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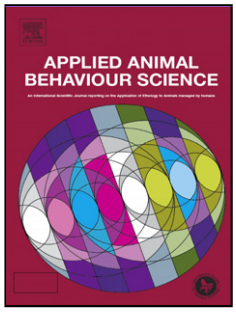

Title: Self-administration by consumption of flunixin in feed alleviates the pain and inflammation associated with castration and tail docking of lambs

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Self-administration by consumption of flunixin in feed alleviates the pain and inflammation associated with castration and tail docking of lambs

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Highlights:

- Analgesia for knife castration and tail docking in lambs was examined
- Voluntary consumption and injection of flunixin were equally effective
- Pain behaviours, abnormal postures and inflammation were reduced by flunixin
- Flunixin in feed is a practical method for alleviating pain in lambs
- Residual signs of pain remained in lambs receiving flunixin

Abstract:

It can be impractical for farmers to provide pain relief to livestock following husbandry procedures such as castration and tail-docking, particularly in pasture-based systems because animals need to be repeatedly gathered to handling facilities and restrained. We investigated whether voluntary consumption by lambs of an analgesic incorporated into feed can achieve pain relief following surgical castration and hot-knife tail-docking. Sixty-four, singleton, male Merino lambs were randomly allocated to one of four treatment groups: sham castration and tail-docking (S), castrated + tail-docked + no pain relief (C), castrated + tail-docked + flunixin in feed (4.0 mg/kg, CF) and castrated + tail-docked + flunixin injection (2 mg/kg, CI). Haematology, cortisol, and plasma haptoglobin concentrations were measured before and up to 48 h after treatment. Lambs also had their scrotal and tail wounds scored based on severity of swelling and wound appearance, with 2 being a healed wound and 8 being severe swelling and evident necrosis and pus. Behaviours were recorded by video for 12 h after treatment. Lambs in the CF and CI groups displayed fewer active pain avoidance behaviours ($P < 0.05$, mean = 3.06 and 3.75 respectively) than C lambs (mean = 6.06) in the first hour following treatment. CF and CI lambs also displayed fewer pain related postures in the 12 h following treatments. All lambs that were castrated and tail-docked had an increase in cortisol

30 mins after treatment (df = 57, $P < 0.05$ for all). The CI group had lower cortisol concentrations by 6 h ($t = 2.17$, 25.02 nmol/L) and CF by 12 h ($t = 1.76$, 33.44 nmol/L) compared with C lambs, however these concentrations were still above basal levels. Flunixin treatment also reduced inflammation, with CF and CI lambs having lower neutrophil/lymphocyte ratios and lower mean wound scores than C lambs. Provision of flunixin in feed was as effective as the flunixin injection in improving behaviour and reducing inflammation in lambs following castration and tail-docking.

Keywords: Pain relief, sheep, welfare, NSAID, animal husbandry, painful husbandry procedures

1. Introduction

Providing pain relief to lambs undergoing painful procedures, such as castration, tail-docking and mulesing is a welfare issue of increasing importance (Phillips et al., 2009). There has been extensive research into using anaesthetics and non-steroidal anti-inflammatory drugs (NSAID) as pain relief for sheep. Lignocaine (Wood et al., 1991; Sutherland et al., 1999), bupivacaine (Graham et al., 1997; Lomax et al., 2008; 2013), carprofen, flunixin (Paull et al., 2007; Paull et al., 2009) and meloxicam (Paull et al., 2012; Small et al., 2014) have been shown to be effective at alleviating the pain associated with painful husbandry procedures. Although NSAIDs are known to be effective, none are registered for use in sheep in the main sheep producing countries in the world. The only pain-relieving drug/substance that is registered for use in sheep in Australia is Tri-Solfen® which is a gel applied to the wound and is currently restricted for use following the mulesing procedure. Tri-Solfen® has been

shown to be effective for alleviating pain in lambs that have been surgically castrated and tail-docked (Lomax et al., 2010) but it is ineffective against ring castration and tail-docking (Paull et al., 2009), due to local anaesthetics having poor skin penetration.

An important consideration is the duration of pain relief provided by local anaesthetics and analgesics. Pain caused by castration and tail-docking lasts several days (Chapman et al., 1994; Melches et al., 2007) whereas the pharmacological effects of most local anaesthetics last only a few hours (Morishima et al., 1979; Mather et al., 1994) while NSAIDs have half-lives ranging between 3 and 30 h (Welsh et al., 1992; Welsh et al., 1993). Therefore, in order to provide adequate pain relief to lambs, it is likely that repeated administration of drugs over several days would be required, which is logistically difficult when large numbers of animals managed in extensive systems are involved.

Currently the lack of registered anaesthetics and analgesics is the biggest limitation in providing sheep with pain relief in Australia but ease of administration is also an obstacle for farmers, as anaesthetics and NSAIDs are commonly administered parenterally and can require veterinarian involvement. An alternative option is buccal administration of analgesics which has been shown to be an effective method of providing pain relief to lambs (Small et al., 2014). However both the methods may require repeated mustering and restraint which is also stressful for the animals.

Administration of analgesics in feed is a potentially practical method for farmers to provide pain relief to lambs over several days. This method of drug administration has been previously explored with NSAIDs in chickens (Danbury et al., 2000; Siegel et al., 2011) and cattle (Odensvik, 1995). We recently showed that sheep have no aversion to consumption of the NSAID flunixin when it is incorporated into a pelleted complete mixed ration and that inferred therapeutic concentration in blood can be reached within 2 h of consumption of the

medicated feed (Marini et al., 2016). Flunixin is a potent NSAID and has been shown to reduce temperature, signs of inflammation and improve behaviour in cows with mastitis (Anderson et al., 1986; Zimov et al., 2011).

The aim of the current research was to test the analgesic and anti-inflammatory efficacy of flunixin administered in a complete mixed ration pelleted feed following castration and tail-docking of lambs. Flunixin injection was used as a positive control to confirm that the computed daily dose of flunixin could elicit behavioural changes that were detectibly different from castrated lambs that received no analgesic. Intramuscular injection is the current method of administration of this drug for most livestock species, however, lambs would require repeat handling for multiple injections to be provided adequate pain relief. We hypothesized that administering flunixin in feed would be as affective in alleviating pain associated with castration and tail-docking as parenteral administration of flunixin.

2. Method and Materials:

The experiment was undertaken at CSIRO's FD McMaster Laboratory, Armidale, New South Wales (NSW), Australia. The protocol and conduct of the experiment was approved by The CSIRO Chiswick Animal Ethics Committee under the NSW Animal Research Act, 1985 (approval ARA 14/21).

2.1. Animals and Treatments

Sixty-four, singleton, male Merino lambs aged approximately 10 weeks were used in this study. Lambs were ear tagged at birth and ewe-lamb pairs allocated to four cohorts based on the lambs' birthdate, with the first 16 lambs born allocated to cohort 1, then next 16 lambs assigned to cohort 2, and so on. Animals were group housed with their mothers and acclimatised to indoor housing for 2 weeks, during which time lambs were caught and handled once a day to reduce subsequent handling stress. After the acclimation period, lambs

were randomly allocated to a treatment balanced for weight and moved with their mothers to smaller pens (4.4 m × 3.0 m). Each pen had four lambs of the same treatment group and for each cohort the treatments were systematically applied to each pen. Lambs within a pen received the same treatment to permit administration of flunixin in feed to all animals within the pen. Previous research has indicated that grouping lambs by castration treatment within a pen, or mixing castration treatments within a pen does not affect the behavioural responses of lambs to castration in this experimental model (Colditz et al., 2012). Castration and tail-docking occurred outside the pen; during the procedure lambs were placed in a restraint cradle for castration and tail-docking which took approximately 1 min to complete. The treatments were as follows:

1. Sham castration and tail-docking (S): The scrotum was handled to simulate surgical castration and the tail handled to simulate gas-knife tail-docking (for 1 min)
2. Castration and Tail-docking (C): Knife castration was performed by cutting off the lower half of the scrotum with a knife then pulling out the testes with the aid of a hook on the knife. Tail docking was performed below the third palpable joint with a gas heated tail docking knife (Scissor action LPG hot knife, TePari Products, Oamaru, New Zealand).
3. Flunixin applied to feed + Castration and Tail-docking (CF); Castration and tail-docking were performed as for C but lambs were provided with feed containing flunixin (4.0 mg/kg, BOVA, New South Wales, Australia) 24 h and 1 h before the procedure. The dose of flunixin was calculated based on the heaviest lamb in the group and the assumption that 1.6kg of a standard pelleted ration was consumed by each ewe-lamb pair. The feed was mixed with the required amount of liquid flunixin to allow an assumed intake of 4mg/kg bodyweight of the lamb. Flunixin was applied

directly to the pellets and mixed through, incorporation being identified by the change in pellet colour.

4. Flunixin injected + Castrations and Tail-docking (CI); Castration and tail-docking were performed as for C but lambs were given an intramuscular injection of flunixin (2.0 mg/kg, Flunixon Injection, Norbrook, Victoria, Australia) 1 h before the procedure.

2.2. Behavioural observations

Behaviours were recorded for 12 h on the day of treatment using two cameras that were mounted to rafters at the end of each pen. The cameras were connected to digital video recorders (WV-CP504, Panasonic Security Camera, North America) and captured by Smartguard software (Pacific Communications, Victoria, Australia). One trained observer conducted the observations and was partially blinded. The observer was aware of the experimental design but did not know the allocation of individual animals to treatment groups at the time behaviours were scored. To identify the lambs, three lambs from each pen were marked with a different coloured paint and one lamb left unmarked. Across pens, marks were randomly associated with treatments. Assessment of behaviour post-treatment was classified into pain avoidance behaviour and postural behaviour (Table 1) using The Observer XT software package (Noldus Ltd., Wageningen, The Netherlands). The pain avoidance behaviour assessment took place every 5 min for 1 min duration during the first hour post castration and tail docking, or sham treatment. Postural behaviour assessment took place every 15 min for 12 h on the day of treatment and were summed within three periods of 4 h duration for analysis. Observation time points were synchronized to each lamb's individual treatment time. The active pain avoidance behaviours and postural behaviours used in this study had been previously validated as indicators of pain in lambs following painful husbandry procedures (Lester et al., 1996; Molony et al., 2002; Paull et al., 2012).

2.3. Blood sampling, cortisol and haptoglobin analysis

Blood was collected by jugular venepuncture using 21 gauge needles into 4.5 mL vacutainers containing EDTA. Samples were collected prior to treatment (0 h) and 30 min, 6 h, 12 h, 24 h and 48 h post-treatment. Neutrophil and lymphocyte counts in whole blood were determined with an automated haematology analyser (Cell Dyn 3500R, Abbott Diagnostics, Illinois, U.S.A). The blood samples were then centrifuged at 2000 × g for 15 min at 5^{o}C and plasma was separated into three aliquots which were then stored at -20^{o}C until assayed for cortisol and haptoglobin concentrations. Plasma cortisol concentrations were determined using a commercial radioimmunoassay (Plasma Cortisol RIA, California, U.S.A.) which has been previously validated for use in sheep (Paull et al., 2007). Coefficients of variation on the quality control plasma samples (50.3, 101.1, 211.7 nmol/L cortisol) were 14.0, 10.6, 11.7% for intra-assay and 16.0, 8.1 and 7.3% for inter-assay, respectively. Haptoglobin was analysed as previously described (Paull et al., 2008b); the inter-plate coefficients of variation for control samples containing (0.120 mg/mL), (0.059 mg/mL) and (0.029 mg/mL) were (4.95%), (6.64%) and (7.55%), respectively.

2.4. Clinical observations

Tail and scrotal wounds were scored and temperature of the wound sites were recorded immediately after every blood sampling time-point. Tail and scrotal wounds were scored individually using a 4-point scale for appearance and swelling (Table 2). The wound temperature was measured using an infrared thermometer (TFA Scantemp 380, TFA, Germany) with a resolution of 0.1 ºC and held 300 mm from the wound surface. These measurements were only conducted on castrated and tail-docked lambs as S lambs did not have a wound or corresponding site to take measurements from.

2.5. Statistical analysis

All data were analysed using R (R Development Core Team, Boston, Massachusetts) and the packages *nlme* (Pinheiro et al., 2015), *pgirmess* (Giraudoux, 2016), *pscl* (Zeileis et al., 2008) were used. Treatment, pen, cohort and interactions were included as fixed effects and animal was fitted as a random effect where appropriate. Data were tested for normality using the Shapiro-Wilk test and visual inspection of residual plots and transformed where necessary. $P < 0.05$ was considered statistically significant and $0.1 > P > 0.05$ was considered a statistical tendency.

2.5.1. Behaviour analysis

Active pain avoidance behaviours were analysed using a general linear model using Poisson regression. For the analysis of data for the category of restless hindquarters, a zero-inflated Poisson model was used due to a high amount of zeros in the data. Postural behaviours were analysed in the same way using non-linear mixed effects model using repeated measures. The frequency of occurrence for individual postures was too low for data analysis, therefore categories had to be combined as given in Table 1.

2.5.2. Blood parameters

Cortisol concentrations required log transformation and were analysed using a repeated measures analysis to determine the relationship between treatment and time-point, fitting pre-treatment values (time 0 h) as a covariate and animal as a random effect. All data from animal 8031 from cohort 1, C treatment were excluded as the base measurement at 0 h was 208.64 nmol/L which was inconsistent with other animals at time 0 h and with other time measurements for that animal. The 48 h measurement (260.82 nmol/L) was also removed for animal 8091 cohort 2, CI treatment as it was inconsistent with previous measurements. The normality of neutrophil/lymphocyte and haptoglobin data was not improved by transformation so those data were analysed by the Kruskal-Wallis test at each time point.

2.5.3. Wound analysis

Wound temperature data were analysed using a repeated measures analysis of the relationship between treatment and time-point. Wound score data could not be normalised by transformation and were analysed using the Kruskal-Wallis rank sum test to test the effect of treatment on wound score.

3. Results:

3.1 *Behaviour post-castration*

Restless hindquarters behaviour was affected by treatment, with lambs in the C group exhibiting this behaviour more (mean = 1.29, $Z = 9.80$, $P < 0.05$) than CF (mean = 0.80, $Z = 4.06$, $P = 0.04$) and S (mean = 0, $Z = -0.73$, $P < 0.05$) lambs. All other active pain avoidance behaviours had to be combined for analysis. In the first hour following castration and tail-docking treatment had a significant effect ($P < 0.001$). Lambs in the S group showed significantly fewer pain avoidance behaviours (mean = 1.50, $Z = 6.89$, $P < 0.05$) than C (mean = 6.06), CF (mean = 3.06) and CI (mean = 3.75). Lambs treated with flunixin in both the CF and CI groups exhibited fewer pain avoidance behaviours (mean = 3.06 and 3.75, Z = -3.89 and -2.93 respectively, $P < 0.05$) compared with lambs receiving no pain relief (mean = 6.06).

Similar results were seen for the abnormal postural behaviours where there was an effect for pen ($P = 0.02$), time ($P = 0.03$) and treatment ($P < 0.001$) but no interaction between time and treatment ($P = 0.15$). Summed across all time periods, C lambs displayed significantly more abnormal postures (mean = 20.7) than CF (mean = 13.6, $Z = -3.35$, $P < 0.001$), CI (mean =12.8, $Z = -3.22$, $P < 0.001$) and S lambs (mean = 9.5, $Z = -3.90$, $P < 0.001$). There was a time by treatment interaction for total lying behaviours ($P = 0.05$), there was no time by treatment interaction for abnormal lying ($P = 0.82$) and total standing ($P = 0.18$) (Table 3).

3.2 *Blood parameters*

There was a treatment effect ($F_{3, 57}$ = 21, P < 0.001) as well as a significant treatment by time interaction ($F_{3, 303}$ = 5, P = 0.003) for cortisol concentrations. At 30 min following treatment, groups C, CF and CI all showed significant increases in plasma cortisol concentration of 10.7, 12.7 and 6.6 nmol/L respectively (df = 57, $P < 0.05$ for all) compared with 0 h (before treatment). S lambs did not show an increase in their cortisol concentration (increase of 1.1 nmol/L, $P = 0.8106$ compared to baseline), and they had significantly lower cortisol concentrations (17.0 nmol/L) compared with C (27.1 nmol/L, $t_{57} = 10.91$, P <0.001), CF (26.8 nmol/L, $t_{57} = 10.88$, P <0.001) and CI (24.7 nmol/L, T = 9.75, P <0.001) lambs 30 min after treatment application. Lambs in the CF group had lower cortisol concentrations than C lambs at 12 h (25.0 nmol/L vs 44.3 nmol/L, $t_{57} = 2.17$, $P = 0.009$) and tended to have lower concentrations at 24 h (33.4nmol/L vs 48.4 nmol/L, $t_{57} = 1.76$, $P = 0.084$). CI lambs had lower cortisol concentrations than C lambs at 6 h (31.8 nmol/L vs 61.6 nmol/L, $t_{57} = 3.16$, $P = 0.002$) but were not significantly different from CF lambs (44.3 nmol/L $t_{57} = 1.58$,). Lambs in the C, CF and CI groups maintained higher concentrations of cortisol than S lambs until 48 h, where S lambs had a significantly increased concentration compared with their baseline (1.9 nmol/L, $t_{287} = 3.24$, $P = 0.0013$, Fig. 1).

For the neutrophil lymphocyte ratio, both treatment (H (3) = 40, P = < 0.05) and time (H (5) = 100, P = < 0.05) had an effect (Fig. 2). All lambs that were castrated and tail-docked had higher neutrophil/lymphocyte ratios at 6 h compared with S lambs (P = < 0.05). At 12 and 24 h, C lambs had significantly higher neutrophil/lymphocyte ratio compared with CF lambs (*difference* = 21.8 and 15.9, respectively) with a critical difference ($\alpha = 0.05$) of 15.8.

Treatment had a significant effect on haptoglobin concentrations (H (3) = 40, P = < 0.05). Lambs in the castrated and tail-docked groups had significantly higher haptoglobin concentrations (*difference* = 78.0 for C; *difference* = 67.3 for CF; and *difference* = 66.1 for CI lambs) when compared with S lambs. The critical difference (α = 0.05) for S and C lambs was 34.8 and for S, CF and CI lambs it was 34.9. The difference between the treatment groups was seen at 24 and 48 h following treatment (H (3) = 30, P = < 0.05 for both time points, Fig. 3) were observed.

3.3 *Wounds*

There was no treatment by time interaction and no treatment effect for tail and scrotal wound temperature (P = 0.90) but there was a time effect for testes wound temperature ($F_{4, 180}$ = 19, P < 0.001). All castrated groups had a significant increase in wound temperature compared to 30 min at 6 h (3.53^{o}C for C; 4.34^{o}C for CF; and 5.39 ^{o}C for CI) but the increase did not differ between treatments. At 12 h and 24 h lambs in the CI group had scrotal wound temperatures that were higher (t_{180}= 2.8, +2.64 ^{o}C, P = 0.006 and t_{180}= 2.1, +1.96 ^{o}C, P = 0.04, respectively) compared with the temperature at 30 min (26.52 ^{o}C). There were no differences in tail wound temperature (Table 4).

For scrotal wound scores there was no treatment by time interaction (P > 0.05); however, an overall treatment effect was seen. Scrotal wound score was significantly affected by treatment (H (2) = 10, P = 0.009). The focused comparisons of the mean ranks between the groups showed that scrotal wound scores for CF lambs were significantly lower (*difference* = 32.8) compared with C lambs (H (2) = 9, P = 0.009). For scrotal wound score the critical difference (α = 0.05) was 24.6. For tail wound scores a time effect (P < 0.001) and treatment effect (P = 0.005). Compared to the 30 min scores (critical difference (α = 0.05) was 35.4), scores at 24 h and 48 h were higher (49.6 and 85.3 respectively). For treatment both CF

(*difference* = 31.1) and CI (*difference* = 31.0) had significantly lower scores than C lambs (*H* (2) = 10, *P* = 0.005). The critical difference (α = 0.05) was 24.6 (Table 4).

4 Discussion

The results of this study indicated that voluntary consumption of flunixin in a complete mixed ration was as effective as flunixin administration by intramuscular injection for reducing the pain and inflammation associated with castration and tail-docking. Flunixin treatments (CF and CI) ameliorated physiological response over the 48 h post-treatment period and ameliorated behavioural responses for the 12 h post-treatment period examined in the study. Nonetheless, residual signs of pain were evident as indicated by CF and CI lambs having elevated cortisol concentrations compared to baseline and displaying more abnormal behaviours compared with S lamb, during the period when maximal blood concentrations of the drug would have occurred (30 min - 2 h) (Welsh et al., 1993; Marini et al., 2016) .

Lambs in CF and CI groups exhibited less pain avoidance behaviours in the hour following treatment compared with C lambs, but their behaviour still differed from S lambs. For postural behaviours, lambs in the CF group spent more time lying in the first 4 h following treatment and their behaviour was similar to S lambs, however CF lambs displayed significantly more abnormal postures compared with S lambs in the first 4 h. In the 4 to 8 h following treatment CF and CI lambs lay down more than C lambs. Normal lying is considered to be a sign of comfort, whereas standing following surgical castration and tail-docking is seen as an attempt to avoid pain (Molony et al., 1993). Previous pen and field studies have also shown increased lying in lambs that received NSAIDs following surgical castration (Paull et al., 2009; Small et al., 2014).

There was a large increase in cortisol concentrations at 30 min in all castrated groups, as previously seen following knife castration (Paull et al., 2008a; Paull et al., 2009). In accord

with these previous studies on the effects of NSAIDs on the acute cortisol response to castration, flunixin treatments had no effect on this acute cortisol response in the current study. A similar lack of effect of flunixin on cortisol responses has also been seen following mulesing (Paull et al., 2007). Lambs in the CF and CI groups had a quicker reduction in cortisol concentration compared with C lambs, however they did not return to pre-castration baseline within the study period. This may have been due to repeated blood sampling, as S lambs also showed a gradual rise in plasma cortisol concentrations to 48 h post-treatment. It has previously been observed that the acute cortisol increase following mulesing is reduced by a combination of flunixin (2.5 mg/kg) with topical local anaesthetic and is abolished by a combination of the NSAID carprofen (4.0 mg/kg) with topical local anaesthetic (Paull et al., 2007). Similar results have been found following surgical castration in cattle where the use of ketoprofen or flunixin alone was not as effective at reducing or preventing the cortisol response as the combination of these NSAIDs with a local anaesthetic (Earley and Crowe, 2002; Webster et al., 2013). Together the results in mulesing in sheep and castration in cattle demonstrate that cortisol is an important indicator of the effectiveness of analgesia following surgical husbandry practices and that failure of flunixin to reduce the cortisol peak at 30 min in the current study is one indicator of residual pain in these lambs.

Neutrophil/lymphocyte ratios are often used as an indicator of inflammation in sheep following painful husbandry procedures. In a study conducted by Paull et al. (2009) administration of an NSAID was shown to reduce the neutrophil/lymphocyte ratio following castration and tail-docking. Flunixin is a cyclooxygenase inhibitor (Bryant et al., 2003) which leads to a reduction in the production of the pro-inflammatory mediator $PGF_2\alpha$ (Cheng et al., 1998; Ricciotti and FitzGerald, 2011). In our study, lambs in the CF group also had a reduced neutrophil/lymphocyte ratio at 12 and 24 h post-treatment compared with C lambs. The reduction in neutrophil/lymphocyte ratio was not significant for CI lambs and it is possible

that the differences between CF and CI can be attributed to the higher dose given to lambs in the flunixin oral group or the effect of continued consumption of flunixin in feed on blood concentrations of the drug. Reduction in inflammation in lambs receiving flunixin compared with C lambs was also indicated by the lower scrotal wound score for CF lambs and tail wound score for both CF and CI lambs. The increase in the acute phase protein, haptoglobin in all groups that were castrated and tail-docked at 24 and 48 h following the procedure, indicated that the animals were affected by tissue damage, inflammation and stress of the procedure (Pfeffer and Rogers, 1989; Petersen et al., 2004).

The lambs that received flunixin through feed were offered the feed 24 and 1 h before castration and tail-docking at double the dose rate recommended for intramuscular administration (4.0 mg/kg vs 2.0 mg/kg). The parenteral dose rate was doubled for oral administration as the rumen can reduce the bioavailability of NSAIDs up to 40% (Odensvik, 1995; Mosher et al., 2012). Lambs were still drinking milk from their mothers at the time of the experiment and had access to their mothers following castration and tail docking, thus transfer of flunixin in milk might have been an additional source of drug for the lambs. The 5-hydroxy metabolite of flunixin is found in milk (38 ppb) following systemic treatment of dairy cows (2.0 - 2.1 mg/kg), although concentrations in milk decline rapidly (Feely et al., 2002). Kissell et al. (2012) reported flunixin metabolite concentrations in milk of 0.044 ug/mL at 1.5 h after intravenous administration in dairy cows with none being detected at 48 h. However, following intramuscular and subcutaneous administration (1.1 mg/kg) at 48 h, concentrations of 0.002 ug/mL were still detected in milk (Kissell et al. (2012). It seems likely therefore that ewes' milk was not a significant source of flunixin for lambs in the current study and that milk of ewes dosed with flunixin would not be an effective route for administering this analgesic to sucking lambs.

Although there was indication of reduced pain through the increased display of normal behaviours in lambs receiving flunixin, the continued display of active pain avoidance behaviours in the CF and CI lambs and postural pain behaviours by the CI lambs indicates that they were still experiencing some pain. This was also confirmed by the increase in cortisol in all animals that had been castrated and tail-docked and, although the cortisol concentrations in the CF and CI lambs decreased quicker than in animals without pain relief, they still did not return to baseline during the 48 h of this study. Thus, even though flunixin was effective in alleviating pain associated with castration and tail-docking it did not completely eliminate the pain. In order to improve the effectiveness of analgesics to provide adequate pain-relief to livestock it may be necessary to use a combination of local anaesthetics with NSAIDs prior to the procedures as was demonstrated by Earley and Crowe (2002), Paull et al., (2007) and Webster et al., (2013).

5 Conclusions

Following castration and tail-docking, lambs that consumed flunixin voluntarily as a component of a total mixed ratio or received flunixin via injection exhibited less pain-related behaviour and had reduced inflammation compared with lambs receiving no pain relief. Some behavioural and physiological indications of residual pain, however, remained in these animals. The results suggest that provision of flunixin in feed could be a practical and effective method for relieving pain in lambs. A combination of a NSAID and local anaesthetic administration, however, may be needed to eliminate all pain and their use should be considered for painful husbandry procedures.

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Figures:

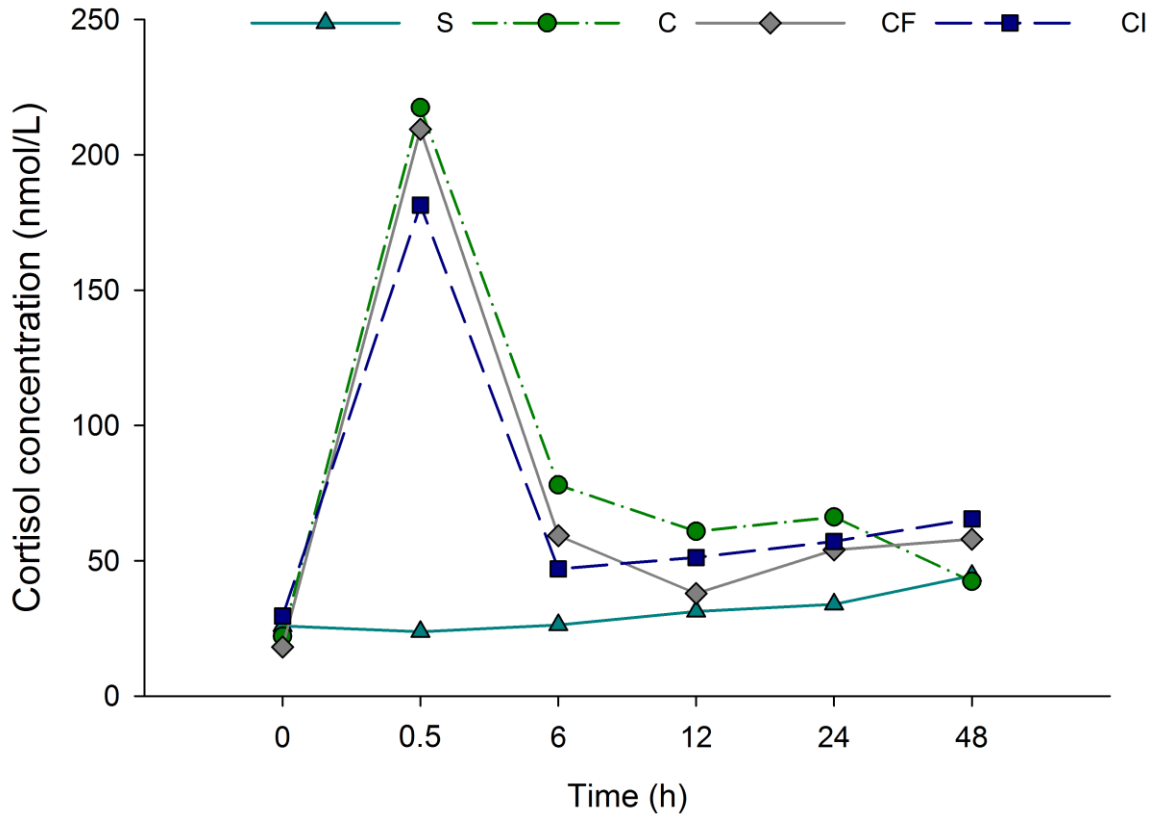

Fig. 1: Raw data of the mean plasma cortisol concentration for all treatment groups before castration and tail-docking or sham treatments and in the 48h period following. There was a treatment effect as well as a significant interaction of treatment by time. S (sham castration and tail-docking), C (castration and tail-docking with no pain relief), CF (castration and tail-docking with flunixin in feed) and CI (castration and tail-docking with flunixin injected). At 30 min following treatment, groups C, CF and CI all showed significant increases in plasma cortisol concentration of compared with baseline. Lambs in the C, CF and CI groups maintained higher concentrations of cortisol than S lambs until 48 h.

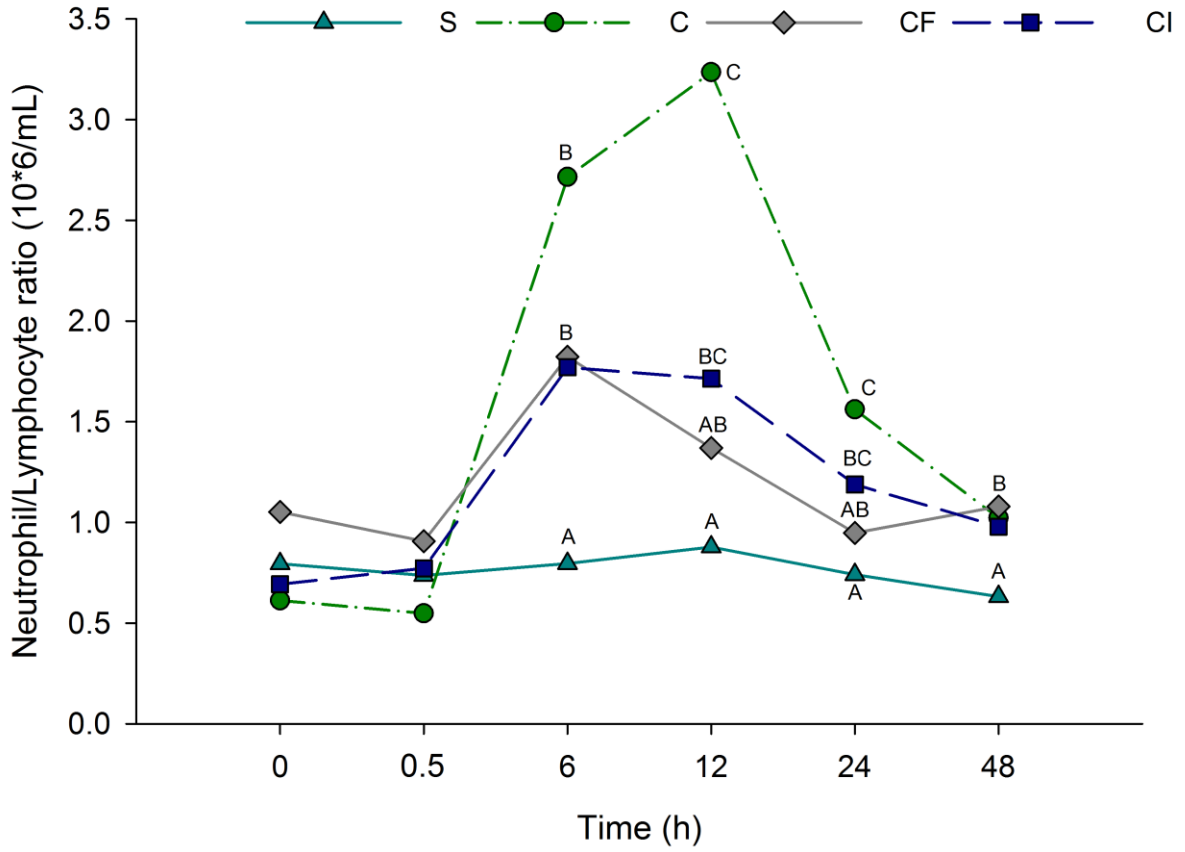

Fig. 2: Raw data of the mean neutrophil/lymphocyte (N:L) ratio for all treatment groups, S (sham castration and tail-docking), C (castration and tail-docking with no pain relief), CF (castration and tail-docking with flunixin in feed) and CI (castration and tail-docking with flunixin injected) before castration and tail-docking or sham treatments and in the 48h period following. There was a treatment and time effect. All lambs that were castrated and tail-docked had higher NL ratio at 6 h compared with S lambs. At 12 and 24 h, C lambs had significantly higher neutrophil/lymphocyte ratio compared with CF lambs. Means without a common superscript are significantly different ($P < 0.05$)

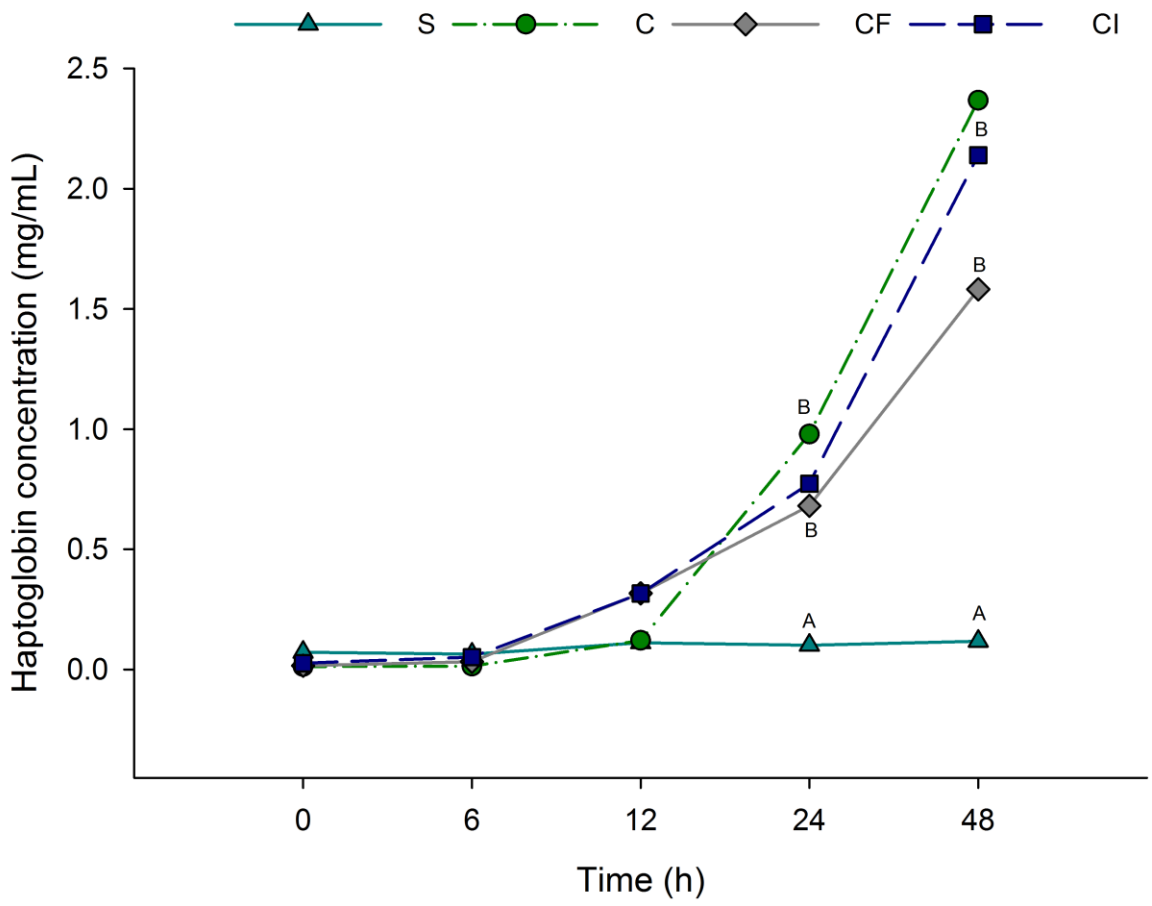

Fig. 3: Raw data of the mean plasma haptoglobin concentration for all treatment groups before castration and tail-docking or sham treatments and in the 48h period following. There was a significant treatment effect seen at 24 and 48 h between sham castration and tail-docking (S) and lambs in the groups that were castrated and tail-docked with no pain relief (C), castration and tail-docking with flunixin in feed (CF) and castration and tail-docking with flunixin injected (CI). Means without a common superscript are significantly different ($P < 0.05$).

Table 1: Ethogram used for behavioural observations of lambs sham castrated and tail-docked, or castrated and tail-docked without pain relief, or receiving flunixin in feed or by intramuscular injection.

Behaviour	Description
Active pain avoidance	
Restlessness	Number of times lamb stood up and laid down
Kicking/foot stamping	Limb was lifted and forcefully placed on the ground while standing or used to kick
Rolling	Lamb rolled from lying on one side to the other without getting up
Jumping	Lamb moved forward using bunny hops with its hind limbs
Licking/biting wound site	Head moved beyond the shoulder, including looking and touching at wound
Restless hindquarters	Weight was shifted slowly between hindquarters, without walking
Easing Quarters	Abnormally lowers rear quarters or attempts to keep quarters off the ground
Pain behaviours	All active pain avoidance behaviours pooled.
Postural behaviours	
Normal ventral lying	Lay on sternum with legs tucked in and head up or down
Abnormal ventral lying	Ventral lying with hind limbs partially or fully extended or keeping scrotal region off the ground (dog sitting).
Ventral lying other	Lamb was lying ventrally but unable to clearly categorise the lying posture.
Lateral lying	Lateral (on side) with one shoulder on ground, extension of hind limbs with head up or down.
Abnormal lying	Abnormal lying categories combined (abnormal ventral lying and lateral lying)
Total lying	All lying categories combined
Normal standing	Standing with no apparent abnormalities
Statue standing	Immobile standing with an obvious withdrawal from interaction with other pen members and outside stimuli. Legs positioned further back than normal. Can show arched back.
Abnormal standing	Standing hunched or unsteadily, often associated with foot stamping, kicking and tail wagging.
Standing other	Lamb was standing but unable to clearly categorise the standing posture.
Normal walking	Walking with no apparent abnormalities
Abnormal walking	Walking unsteadily or stiffly, includes walking backwards, on knees, moving forward with bunny hops, circling, leaning or falling.
Walking other	Lamb was walking but unable to clearly categorise the walking type.
Total Standing	All standing and walking categories combined (including all normal, abnormal and unknown)
Total abnormal behaviours	All abnormal posture categories combined (abnormal ventral lying, lateral lying, abnormal standing, statue standing and abnormal walking)

Table 2: Wound score descriptors, score increases when wound condition worsens and then decreases as the wound heals. Wound score is the total of swelling description and wound appearance.

Swelling descriptor	**Score**	**Wound appearance**	**Score**
No swelling	1	Edges close together, dry scab	1
Slight swelling along wound edges (up to 5mm either side)	2	Small area (<1cm) wet and oozing, no visible pus	2
Large area swelling, but soft	3	Medium area wet and oozing (1-5cm); small amount pus	3
Large area hard swelling; pitting oedema (thumb impression can be made)	4	Large area wet (>5cm); necrotic; copious pus draining	4
Reducing hard swelling with loose cover (healing phase)	3	Granulation tissue forming, but still oozing (healing); watery exudate	3
Scarring or nodule (healed)	2	New skin evident, shiny, not oozing (healed)	2

Table 3: Count (Means +/- SD) of postural behaviours in consecutive 4h observation periods in the 12 h following treatment for sham castrated and tail-docked lambs (S); castrated + tail-docked + no pain relief (C); castrated + tail-docked + flunixin in feed (4.0 mg/kg, CF); and castrated + tail-docked + flunixin injection (2 mg/kg, CI). AB indicates significance within a time point and behaviour, means without a common superscript are significantly different ($P < 0.05$)

Behaviours	0 – 4 h				4 – 8 h				8 – 12 h				Summed			
	S	C	CF	CI	S	C	CF	CI	S	C	CF	CI	S	C	CF	CI
Abnormal lying	1.9±1.44	2.1±2.24	2.6±2.33	1.8±1.95	3.7±2.21	2.9±2.46	3.6±2.90	2.7±2.27	1.8±1.94	2.6±2.73	2.9±2.05	1.4±1.93	7.1±1.00	7.4±1.45	9.3±1.38	6.5±1.04
Total lying	7.4±2.2^{A}	5.2±3.37^{C}	7.6±2.71^{AB}	5.3±2.62^{BC}	9.3±2.14^{A}	4.5±3.46^{C}	7.3±2.54^{AB}	6.9±2.43^{AB}	8.2±2.26	4.7±3.70	7.9±3.76	8.9±1.95	25.0±0.84^{A}	14.4±2.08^{B}	22.7±1.85^{A}	21.1±1.40^{B}
Total Standing	5.1±1.96	8.9±3.19	6.1±2.68	8.3±2.77	4.8±1.77	9.0±2.99	6.0±2.90	6.8±2.51	6.2±2.26	10.3±3.63	6.0±3.43	6.2±2.10	16.1±0.93^{A}	28.3±1.96^{B}	18.1±1.96^{A}	21.3±1.45^{B}
Total abnormal behaviours	3.7±3.40	6.9±2.62	4.1±2.55	4.3±2.24	5.0±3.31	7.4±2.22	5.1±2.69	4.3±2.08	2.6±1.82	6.9±2.99	4.8±2.46	3.4±2.22	9.5±1.11^{A}	20.7±1.37^{C}	13.6±1.17^{B}	12.9±1.03^{B}

Table 4: Raw value means for wound score and temperature for the 48 h following castration and tail-docking. For C (castration and tail-docking no pain relief), CF (castration and tail-docking, flunixin in feed) and CI (castration and tail-docking, flunixin injected). S lambs were not included in wound scoring and wound temperature due to the absence of a wound.

Variable	C	CF	CI
Scrotal temperature (°C)			
30 min	27.8	26.9	26.5
6 h	31.4	31.3	31.9
12 h	29.0	28.5	29.1
24 h	27.9	28.1	28.4
48 h	28.6	27.4	27.9
Tail temperature (°C)			
30 min	29.0	28.5	30.8
6 h	33.8	33.5	33.6
12 h	29.8	30.5	30.0
24 h	30.6	30.8	29.4
48 h	29.3	30.7	30.4
Scrotal wound score			
30 min	4	4	4
6 h	4	3	3
12 h	4	3	3
24 h	4	4	4
48 h	4	3	4
Tail wound score			
30 min	4	4	4
6 h	4	4	4
12 h	5	4	4
24 h	5	4	4
48 h	6	5	5