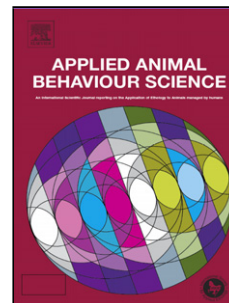## Accepted Manuscript

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

Author: J. Carol Petherick Alison H. Small David J. Reid Ian G. Colditz Drewe M. Ferguson

1 **Welfare outcomes for 3- and 6-month-old beef calves in a tropical environment**

2 **castrated surgically or by applying rubber rings**

3

4 **J. Carol Petherick[a]***, **Alison H. Small[b]**, **David J. Reid[c]**, **Ian G. Colditz[b]** and **Drewe M.**

5 **Ferguson[b]**

6

7 *[a]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation,*

8 *PO Box 6014, N. Rockhampton, QLD 4701, Australia*

9 *[b]CSIRO Agriculture Flagship, Armidale, NSW, Australia*

10 *[c]Dept Agriculture, Fisheries and Forestry, N. Rockhampton, QLD, Australia*

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14 ABSTRACT

15 Castration of cattle using rubber rings is becoming increasingly popular due to the perceived

16 ease of the procedure and greater operator safety when compared with surgical castration.

17 Few comparative studies have investigated the effects of different castration methods and

18 calf age on welfare outcomes, particularly in a tropical environment. Thirty Belmont Red (a

19 tropically adapted breed), 3-month-old (liveweight 71 to 119 kg) and 30, 6-month-old

20 (liveweight 141 to 189 kg) calves were assigned to a two age x three castration (surgical,

21 ring and sham) treatment factorial study (Surg3, Surg6, Ring3, Ring6, Sham3 and Sham6, n

22 = 10 for each treatment group). Welfare outcomes were assessed post-castration using:

23 behaviour for 2 weeks; blood parameters (cortisol and haptoglobin concentrations) to 4

24 weeks; wound healing to 5 weeks; and liveweights to 6 weeks. More Surg calves struggled

25 during castration compared with Sham and Ring ($P < 0.05$, $90 \pm 7\%$ vs. $20 \pm 9\%$ and $24 \pm$

26 10%) and performed more struggles (1.9 ± 0.2, 1.1 ± 0.3 and 1.1 ± 0.3 for Surg, Sham and

27 Ring, respectively), suggesting that surgical castration caused most pain during

28 performance of the procedure. A significant ($P < 0.05$) time x castration method x age

29 interaction for plasma cortisol revealed that concentrations decreased most rapidly in Sham;

30 the Ring6 calves failed to show reduced cortisol concentrations at 2 h post-castration, unlike

31 other treatment groups. By 7 h post-castration, all treatment groups had similar

32 concentrations. A significant ($P < 0.01$) interaction between time and castration method

33 showed that haptoglobin concentrations increased slightly to 0.89 and 0.84 mg/mL for Surg

34 and Ring, respectively over the first 3 days post-castration. Concentrations for Surg then

* Corresponding author.

E-mail address: c.petherick@uq.edu.au; carol.petherick@daf.qld.gov.au

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35   decreased to levels similar to Sham by day 21 and, although concentrations for Ring

36   decreased on day 7 to 0.76 mg/mL, they increased significantly on day 14 to 0.97 mg/mL

37   before reducing to concentrations similar to the other groups (0.66 mg/mL) by day 21.

38   Significantly ($P < 0.05$) more of the wounds of the 3-month compared with the 6-month

39   calves scored as 'healed' at day 7 (74% vs 39%), while more ($P = 0.062$) of the Surg than

40   Ring scored as 'healed' at day 21 (60% vs 29%). At day 14 there were significantly ($P <$

41   0.05) fewer healed wounds in Ring6 compared with other treatment groups (13% vs 40-

42   60%). Liveweight gain was significantly ($P<0.05$) greater in 3-month (0.53 kd/day) than in 6-

43   month calves (0.44 kg/day) and in Sham calves ($P<0.001$, 0.54 kg/day), than in Ring (0.44

44   kg/day) and Surg (0.48 kg/day) calves. Overall, welfare outcomes were slightly better for

45   Surg than Ring calves due to reduced inflammation and faster wound healing, with little

46   difference between age groups.

47   **1. Introduction**

48   Castration is one of the most common routine husbandry procedures conducted in

49   beef production systems and some of the largest of these systems (e.g. North and South

50   America, and Australia) rear calves on extensive rangelands where interactions with people

51   are infrequent. In northern Australia, for example, calves are likely to be mustered

52   (gathered) from paddocks to yards for handling once or twice a year. In addition, many of

53   the large beef producing areas are located in the tropics and sub-tropics where highly

54   seasonal rainfall can temporarily restrict or prevent access to cattle and interfere with the

55   timing of routine management procedures, such as castration (Petherick, 2005). Also,

56   rearing of cattle in tropical environments has resulted in the increased use of tropically

57   adapted breeds, such as Zebu (*Bos indicus*) and Sanga (*Bos taurus africanus*) cattle, and

58   their crosses. There is evidence of differences between cattle breeds in physiological (e.g.

59   Ledger, 1959; Phillips et al., 1987; Arthington et al., 2004) and production (e.g. Hammond et

60   al., 1996; Frisch and O'Neill, 1998) parameters, and behavioural responses (e.g.

61   approachability, Murphey et al., 1980; temperament or reactivity, Hearnshaw and Morris,

62   1984; Fordyce et al., 1988). Thus, it is not unreasonable to suggest that breeds will differ in

63   their responses to routine husbandry procedures.

64   The majority of studies on the welfare impacts of calf castration have been conducted

65   in temperate climates using dairy calves and *Bos taurus* crossbreeds derived from British

66   and European parent stock (e.g. see reviews by Bretschneider, 2005; Coetzee, 2011).

67   Furthermore, from the review by Bretschneider (2005), most experiments on castration have

68   used calves accustomed to confinement, people and handling. Such studies on British or

69   Continental breeds of calf may not provide an appropriate model for investigating the

70   impacts of castration on the welfare of commercial beef calves reared in tropical and sub-

71   tropical environments. Thus, one aim of this experiment was to assess the welfare impacts

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72   of castration on calves that are representative of extensive beef cattle production systems

73   located in the tropics and sub-tropics.

74   The application of rubber rings is perceived to be "a simple, inexpensive and effective

75   method of castration" (Becker et al., 2012) and, as a consequence, the method is, at least in

76   Australia, increasingly being used. Furthermore, manufacturers and retailers promote rings

77   as being the least stressful method of castration (e.g. see

78   http://www.thecattleshop.co.au/category17_1.htm) and also encourage beef producers to delay

79   castration (to 5 to 8 months of age) to exploit the superior liveweight gains and musculature

80   of bulls compared with castrates (e.g. see

81   http://horsleywholesale.com.au/products/Jumbo_Castration_Bander_Delivery-487-113.html).

82   Currently in some parts of Australia it is legally permissible to castrate cattle of any age

83   without the use of anaesthetics or analgesics, although in some States and Territories it is

84   illegal to castrate an animal older than 6 months of age unless it is undertaken by a

85   veterinarian (Primary Industries Standing Committee (PISC), 2004). It is incongruous,

86   therefore, that this same Welfare Code of Practice (PISC, 2004) stipulates that "castration

87   with rubber rings is only recommended for calves up to 2 weeks of age." Thus, the second

88   aim of this study was to compare the welfare outcomes for different ages of calves castrated

89   using rings and surgical castration, the latter being the most common method used in both

90   the USA (Coetzee et al., 2010) and Australia (Meat and Livestock Australia, pers. comm.).

91   Herd management practices in tropical Australia made it impractical to study calves less

92   than 2 weeks of age. In this environment, however, some commercial enterprises castrate

93   calves of 2 to 3 months of age without known adverse effects on welfare, although most

94   enterprises routinely castrate calves at around 6 months of age. Therefore, the age

95   comparison comprised 3 months and 6 months.

96   To assess welfare status we used a combination of measures in line with our

97   previous research on cattle castration (Petherick et al., 2014a, b) and that of other authors

98   (e.g., Robertson et al., 1994; Molony et al., 1995; Stafford et al., 2002). Behavioural

99   responses to pain can be difficult to interpret in isolation due to their variability both between

100   and within individuals (Mellor et al., 2000) and so are best supported by other measures.

101   Although there can be difficulties with interpretation of pain vs. generalised stress (Mellor et

102   al., 2000), plasma cortisol concentration and liveweight changes have been measured to

103   assess the pain and stress associated with castration in many studies (e.g., see review by

104   Bretschneider, 2005). Increases in plasma concentrations of creatine kinase (CK) are

105   associated with muscle damage (Radostits et al., 2007) and changes in total protein (TP)

106   and pack cell volume (PCV) reflect dehydration and blood loss (Carlson, 1997). Thus, we

107   included these measures anticipating that they would assist with assessing welfare status in

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108 animals subjected to a castration method that involves cutting tissue (surgical) and one that

109 does not (application of rubber rings).

110   Our specific hypothesis was that welfare outcomes, as assessed by changes in

111 behaviour, certain blood parameters, wound healing rates and liveweight, would be no

112 different for 3-month-old or 6-month-old calves castrated surgically or using rings.

113

114 **2. Method**

115   The use of the cattle in this experiment was approved by the CSIRO (Queensland)

116 Animal Ethics Committee (approval A1-2012).

117

118 *2.1 Location and experimental design*

119   The experiment was conducted at Belmont Research Station, approximately 26 km

120 north of Rockhampton, Queensland, Australia (150° 22' 57'' E, 23° 13' 26'' S) during the late

121 wet season to early dry season (26 February – 13 April), a time at which commercial

122 enterprises in the region would castrate calves. The range of mean minimum and maximum

123 temperatures were 15.1 – 24.4°C and 25.4-34.7°C respectively, with 218 mm of rainfall (14

124 wet days) during the experimental period.

125   Belmont Red (a stabilised African Sanga X *Bos taurus* hybrid) calves that were born

126 on Belmont Research Station between 30 August and 20 December of the previous year

127 were used for the experiment. The calves were ear-tagged within 24 h of birth and branded

128 in early January, but were not dehorned to eliminate potential confounding due to

129 experience of restraint and pain. Sixty calves were assigned to six treatment combinations

130 (n = 10 per treatment group) according to birth date (3-month or 6-month age group), and

131 stratified within age group by liveweight and flight speed as measured at the time of

132 branding.   Flight speed was measured according to a validated method (Burrow et al., 1988)

133 using specially manufactured equipment (Ruddweigh-Gallagher Animal Management

134 Systems, Campbellfield, Vic, Australia). Three flight speeds, taken in succession, were

135 recorded but as has been found previously, the first speed was poorly correlated with the

136 others (Petherick et al., 2009a), thus a mean of the second and third was used. It was

137 considered important to take into account flight speed in the allocation of the calves to the

138 treatments, as previous work has found relationships between flight speed and stress

139 responses and liveweight gains (Petherick et al., 2002, 2009b).

140   There were six treatment combinations of age of calf (two levels) and castration

141 method (three levels) : sham castration of 3-month-old calves (Sham3); surgical castration of

142 3-month-old calves (Surg3); ring castration of 3-month-old calves (Ring3); sham castration of

143 6-month-old calves (Sham6); surgical castration of 6-month-old calves (Surg6) and ring

144 castration of 6-month-old calves (Ring6). Due to limitations on calf numbers from which to

4

145 select the experimental animals, there was a range of ages within the two age groups; the 6-

146 month-old calves ranged in age from 5 to 7 months and the 3 month-old calves ranged from

147 2.5 to 4 months. Liveweights averaged 163.3 kg (range 141-189 kg) and 93.7 kg (range 71-

148 119 kg) for the 6- and 3-month-old groups, respectively.

149 Due to time and daylight constraints, 30 calves were castrated on 2 successive days

150 (day 0). Calves were allocated to 10 blocks, each containing one animal from each

151 treatment. Five blocks (randomly selected) were treated on each day (batch A and B) with

152 the procedures for the five blocks starting at approximately 7:00, 8:00, 8:45, 9:45 and 11:00

153 h, respectively on both days.

154 *2.2 Procedures*

155 On the day before the experiment started, the calves and their mothers were

156 mustered from their paddock, walked to the yards and cows and calves sorted into the two

157 batches. Calves were weighed and returned to their dams. Batch B cows and calves were

158 returned to their home paddock and batch A held in a small paddock adjacent to the yard

159 complex.

160 On the day of castration (day 0), cows and calves in the batch (A and B) were walked

161 to the yards complex and calves separated from their mothers and sorted into the five block

162 groupings. The cows were retained in the yard complex; the calves could hear, but not see

163 them. Calves were moved individually into a calf cradle, tipped onto their left side and two

164 blood samples (approximately 8 and 4 mL) were taken via a single jugular venipuncture via a

165 20 G needle into vacutainers. Scrotal circumference was measured (Entwistle and Fordyce,

166 2003) and an IceTag3D™ motion sensor device (data logger) was fitted to the right hind leg

167 in accordance with the manufacturer's recommendations (IceRobotics, Roslin, Midlothian,

168 Scotland). All calves were then castrated by the pre-assigned method by one operator.

169 *2.2.1 Surgical castration*

170 Calves were individually restrained in the calf cradle, with additional manual restraint

171 by a person holding the right hind leg. Using a hand-held scalpel blade, the operator

172 conducted the castration according to a beef industry 'best practice' guide (Newman, 2007),

173 using a cut to the scrotum for each testicle. After incision, the scrotum was pulled back to

174 expose the testicle, and the spermatic fascia incised to expose the testis. Once the testis

175 was exposed, the cremaster muscle and proper ligament of the testis were separated from

176 the testis. The testis was then pulled away from animal's body to expose as much of the

177 spermatic cord (incorporating the ductus deferens and the testicular artery and vein) as

178 possible. The cord was roughly severed (to minimise blood loss) as close to the animal's

179 body as possible and proximal to the testicle, away from where a high density of blood

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180 vessels were clearly obvious. Once both testes had been removed, the animal was
181 immediately righted and released to a grassed yard, with the entire procedure (from the start
182 to end of restraint) taking approximately 1 min.

183 *2.2.2 Ring castration*

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

197 *2.2.3 Sham castration*

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

200 *2.2.4 Post-castration management*

201   When all six calves in a block had been treated, the group was either moved into a
202 holding pen at the end of the race to return to the calf cradle for the second blood sample (at
203 30 min post-treatment), or moved to a "home" yard (approximately 50-70 m^2) with shade,
204 and lucerne hay and water available *ad libitum,* until a few min before the next blood sample
205 was due. For the second blood sample the animals were kept in the order in which they had
206 been castrated, but for subsequent blood samples they were bled in the order that they
207 entered the crush. After blood-sampling they were returned to their home yard. Thus, each
208 block of six calves was maintained as a group in a separate yard on the day of castration.
209   After their final blood sample on the treatment day, blocks of calves were returned to
210 the cows being held in the yard complex.   Once all calves were returned to their mothers
211 they were given time to mother-up and then cows and calves were released to a small
212 paddock (approximately 1 ha) adjacent to the yard complex, with pasture and water
213 available *ad libitum*. The following day, cows and calves were walked to the yard complex

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214 and calves separated from their dams for their day 1 blood sample and then returned to their

215 mothers and to the original home paddock. This process was repeated for days 2 and 3 for

216 both batches of cattle, with the batch A being returned to their home paddock on each

217 occasion and batch B being held in small paddocks (each approximately 2.5 ha) adjacent to

218 the yards. After the day 3 blood sample for batch B, the batches were combined into a 6.85

219 ha paddock consisting of Rhodes Grass (*Chloris gayana* var. Callide) pasture. Dry matter

220 availability was estimated to be above 2000 kg/ha at all times and exceeded 4000 kg/ha at

221 first grazing. The cows and calves were rotated through four, similar (in terms of area,

222 pasture-type and DM availability) contiguous paddocks during the period of the experiment.

223 *2.2.5 Blood sampling*

224 Blood samples were taken on restraint (time 0) and at 30 min, 2 h and 7 h post-

225 castration. Samples were collected into EDTA and sodium heparin vacutainers (Becton

226 Dickinson, North Ryde, NSW, Australia) and refrigerated (4°C) until processed. Whole blood

227 samples (those collected into the EDTA tubes) were measured on site, immediately post-

228 collection, for packed cell volume (PCV). Blood was drawn into duplicate micro-haematocrit

229 tubes (Clinilab, Herley, Denmark) and sealed with Seal-Ease (Becton Dickinson, North

230 Ryde, NSW, Australia). The micro-haematocrit tubes were centrifuged (Hawksley, Sussex,

231 UK) for 20 min and the average PCV concentrations calculated from duplicate samples

232 (Hawksley, Sussex, UK). Total protein (TP) and creatine kinase (CK) concentrations were

233 analysed using an automated biochemical analyser (Olympus Reply Biochemistry Analyser,

234 Sydney, NSW, Australia). The sodium heparin vacutainers were centrifuged on the day of

235 collection at 2500 rpm for 20 min and plasma extracted and stored at -20 ºC until plasma

236 cortisol and haptoglobin assays were performed. Haptoglobin concentrations were assayed

237 in the same biochemical analyser indicated above using Tridelta haptoglobin kits (Tridelta

238 Development Ltd., Maynooth, Co. Kildare, Ireland). Plasma cortisol concentrations were

239 determined using a commercial radioimmunoassay (Spectria Cortisol RIA, Orion

240 Diagnostica, Espoo, Finland), adapted and validated for bovine plasma, as described

241 previously (Paull et al., 2007). The detection limit of the assay was 5.0 nmol/L. The intra-

242 assay coefficients of variation (CV) for samples containing 34.6, 80.4 and 149.8 nmol/L

243 cortisol were 10.3, 11.0 and 9.1%, respectively. The inter-assay CVs for the same samples

244 were 12.5, 10.8 and 10.8%.

245 Blood samples were also taken on days 1, 2, 3, 7, 14, 21 and 28 post-castration.

246 With the exception of the day 28 sample, the calves were restrained in the calf cradle. For

247 the final sampling occasion, the calves were restrained via the head-bail in a veterinary

248 crush. This change was made because of the difficulty in restraining some of the 6-month-

249 old calves in the calf cradle, due to their size. Furthermore, three of these older calves (two

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250   Ring6 and one Surg6) had previously sustained leg/shoulder injuries, apparently from

251   vigorous struggling in the cradle. The injury to one Ring6 calf was sufficiently severe that it

252   was not restrained and sampled on days 21 and 28.

253   On these blood sampling occasions, a single sample was collected (into a sodium

254   heparin vacutainer) and samples were handled and stored as described above, for plasma

255   haptoglobin and cortisol assays. Although the two batches of cattle were mixed after day 3

256   they were blood-sampled on successive days for the day 7 sample. Thereafter, the cattle

257   were treated as a single group and, thus, samples taken on days 14, 21 and 28 were

258   technically days 13, 20 and 27 for the batch B calves, but for simplicity, these were

259   considered to be 14, 21 and 28 days post-castration for all calves.

260   *2.2.6 Behavioural recording*

261   Behaviour was recorded during castration by direct observation. Being restrained in

262   a calf cradle, the calves were limited in what behavioural patterns they could show. Counts

263   were scored for individual vocalisations; struggles (a movement back and forth and/or side-

264   to-side in the cradle with head and legs flailing; kicks (a movement of one or both hind legs

265   to the rear, even if manually restrained); and tail flicks (a sideways movement of the tail from

266   vertical and return to vertical).

267   Post-castration on Day 0, blocks of animals were directly recorded, in the yard

268   complex, by 5-min focal animal sampling by two observers using an ethogram developed for

269   studies on the castration of *Bos indicus* bulls (Petherick et al., 2014a; Table 1). Those

270   behaviours having a duration of 5 s or more were categorised as states and other

271   behaviours (lasting less than 5 s) classified as events. States were mutually exclusive and

272   total durations (s) were calculated for each state and the proportion of the total time (300 s)

273   spent in each state determined. Counts of all events were summed for each 5-min

274   observation period. Additionally, the number of 'transitions' between states was scored by

275   counting, for every animal observation, when there was a change of behavioural state.

276   Three calves within a block were randomly allocated to each observer on each

277   occasion, to minimise any bias that may have occurred from the same observer always

278   recording the same calves. The order in which individuals in a block were observed was as

279   they were individually identified by the observers. There was no fixed schedule of

280   observations for each block, rather blocks were observed opportunistically to fit with the

281   blood sampling schedule and the movement of cattle through the yard system. Furthermore,

282   rainfall prevented some block data from being collected. Each block was, however,

283   observed on four to six occasions from immediately post-castration to immediately after the

284   final blood sample at 7 h post-castration. Inspections of plots of the observation times made

285   it clear that there was no bias in the times post-castration that the observations were made.

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286   Behaviour was also recorded by 5-min focal animal sampling on days 1 to 3 post-
287   castration when the cattle were in the two batches in paddocks. With one exception, when
288   recording on day 0 for batch B and day 1 for batch A clashed, these observations were
289   conducted by a single observer. The order in which the animals were recorded was on the
290   basis of locating individuals. Observations were fitted-in with blood sampling and so some
291   were conducted post-blood sampling. Observations were conducted between 7:00 and
292   10:45 h on day 1; 12:45 and 17:00 h on day 2; and 6:45 and 12:45 h (with blood sampling
293   between about 9:00 and 11:00 h) on day 3, for both batches.

294   The percentage of time spent standing and lying and the number of steps taken were
295   automatically determined from the IceTag3D™ data. Some of the loggers (23) were
296   removed at day 14 post-castration because minor lesions had developed under the straps
297   (probably due to the wet weather) and we did not wish to risk leg infections and lameness.
298   The remainder were removed at day 21 post-castration. Three loggers failed to record any
299   data (two Sham3 and one Sham6) and two provided partial recordings (one Ring3 and one
300   Sham3).

301   *2.2.7 Liveweight and wound healing recording*

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

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

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321   Scrotal circumferences were measured (as an indicator of oedema and shrivelling) to

322 day 28 post-castration, at which time the scrotums of all but one ring-castrated calf had

323 dehisced and those of the surgically castrated calves, with one exception, had been scored

324 as fully-healed (score 1).

325 *2.3 Statistical analyses*

326   One calf died and two became lame (see 3.1 below). The calf that died contributed

327 few data; one lame calf was an outlier due to substantial weight loss during the first week

328 post-castration; and the other lame calf was sufficiently injured that we did not collect data

329 after day 14. All data for these three animals were removed.

330 *2.3.1 Behaviour at castration*

331   Counts of vocalisations and struggles during castration were analysed by a two-stage

332 analysis of zero-inflated data. The presence / absence of the behaviour was modelled as a

333 Generalised Linear Model (GLM) with binomial error and logit link function with dispersion

334 fixed at unity. Counts of behaviours for animals exhibiting the behaviour (present) were then

335 modelled as a GLM with Poisson error and log link function with the dispersion parameter

336 estimated. Models included the effects of castration method and age group. As only eight

337 and 11 animals were observed performing tail flicking and kicking, respectively, these

338 behaviours were not analysed.

339 *2.3.2 Behaviour by direct observations on the day of castration*

340   The number of occurrences of behavioural events and the time spent in behavioural

341 states during a sampling period was recorded at various times in the 7 h following castration

342 with times grouped into three periods: 0 to 40 min (0-1 h), 68 to 230 min (1-4 h) and 238 to

343 440 min (4-7 h) post-castration.

344   Behavioural states of walking forward and standing within each time period were

345 modelled with a GLM assuming a binomial error distribution, a logit link function and binomial

346 totals of the recording time in the sampling period (300 s) with the dispersion parameter

347 estimated. Other behavioural states did not occur on sufficient occasions to be analysed.

348   Behavioural events of tail movements (primarily tail flicking), leg movements

349 (primarily leg lifting) and vocalising within each time period post-castration were analysed by

350 a two-stage analysis of zero-inflated data. The presence / absence of the behaviour was

351 modelled as a GLM with binomial error and logit link function with dispersion fixed at unity.

352 Counts of the behaviour for animals exhibiting the behaviour (present) were then modelled

353 as a GLM with Poisson error and log link function with the dispersion parameter estimated.

354 Models included the effects of castration method and age group. Two tail movement records

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355   and a leg movement record were identified as extreme outliers and were excluded from

356   analyses. Other behavioural events did not occur in sufficient numbers within time periods to

357   be analysed.

358   The number of transitions between behavioural states in each of the time periods 0-

359   1, 1-4 and 4-7 h was modelled as a GLM with Poisson error and log link function with the

360   dispersion parameter estimated.

361   *2.3.3 Behaviour by direct observations on days 1 to 3 post-castration*

362   Behavioural states of walking forward, standing (primarily standing alert), lying and

363   feeding were totalled for the 3 days and modelled as a GLM assuming a binomial error

364   distribution, a logit link function and binomial totals of the total sampling time (900 s) with the

365   dispersion parameter estimated. Other behavioural states did not occur on sufficient

366   occasions to be analysed.

367   The behavioural event of tail movements (primarily tail flicking) and the number of

368   transitions were totalled for 3 days and modelled as a GLM with Poisson error and log link

369   function and the dispersion parameter estimated. Other behavioural events did not occur in

370   sufficient numbers within time periods to be analysed.

371   *2.3.4 Behaviour via IceTags*

372   Examination of the data from the IceTag of a Surg6 calf revealed that it was an

373   extreme outlier on all measures recorded (extremely large quantities of data compared with

374   all others) and so the data were not used in the analysis. After 14 days post-castration,

375   there were small experimental numbers for some treatments because the IceTags had been

376   removed from some calves, so analysis was conducted on only the data collected to day 14.

377   IceTag data (percentage time standing, percentage time lying and number of steps) were

378   exported on a per hour basis and further summarised as averages for three periods on the

379   day of castration (0-1, 1-4 and 4-7 h post-castration, in line with direct observations) and for

380   13 consecutive 24-h periods post-castration, where the first period for each animal was the

381   first 24 h post-castration (i.e. the first period included the data from the day of castration).

382   Percentage time standing and lying are reciprocal data, so only percentage time standing

383   data were analysed and are reported.

384   The three periods on the day of castration were analysed separately using restricted

385   maximum likelihood (REML), with a model including the effects of castration method and age

386   group. Data for the 13, 24-h periods post-castration were analysed as repeated measures

387   using REML and modelling the variance-covariance matrix with an unstructured correlation

388   structure. Numbers of steps were log-transformed and percent time standing was arcsine-

389   transformed prior to analysis.

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390 *2.3.5 Blood parameters*

391   Blood parameters on the day of castration (PCV, TP, CK and cortisol) were analysed

392 as repeated measures using REML and modelling the variance-covariance matrix to account

393 for the correlation structure induced by the repeated sampling. A general unstructured

394 covariance matrix was used. Similarly, haptoglobin and cortisol concentrations on the days

395 following castration were analysed as repeated measures using REML with a general

396 unstructured covariance matrix. The corresponding initial blood concentration (time 0

397 sample) was included in the models as a covariate. Inspection of residual plots revealed that

398 CK data were skewed, so were log-transformed prior to analysis. A cortisol concentration

399 was identified as an outlier (Ring6 at 120 min post-castration) because the value was

400 inconsistent with other values for that calf and also about 30 units less than the mean of the

401 treatment group. It was, therefore, treated as missing data. Haptoglobin concentrations for

402 one calf on day 14 and another on day 28 (both Sham6 treatment) were identified as outliers

403 because the values were about twice and three times, respectively, greater than the group

404 means for those days and were inconsistent with the remaining pattern of values for those

405 individuals. These were, also, treated as missing data.

406 *2.3.6 Liveweight, scrotal circumference and wound scores*

407   Liveweight gains from the initial liveweight were calculated for all weights following

408 castration. Liveweight gains and scrotal circumferences post-treatment were analysed as

409 repeated measures using REML and modelling the variance-covariance matrix to account

410 for the correlation structure induced by the repeated sampling. An antedependence structure

411 of order 1 was used to model the correlation structure for both liveweight gains and scrotal

412 circumferences. Initial liveweight or scrotal circumference was included as a covariate.

413   Wound scores for Ring and Surg calves were summarised into two categories: 1 =

414 normal wound healing (scores 1-3) and 2 = delayed wound healing/infection (scores 4-6).

415 Data were then subjected to logistic regression using a GLM with binomial error and logit link

416 function.

417 **3. Results**

418 *3.1 Mortalities and morbidity*

419   One Ring3 calf died between days 2 and 3, after being detected as unwell on the

420 morning of day 2. Post-mortem examination showed nothing overtly abnormal, suggesting

421 that it was unlikely that the treatment or the blood sampling *per se* were the cause of death.

422   Castration wound inflammation and infection was sufficiently severe for three calves

423 to warrant treatment with penicillin (Norocillin L.A., Norbrook Laboratories Australia Pty Ltd.,

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424   Tullamarine, VIC, Australia), injected intramuscularly into the neck at a rate of 4 mL/100 kg

425   liveweight, according to manufacturer recommendations. One Surg6 calf was treated at

426   days 7 and 14 post-castration, and one each of Surg3 and Ring6 at day 21.

427   Two calves (one each of Ring6 and Surg6) were treated for lameness with

428   ketoprofen (Ilium Ketoprofen, Troy Laboratories Pty., NSW, Australia) injected into the

429   anterior of the neck at a rate of 3 mg/100 kg liveweight, according to manufacturer

430   recommendations at day 7 post-castration. As indicated above (2.3), data for the Ring6 calf

431   were removed from analysis, as it lost a large amount of weight during the first week when it

432   was lame, but the Surg6 calf showed no sign of being an outlier. Another Ring6 calf was no

433   longer restrained and sampled after day 14 because of an apparent back injury which

434   caused lameness. These three calves had allocation flight times (time to cover 1.8 m) of

435   between 0.65 and 0.73 s, which were in the fastest 10% of calves.

436   *3.2 Behaviour at castration*

437   The percentage of animals vocalising during castration (29%) did not differ (P > 0.10)

438   among castration treatments, but did differ (Wald = 4.93, 1 df; P < 0.05) between age

439   groups; more 3-month-old calves vocalised than the 6-month-old calves (data presented are

440   mean ± s.e. unless otherwise stated; 41 ± 9% vs. 14 ± 7%). For those that did vocalise,

441   there was no difference (P > 0.10) in the number of vocalisations among castration

442   treatments (average of 2.8 vocalisations) while there was a weak difference (Wald = 4.49, 1

443   df; P = 0.056) between age groups (2.1 ± 0.5 and 5.1 ± 1.6 vocalisations for the 3- and 6-

444   month-old calves, respectively). More Surg calves struggled than Sham and Ring (Wald =

445   16.98, 2 df P < 0.001, 90 ± 7% compared with 20 ± 9% and 24 ± 10%, respectively) and, if

446   they struggled, they performed more struggles (Wald = 6.84, 2 df; P = 0.051; 1.9 ± 0.2, 1.1 ±

447   0.3 and 1.1 ± 0.3 for Surg, Sham and Ring, respectively).

448   *3.3 Behaviour post-castration*

449   In the first hour post-castration, there was a significant castration method x age

450   interaction (Wald = 7.53, 2 df; P < 0.05) on the number of leg movements for calves that

451   performed the behaviour. Surg3 (2.7 ± 1.3) and Ring3 (3.3 ± 1.4) calves performed less leg

452   movements than the Sham3 (6.0 ± 1.2) calves, but Surg6 (3.8 ± 1.1) and Ring6 (5.3 ± 1.5)

453   calves performed more than the Sham6 calves (1.6 ± 1.8). The data from the IceTags

454   revealed that castration method significantly affected the number of steps/h ($F_{2,48}$ = 6.99; P <

455   0.05) and the percentage of time spent standing ($F_{2,48}$ = 27.13; P < 0.001). The Ring calves

456   took more steps/h than the Surg calves, with the Sham intermediate (6.19 (back transformed

457   485), 5.83 (338) and 5.91 (366), respectively; l.s.d. = 0.29). Sham and Surg calves spent

458   about 100% of the time standing compared with the Ring calves that spent about 86% of the

13

459 time standing (transformed means 1.57, 1.51 and 1.19, respectively; l.s.d. = 0.11). More

460 (Wald = 4.52, 1 df; P < 0.05) transitions between behavioural states were performed by 3-

461 month-old than 6-month-old calves (16.3 ± 1.3 vs. 12.6 ± 1.1).

462 In the 1-4 h period post-castration there was a significant interaction between

463 castration method and age for numbers of tail movements (Wald = 7.48, 2 df; P < 0.05) for

464 those calves performing the behaviour (Table 2).   Ring3 calves performed more tail

465 movements (48.7 ± 10.8) than Surg3 (21.3 ± 6.3) and Sham3 (22.7 ± 6.5), but there was no

466 difference in castration method for the 6-month-old calves (19.6 ± 6.8, 30.6 ± 7.5 and 36.7 ±

467 8.2 for Ring, Surg and Sham, respectively). Ring3 calves also performed more tail

468 movements than Ring6 calves. There was a tendency ($F_{2,49}$ = 2.99; P = 0.060) for the Sham

469 calves to spend a greater percentage of time standing (as determined from the IceTag data)

470 compared with both the Surg and Ring calves (1.36 (back-transformed 95%), 1.11 (80%)

471 and 1.14 (82%), respectively; l.s.d. = 0.22).

472 In the 4-7 h period post-castration, castration method significantly (Wald = 8.04, 2 df;

473 P < 0.05) affected the percentage of calves vocalising, with less Surg (58 ± 7.1%) and Ring

474 calves (63 ± 6.9%) compared with Sham (84 ± 5.2%). There was also a tendency (Wald =

475 5.71, 2 df; P = 0.061) for castration method to affect the percentage of time spent walking

476 forwards, with less time spent by the Surg calves (5 ± 1.5%) than both the Sham (11 ± 2.1%)

477 and Ring calves (11 ± 2.2%). There were no effects of treatment on the IceTag-recorded

478 data in this period.

479 During days 1 to 3 post-castration, there were significant castration method x age

480 interactions (P < 0.05) for both the percentage of time spent walking forward (Wald = 6.48, 2

481 df) and the number of tail movements (Wald = 8.24, 2 df) for those calves performing the

482 behaviours (Table 2). The Surg3 calves spent less time walking forward than the Ring3 and

483 Sham3 calves, but there were no differences between castration methods in the 6-month-old

484 animals. Similarly, Surg3 calves performed more tail movements than the Ring3 and Sham3

485 calves with no differences among castration methods in the 6-month-old calves. The IceTag

486 data revealed that Ring calves spent less ($F_{2,49}$ = 4.72; P < 0.05) time standing than Sham or

487 Surg calves (0.814 ± 0.007 (back-transformed 53%), 0.840 ± 0.007 (55%) and 0.838 ±

488 0.007 (55%), respectively) with no difference (P > 0.10) among castration methods in the

489 number of steps taken. Three-month-old calves spent less ($F_{1,49}$ = 13.11; P < 0.001) time

490 standing (0.817 ± 0.006 (back-transformed 53%) vs 0.844 ± 0.006 (56%)) and tended ($F_{1,49}$ =

491 3.66; P = 0.062) to take more steps (5.34 ± 0.03 (208 steps/h back-transformed) vs 5.27 ±

492 0.03 (193 steps/h)) than 6-month-old calves averaged over time periods and castration

493 methods.

14

494   *3.4 Blood parameters*

495   For PCV, TP and CK concentrations, there were no significant interactions between

496   treatments (i.e. castration methods and age groups) and time, or between castration method

497   and age group, and no differences between age groups or among castration methods. Each

498   parameter, however, increased with time, being greatest at 7 h post-treatment. Overall

499   means were 35.55% ± 0.25%, 70.20 ± 0.25 g/L and 6.54 ± 0.09 (691.3 U/L) for PCV, TP and

500   CK, respectively.

501   Cortisol profiles on the day of castration are given in Fig. 1. There was a significant

502   ($F_{4,59}$ = 2.95; P < 0.05) time x castration method x age interaction; concentrations decreased

503   most rapidly in the Sham calves, the Surg and Ring3 calves showed similar, but less rapid

504   declines in cortisol concentrations, while the Ring6 calves failed to show a reduced cortisol

505   response at 2 h post-castration. By 7 h post-castration, however, all treatment groups had

506   similar concentrations (20.4 nmol/L). On days 1 to 28 post-castration there were no

507   significant (P > 0.05) interactions involving time, castration methods and age groups, or

508   differences among castration methods (23.7 ± 1.2, 25.6 ± 1.2 and 26.6 ± 1.3 nmol/L for

509   sham, surgical and ring methods, respectively). Cortisol concentrations were greater ($F_{1,52}$ =

510   8.09; P < 0.01) for 3-month-old than 6-month-old calves (27.2 ± 1.0 vs 23.4 ± 1.0 nmol/L,

511   respectively).

512   Haptoglobin profiles on days 1 to 28 post-castration are shown in Fig. 2. There was

513   a significant ($F_{12,74}$ = 2.47; P < 0.01) interaction between time and castration method;

514   haptoglobin concentrations decreased slightly over time for the Sham calves while levels

515   increased slightly (but not statistically) to 0.89 and 0.84 mg/mL for Surg and Ring over the

516   first 3 days post-castration. Haptoglobin levels for the Surg calves then decreased steadily to

517   levels similar to the Sham calves by day 21. Although the levels for the Ring calves

518   decreased on day 7 to 0.76 mg/mL, they increased significantly on day 14 to 0.97 mg/mL

519   before reducing to levels similar to the other groups by day 21 (0.66 mg/mL).

520   *3.4 Wounds*

521   As anticipated, the number of scrotums present on the Ring calves declined over

522   time with all gone by day 35. All were present at day 3 and day 7, although at day 7 three

523   were broken and the contents lost. At days 14, 21 and 28, 6/19 (31.6%), 14/19 (73.7%) and

524   18/19 (94.7) had dehisced, respectively.

525   Wound scores on day 3 (approximately 90% in category 1, indicative of normal

526   healing) and on days 28 and 35 (all in category 1) had insufficient variation to be analysed.

527   There was no interaction between castration method and age group for wound score at days

528   7 and 21. Significantly (Wald = 4.57, 1 df; P < 0.05) more of the 3-month age group were in

529   category 1 at day 7 than the 6-month age group (74 ± 10% vs 39 ± 11%), while more (Wald

15

530   = 3.48, 1 df; P = 0.062) of the Surg calves had wounds in category 1 at day 21 than the Ring

531   calves (60 ±11% vs 29 ± 11%). Wound score at day 14 differed (Wald = 3.66, 1 df; P =

532   0.056) with both castration method and age group, with fewer of the Ring6 calves in

533   category 1 than the other treatment groups (13% vs 40-60%; Fig. 3).

534   The effect of castration treatment on scrotal circumference differed ($F_{7,89}$ = 27.75; P <

535   0.001) over time (Fig. 4). Scrotal circumferences of Sham calves increased slightly (approx

536   0.5 cm) over the first 21 days post-castration. Circumferences of both the Surg and Ring

537   calves decreased by approximately 4 cm over this period, but the circumferences of the Surg

538   calves were about 4 cm greater than the Ring calves at all times.

539   *3.5 Liveweight gains*

540   Across the 45 days post-castration, liveweight gain differed ($F_{1,59}$ = 6.53; P < 0.05)

541   between age groups, with greater gains in 3-month-old (0.53 ± 0.02 kg/day) than in 6-month-

542   old calves (0.44 ± 0.02 kg/day). Liveweight gain also differed ($F_{2,59}$ = 11.62; P < 0.001)

543   among castration treatments, with greater gains in Sham calves (0.54 ± 0.01 kg/day) than

544   castrated calves, but with no difference between Ring (0.44 ± 0.02 kg/day) and Surg calves

545   (0.48 ± 0.01 kg/day). The castration treatment by age interaction was not significant, nor

546   were interactions between time (the weekly weighings) and method or age (Fig. 5).

547   **4. Discussion**

548

549   Our findings indicated few differences in welfare outcomes between castrated 3-

550   month-old and 6-month-old calves, but the castration method used did affect welfare. During

551   the castration procedures it was clear, from the extent of struggling, that surgical castration

552   caused more pain and discomfort than ring application, which is consistent with findings from

553   other studies (Fell et al., 1986; Thüer at al., 2007) and our own work on tension-banding

554   castration of beef cattle (Petherick et al., 2014a, b).

555   On the day of castration there was evidence from behavioural and cortisol responses

556   that both ring and surgical castration caused pain. Active behavioural responses (e.g.

557   walking and leg and tail movements) tended to be evoked with rings compared with

558   stationary behaviours (e.g. standing) with surgical castration, which agree with our findings

559   comparing surgical and tension-banding castration of beef cattle (Petherick et al., 2014a, b).

560   Interestingly only the Ring6 calves showed increased cortisol concentrations at 2 h post-

561   castration, although this did not coincide with any notable pain-related behavioural

562   responses (both Ring6 and Surg6 showed more leg movement than Sham6 during the first h

563   post-castration). This finding of an elevated cortisol response at 2 h post-castration in the 6-

564   month ring castrates contradicts other studies that have found higher cortisol plasma

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565 concentrations on the day of castration in surgically compared with ring castrated calves

566 (Fell et al., 1986; Robertson et al., 1994; Molony et al., 1995). The calves in these studies

567 were, however, much younger (5 days to 11 weeks of age) than the 6-month age group used

568 in the present study. Furthermore, the relative amounts of pulling and cutting of spermatic

569 cord tissue during surgical castration are likely to contribute to differences observed in the

570 cortisol response in different studies (Stafford et al., 2002). In the current study, peak

571 cortisol concentrations for both castration methods were mostly similar to the averages

572 reported for ring (45 nmol/L), or below those for surgical castration (129 nmol/L; Coetzee,

573 2011), being about 47 nmol/L for Ring and 52 nmol/L for Surg at 30 min post-castration.

574 Concentrations had returned to pre-treatment levels by 7 h post-castration, which is in broad

575 agreement with other research (Fell et al., 1986; Molony et al., 1995; Stafford et al., 2002;

576 Thüer et al., 2007).

577 On the day of castration, due to the degree of tissue damage and blood loss, we had

578 anticipated finding evidence of greater muscle damage (CK, Radostits et al., 2007), and

579 dehydration/blood loss (measures of TP and PCV, Carlson, 1997) in the surgically castrated

580 calves compared with the ring castrates, but this was not the case. There were increasing

581 CK concentrations during the day for all treatments groups and the mean value of 691 U/L

582 greatly exceeded the upper limit of normal values (35-280 U/L for *Bos taurus* cattle,

583 Radostits et al., 2007). It is likely that the high CK concentrations were due to the repeated

584 movement of the calves through the yard complex, tipping and restraint in the calf cradle,

585 and blood sampling. The increasing values of TP and PCV were unexpected, as there was

586 no indication of decreased drinking during the day. As mean values were, however, within

587 normal ranges (PCV 24-46% and TP 57-81 g/L; Radostits et al, 2007), these increases are

588 of little biological significance. Other work has found TP and PCV to be unaffected by

589 surgical or chemical castration (Cohen et al., 1990).

590 We found no differences between treatments in cortisol response on the days after

591 castration, but behavioural responses suggested that the surgically castrated 3-month-old

592 calves were in greater pain and discomfort than the Ring (and Sham) calves on days 1-3

593 post-castration. In contrast, there were no differences in the 6-month-old calves. Coincident

594 with the behavioural responses in the surgical castrates was a rise in haptoglobin

595 concentrations indicative of a systemic inflammatory response, although this occurred with

596 both castration methods and regardless of age. In the longer-term, whilst haptoglobin

597 concentrations steadily declined in the surgical castrates, they were significantly elevated at

598 14 days post-castration in the Ring calves of both age groups. This difference between

599 methods in the pattern of the inflammatory response is supported by the findings of others

600 (Fenton et al., 1958; Molony et al, 1995; Carragher et al., 1997; Warnock et al., 2012). The

601 inflammatory response appeared related to the rate of wound healing as, at days 14 and 21,

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602 there were indications of infection and poor healing in the Ring calves and particularly in the

603 6-month-old calves. This finding that wound healing was faster in the surgical compared with

604 the ring castrates is also supported by other work with 7-week-old (Fenton et al., 1958) and

605 2 to 4-month-old calves (Stafford et al., 2002). Our finding of greater inflammation and

606 poorer wound healing in the Ring6 compared with Ring3 calves may have been a

607 consequence of the rings exerting insufficient pressure on the scrotal neck to create an

608 effective seal and cut off the blood supply in the older, larger calves, as has been suggested

609 by others (Molony et al., 1995; Bretscheider, 2005; Thüer et al., 2007). Other factors,

610 however, are also likely to influence wound development and healing rates, such as climatic

611 conditions and the environment in which the cattle are kept post-castration, which could

612 influence the propensity for contamination and infection of wounds. The numbers of calves

613 requiring treatment for wound inflammation and infection were too small to determine any

614 relationship with calf age or castration treatment. We observed physical damage (punctures

615 and tears) to some scrotums of the ring castrated calves at 7 days post-castration, although

616 these sacs appeared to be among the first to dry-out and dehisce. The increase in the

617 circumference of the scrotal sacs of the Sham calves probably reflected normal testicular

618 growth in contrast to the decrease in size in both the Surg and Ring treatment groups. The

619 size difference (of 4 cm) between these two groups was probably a consequence of both the

620 scrotal sac and contents drying and shrivelling in the Ring calves compared with removal of

621 the testes, but retention of healthy, living scrotal sac tissue in the Surg calves.

622 Normal concentrations of haptoglobin are reported to be less than 0.35 mg/mL

623 (Horadagoda et al., 1999), but in both age groups and throughout the 28 days post-

624 castration, concentrations were above this. Indeed, even the pre-treatment concentrations

625 were elevated above normal. Although haptoglobin is reported to be a sensitive acute-

626 phase protein in cattle indicative of systemic inflammation (Horadagoda et al., 1999), it has

627 been found to be elevated by social and psychological stressors in some species, although

628 not yet determined in cattle (Maes et al., 1997). The temporary separation of calves from

629 their mothers, required for data collection in this study was likely, in itself, to be stressful for

630 the calves (King et al., 1991; Enriquez et al., 2011). It is possible, therefore, that the

631 elevated haptoglobin concentrations we found were a consequence of social stress.

632 Castration reduced liveweight gains compared with the sham castrates, but there

633 was no difference between the surgical and ring methods. This is in agreement with studies

634 previously reviewed (Bretschneider, 2005) and others not included in that review (Fenton et

635 al., 1958; Fell et al., 1986; Warnock et al., 2012). Another study not included in that review

636 reported significantly superior ADG (by about 0.3 kg/day) to weaning (timing not reported) in

637 calves ring castrated at 2 to 3 months of age compared with calves surgically castrated or

638 left intact (Lents et al., 2001). In the current study, 3-month-old calves had superior gains

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639   compared with the 6-month-old calves, which was expected for the castrated calves;

640   Bretschneider (2005) analysed liveweight changes from a number of castration studies

641   which indicates an expected liveweight loss of about 0.15 kg/day for 3-month-old calves and

642   0.3 kg/day loss for 6-month-old calves during the first month post-castration. It is less

643   apparent why we found higher weight gains for the Sham3 compared with the Sham6

644   calves, but it was perhaps related to nutritional plane. A study on milk yield of grazing,

645   primiparous beef cows indicates a decline in milk yield after about 100 days in milk (Grings

646   et al., 2008). Thus, the 3-month-old calves would have been obtaining a greater proportion

647   of their nutritional requirements from milk than the 6-month-old calves. It may have been

648   expected, however, that the 6-month-old calves would have compensated for the reduced

649   nutrient supply from milk by consuming more forage and would have grown at a similar rate

650   to the younger calves (Tedeschi and Fox, 2009).

651   In the current study, one calf died and three others experienced injuries that required

652   treatment, but all appeared unrelated to castration method *per se*. The experiment was

653   conducted during hot, humid and wet conditions, although not necessarily atypical of the

654   weather during which calves may be castrated in northern Australia. The calf that died was

655   the youngest in the experiment, although was not the lightest. It is possible that the

656   combination of the weather, castration (ring) and the repeated restraint and blood sampling

657   were sufficiently stressful that the calf failed to cope. The three calves that were injured

658   were amongst the fastest (flight speed) 10% of the calves, suggesting that their poor

659   temperament may have contributed to their injuries, probably due to their extremely agitated

660   response to being handled and restrained.

661   Some behavioural indicators of restlessness/activity were influenced by calf age,

662   being greater in 3-month-old than 6-month-old calves. Further, during the 4-7 h period post-

663   castration, by which time calves had been separated from their mothers for at least 4 or 5 h,

664   there were higher levels of vocalisation by the Sham calves compared with the castrated

665   calves. Higher levels of restlessness in the younger compared with older calves on the day

666   of castration may have been due to higher motivation to establish contact with their mothers,

667   as a consequence of their greater dependency on the mother for their food supply (Enriquez

668   et al., 2011). The Sham calves possibly vocalised more during the 4-7 h period compared

669   with the Surg and Ring calves as they were experiencing least pain and were, therefore,

670   behaving the most 'normal' of the groups in trying to establish contact with their mothers.

671   Throughout the day, pain-related behavioural responses may have been confounded by

672   competing motivational states; although not studied in cattle, competing motivational states

673   and attentional shifts have been shown to reduce the performance of pain-related

674   behaviours in poultry (Gentle, 2001). Thus, temporary separation of calves from cows may

675   have attenuated the castrated calves' pain-related behavioural responses because the

19

676 calves were motivated to reunite with their dams and had their attention shifted from the

677 pain.

5. Conclusion

679   Castration should be conducted by the procedure that has the least adverse impacts

680 on cattle welfare. Both ring and surgical castration cause pain and stress post-castration

681 and reduce liveweight gains, and there is little evidence of differences between 3- and 6-

682 month-old calves. Thus, provision of pain relief is the preferred option for castration by both

683 methods and for both ages. With ring castration there is evidence of systemic inflammation

684 at about 2 weeks post-castration, but it is not clear if this is painful. In experimental

685 situations where unweaned calves are temporarily separated from their mothers for data

686 collection, pain-related behavioural responses may be attenuated due to calves being

687 motivated to re-establish contact with their dams, which may switch their attention from pain.

688 Competing motivational states and attentional shifts, thus, require consideration when

689 interpreting pain-related behaviours.

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823   Figure captions

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825   **Fig. 1** Profiles of predicted cortisol concentrations on the day of castration, adjusted for pre-castration

826   cortisol concentrations (given at time 0), for 3- and 6-month-old calves sham, surgically or ring

827   castrated. The vertical bar represents the average l.s.d. at P = 0.05.

828   **Fig. 2** Predicted mean haptoglobin concentrations during days 1 to 28 post-castration, adjusted for

829   pre-castration haptoglobin concentrations, for calves sham, surgically or ring castrated. The vertical

830   bar represents the average l.s.d. at P = 0.05.

831   **Fig. 3** Proportion of wounds scored as healing (as opposed to delayed or abnormal healing) during a

832   4-week period post-castration for 3- and 6-month-old calves surgically or ring castrated.

833   **Fig. 4** Predicted mean scrotal circumferences, adjusted for pre-treatment circumference (given at

834   time 0), to day 28 post-castration for 3- and 6-month-old calves sham, surgically or ring castrated. The

835   vertical bar represents the average l.s.d. at P = 0.05.

836   **Fig. 5** Predicted mean liveweight gains, adjusted for initial liveweight, to 46 days post-castration for

837   3- and 6-month-old calves sham, surgically or ring castrated. The vertical bar represents the average
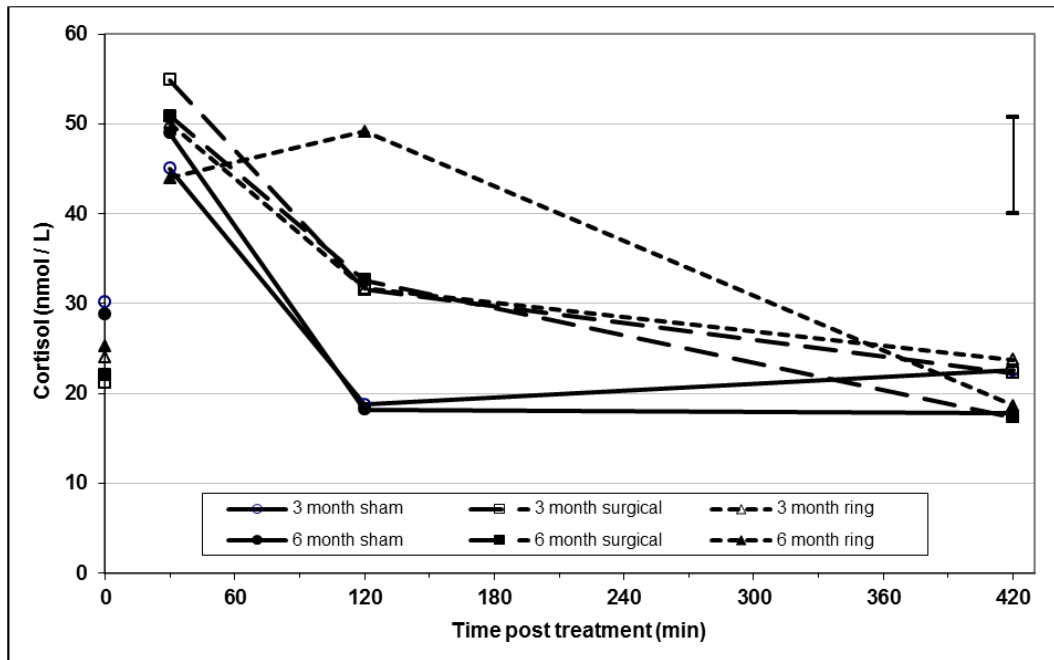
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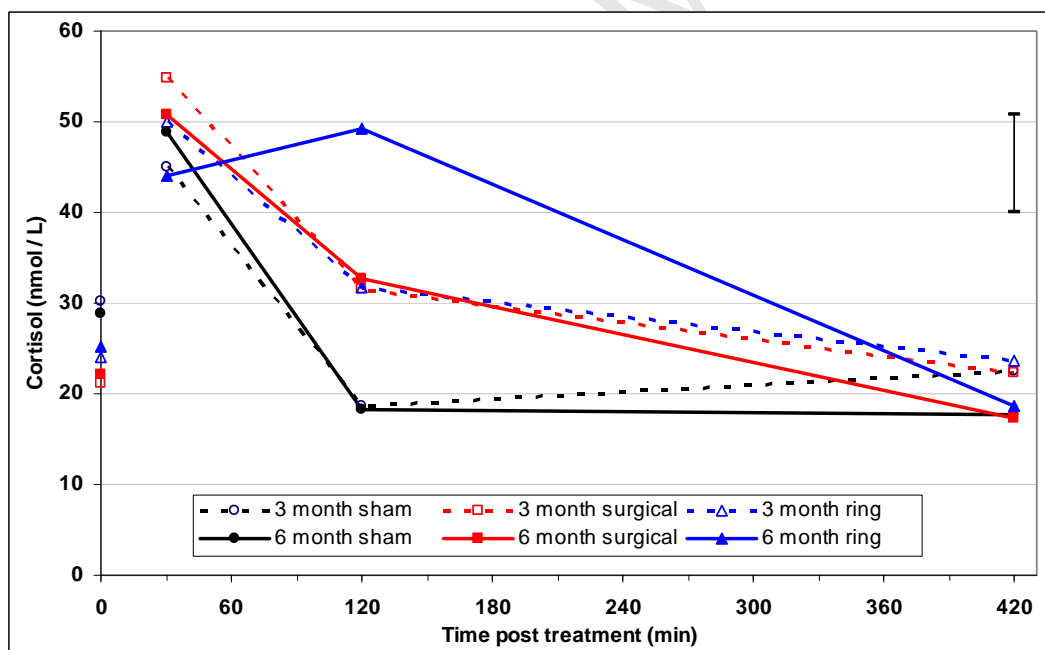
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843   **Fig. 1**
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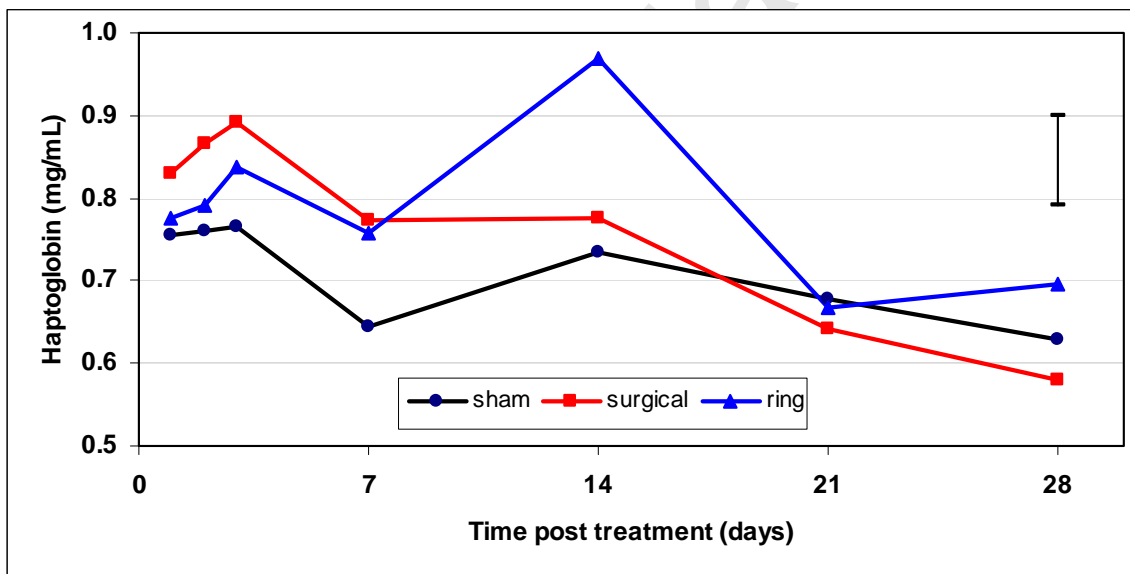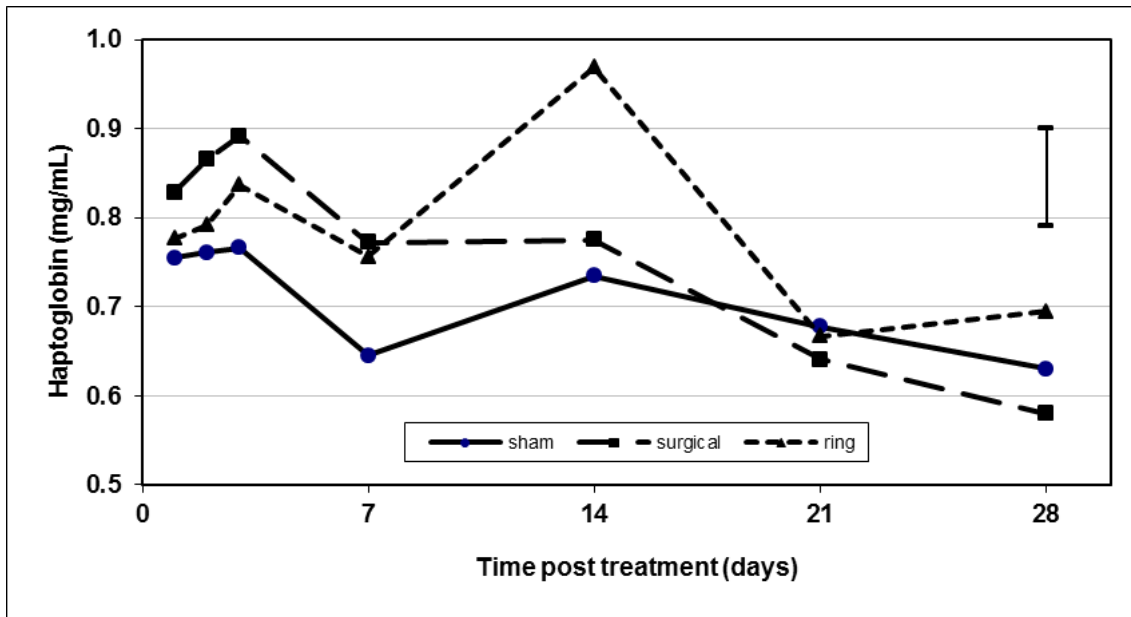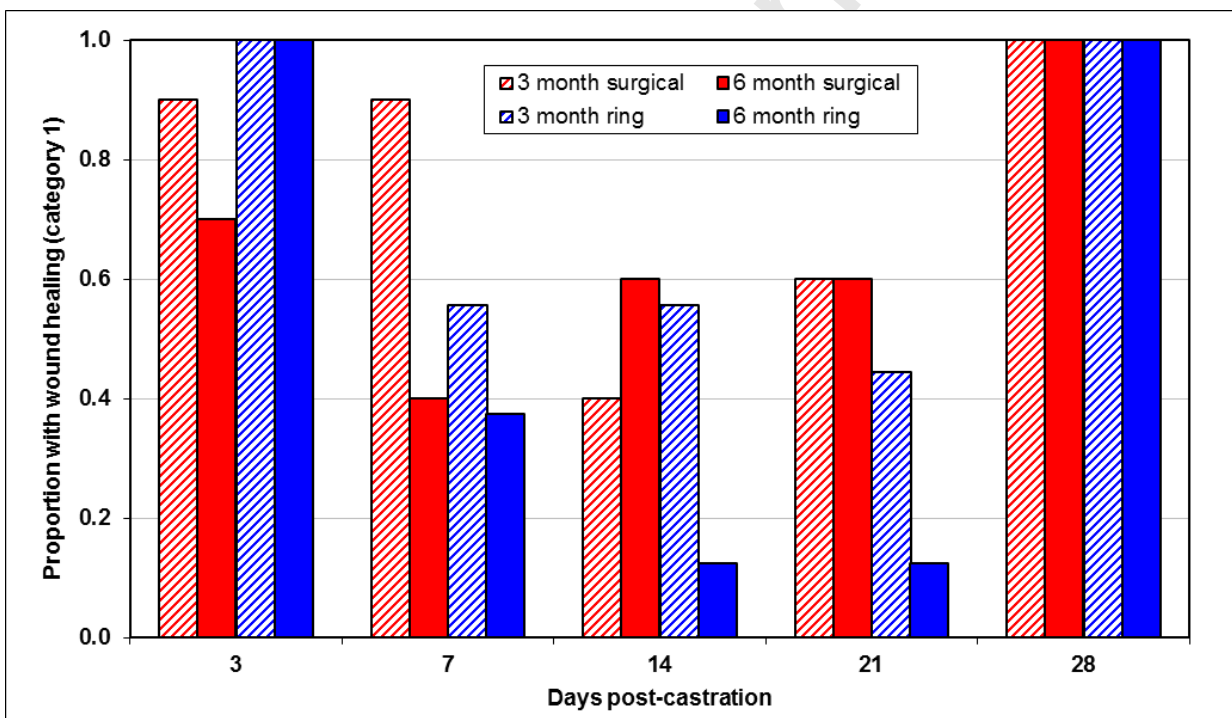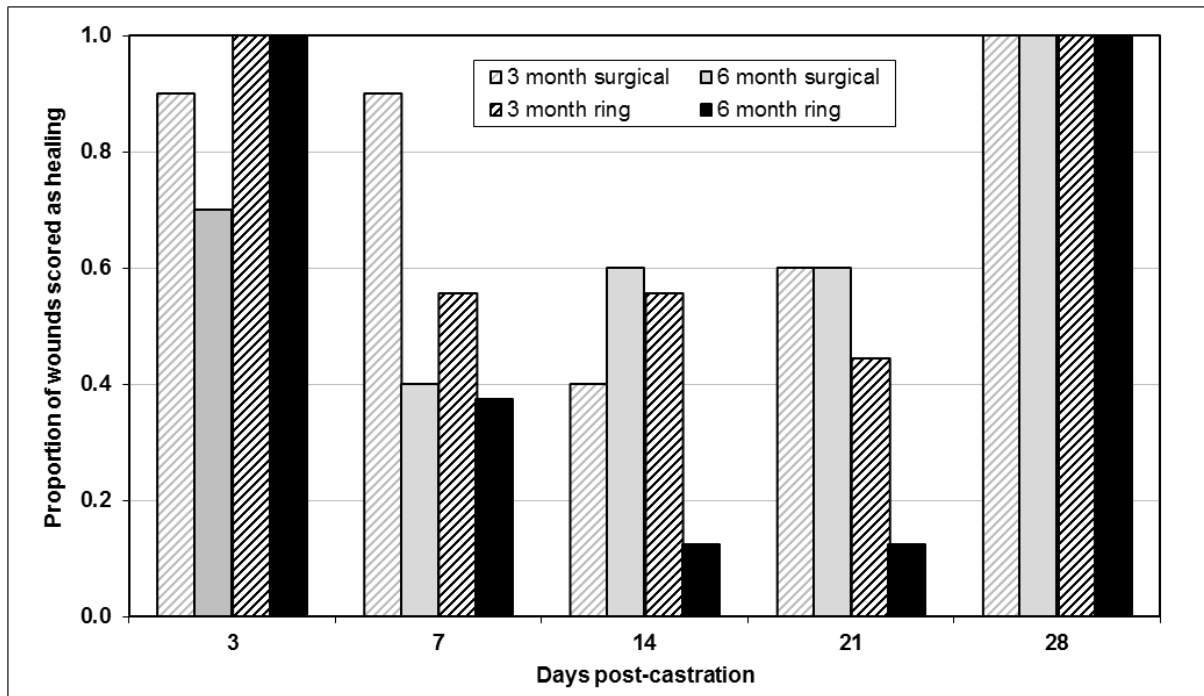852
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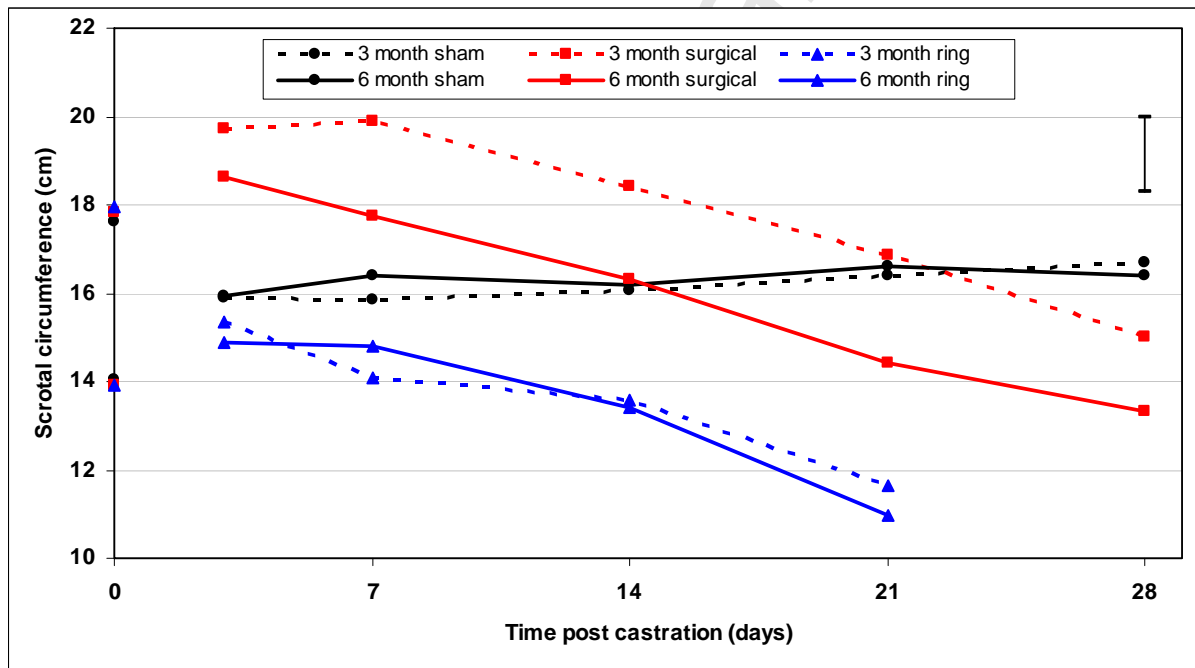
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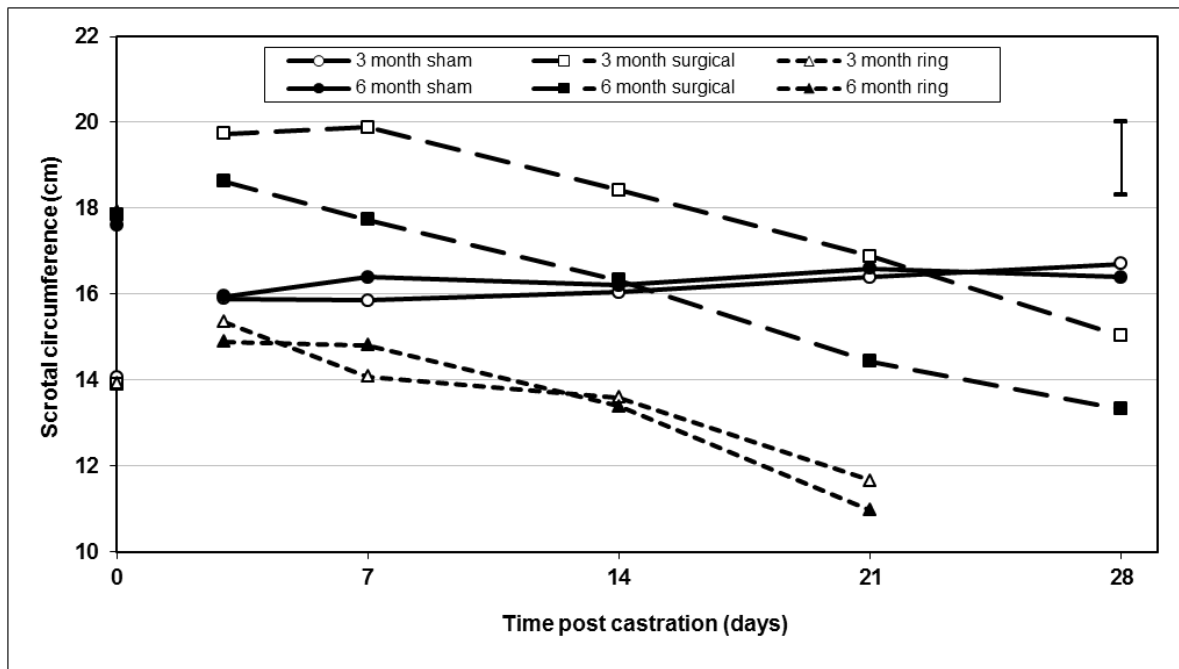
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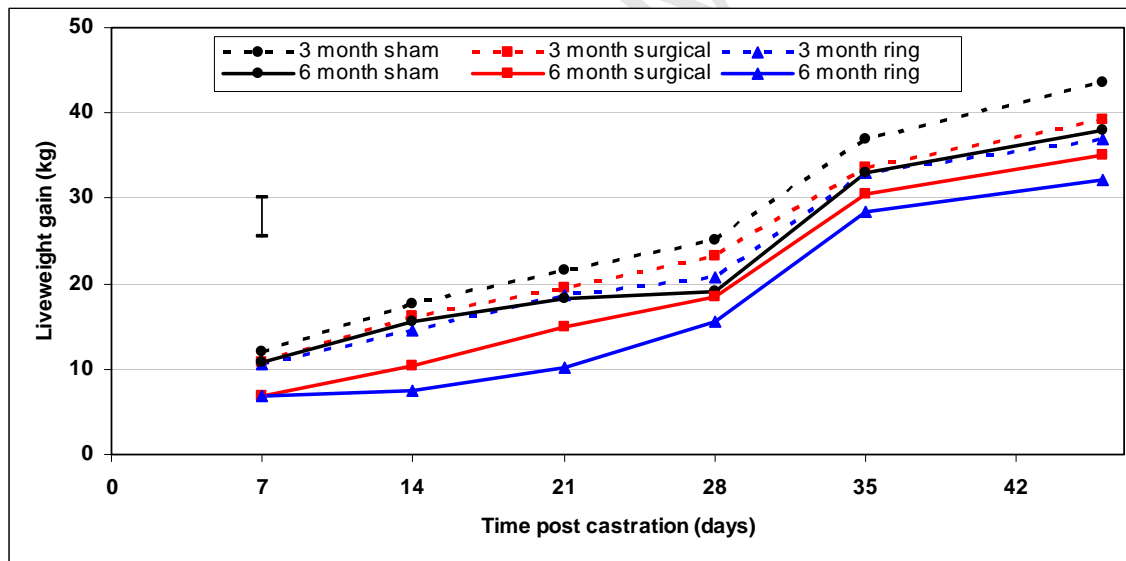
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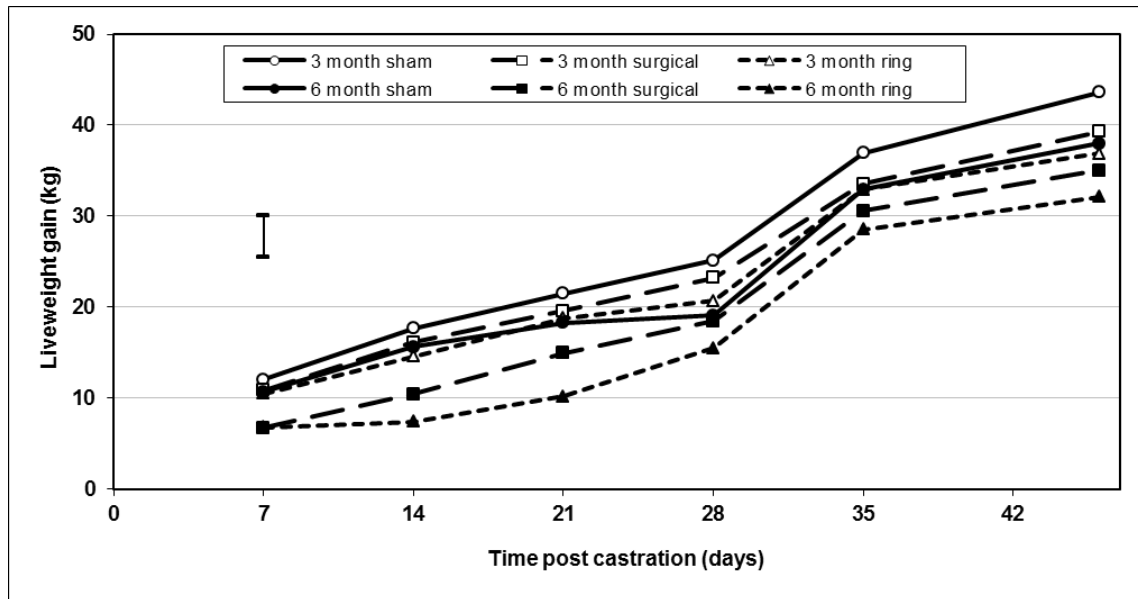
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912   Table 1. Ethogram developed for observations conducted on calves post-castration
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Behaviour	Description	Category
States (durations)		
Stand alert	Standing with muscles tense, head held high, ears pricked, apparently looking at something	Stand
Stand relaxed	Standing with muscles relaxed, head held relaxed, ears loose, apparently not focusing visually	
Stand head down	Standing with head below brisket, looking "depressed" e.g. ears drooped, little/no response to external stimuli	'Abnormal' standing
Stand shaking	Standing with muscle and body tremors	
Lie alert	Lying with muscles tense, head held high, ears pricked, apparently looking at something	Lie
Lie relaxed	Lying on sternum with muscles relaxed, head held relaxed, ears loose, apparently not focusing visually	
Stand ruminating	Standing with slow chewing movements and regurgitations	Ruminate
Lie ruminating	Lying on sternum with slow chewing movements and regurgitations	
Lateral lying	Lying recumbent on side	'Abnormal' lying
Lie neck extended	Lying on the sternum with head and neck extended on the ground	
Walk forward	Forward locomotion (mainly walk, but occasionally trot or gallop)	
Walk backwards	Backwards locomotion (walk)	
Feed	Ingestion (eating hay, grazing, browsing and sucking dam's teats)	
Drink	Ingesting water	
Events (counts)		
Tail flick	Sideways movement of the tail from vertical and return to vertical	Tail movement
Tail tuck	Standing or lying, tail pulled tight between the hind legs and released	
Tail lift	Tail raised and lowered	
Leg lift	Raising and lowering of front or hind foot, may involve a "stamp"	Leg movement

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Kick	Rapid movement of one or both hind legs to the rear or the belly of the animal
Leg Shake	Rapid side-to-side and/or up-and-down motion of a single leg (usually hind)
Vocalisation	Calls emitted by a calf

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921 Table 2. Mean behaviours (± s.e.) performed during days 1-3 post-castration by calves of two ages
922 sham, surgically or ring castrated
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	Sham		Surgical		Ring	
	3 month	6 month	3 month	6 month	3 month	6 month
Time walking forward (% time)	13 ± 3.1	7 ± 2.5	4 ± 1.8	9 ± 2.6	11 ± 3.1	4 ± 2.0
Tail movement (no.)	69 ± 22	66 ± 22	235 ± 41	116 ± 29	47 ± 19	121 ± 33

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928 • surgical and ring castration compared in unweaned 3- and 6-month-old beef calves

929 • during procedures surgical castration was more painful/stressful than applying rings

930 • inflammation and wound healing indicated better welfare in surgical vs ring castrates

931 • few differences between age groups

932 • calf behavioural responses may be confounded by temporary separation from dam

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