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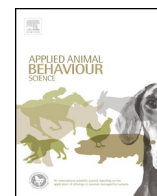
A comparison of welfare outcomes for weaner and mature *Bos indicus* bulls surgically or tension band castrated with or without analgesia: 2. Responses related to stress, health and productivity

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A R T I C L E I N F O

A B S T R A C T

Tension banding castration of cattle is gaining favour because it is relatively simple to perform and is promoted by retailers of the banders as a humane castration method. Two experiments were conducted, under tropical conditions using *Bos indicus* bulls comparing tension banding (Band) and surgical (Surgical) castration of weaner (7–10 months old) and mature (22–25 months old) bulls with and without pain management (NSAID (ketoprofen) or saline injected intramuscularly immediately prior to castration). Welfare outcomes were assessed using a range of measures; this paper reports on some physiological, morbidity and productivity-related responses to augment the behavioural responses reported in an accompanying paper. Blood samples were taken on the day of castration (day 0) at the time of restraint (0 min) and 30 min (weaners) or 40 min (mature bulls), 2 h, and 7 h; and days 1, 2, 3, 7, 14, 21 and 28 post-castration. Plasmas from day 0 were assayed for cortisol, creatine kinase, total protein and packed cell volume. Plasmas from the other samples were assayed for cortisol and haptoglobin (plus the 0 min sample). Liveweights were recorded approximately weekly to 6 weeks and at 2 and 3 months post-castration. Castration sites were checked at these same times to 2 months post-castration to score the extent of healing and presence of sepsis. Cortisol concentrations (mean ± s.e. nmol/L) were significantly ($P < 0.05$) higher in the Band (67 ± 4.5) compared with Surgical weaners (42 ± 4.5) at 2 h post-castration, but at 24 h post-castration were greater in the Surgical (43 ± 3.2) compared with the Band weaners (30 ± 3.2). The main effect of ketoprofen was on the cortisol concentrations of the mature Surgical bulls; concentrations were significantly reduced at 40 min (47 ± 7.2 vs. 71 ± 7.2 nmol/L for saline) and 2 h post-castration (24 ± 7.2, vs. 87 ± 7.2 nmol/L for saline). Ketoprofen, however, had no effect on the Band mature bulls, with their cortisol concentrations averaging 54 ± 5.1 nmol/L at 40 min and 92 ± 5.1 nmol/L at 2 h. Cortisol concentrations were also significantly elevated in the Band (83 ± 3.0 nmol/L) compared with Surgical mature bulls (57 ± 3.0 nmol/L) at weeks 2–4 post-castration. The timing of this

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elevation coincided with significantly elevated haptoglobin concentrations (mg/mL) in the Band bulls (2.97 ± 0.102 for mature bulls and 1.71 ± 0.025 for weaners, vs. 2.10 ± 0.102 and 1.45 ± 0.025 respectively for the Surgical treatment) and evidence of slow wound healing and sepsis in both the weaner (0.81 ± 0.089 not healed at week 4 for Band, 0.13 ± 0.078 for Surgical) and mature bulls (0.81 ± 0.090 at week 4 for Band, 0.38 ± 0.104 for Surgical). Overall, liveweight gains of both age groups were not affected by castration method. The findings of acute pain, chronic inflammation and possibly chronic pain in the mature bulls at least, together with poor wound healing in the Band bulls support behavioural findings reported in the accompanying paper and demonstrate that tension banding produces inferior welfare outcomes for weaner and mature bulls compared with surgical castration.

1. Introduction

As indicated in the first paper of this pair (Petherick et al., 2014), tension-banding castration of bulls is gaining favour and this may be a consequence of claims made by manufacturers and retailers of banding devices that banding is humane and less stressful than surgical castration. The experiments were conducted to determine whether this is, indeed, the case by comparing the welfare outcomes for weaner and mature Brahman bulls from castration by surgery and tension banding with or without analgesia. We have found that for farmers to accept scientific findings and modify practices accordingly, they need to perceive that the research is directly relevant to them i.e. it used the same "type" of animal that they farm and was performed in the same "type" of environment in which they farm. Thus, whilst there have been previous evaluations of tension banding of bulls, none have previously used *Bos indicus* cattle in a tropical environment and measured as broad a range of welfare-related parameters as in this study.

In the first paper we reported on the behavioural responses of the bulls to castration by tension banding and surgery, with and without analgesia (a non-steroidal anti-inflammatory drug, NSAID) and drew conclusions about relative welfare based solely on those behavioural responses (Petherick et al., 2014). In this paper we report on other measures that are widely accepted in the scientific community as being indicative of welfare status, in order to augment the behavioural findings. Plasma cortisol concentrations have previously been used as an indicator of pain associated with invasive livestock husbandry procedures (Mellor et al., 2000) and castration of cattle specifically (Bretschneider, 2005). Creatine kinase (CK) is an indicator of muscle damage, stress and fatigue (Braun et al., 1993; Garcia-Belenguer et al., 1996; Knowles and Warriss, 2007) and decreases in both total protein (TP) and pack cell volume (PCV) are indicative of blood loss (Carlson, 1997). We predicted that, due to the cutting and tearing of tissue, cortisol and CK concentrations would initially be higher in surgical than band castrates due to the greater pain and stress, and TP and PCV would decline only in the surgical castrates. Further, although this could not be tested statistically because separate experiments were conducted for the two age cohorts, we predicted greater pain, stress, tissue damage and blood loss in the mature than weaner bulls due to their greater size. We were unsure of the likely longer-term impacts of castration methods on cortisol responses, although there is some behavioural evidence of chronic pain in tension-banded calves (Gonzalez et al., 2010) which would suggest that cortisol concentrations may be elevated for some weeks post-tension banding.

We used ketoprofen as the NSAID as it has previously been shown to be effective for the alleviation of castration-related pain in cattle (Earley and Crowe, 2002; Stafford et al., 2002). We chose to administer it immediately prior to castration in order to simulate what would likely happen in a commercial situation, as this would be a practicable method that would also minimise repeated handling and restraint of cattle unaccustomed to those procedures and allow castration of large numbers of cattle in a short a time as possible. We were aware that it would take some time for analgesia to develop and, thus, there may be no effects due to pain alleviation during castration, and for up to about 1 h post-castration. It was also improbable that analgesia would last for more than 12–24 h post-castration (Landoni et al., 1995) and, thus, it would not impact on the measures after this time.

Haptoglobin is an acute-phase protein indicative of inflammation (Horadagoda et al., 1999). Due to the difference in tissue damage from the castration methods, we predicted greater inflammation with surgical than tension banding castration during the first few days post-castration. Thereafter, haptoglobin concentrations would likely reflect the healing process and any wound infections. We had no reason to believe that these would differ with castration method and, therefore, expected no differences in haptoglobin concentrations or the wound healing process.

Liveweight changes are an important measure as they have implications for the profitability of livestock operations and may, thus, influence adoption of practices. In addition, liveweight changes are also welfare indicators because states such as pain and stress may reduce feeding behaviour e.g., due to a reluctance of cattle to walk (e.g. see Gonzalez et al., 2010), or alter energy partitioning for anabolism and catabolism (Elsasser et al., 2000). We anticipated no difference between the two castration methods given that previous studies comparing tension banding and other castration methods (mainly surgical and burdizzo) have produced mostly consistent findings of no differences in liveweight or average daily gains in bulls (e.g. ZoBell et al., 1993; Chase et al., 1995; Knight et al., 2000; Fisher et al., 2001; Stafford et al., 2002; Pang et al., 2008; Repenning et al., 2013).

2. Materials and methods

2.1. Animals, treatments and general procedures

Full details of the cattle and procedures are given in the accompanying paper (Petherick et al., 2014) but, briefly, studies comparing surgical and tension-band castration of weaner (7–10 months old, mean liveweight ± s.e. at allocation, 217.8 ± 2.93 kg) and mature (22–25 months old, mean liveweight ± s.e. at allocation, 401.6 ± 5.80 kg) bulls were conducted successively in a tropical environment near Rockhampton, Queensland, Australia. The weaner bulls had been handled through the yard complex once prior to the experiment, but the mature bulls had been used in a previous experiment and had been handled through the yard complex at least four times prior to the experiment. On three of these occasions they were restrained in a veterinary crush/chute and given a Bull Breeding Soundness Examination (Entwistle and Fordyce, 2003) which involved semen collection by electro-ejaculation.

For both experiments, bulls were assigned to treatments on the basis of liveweight, scrotal circumference and flight speed (a measure of temperament). There were four treatment combinations of castration method and pain management ($n = 8$ per treatment combination): tension band castration and an intramuscular injection of saline (Band + saline); tension band castration and an intramuscular injection of a non-steroidal anti-inflammatory drug (Band + NSAID); surgical castration and an intramuscular injection of saline (Surgical + saline); and surgical castration and an intramuscular injection of a non-steroidal anti-inflammatory drug (Surgical + NSAID). The tension banding was conducted using the Callicrate Bander (No-Bull Enterprises, St. Francis, Kansas, USA), and surgical castration was conducted according to industry best practice (Newman, 2007), with emasculators used on the mature bulls in order to prevent excessive blood loss. 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, immediately prior to castration.

Due to daylight constraints, castrations were conducted on 2 consecutive days for both the weaner and mature bull experiments (the 16 bulls castrated each day being termed a 'batch'). On the day of castration (day 0), the bulls were maintained in groups of four (blocks) with each block containing one bull of each treatment. Bulls were moved individually into a veterinary crush/chute and restrained by head-bailing and two blood samples (both approximately 8 mL) were taken, via a single jugular venipuncture using 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 (IceTag data logger) was fitted to the left hind leg in accordance with the manufacturer recommendations (IceRobotics, Roslin, Midlothian, Scotland) to remotely monitor aspects of behaviour (these data are presented in the accompanying paper). The bulls were then castrated by the pre-assigned method. When all four animals in the block had been castrated they were moved into small yards with shade, and hay and water

available ad libitum, where they remained until it was time for their next blood sample to be taken, which was 30 min post-castration for the weaners and 40 min post-castration for the mature bulls (the difference was because we were uncertain as to the time needed to restrain the bulls' heads for blood-sampling). For this (2nd) sample, the bulls were kept in the order in which they had been castrated, but for subsequent samples, at 2 h and 7 h post-castration, they were blood sampled in the order that they entered the crush. After blood-sampling they were returned to the same small yard.

At the end of day 0, the blocks were combined and the cattle walked to a small holding paddock 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 batches held in separate holding paddocks. After the day 3 blood sample for the second batch, the batches were combined and grazed paddocks with ample forage of good quality and water available at all times.

2.2. Blood samples

Blood samples were taken on restraint (time 0) and at 30 or 40 min, 2 h and 7 h 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 (in EDTA tubes) 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. Total protein (TP) concentrations were calculated from the mean of the duplicate micro-haematocrit tubes which were read from a refractometer (Bellingham and Stanley Ltd., Tunbridge Wells, Kent, UK). 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 °C 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 according to the method described by Paull et al. (2007). The detection limit of the assay was 5.0 nmol/L. The intra-assay coefficient of variation (CV) for samples containing 34.6, 80.4 and 149.8 nmol/L cortisol respectively, were 10.3, 11.0 and 9.1%. The inter-assay CVs

for the same samples were 12.5, 10.8 and 10.8%, 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 batch B, but for simplicity, these dates will be considered to be 2, 3 and 4 weeks post-castration for all animals.

2.3. Liveweights and wound healing

Liveweights were recorded on days 7, 14, 21, 28 (1 month), 34 (5 weeks), 42 (6 weeks), 56 for the weaners and 57 for the mature bulls (2 months), and 84 (3 months) days post-castration (a day less on each occasion for the second batch of cattle in each experiment). 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 bulls, 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; and 5, wound fully open, moist and pus present. As two cuts were made in the surgically castrated bulls, a score corresponding to the state of the least-healed cut was given e.g. if one cut was part-closed and had pus present then the animal was given a score of 3, even if the other cut was part-closed, dry and without pus.

2.4. Statistical analyses

Generalised linear models (McCullagh and Nelder, 1989) were used to analyse the data, in GenStat (GenStat, 2009). For the analyses, the distributions of the residuals were checked for normality and homogeneity of variances via four-part residual plots (histogram of the residuals, Normal plot, half-Normal plot, and residuals vs. fitted-values plot). For the blood parameters and liveweights over days, the time-series nature was taken into account by an analysis of variance of repeated measures (Rowell and Walters, 1976), 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, TP, 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.

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 75% (12 of 16) of the individual tests (the random expectation for significance would be 5%), and, hence, these time-patterns form the main focus in the results. These analyses were, however, less conclusive for the effect of NSAID. Research findings (Landoni et al., 1995) and manufacturer recommendations on the frequency of administration of ketoprofen indicate that the analgesic effect would likely be present only during the first 12–24 h post-administration. Thus, the cortisol concentrations were re-analysed using data up to 24 h only. These analyses, in addition to those for the parameters that were only measured during the first 24 h, 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% (six of 32) of the individual tests, considerably more than would be expected from random chance alone. Hence, the result of the NSAID effect are presented only to 24 h; thereafter the two levels (NSAID and saline) are pooled as replicates for the castration method treatment, to better determine these patterns over times.

3. Results

3.1. Weaner bulls

3.1.1. Blood/plasma parameters

There was a significant castration method × time interaction ($F_{9,252} = 2.76$; $P = 0.033$; Fig. 1a) for cortisol concentrations; at 2 h post-castration, concentrations were significantly higher in the Band compared with the Surgical animals. At 24 h post-castration, this trend was reversed, with the cortisol concentrations in the Surgical weaners being significantly greater compared with the Band weaners. Thereafter, there were no significant differences between the groups.

There was a significant ($F_{6,167} = 14.43$; $P < 0.001$; Fig. 1b) castration method × 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 bulls. Haptoglobin concentrations in the Band bulls then rose above those of the Surgical at week 2, peaked at 3 weeks post-castration and remained significantly greater than those in the Surgical bulls at 4 weeks post-castration.

Time significantly affected concentrations of CK ($F_{2,56} = 24.31$; $P < 0.001$; Fig. 1c), with increases in all treatment combinations during day 0. At 7 h post-castration both NSAID treatments had significantly higher concentrations of CK compared with the Surgical + saline treatment, with Band + saline being intermediate and not significantly different to any other.
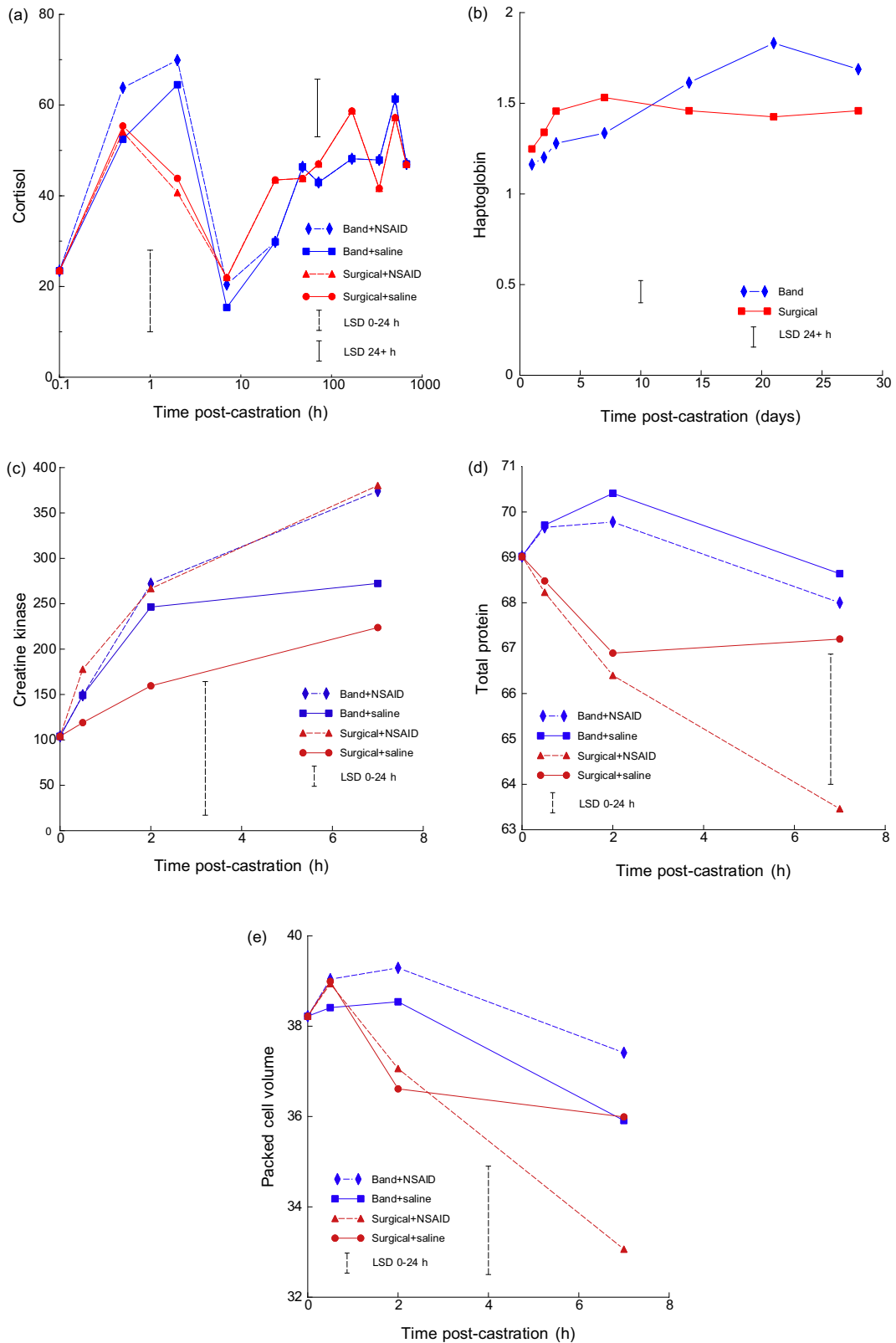
Fig. 1. Mean plasma concentrations in weaner bulls of blood parameters post-castration by tension banding (Band) or surgery (Surgical) and with or without the administration of a non steroidal anti-inflammatory drug (NSAID) immediately prior to castration: (a) cortisol (nmol/L) and (b) haptoglobin (mg/mL) to 4 weeks, and (c) creatine kinase (U/L), (d) total protein (g/L) and (e) packed cell volume (%) on the day of castration.

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

There was a significant castration method × time interaction ($F_{2,56} = 4.44$; $P = 0.027$; Fig. 1e) 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 h post-castration PCV was significantly greater in the Band + NSAID than the 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 h post-castration, the PCV in the Surgical + NSAID cattle was significantly lower than the other three treatments, with no difference between these.

3.1.2. Wounds

There was a significant time × castration method interaction on scrotal circumference ($F_{5,120} = 19.77$; $P < 0.001$; Fig. 2a); 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 ($P < 0.05$) for the first 3 weeks post-treatment before stabilising at a mean of 16 cm for the remainder of the 2 months of measurement.

As anticipated, time affected the number of scrotums present on the Band bulls ($F_{6,91} = 17.12$; $P < 0.001$). The mean (±s.e.) proportion of bulls with scrotums for weeks 1–6 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.

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

There was a significant time × castration method interaction on the proportion of animals with scores of 3–5 ($F_{6,195} = 6.76$; $P < 0.001$) which are indicative of slow healing and/or the presence of infection (Table 1). Proportions were greater for the Band bulls compared with the Surgical bulls at weeks 2–5 post-castration.

3.1.3. Liveweight and liveweight changes

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
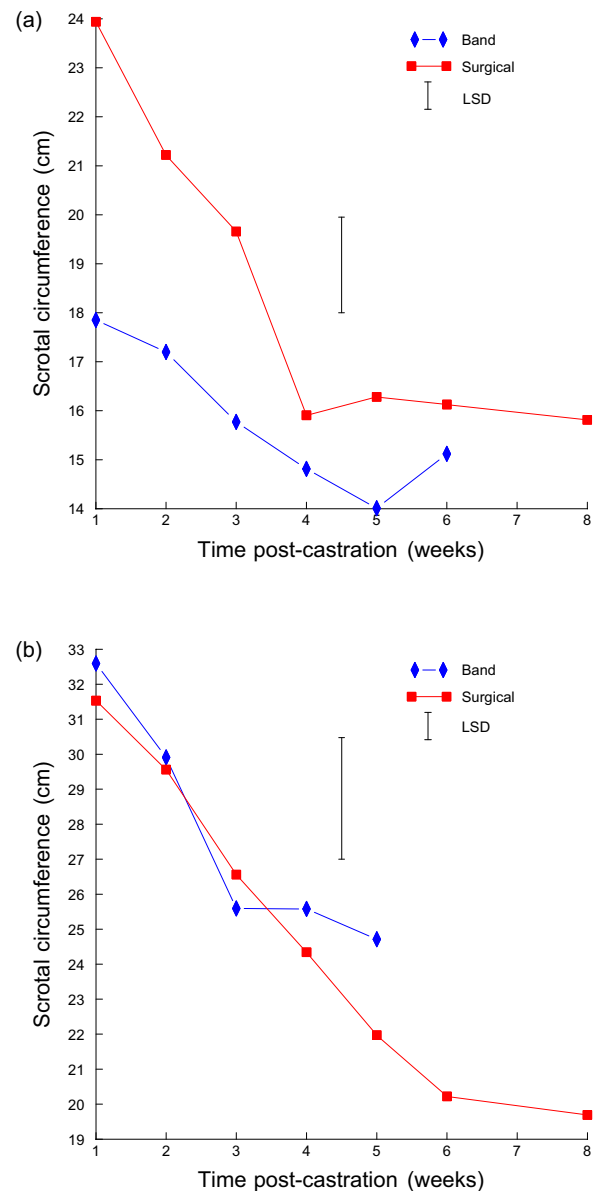
Fig. 2. Mean scrotal circumference of (a) weaner and (b) mature bulls post-castration by tension banding (Band) or surgery (Surgical).

daily gains). Initial liveweight was a significant covariate for final liveweight ($P < 0.001$) and when adjusted for initial liveweights, final liveweights were similarly unaffected by the treatments ($P = 0.40$ for castration method and 0.70 for NSAID administration). As expected, there was a significant effect of time ($P < 0.001$) on adjusted liveweights (Fig. 4a).

3.2. Mature bulls

3.2.1. Blood/plasma parameters

There was a significant NSAID × castration method × time interaction on cortisol concentrations ($F_{9,252} = 2.96$; $P = 0.016$; Fig. 5a). Cortisol concentrations
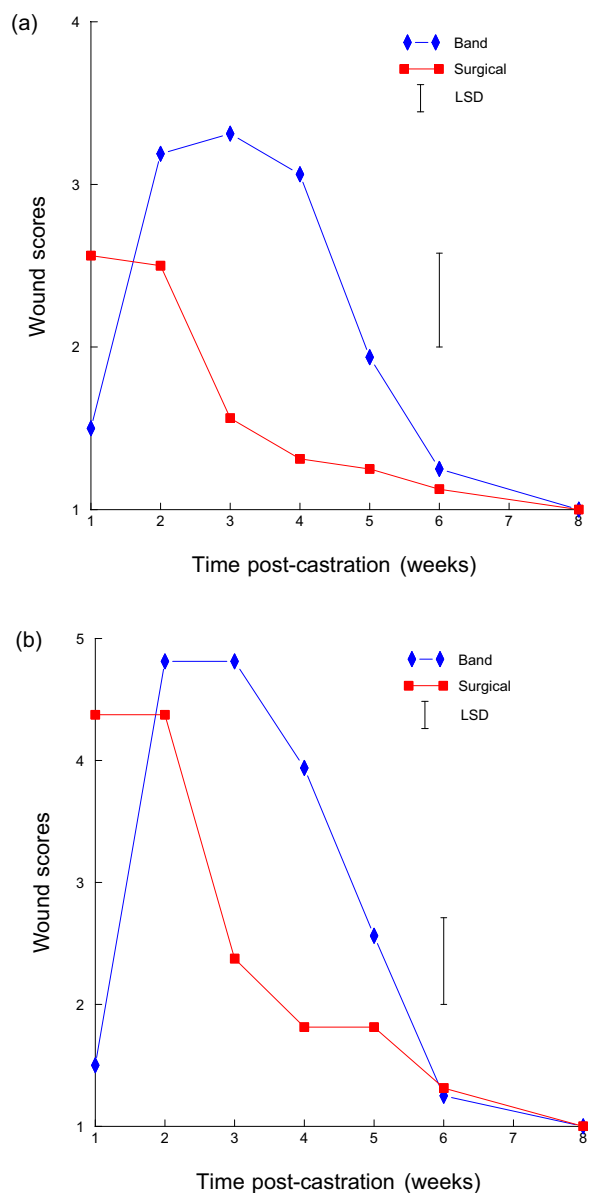
Fig. 3. Scores of castration wounds of (a) weaner and (b) mature bulls castrated by tension banding (Band) or surgery (Surgical). ((1) Wound closed/scabbed, dry and no pus; (2) wound part-closed, dry and no pus; (3) wound part-closed, moist and pus present; (4) wound fully open, moist and no pus present; and (5) wound fully open, moist and pus present).

Table 1

Mean proportion (±s.e.) of tension-banded and surgically castrated weaner and mature bulls with wound scores of 3–5[a] during 8 weeks post-castration.

Week	Tension banded	Surgical
Weaner bulls		
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
Mature bulls		
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

[a] 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.

Concentrations of haptoglobin showed a significant castration method × time interaction ($F_{6,168} = 14.94$; $P < 0.001$; Fig. 5b). 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 2–4 inclusive.

There was a significant NSAID × time interaction for CK concentrations ($F_{2,56} = 4.44$; $P = 0.040$; Fig. 5c). CK concentrations increased during day 0 for all groups, but the rise was greatest in the Band + NSAID bulls, such that, at 7 h post-castration, the concentrations in these animals were significantly greater than in the other three treatments.

Concentrations of TP showed a significant castration method × time interaction ($F_{2,56} = 6.62$; $P = 0.010$; Fig. 5d); at 40 min post-castration the Band + NSAID bulls 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 h post-castration, concentrations were greater in the Band treatments compared with the Surgical treatments.

PCV also showed a significant castration method × time interaction ($F_{2,56} = 4.60$; $P = 0.019$; Fig. 5e); at 2 h 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 h 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.

3.2.2. Wounds

There was a significant time × castration method interaction on scrotal circumference ($P < 0.016$; Fig. 2b); 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 2 months of measurement.
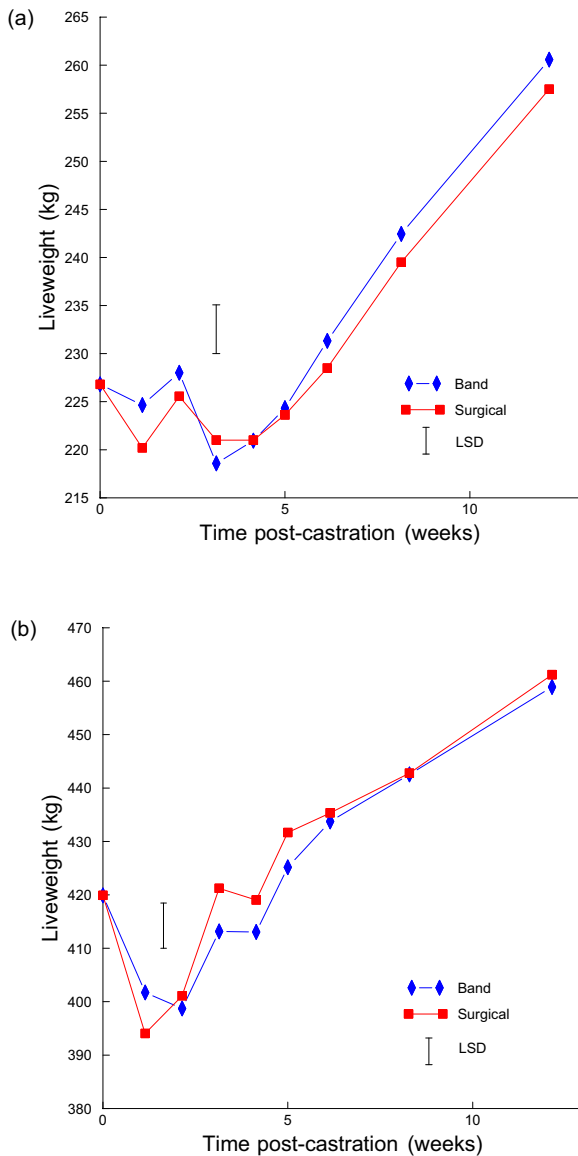
at 40 min post-castration were lowest in the Surgical + NSAID group and highest in the Surgical + saline, with the others intermediate. There were, however, no significant differences between concentrations in Surgical + saline, Band + saline and Band + NSAID, or between Surgical + NSAID, Band + NSAID and Band + saline. At 2 h post-castration, however, there was a significant effect of the ketoprofen in the Surgical + NSAID bulls, 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 bulls.

(a)

(b)

Fig. 4. Mean liveweight changes of (a) weaner and (b) mature bulls post-castration by tension banding (Band) or surgery (Surgical).

As anticipated, time affected the number of scrotums present on the Band bulls ($F_{6,91} = 18.90$; $P < 0.001$). The mean ($\pm$s.e.) proportion of bulls with scrotums for weeks 1–5 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.

There was also a significant time × castration method interaction for wound scores ($F_{6,168} = 33.26$; $P < 0.001$; Fig. 3b). 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 3–5 the score was significantly higher in the Band than Surgical bulls ($P < 0.05$). The Surgical bulls showed a significant

decrease in score from weeks 2–3 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 Band bulls.

There was a significant time by castration method interaction on the proportion of animals with scores of 3–5 ($F_{6,195} = 9.53$; $P < 0.001$) which are indicative of slow healing and/or the presence of infection (Table 1). Proportions were greater for the Band bulls compared with the Surgical bulls at weeks 3 and 4 post-castration.

3.2.3. Liveweight and liveweight changes

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; Fig. 4b). 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 × time interaction ($F_{7,196} = 1.39$; $P = 0.005$) on mean liveweights. Mean liveweight for the bulls given the NSAID (421.2 ± 2.18 kg) was lower than those given saline (428.0 ± 2.18 kg).

4. Discussion

The patterns of plasma cortisol concentrations indicated that, on the day of procedures, tension banding was more painful for the mature bulls than surgical castration; ketoprofen reduced cortisol concentrations in the surgical castrates at 40 min and by 2 h post-castration they were within the normal reference value range (0.47–0.75 μg/dL (approximately 13–21 nmol/L); Radostits et al., 2007). Other mature bull treatment groups and the weaners did not return to these levels until 7 h post-castration. The beneficial effect of the NSAID indicates that the cortisol response was pain-related, rather than a generalised stress response to castration. The lack of an effect of ketoprofen on the cortisol response in the tension-banded mature bulls indicates either its ineffectiveness in relieving pain, or that tension banding evokes a noxious experience which is not wholly due to pain per se. The fact that this noxious experience was not attenuated by ketoprofen has major implications for welfare and indicates that alternative methods for minimising the impact of this procedure need to be developed if tension banding is to be performed on mature bulls. There was also a lack of effect of the NSAID on cortisol concentrations on the day of procedures in the weaner bulls, but at 2 h post-castration, cortisol concentrations were significantly lower in the surgical then tension-banded castrates. This, again, suggests that tension banding was the more noxious process for the weaners and, in contrast to the mature bulls, a generalised stress response may have been involved. The bulls in the two experiments were of the same genotype and had been born and reared in the same environment. Apart from age, the main difference between the cohorts was their experience of handling and restraint; the mature bulls had been moved through the yards, handled and restrained on several
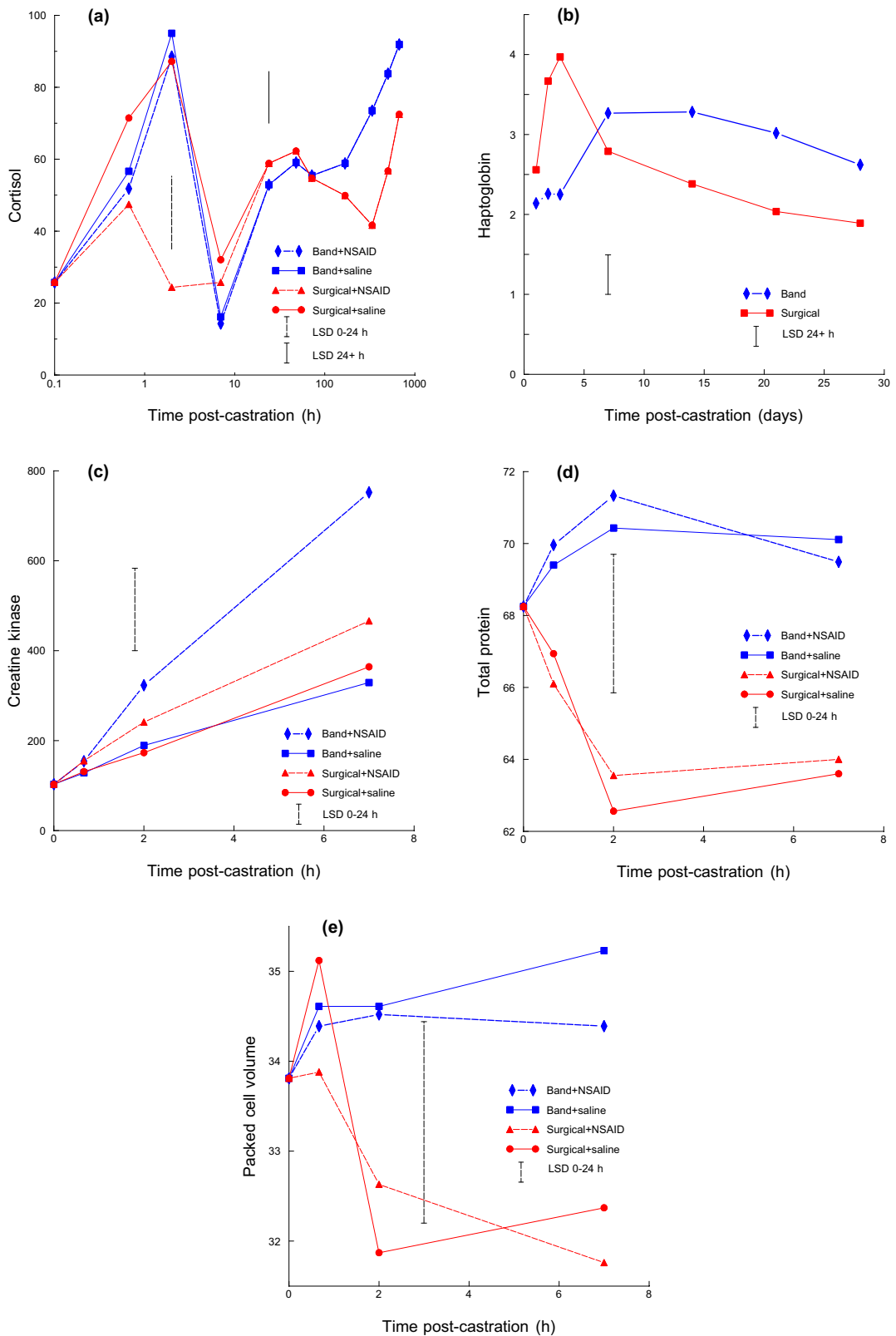
Fig. 5. Mean plasma concentrations in mature bulls of blood parameters post-castration by tension banding (Band) or surgery (Surgical) and with or without the administration of a non-steroidal anti-inflammatory drug (NSAID) immediately prior to castration: (a) cortisol (nmol/L) and (b) haptoglobin (mg/mL) to 4 weeks, and (c) creatine kinase (U/L), (d) total protein (g/L) and (e) packed cell volume (%) on the day of castration.

occasions prior to this study, but the weaners were relatively naïve to handling. 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 (Grandin, 1997) and there is some evidence that cattle unaccustomed to being handled have considerably higher cortisol responses compared with those that are accustomed to it (Mitchell et al., 1988). The cortisol response of the weaner bulls was, thus, likely a combination due to the pain of castration and a stress response to the handling, restraint and blood-sampling.

Our finding for weaners that surgical castration was less noxious than tension banding 2 h post-castration contrasts with the findings of Fisher et al. (2001) who found, using 400 kg bulls, no difference in cortisol concentrations between banded and surgically castrated, or between castrates and entire bulls during the first week post castration. Our findings do, however, agree with the findings of Stafford et al. (2002) who used 100 kg calves. Whilst that study did not make a direct comparison between banding and surgical castration, it did show that peak cortisol concentrations in banded animals (101 nmol/L) were greater than in those surgically castrated with the spermatic cords broken by pulling (68 nmol/L) or by emasculators (56 nmol/L).

Our finding that ketoprofen reduced cortisol concentrations in the surgically castrated, mature bulls is supported by those of others (Earley and Crowe, 2002; Stafford et al., 2002; Ting et al., 2003). The ineffectiveness of ketoprofen in alleviating the pain associated with banding in both age cohorts, however, contrasts with the findings from Stafford et al. (2002) 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. Moya et al. (2011) reported that a single injection of ketoprofen 30 min prior to surgical or banding castration of 6–8.5-month-old calves was ineffective in mitigating pain for both techniques, but experimental details were sparse making the value of the study difficult to interpret.

Subsequent to day 0, when any effect of the NSAID was expected to have worn off, the higher cortisol concentrations found in the surgically castrated weaners at 24 h post-castration indicated greater pain and stress from surgical castration than tension banding at this time. In the mature bulls, however, cortisol concentrations were significantly greater in the tension-banded than surgically castrated bulls at weeks 2–4 post-castration. Previous tension banding castration studies did not find elevated cortisol concentrations in tension-banded cattle at 2 weeks post-castration (Fisher et al., 2001; Gonzalez et al., 2010) and no studies on tension banding, other than the current work, appear to have examined cortisol concentrations beyond 2 weeks post-castration. The elevated cortisol concentrations found in the current study coincided with elevated haptoglobin concentrations. Given that haptoglobin is a sensitive indicator of systemic inflammation in cattle (Horadagoda et al., 1999), it seems highly probable that the bulls were experiencing pain at this time as a consequence of inflammation. Indeed, other research

on ring castration (Molony et al., 1995) and tension banding of calves (Gonzalez et al., 2010) report behavioural changes indicating chronic pain lasting for at least 42 days. We found some behavioural changes in both the weaner and mature bulls at about 3–4 weeks post-castration that we found difficult to interpret (Petherick et al., 2014); the additional data reported here suggest that those behaviours were, indeed, related to chronic pain.

The elevated cortisol and haptoglobin concentrations were reflected in the healing process of the wounds. In both age cohorts and with both castration techniques there were indications of sepsis and delayed healing in some animals, but this was more severe in the banded cattle. The wounds of the banded bulls were scored higher (less healing) than the surgically castrated bulls 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 bulls had dehisced, although areas of bare, granulated skin remained at 8 weeks post-castration in the banded 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 Fisher et al. (2001) 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. Stafford et al. (2002) investigated five different methods of castration and also indicated that wounds healed at slightly different rates, with all those from surgery healed by 9 weeks post-castration in comparison to some banding wounds taking about 13 weeks for full healing.

The onset of inflammation was earlier in the surgical than band castrates; haptoglobin concentrations were higher during the first week post-castration in the surgically castrated cattle compared with the banded bulls and this finding is supported by other work (Fisher et al., 2001; Repenning et al., 2013). The concentrations of haptoglobin measured in the study by Fisher et al. (2001) were extremely low (3–9 µg/mL in the banded cattle and 585–925 µg/mL in surgical castrates) compared to the values we obtained (1–4 mg/mL). Also in contrast to our work, that 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 with the surgically castrated bulls after the first week and remained so at 1 month post-castration. Normal concentrations of haptoglobin are reported to be less than 0.35 mg/mL (Horadagoda et al., 1999), but in both cohorts and throughout the 4 weeks post-castration, concentrations were above this, suggesting on-going inflammation. In the weaner bulls, the initial elevation in haptoglobin coincided with swelling of the scrotum, presumably from inflammation and oedema, although the swelling persisted for 3 weeks. Such swelling has been noted previously and for about the same length of time post-castration (Chase et al., 1995; Rust et al., 2007). Similar swelling was not seen in the surgically castrated mature bulls, but haptoglobin

concentrations were elevated. It is possible that the use of emasculators reduced post-surgical swelling of the scrotum.

The time taken for all scrotums to be lost is variable between studies, and is reported as 5 (Chase et al., 1995), 6 (this study, mature bulls), 7 (this study, weaners), 8 (Knight et al., 2000; Fisher et al., 2001), 9 (Knight et al., 2000) and 12 (Pang et al., 2008) 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 differences in tension achieved with bands (although the model of tension bander we used incorporated a 'notch' to indicate "correct" tension), climatic conditions and environments 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 and 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 with the surgically castrated animals.

The acute cortisol responses support the behavioural responses reported in the accompanying paper (Petherick et al., 2014), although the greater pain in the mature bulls from tension banding compared with surgical castration was not detected through behaviour. Rather, the behavioural responses indicated pain from both methods on the day of procedures, with some evidence of pain and stress continuing on days 1 and 2 post-castration. In line with the cortisol findings, ketoprofen did not affect behavioural responses in the weaner bulls, but had a beneficial effect in the mature surgically castrated bulls. There were behavioural responses in the weaners (tail movements) and the mature tension-banded bulls (reduced time feeding) at week 3 post-castration which were difficult to interpret in isolation, but the cortisol, haptoglobin and wound healing patterns indicate they were likely related to chronic pain.

Regarding other acute (on the day of castration) responses to castration, as CK is an indicator of muscle damage, physical exertion and fatigue in cattle (Braun et al., 1993; Garcia-Belenguer et al., 1996; Knowles and Warriss, 2007), it was unsurprising that concentrations increased on all treatments 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 damage 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; Radostits et al., 2007). It is possible that ketoprofen may

have some specific effect on muscles resulting in the production of CK.

We anticipated that surgical castration would result in blood loss which, if excessive, could have a detrimental impact on welfare. An assessment of TP and PCV is used to evaluate acute fluid and electrolyte changes, with blood loss generally resulting in a decrease in both PCV and TP concentration (Carlson, 1997). In both cohorts TP and PCV declined during the day, but declines were greater in the surgical than band castrates, indicating greater blood loss in the surgical castrates. Although declines also appeared greater in the mature bulls (Fig. 5) than the weaners (Fig. 1) there were not large differences, perhaps indicating the effectiveness of the emasculators in reducing blood loss in the mature bulls. In the weaners, those on 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 (Niemi et al., 1997). 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; Radostits et al., 2007), these declines are of little biological significance. If, however, an animal had low haematocrit before surgical castration then the additional decline in PCV associated with the use of the NSAID could be detrimental.

In this study no differences were found between castration methods in weaner or mature liveweights, which is consistent with other studies that have compared tension banding with other methods (mainly surgical and burdizzo) in bulls ranging from 95 kg (Stafford et al., 2002) to 400 kg (Knight et al., 2000; Fisher et al., 2001; Pang et al., 2008; Repenning et al., 2013) or more (ZoBell et al., 1993; Chase et al., 1995) during experimental periods of 4 (Rust et al., 2007) to 17 weeks (Knight et al., 2000). Inferior liveweight gains in banded compared with surgical castrates in the weeks following castration have been reported for 240 kg bulls (Fisher et al., 2001) and 360 kg bulls (Rust et al., 2007). In just one study, using 275 kg bulls, have superior ADG been found in banded cattle, but this was measured on a carcass weight basis (Booker et al., 2009). The current study did produce an unexpected finding of reduced gains in the ketoprofen-treated mature bulls compared with those given saline. This is difficult to explain given that ketoprofen is effective for a maximum of about 24 h. Furthermore, the finding contrasts with those of Earley and Crowe (2002) who found minimal effects of ketoprofen on intakes and gains in Friesian calves (of approximately 215 kg) and Ting et al. (2003) who 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.

5. Conclusion

Measures relating to pain, stress, morbidity and productivity of Brahman bulls in a tropical environment in response to castration by tension banding or surgery and with, or without analgesia demonstrate that surgical castration delivers superior welfare outcomes for both

weaner and mature bulls compared with tension banding, particularly in relation to chronic inflammation, pain and wound healing. Administration of ketoprofen immediately prior to castration appears to alleviate the acute pain associated with surgical castration, but not the noxiousness of tension banding. Based on these findings, we recommend that tension banding should not be performed on mature bulls unless effective methods can be developed to minimise the noxiousness of the procedure. Cattle that are unfamiliar with handling and restraint show a heightened stress response (as assessed by plasma cortisol concentrations) to castration, restraint and blood sampling which may mask a pain-specific cortisol response and any beneficial effects of an analgesic. Thus, care is needed when interpreting cortisol data collected from minimally handled cattle.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with people or organisations that inappropriately influenced or biased the conduct of the research or the content of this paper.

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References

Booker, C.W., Abutarbush, S.M., Schunicht, O.C., Pollock, C.M., Perrett, T., Wildman, B.K., Hannon, S.J., Pittman, T.J., Jones, C.W., Jim, G.K., Morley, P.S., 2009. Effect of castration timing, technique, and pain management on health and performance of young feedlot bulls in Alberta. Bov. Pract. 43 (1), 1–11.

Braun, J.P., Lefebvre, H.P., Hamliri, A., Kessabi, M., Toutain, P.L., 1993. Creatine kinase in sheep: a review. Rev. Méd. Vét. 144, 659–664.

Bretschneider, G., 2005. Effects of age and method of castration on performance and stress responses of beef male cattle: a review. Livest. Prod. Sci. 97, 89–100.

Carlson, G.P., 1997. Fluid, electrolyte, and acid–base balance. In: Kaneko, J.J., Harvey, J.W., Bruss, M.L. (Eds.), Clinical Biochemistry of Domestic Animals. , 5th ed. Academic Press, San Diego, pp. 485–516.

Chase, C.C., Larsen, R.E., Randel, R.D., Hammond, A.C., Adams, E.L., 1995. Plasma cortisol and white blood cell responses in different breeds of bulls: a comparison of two methods of castration. J. Anim. Sci. 73, 975–980.

Earley, B., Crowe, M.A., 2002. Effects of ketoprofen alone or in combination with local anaesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci. 80, 1044–1052.

Elsasser, T.H., Klasing, K.C., Filipov, N., Thompson, F., 2000. The metabolic consequences of stress: targets for stress and priorities of nutrient use. In: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress. Basic Principles and Implications for Animal Welfare. CABI, Wallingford, pp. 77–110.

Entwistle, K., Fordyce, G., 2003. Evaluating and Reporting Bull Fertility. Aust. Assoc. Cattle Vet., Brisbane, pp. 26–28.

Fisher, A.D., Knight, T.W., Cosgrove, G.P., Death, A.F., Anderson, C.B., Duganzich, D.M., Matthews, L.R., 2001. Effects of surgical or banding castration on stress responses and behaviour of bulls. Aust. Vet. J. 79, 279–284.

Garcia-Belenguer, S., Palacio, J., Gascón, M., Aceña, C., Revilla, R., Mormède, P., 1996. Differences in the biological stress responses of two cattle breeds to walking up to mountain pastures in the Pyrenees. Vet. Res. 27, 515–526.

GenStat, 2009. GenStat for Windows, Release 11.1, 11th ed. VSN International Ltd., Oxford.

Gonzalez, L.A., Schwartzkopf-Genswein, K.S., Caulkett, N.A., Janzen, E., McAllister, T.A., Fieheller, E., Schaefer, A.L., Haley, D.B., Stookey, J.M., Hendrick, S., 2010. Pain mitigation after band castration of beef calves and its effects on performance, behaviour, *Escherichia coli*, and salivary cortisol. J. Anim. Sci. 88, 802–810.

Grandin, T., 1997. Assessment of stress during handling and transport. J. Anim. Sci. 75, 249–257.

Horadagoda, N.U., Knox, K.M.G., Gibbs, H.A., Reid, S.W.J., Horadagoda, A., Edwards, S.E.R., Eckersall, P.D., 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet. Rec. 144, 437–441.

Knight, T.W., Cosgrove, G.P., Death, A.F., Anderson, C.B., Fisher, A.D., 2000. Effect of method of castrating bulls on their growth rate and liveweight. N. Z. J. Agric. Res. 43, 187–192.

Knowles, T., Warriss, P., 2007. Stress physiology of animals during transport. In: Grandin, T. (Ed.), Livestock Handling and Transport. , 3rd ed. CAB International, Wallingford, pp. 312–328.

Landoni, M.F., Cunningham, F.M., Lees, P., 1995. Comparative pharmacodynamics of flunixin, ketoprofen and tolfenamic acid in calves. Vet. Rec. 137, 428–431.

McCullagh, P., Nelder, J.A., 1989. Generalized Linear Models, 2nd ed. Chapman and Hall, London.

Mellor, D.J., Cook, C.J., Stafford, K.J., 2000. Quantifying some responses to pain as a stressor. In: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress. Basic Principles and Implications for Animal Welfare. CABI, Wallingford, pp. 171–198.

Mitchell, G., Hattingh, J., Ganhao, M., 1988. Stress in cattle assessed after handling, after transport and after slaughter. Vet. Rec. 123, 201–205.

Molony, V., Kent, J.E., Robertson, I.S., 1995. Assessment of acute and chronic pain after different methods of castration of calves. Appl. Anim. Behav. Sci. 46, 33–48.

Moya, D., Schwartzkopf-Genswein, K.S., Gonzalez, L.A., Caulkett, N.A., Fireheller, E., Janzen, E., 2011. Effect of a single intramuscular injection of ketoprofen on physiological and behavioural indicators of pain in beef calves following surgical or band castration. Can. J. Anim. Sci. 91, 510 (abstract).

Newman, R., 2007. A Guide to Best Practice Husbandry in Beef Cattle. Branding, Castrating and Dehorning. Meat & Livestock Australia Ltd., Sydney.

Niemi, T.T., Taxell, C., Rosenberg, P.H., 1997. Comparison of the effect of intravenous ketoprofen, ketorolac and diclofenac on platelet function in volunteers. Acta Anaesth. Scand. 41, 1353–1358.

Pang, W.Y., Earley, B., Gath, V., Crowe, M.A., 2008. Effect of banding or burdizzo castration on plasma testosterone, acute-phase proteins, scrotal circumference, growth, and health of bulls. Livest. Sci. 117, 79–87.

Paull, D.R., Lee, C., Colditz, I.G., Atkinson, S.J., Fisher, A.D., 2007. The effect of a topical anaesthetic mixture and systemic flunixin or carprofen on modifying the pain and stress responses of Merino lambs to mulesing. Aust. Vet. J. 85, 98–106.

Petherick, J.C., Small, A., Mayer, D., Colditz, I., Ferguson, D., Stafford, K., 2014. A comparison of welfare outcomes for weaner and mature *Bos indicus* bulls surgically or tension-bander castrated with or without analgesia: 1. Behavioural measures and responses. Appl. Anim. Behav. Sci., http://dx.doi.org/10.1016/j.applanim.2014.05.003.

Radostits, O.M., Gay, C.G., Hinchcliff, K.W., Constable, P.D., 2007. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats, 10th ed. Saunders, Philadelphia.

Repenning, P.E., Ahola, J.K., Callan, R.J., Fox, J.T., French, J.T., Giles, R.L., Peel, R.K., Whittier, J.C., Engle, T.E., 2013. Effects of pain mitigation and method of castration on behaviour and feedlot performance in cull beef bulls. J. Anim. Sci. 91, 4975–4983.

Rowell, J.G., Walters, R.E., 1976. Analysing data with repeated observations on each experimental unit. J. Agric. Sci. 87, 423–432.

Rust, R.L., Thomson, D.U., Loneragan, G.H., Apley, M.D., Swanson, J.C., 2007. Effect of different castration methods on growth performance and behaviour responses of postpubertal beef bulls. Bov. Pract. 41 (2), 111–118.

Stafford, K.J., Mellor, D.J., Todd, S.E., Bruce, R.A., Ward, R.N., 2002. Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. Res. Vet. Sci. 73, 61–70.

Ting, S.T.L., Earley, B., Crowe, M.A., 2003. Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growth, and behaviour. J. Anim. Sci. 81, 1253–1264.

ZoBell, D.R., Goonewardene, L.A., Ziegler, K., 1993. Evaluation of the bloodless castration procedure for feedlot bulls. Can. J. Anim. Sci. 73, 967–970.