the 4-weeks post-castration.

Fig. 35 Motion indices (back-transformed means) from IceTags on mature cattle for the 4 weeks post-castration

No. 9s



3.2.3 Blood/plasma parameters

3.2.3.1 Cortisol

There was a significant NSAID x castration method x time interaction on cortisol concentrations ($F_{9,252}$ = 2.96; P = 0.016; Fig. 36).

Cortisol concentrations at 40 min post-castration were lowest in the Surgical+NSAID group and highest in the Surgical+saline, with the others intermediate. There was, however, no significant differences between concentrations in Surgical+saline, Band+saline and Band+NSAID, or between Surgical+NSAID, Band+NSAID and Band+saline. At 2 hrs post-castration, however, there was a significant effect of the ketoprofen in the Surgical cattle, with no difference between the other three treatment groups. At weeks 2, 3 and 4 post-castration, cortisol concentrations were significantly greater in the Band than Surgical cattle. The results show that the NSAID was effective in alleviating pain in the Surgical, but not the Band treatment. The elevated cortisol levels in the Band cattle compared to the Surgical animals at weeks 2 to 4 post-castration indicate that that the Band cattle were experiencing greater pain/discomfort at these times.

Fig. 36 Mean concentrations of plasma cortisol (nmol/mL) in mature cattle

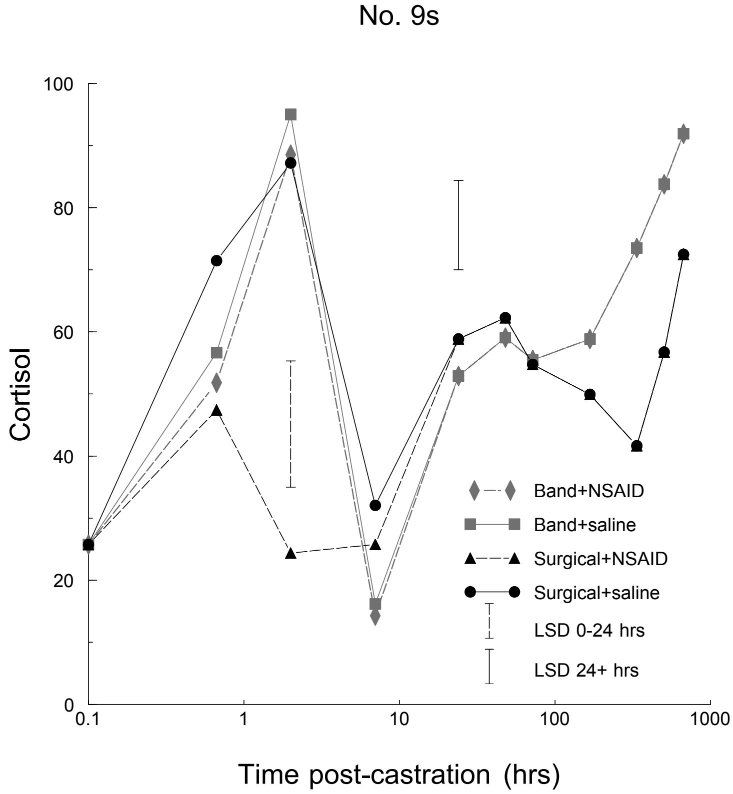
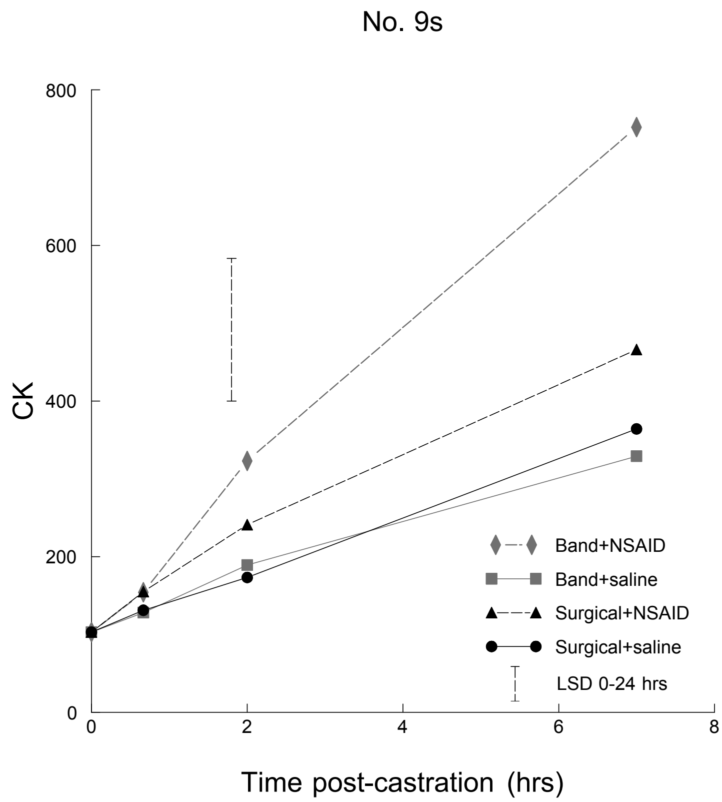


Fig. 37 Mean concentrations of plasma creatine kinase (CK) (U/L) in mature cattle during the day of treatment



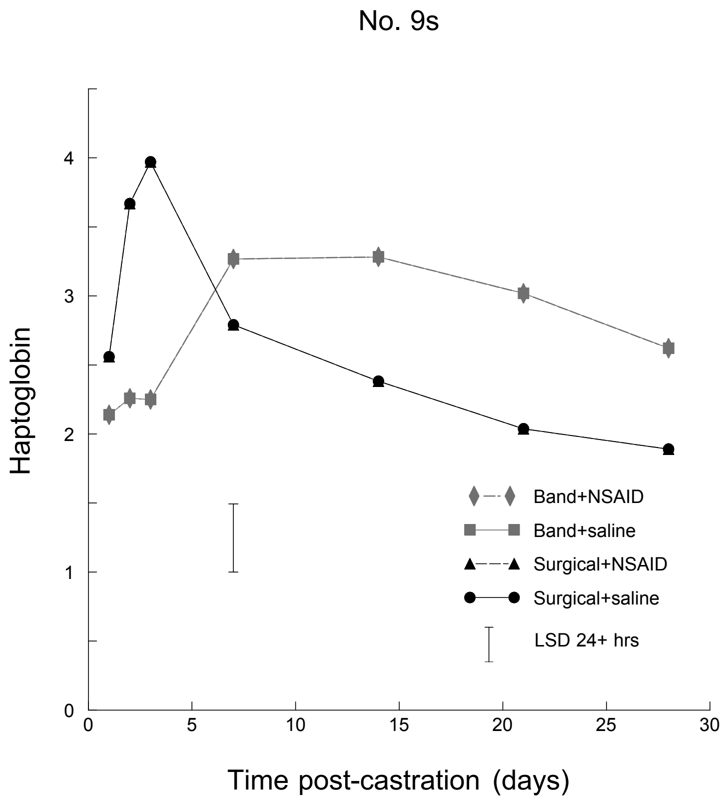
3.2.3.2 Creatine kinase (CK)

There was a significant NSAID x time interaction for CK concentrations ($F_{2,56}$ = 4.44; P = 0.04; Fig. 37).CK concentrations increased during the day of treatment for all groups, but the rise was greatest in the Band+NSAID cattle, such that, at 7 hrs post-castration the concentrations in these animals were significantly greater than in the other three treatments. CK is an indicator of muscle damage/trauma, suggesting that at 7 hrs post-castration, the Band+NSAID cattle were experiencing greater muscle damage than the other treatment groups.

3.2.3.3 Haptoglobin

Concentrations of haptoglobin showed a significant castration method x time interaction ($F_{6,168}$ = 14.94; P < 0.001; Fig. 38). At days 2 and 3 post-castration, concentrations were significantly greater in the Surgical than Band cattle, but the opposite was the case at weeks 2 to 4 inclusive. As haptoglobin is an indicator of inflammation and tissue damage, it is clear that the Surgical cattle experienced greater inflammation than the Band cattle initially, but inflammation in the Band bulls increased and remained elevated at 4 weeks post-castration.

Fig. 38 Mean concentrations of haptoglobin (mg/mL) in mature cattle



3.2.3.4 Total protein

Concentrations of total protein showed a significant castration method x time interaction ($F_{2,56}$ = 6.62; P = 0.010; Fig. 39); at 40 min post-castration Band+NSAID cattle had the greatest concentrations and Surgical+NSAID the lowest, although there was no difference between Band+NSAID, Band+saline and Surgical+saline or between Surgical+NSAID, Surgical+saline and Band+saline. At 2 and 7 hrs post-castration, concentrations were greater in the Band treatments compared to the Surgical treatments.

Fig. 39 Mean concentrations of plasma total protein (g/L) in mature cattle on the day of treatment

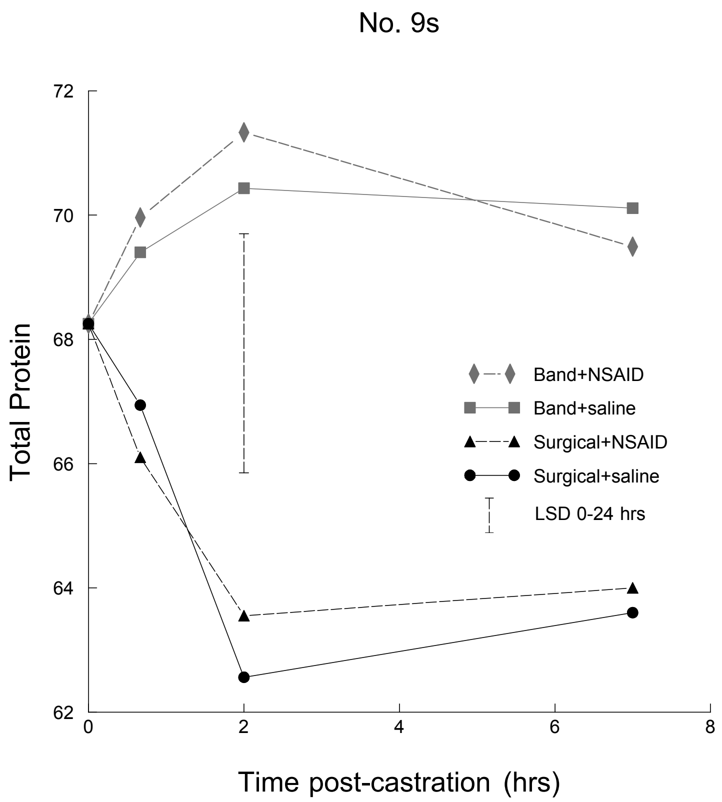
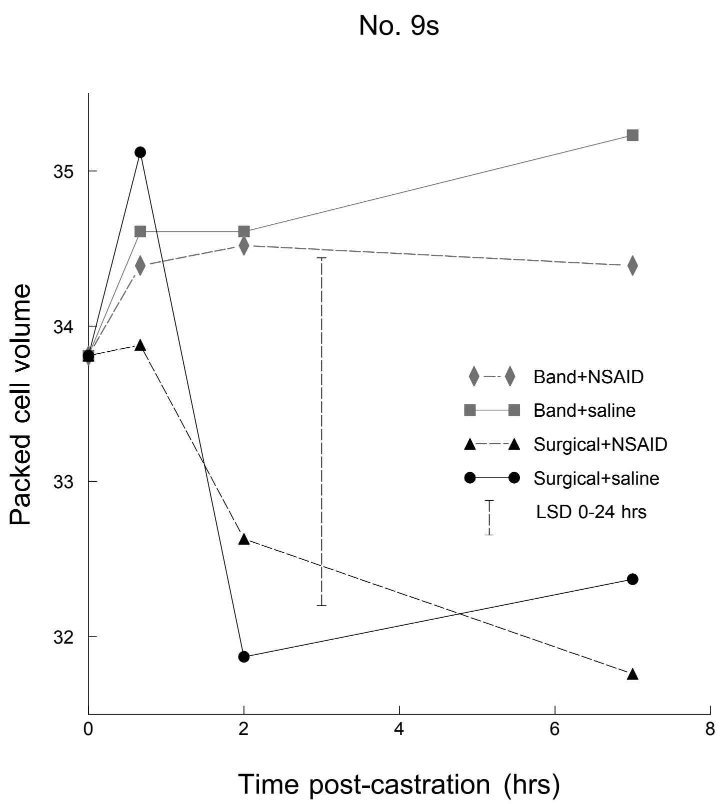


Fig. 40 Mean packed cell volume (%) in mature cattle on the day of treatment



3.2.3.5 *Packed cell volume (PCV)*

PCV also showed a significant castration method x time interaction ($F_{2,56}$ = 4.60; P = 0.019; Fig. 40); at 2 hrs post-castration, the Band treatments had higher PCV than the Surgical treatments, although Surgical+NSAID was not different from the Band treatments or Surgical+saline. At 7 hrs post-castration, the highest PCV was again in the Band treatments and the lowest in the Surgical, although there was no difference between Band+NSAID and Surgical+saline.

3.2.4 Wounds

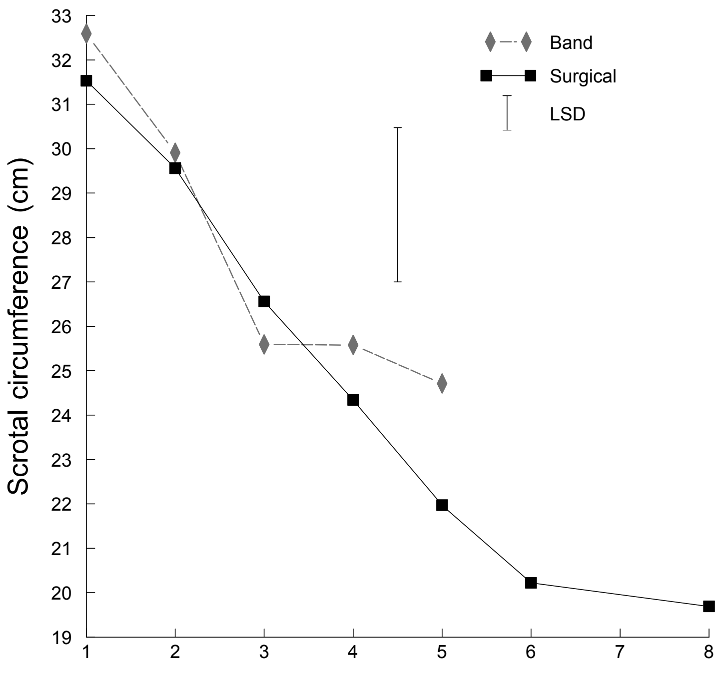
There was a high correlation (r = 0.79; P<0.01) between the pre-experiment scrotal circumference and the weight of the testicular tissue removed by surgical castration.

There was a significant time x castration method interaction on scrotal circumference (P<0.016; Fig. 41; as expected, the scrotums of the Band cattle gradually decreased in size over weeks, as they dried and shrivelled. Similarly, the circumferences of the scrotums of the Surgical animals also decreased during the 8 weeks of measurement.

As anticipated, time affected the number of scrotums present on the Band bulls ($X^2_{6,91}$ = 18.90; P<0.001). The proportion of bulls with scrotums for weeks 1 to 5 were: 1.00 ± 0.000, 1.00 ± 0.000, 0.75 ± 0.095, 0.56 ± 0.099 and 0.13 ± 0.053 respectively, and all had dehisced by week 6.

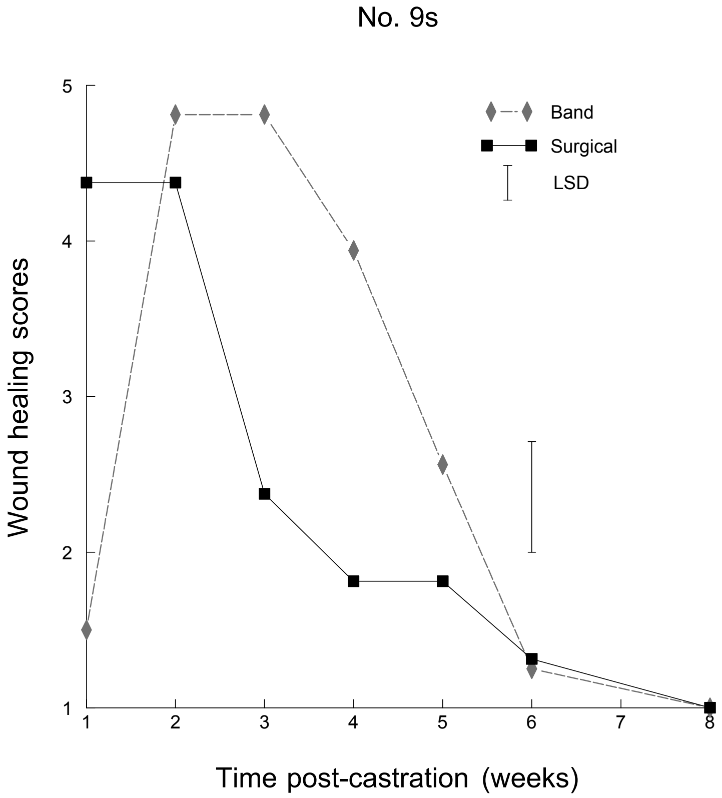
Fig. 41 Scrotal circumference of mature cattle during trial

No. 9s



There was also a significant time x castration method interaction for healing scores ($F_{6,168}$ = 33.26; P<0.001; Fig. 42). The score for the Band cattle at week 1 was significantly lower than for the Surgical bulls (P<0.05), but it increased and showed little change until week 5, when there were significant decreases (P < 0.05) between weeks 4 and 5, and 5 and 6. At week 2 there was no difference between the scores of the treatment groups, but at weeks 3 to 5 the score was significantly higher in the Band than Surgical bulls (P<0.05). The Surgical cattle showed a significant decrease in score from weeks 2 to 3 and then statistically non-significant decreases. At week 8 all wounds were fully healed in both treatment groups although areas of bare, granulated skin remained in the banded cattle. Photographs of wounds are given in Appendix II.

Fig. 42 Healing scores of castration wounds of mature cattle during trial (1. Wound closed/scabbed, dry and no pus; 2. Wound part-closed, dry and no pus; 3. Wound part-closed, moist and pus present; 4. Wound fully open, moist and no pus present; and 5. Wound fully open, moist and pus present)



There was a significant time by castration method interaction on the proportion of animals with scores of 3 to 5 ($X^2_{6,195} = 9.53$; P<0.001) which are indicative of slow healing and/or the presence of infection (Table 4). Proportions were greater for the Band bulls compared to the Surgical bulls at weeks 3 and 4 post-castration.

Table 4. Mean proportion (± s.e.) of tension-banded and surgically castrated mature bulls with healing scores of 3 to 5* during 8 weeks post-castration

Week	Tension-banded	Surgical
1	0.13 ± 0.080	1.00 ± 0.000
2	1.00 ± 0.000	0.94 ± 0.055
3	1.00 ± 0.000	0.63 ± 0.106
4	0.81 ± 0.090	0.38 ± 0.104
5	0.50 ± 0.112	0.38 ± 0.101
6	0.00 ± 0.000	0.06 ± 0.057
8	0.00 ± 0.000	0.00 ± 0.000

* 3. Wound part-closed, moist and pus present
4. Wound fully open, moist and no pus present
5. Wound fully open, moist and pus present

3.2.5 Liveweight and liveweight changes

There was no difference in liveweights between treatments at allocation (castration method P = 0.90; NSAID administration P = 0.82), with the mean weight being (mean ± s.e.) 419.9 ± 5.46 kg. There was no effect of castration method (P = 0.67), NSAID administration (P = 0.15) or an interaction (P = 0.61) on overall liveweight change (determined from average daily gains). Initial liveweight was a significant covariate for final liveweight (P< 0.001) and when adjusted for initial liveweights, final liveweights were unaffected by treatment (P = 0.69 for castration method and P = 0.14 for NSAID administration). There were, however, significant effects of NSAID administration ($F_{1,20}$ = 4.90; P = 0.039) and a castration method x time interaction ($F_{7,196}$ = 1.39; P = 0.005; Fig. 43) on mean liveweights. Mean liveweight for the bulls given the NSAID (421.17 ± 2.180 kg) was lower than those given saline (427.99 ± 2.180 kg). The Band cattle initially lost weight, gained significantly between weeks 2 and 3, but then did not gain until after week 4. The Surgical cattle also lost weight initially, but gained between weeks 1 and 2, and 2 and 3. They stayed the same weight between weeks 3 and 4, then gained, but remained the same between weeks 5 and 6 but had gained at 2 and 3 months post-castration.

Fig. 43 Mean liveweight changes for mature cattle

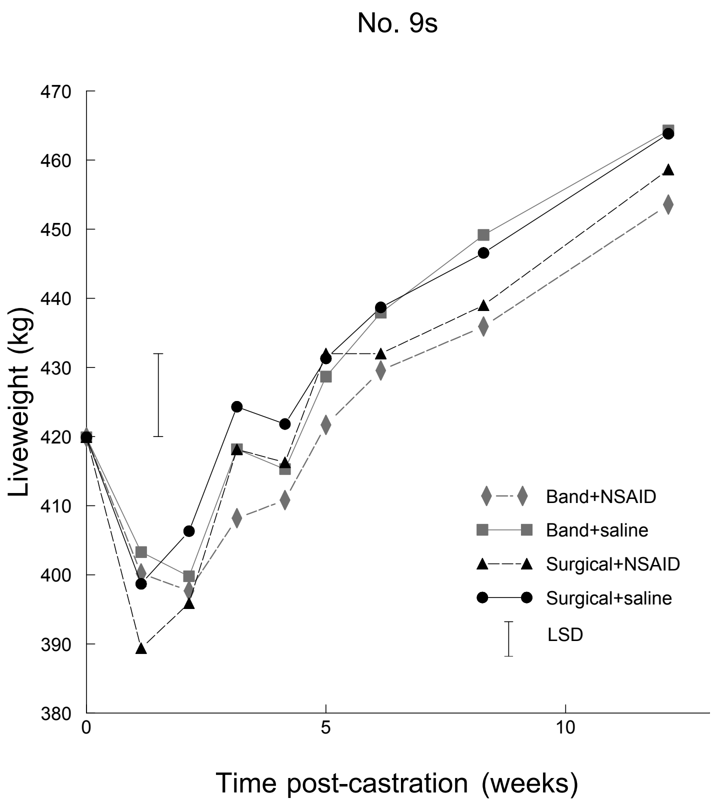


Plate 18 Mature bulls at 3 months post-castration

4 Discussion

There are claims by the manufacturers and retailers of tension-banders that they are the most humane method of castration and they offer improved liveweight gains over other castration methods. The findings of these two trials demonstrate that welfare and production outcomes are not superior for either immature or mature bulls castrated using the tension-bander method compared to surgical castration; behavioural, physiological and health responses indicated poorer welfare in tension-banded cattle compared to surgically castrated animals and liveweight gains were no different. In only one respect did tension-banding appear to provide improved welfare outcomes compared to surgical castration and that was during the conduct of the procedures. This finding agrees with those of other researchers, who reported more vocalisation during surgical compared to tension-banding castration of 360 kg bulls [36] and others who used rubber rings for castration, rather than tension-banding[37].

It is recognised that behavioural and physiological responses allow the presence or absence of pain to be indicated, but because pain is subjective, caution in the interpretation of the responses is required[38]. It is also recognised that, as a prey species, cattle may conceal symptoms of pain, and this may be particularly true of rangeland-reared animals that are unaccustomed to humans[39]. The current experiment, however, documented behavioural responses that have previously been reported as being indicative of pain in cattle e.g. repetitive tail movements[40] [41] [42] [43], leg movements[44] [45] and a suppression of feeding behaviour[46] [47] [48] [49] and rumination[50] [51] [52] [53]. We also recorded the suppression of other behavioural patterns that are part of the normal cattle repertoire[54], such as comfort behaviours (self-grooming, scratching and rubbing) and head movements. Standing, lying and walking are difficult to interpret with regard to pain; some researchers suggest a reluctance to move is indicative of pain[55] [56], others have found castration by tension-banding leads to reduced time lying[57], whilst others suggest that cattle in pain are more restless[58]. We saw a difference in the responses of the cattle within 24 hrs of castration, with the surgically castrated animals standing stationary more than the tension-banded cattle and higher levels of walking in the banded than surgically castrated animals. In contrast, we found greater levels of locomotion and activity in the surgically compared to the bander castrated mature bulls in the 4 weeks post-castration. It is likely that standing, lying and

[36] Rust et al. (2007) Bov Prac 41(2), 111-118
[37] Fell et al. (1986) Aust Vet J 63, 16-18
[38] Stafford & Mellor (2010) In: Improving Animal Welfare: A Practical Approach (Grandin, ed.)
[39] ibid.
[40] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48
[41] Graf & Senn (1999) Appl Anim Behav Sci 62, 153-171
[42] Fisher et al. (2001) Aust Vet J 79, 279-284
[43] Sylvester et al. (2004) Aust Vet J 82, 697-700
[44] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48
[45] Fisher et al. (2001) Aust Vet J 79, 279-284
[46] Graf & Senn (1999) Appl Anim Behav Sci 62, 153-171
[47] McMeekan et al. (1999) NZ Vet J 47, 92-96
[48] Fisher et al. (2001) Aust Vet J 79, 279-284
[49] Almeida et al. (2008) Dom Anim Endocrin 34, 89-99
[50] McMeekan et al. (1999) NZ Vet J 47, 92-96
[51] Sylvester et al. (2004) Aust Vet J 82, 697-700
[52] Almeida et al. (2008) Dom Anim Endocrin 34, 89-99
[53] Kolkman et al. (2010) Reprod Dom Anim 45, 160-167
[54] Hafez & Bouissou (1975) In: The Behaviour of Domestic Animals 3rd Edn. (Hafez, ed.)
[55] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48
[56] Stafford & Mellor (2005) NZ Vet J 53, 271-278
[57] Gonzalez et al. (2010) J Anim Sci 88, 802-810
[58] Mellor et al. (1991) Res Vet Sci 51, 149-154

walking can all be indicative of pain because, as noted[59], different castration methods affect different tissues in different ways which would likely result in different behaviours being elicited. Indeed, it has been stated that both restlessness and immobility are abnormal behaviours associated with pain[60].

A form of standing (with head below the brisket) is one that we have documented as being associated with pain previously[61]. We recorded this infrequently in the weaner bulls, but in the mature animals it was markedly reduced in the NSAID treatment animals and increased markedly in the Surgical+saline bulls at 1.5 to 3 hrs post-castration. Walking backwards is a form of locomotion that has been recorded as being associated with pain[62] [63] and which was at significantly higher levels in the banded than surgically castrated cattle and was also reduced by the NSAID. Lateral lying is another response that we recorded and it has been reported to be associated with pain in cattle[64] [65] [66] and work has been cited for cattle castrated with 'heavy rubber bands' lying on the ground in 'strange, contorted postures'[67]. More 'abnormal lying' in young (1 week-old) calves castrated with rings compared to other castration methods has also been reported[68]. What was unexpected in the current study was that lateral lying was seen almost entirely in the Band+NSAID treatment, which makes us suspect that it was exacerbated by the ketoprofen mode of action. The basis for this effect of ketoprofen is unclear.

It is difficult to compare the findings from the current study with others, as very few studies have directly compared surgical and tension-banding castration. In some studies that have made the comparison it is not stated how the cattle were banded [69] [70] (i.e. whether tension-banded or even whether rings, as opposed to bands, were used), which would make any comparison to the current study of doubtful value. Furthermore, only one study comparing surgical and banding castration has examined behavioural responses in an objective, systematic way[71], but the researchers did not record behaviour to the same detail as the current study. One other study examined castration by tension-banding and recorded behaviour, but the study compared tension-banding to sham castration[72]. Another comparative study used subjective scoring of "attitude", "gait and posture", "appetite" and lying posture [73], making it impossible to directly compare our findings with theirs.

Our findings on behavioural changes contrast with those in a comparable study[74] which found that surgically castrated 14-month-old *Bos taurus* bulls showed more leg and tail movements than banded bulls of the same age. Studies on young calves, however, provide some support for our findings, with more leg movements shown by 1-week-old calves castrated by rings than by those castrated surgically [75] and more tail, foot and head movements in calves of between 6 and 42 days of age castrated by rings compared to burdizzo and surgical methods[76].

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[59] Stafford & Mellor (2005) NZ Vet J 53, 271-278
[60] Mellor et al. (1991) Res Vet Sci 51, 149-154
[61] McCosker et al. (2007) Final Report AHW.143, MLA
[62] Robertson et al. (1994) Res Vet Sci 56, 8-17
[63] Graf & Senn (1999) Appl Anim Behav Sci 62, 153-171
[64] Mellor et al. (1991) Res Vet Sci 51, 149-154
[65] Robertson et al. (1994) Res Vet Sci 56, 8-17
[66] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48
[67] Stafford & Mellor (2010) In: Improving Animal Welfare: A Practical Approach (Grandin, ed.)
[68] Molony et al. (1995) Appl Anim Behav Sci 46, 33-48
[69] Baker et al. (2000) Bov Prac 34(2), 124-126
[70] Berry et al. (2001) Agric Expt Stn, Oklahoma State Univ. P-986
[71] Fisher et al. (2001) Aust Vet J 79, 279-284
[72] Gonzalez et al. (2010) J Anim Sci 88, 802-810
[73] Rust et al. (2007) Bov Prac 41(2), 111-118
[74] Fisher et al. (2001) Aust Vet J 79, 279-284
[75] Molony et al (1995) Appl Anim Behav Sci 46, 33-48
[76] Robertson et al. (1994) Res Vet Sci 56, 8-17

Our findings on changes in time spent feeding, lying and walking also contrasted with the above mentioned study[77] which found that surgical castrates spent less time grazing than banded animals. We, however, found no difference in time spent feeding between castration methods, although NSAID administration increased feeding in the weaners. That same study also found no differences between banded and surgically castrated bulls in lying and walking[78], but we found an increase in walking initially in the banded bulls of both cohorts, and a greater time standing and reduced time lying in the surgically castrated, mature bulls on the day of treatment. These latter contrasting results may reflect the greater age of the mature bulls compared to those used by these researchers[79]; the tissue removed from the mature bulls was much greater than from the weaners, which were of a more similar age to those used by those researchers. It is highly probably that there was more tissue damage and, thus, greater pain induced in the mature compared to younger, smaller bulls, resulting in the differences in behavioural responses. Genotype could have also contributed to the differences in behaviour given that *Bos indicus* cattle are reported as being behaviourally and physiologically more reactive to handling than *Bos taurus* animals[80 81].

Plasma cortisol concentrations are commonly measured in studies of pain induced by husbandry procedures, but do not measure pain per se but, rather, allow the assessment of the overall unpleasantness or noxiousness of the procedures[82]. In both cohorts of cattle the relationship between behavioural and cortisol responses on the day of treatment were not completely aligned. Behaviours of the weaners indicated that banding caused more pain than surgical castration and that ketoprofen alleviated the pain during the 1.5 to 3 hr period post-castration. Cortisol concentrations were no different between any of the treatments until 2 hrs post-castration, in line with the behavioural responses, but at this time the values of the surgical treatments were significantly lower than those of the banded cattle, regardless of the NSAID. Also, although no behavioural differences were evident, at 24 hrs post castration, the plasma cortisol concentrations were significantly higher in the surgical than banded cattle, but there were no differences thereafter. The patterns of changes were also different between the cohorts. Again, there was no difference between the treatments until the 2 hr post-castration sample, but at this time the cortisol concentrations of the Surgical+NSAID were significantly lower than the other treatments, which were no different to each other. Furthermore, whilst there was no difference at 24 hrs post-castration, at 2 to 4 weeks cortisol concentrations were significantly higher in the banded compared to surgically castrated cattle.

A review of the effects of age of cattle and castration on the cortisol response has been published[83] and six studies are listed as investigating castration by banding. When these studies are explored, however, half of these refer to ring castration and are, thus, of limited value for comparison with our work. Our finding in the weaner bulls of no difference between banding and surgical castration is in agreement with an experiment that found no differences between 400 kg bulls banded and surgically castrated or between castrates and entire bulls during the first week post castration[84]. Both this experiment and the current study, however, contrast with the findings of a study that used 100 kg calves and, whilst a direct comparison between banding and surgical was not made, the study did show that peak cortisol concentrations in banded animals (101 nmol/L) were greater than those surgically castrated with the spermatic cords broken by pulling (68 nmol/L) or by emasculators (56 nmol/L)[85]. In

[77] Fisher et al. (2001) Aust Vet J 79, 279-284
[78] ibid.
[79] ibid.
[80] Fordyce et al. (1988) Aust J Exp Agric 28, 683-687
[81] Zavy et al. (1992) Am J Vet Res 53, 551-557
[82] Mellor et al. (2000) In: The Biology of Animal Stress (Moberg & Mench, eds.)
[83] Bretschneider (2005) Livest Prod Sci 97, 89-100
[84] Fisher et al. (2001) Aust Vet J 79, 279-284
[85] Stafford et al. (2002) Res Vet Sci 73, 61-70

contrast, another experiment showed significantly lower cortisol concentrations in banded compared to surgically cattle on the day of castration[86], but close examination of the method reveals that this significant difference was determined by the rise in cortisol from blood samples taken some unspecified minutes before castration and ones taken about 2 min after the procedures. At best, the cortisol change indicates that the castration process *per se* was more noxious for the surgically castrated animals than the banded ones (which agrees with our findings), but tells us nothing about the post-castration pain and stress. It is of interest to note that the review to which reference is made above[87] uses this study and another that used rings to demonstrate and conclude that surgical castration results in the highest stress response, with intact cattle the lowest and 'banding' intermediate. This conclusion would appear inaccurate.

No studies on banding, other than the current work, have examined cortisol concentrations beyond 2 weeks post-castration and, so, we are unable to say whether the elevated concentrations we found in the mature bulls are a typical response. They do, however, coincide with the elevated haptoglobin concentrations in these animals and may be indicative of chronic pain. Certainly others[88] have cited behavioural changes in calves castrated by rings as being suggestive of chronic pain, lasting for at least 42 days. Neither of two tension-banding studies[89] [90] found elevated cortisol concentrations in tension-banded cattle at 2 weeks post-castration, but one found significantly lower cortisol in control bulls compared to surgically castrated animals at 2 weeks[91].

Our finding that ketoprofen reduced cortisol concentrations in the surgically castrated, mature bulls is supported by those of others[92] [93] [94]. One of these experiments used a local anaesthetic in conjunction with the NSAID[95], but another found ketoprofen to be more effective than local anaesthetic in reducing cortisol concentrations[96]. The ineffectiveness of ketoprofen in alleviating the pain from banding in both cohorts, however, contrasts with the findings from one study[97] and it is unclear why, although the use of ketoprofen in conjunction with a local anaesthetic in that study and differences between the experimental cattle in age, liveweight, genotype and handling experiences could all be contributing factors.

An obvious question about our findings is why was ketoprofen effective at reducing the cortisol response in the surgically castrated mature bulls, but not the surgically castrated weaners? In some ways, the result is counter-intuitive; the weaners had smaller testicles, as evidenced by the differences in weights of tissues removed, and would likely have experienced less trauma and inflammation than the mature bulls and, so, should have experienced less pain and stress. In addition, the animals were of the same genotype and had been born and reared in the same environment. The major difference between the two cohorts was their age and the consequent differences in handling experiences. As stated above, plasma cortisol concentrations reflect the overall stressfulness or noxiousness of a procedure and do not discriminate pain *per se*. This is apparent from the inexact correlation between the behavioural and cortisol responses. Thus, it appears that the weaners found the whole experience of castration (inclusive of the handling, restraint and blood-sampling) more aversive and unpleasant than did the mature bulls that had been moved through the

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[86] Chase et al. (1995) J Anim Sci 73, 975-980
[87] Bretschneider (2005) Livest Prod Sci 97, 89-100
[88] Molony et al (1995) Appl Anim Behav Sci 46, 33-48
[89] Fisher et al. (2001) Aust Vet J 79, 279-284
[90] Gonzalez et al. (2010) J Anim Sci 88, 802-810
[91] Fisher et al. (2001) Aust Vet J 79, 279-284
[92] Earley & Crowe (2002) J Anim Sci 80, 1044-1052
[93] Stafford et al. (2002) Res Vet Sci 73, 61-70
[94] Ting et al. (2003) J Anim Sci 81, 1253-1264
[95] Stafford et al. (2002) Res Vet Sci 73, 61-70
[96] Earley & Crowe (2002) J Anim Sci 80, 1044-1052
[97] Stafford et al. (2002) Res Vet Sci 73, 61-70

yards, handled and restrained on many occasions prior to this study. The 'generalised stress response' of the weaner bulls may have effectively masked or over-rode any specific cortisol response to pain, which meant that any effect of the NSAID was also concealed. It has been stated that animals that are accustomed to handling and close contact with people are usually less stressed than those that are not [98] and there is evidence that cattle unaccustomed to being handled have considerably higher cortisol responses compared to those that are accustomed to it [99]. Moreover, research on hot-iron branding highlighted the magnitude of the cortisol response in cattle unaccustomed to being handled and restrained[100] [101] compared to dairy cattle that were accustomed to it[102]. There is additional support for this hypothesis from recent dehorning work conducted on weaner cattle (heifers) from this same herd. An experiment demonstrated the effectiveness of a cornual block, using lignocaine, and an NSAID in reducing the behavioural responses to dehorning, but the combination had no effect on the cortisol response, with concentrations being no different to weaners dehorned without anaesthetic[103]. It may be possible to use plasma cortisol concentrations to assess the pain component of a procedure by using various control groups e.g. for handling, for local anaesthetic administration, analgesic administration, etc.

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[104]. Although not yet determined in cattle, social and psychological stressors can also elevate haptoglobin in some species[105], but to a much lesser degree than does inflammation. Our finding of haptoglobin concentrations being greater initially (during the first week post-castration) in the surgically castrated cattle compared to the banded animals is supported by other work[106], although the concentrations of haptoglobin measured in that study were extremely low (3 to 9 µg/mL in the banded cattle and 585 to 925 µg/mL in surgical castrates) compared to the values we obtained (1 to 4 mg/mL). Also in contrast to our work, this study found no differences between castration methods after day 4 (to day 56) and haptoglobin concentrations were negligible. In both cohorts in the current study, haptoglobin levels were significantly elevated in the banded compared to the surgically castrated bulls after the first week and remained so at 4 weeks post-castration.

Normal concentrations of haptoglobin are reported to be less than 0.35 mg/mL[107], but in both cohorts and throughout the 4 weeks post-castration, concentrations were above this. In the weaner bulls, the initial elevation in haptoglobin coincided with swelling of the scrotum in the weaner bulls, although the swelling persisted for 3 weeks. Such swelling has been noted previously and for about the same length of time post-castration[108] [109]. Similar swelling was not seen in the surgically castrated mature bulls, but haptoglobin concentrations were also elevated. In both cohorts the elevation in haptoglobin above normal levels indicates an ongoing inflammatory response, with inflammation being significantly greater in the banded than in the surgical castrates. As indicated above, this inflammation was associated with elevated cortisol concentrations in the mature animals, which may indicate chronic pain.

The inflammatory response was reflected in the healing process of the wounds. In both cohorts and both techniques there were indications of sepsis and delayed healing in some

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[98] Grandin (1997) J Anim Sci 75, 249-257
[99] Mitchell et al. (1988) Vet Rec 123, 201-205
[100] Lay et al. (1992a) J Anim Sci 70, 330-336
[101] Lay et al. (1992b) Appl Anim Behav Sci 33, 137-147
[102] Lay et al. (1992c) J Anim Sci 70, 1121-1125
[103] Sinclair et al. (2009) *Proc. 43nd Cong ISAE*, p.73
[104] Horadagoda et al. (1999) Vet Rec 114, 437-441
[105] Maes et al. (1997) Psychoneuroendocrinol 22,397-409
[106] Fisher et al. (2001) Aust Vet J 79, 279-284
[107] Horadagoda et al. (1999) Vet Rec 114, 437-441
[108] Chase et al. (1995) J Anim Sci 73, 975-980
[109] Rust et al. (2007) Bov Prac 41(2), 111-118

animals, but this was more severe in the banded cattle. The wounds of the banded cattle were scored higher (less healing) than the surgically castrated cattle during at least weeks 3 and 4 post-castration. Most wounds from both techniques were resolved by 6 weeks post-castration, when the scrotums of the banded animals had dehisced, although areas of bare, granulated skin remained at 8 weeks post-castration in these cattle. Most surgical wounds were healed by 4-weeks post castration, although a small percentage (13%) of those in the weaners and a larger percentage (38%) of the mature animals took longer to heal. These findings are in broad agreement with others[110] who reported that banded cattle had lost their scrotums by 8 weeks post-castration, but said that the wounds took 'several weeks' further to heal. These authors also found that surgical wounds were healed by 4 weeks, but with some wounds (15%) taking to 8 weeks to heal. A study that investigated five different methods of castration also indicated that wounds healed at slightly different rates, with all those from surgery healed by 9 weeks post-castration in comparison to some banded wounds taking about 13 weeks for full healing[111].

The time taken for all scrotums to be lost is variable between studies, and are reported as 5[112], 6 (this study, mature bulls), 7 (this study, weaners), 8[113 114], 9[115] and 12[116] weeks post-castration. From the limited information provided in these studies, the variability does not appear to be correlated with liveweight, initial scrotal size or genotype. It may be that variation results from climatic conditions and environment in which the cattle were kept post-castration e.g. whether they were grazed at pasture or lot-fed. These factors could also influence the propensity for contamination and infection of wounds and the rate of healing. Certainly we observed physical damage (punctures and tears) to some scrotums at weeks 1 and 2 post-castration (see Appendices), although these appeared to be among the first to dry-out and dehisce. In the current study, due to an exceptional wet season, the mature bulls were castrated at a less than optimal time, but the weaners were castrated during dry conditions and at the time of year that they would frequently be castrated in northern Australia. In both cohorts, however, the wounds of the tension-banded cattle were more inflamed, took longer to heal and showed higher levels of potential infection compared to the surgically castrated animals.

Castration, both by open (surgical) and closed (e.g. banding, burdizzo) methods, carries a risk of tetanus infection. Anecdotal evidence suggests that the risk is greater for closed methods, although there appear to be no publications in the scientific literature that address the relative risks of infection for different castration methods. A Canadian case report on a feedlot illustrates the potential for a tetanus outbreak in unvaccinated calves castrated with a bander[117]. In view of this risk, the manufacturer and retailers of the Callicrate Bander specify the need for protection against tetanus through vaccination prior to castration, although it should be noted that vaccination with tetanus toxoid (or a multivalent clostridial vaccine including tetanus toxoid) is also recommended before surgical castration by vaccine manufacturers and best practice guides. In the absence of vaccination, protection against tetanus can be provided by prophylactic administration of tetanus antitoxin. This is, however, an expensive option relative to the cost of prior vaccination.

An assessment of total protein and packed cell volume is used to evaluate acute fluid and electrolyte changes, with blood loss generally resulting in a decrease in both PCV and TP

[110] Fisher et al. (2001) Aust Vet J 79, 279-284
[111] Stafford et al. (2002) Res Vet Sci 73, 61-70
[112] Chase et al. (1995) J Anim Sci 73, 975-980
[113] Knight et al. (2000) NZ J Agric Res 43, 187-192
[114] Fisher et al. (2001) Aust Vet J 79, 279-284
[115] Knight et al. (2000) NZ J Agric Res 43, 187-192
[116] Pang et al. (2008) Livest Sci 117, 79-87
[117] O'Connor et al. (1993) Can vet J 34, 311-312

concentration[118]. In both cohorts TP and PCV declined during the day, but were greater in the surgical than band castrates, indicating greater blood loss in the surgical castrates. In the weaners, those in the Surgical+NSAID treatment had the significantly lowest levels of both TP and PCV, suggesting that the ketoprofen resulted in greater blood loss in this class of animal. Ketoprofen has been shown to impair platelet function and increase the risk of bleeding, at least in humans[119]. As all values of PCV and TP at all times in the current study were, however, within normal ranges (PCV 24-46% and TP 57-81 g/L[120]), these declines are of little biological significance. If an animal had low haematocrit before surgical castration, however, then the additional decline in PCV associated with the used of the NSAID could be detrimental. We have found only one other report of TP being measured in work on tension banding, although the study compared banding and burdizzo castration. Concentrations were found to be no different between methods at 28 days post-castration, but were higher than controls[121]. As no measure of PCV was included, the results are difficult to interpret.

Damage to muscle tissue due to mechanical trauma or high muscular activity can be determined by measuring plasma concentrations of creatine kinase (CK)[122]. It was not surprising that levels increased on all treatments during the course of the day of castration, as cattle were repeatedly moved through the yard complex, restrained and blood sampled. Intuitively, a greater increase in the surgically castrated than banded cattle may have been anticipated because of differences in the extent of trauma to tissues involved with the different castration procedures. This was not the case; in both cohorts the NSAID treatment groups had higher concentrations than the saline-treated and exceeded the upper limit for normal values (35-280 U/L for *Bos taurus* cattle[123]). The effectiveness of the NSAID in pain alleviation may have resulted in these cattle moving around more than those not given ketoprofen, although this increased movement is not apparent in the behavioural data. Only one other study on tension-banding has evaluated CK and that was in a comparison of banding and burdizzo castration[124], so the findings are unlikely to be directly relevant to the current study; at 28 days post-castration CK concentrations were lower in banded than burdizzo castrated cattle, with the latter at about the upper limit of normal values.

Results on liveweight changes from comparative studies of tension-banding and other castration methods (mainly surgical and burdizzo) have produced mostly consistent findings of no differences in liveweight or average daily gain (ADG) in bulls ranging from 95 kg[125] to 400kg[126] [127] [128] or more[129] [130] during experimental periods of 4[131] to 17 weeks[132]. In some of the studies there were differences in gains during parts of the trials, generally with banded cattle showing lower gains[133] [134]. In one, however, lower ADG was found in the surgical compared to the banded castrates, but overall there was no difference between them over a 16-week period[135]. In a study on 400 kg bulls[136] [137], ADG was reduced in banded cattle

[118] Carlson (1997) In: Clinical Biochemistry of Domestic Animals (Kaneko et al., eds.)
[119] Niemi et al. (1997) Acta Anaesth Scand 41, 1353-1358
[120] Radostits et al. (2007) Veterinary Medicine 10th Edn.
[121] Pang et al. (2008) Livest Sci 117, 79-87
[122] Radostits et al. (2007) Veterinary Medicine 10th Edn.
[123] ibid.
[124] Pang et al. (2008) Livest Sci 117, 79-87
[125] Stafford et al. (2002) Res Vet Sci 73, 61-70
[126] Knight et al. (2000) NZ J Agric Res 43, 187-192
[127] Fisher et al. (2001) Aust Vet J 79, 279-284
[128] Pang et al. (2008) Livest Sci 117, 79-87
[129] ZoBell et al. (1993) Can J Anim Sci 73, 967-970
[130] Chase et al. (1995) J Anim Sci 73, 975-980
[131] Rust et al. (2007) Bov Prac 41(2), 111-118
[132] Knight et al. (2000) NZ J Agric Res 43, 187-192
[133] Pang et al. (2006) J Anim Sci 84, 351-359
[134] Pang et al. (2008) Livest Sci 117, 79-87
[135] ZoBell et al. (1993) Can J Anim Sci 73, 967-970
[136] Knight et al. (2000) NZ J Agric Res 43, 187-192

compared to surgically castrated cattle in the 4 weeks post-castration but no differences in liveweight were found at the end of the trial (15 weeks). With 240 kg bulls there were reduced gains in the banded bulls during the 5 weeks post-castration leading to lower liveweights in the banded than surgical castrates at 17 weeks. Superior ADG and FCE in surgical castrates compared to banded cattle were also found in a 4-week experiment by with 360 kg bulls[138]. In only one study using 275 kg bulls entering a feedlot have superior ADG been found in banded cattle and this was as measured on a carcass weight basis[139]. Most of these finding are consistent with those from the current study where no differences were found between surgical and band castrated bulls in liveweights or average daily gains.

The current study did, however, produce an unexpected finding of reduced gains in ketoprofen-treated mature cattle compared to those given saline. This is difficult to explain given that ketoprofen is effective for a maximum of about 24 hrs. Our finding also contrasts with the those of others who found minimal effects of ketoprofen on intakes and gains in Friesian calves (of approximately 215 kg)[140] and another study that found no difference in intakes and gains over a 34-35 d period of 300 kg *Bos taurus* surgically castrated bulls with and without the administration of ketoprofen[141].

[137] Fisher et al. (2001) Aust Vet J 79, 279-284
[138] Rust et al. (2007) Bov Prac 41(2), 111-118
[139] Booker et al. (2009) Bov Prac 43(1), 1-11
[140] Earley & Crowe (2002) J Anim Sci 80, 1044-1052
[141] Ting et al. (2003) J Anim Sci 81, 1253-1264

5 Conclusions

In *Bos indicus* cattle, castration by tension-banding causes less pain and discomfort during the procedure than does surgical castration. It does, however, cause more pain and stress immediately post-castration compared to surgical castration. The pain can be reduced but not eliminated, by the administration of an appropriate non-steroidal, anti-inflammatory drug. The drug, however, needs to be administered about 20-30 min prior to castration and carries some risk of increased blood loss following surgical castration. Tension-banding results in large wounds that remain inflamed for at least 4 weeks post-castration and which may cause chronic pain, particularly in mature bulls, perhaps as a consequence of the size of the wounds. The wounds from tension-banding are also slower to heal than those from surgical castration. Tension-banding compared to surgical castration appears not to offer improvements in liveweight gains in either immature or mature bulls. Thus, the results from this study clearly do not support the manufacturer's and retailers' claims that tension-banding gives superior welfare and production outcomes compared with other castration methods.

Whilst the design of this study does not allow for direct comparisons between the age groups, it would appear that, from absolute concentrations of plasma cortisol and haptoglobin, the extent of delayed wound healing and liveweight losses, castration had a greater adverse impact on the welfare of the mature bulls compared to the weaners. For a more definitive conclusion, however, a study would need to be conducted where different ages of bulls are castrated at the same time and managed as a single cohort.

The outcomes of this work highlight the need for further work on the impacts on welfare of ring castration, given that tension-banding is reportedly superior to rings in preventing blood flow, contamination and sepsis. It is important to establish the impacts in different ages of calves, as there are anecdotal reports of rings being used for weaners of a similar age and weight to those used in the current study, even though the Cattle Code indicates they should not be used in calves of more than 2 weeks of age.

Our overall recommendation is that castration should be conducted by the procedure that has the least adverse impacts on cattle welfare. Thus, based on the evidence from this study, surgical castration is preferred over tension-banding and use of analgesia is preferable to non-use. Non-preferred procedures should only be used if there are compelling reasons. For example, it may be argued that analgesics cannot be administered without veterinary supervision, or that the risks of blood loss for some cattle (e.g. those suspected of having low haematocrit) outweigh the benefits of surgery over banding.

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