Final report

Extended Commercial Trials of NUMNUTS®

Project code: B.AWW.0263

Prepared by: Alison Small*, Jim Lea*; Danila Marini*; Carolina Munoz Gallardo# and Ellen Jongman#

*Commonwealth Scientific and Industrial Research Organisation; Animal Welfare Science Centre; # University of Melbourne

Date published: 21 November 2022

PUBLISHED BY
Meat & Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

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

Abstract

The NUMNUTS® device, which delivers local anaesthetic to the site of ring application at the time of ring castration and/or tail docking was launched in Australia in 2020. Controlled studies have demonstrated reductions in pain-related behaviour in lambs when the NUMNUTS® device is used. However, large scale commercial studies have not been conducted. This project involved on-farm studies assessing producer observations of pain-related behaviours, a producer survey on attitudes to pain relief at marking and a controlled study assessing the benefits of NUMNUTS® for castration. By validating the commercial application and animal safety of the NUMNUTS® device and confirming its efficacy for castration, this project provides evidence that reductions in pain-related behaviours in lambs are observed in a commercial setting, provided the opportunity for a group of producers to try out the NUMNUTS® device with support from research staff, and identified some of the challenges to wider adoption of pain relief at marking.

Executive summary

Background

The NUMNUTS® device, which delivers local anaesthetic to the site of ring application at the time of ring castration and/or tail docking was launched in Australia in 2020. Controlled studies have demonstrated reductions in pain-related behaviour in lambs when the NUMNUTS® device is used. However, large scale commercial studies have not been conducted. By validating the commercial application and animal safety of the NUMNUTS® device and confirming its efficacy for castration, this project provides livestock producers with the evidence needed to build trust in the product and confidence in its use. In so doing, this project aims to ultimately provide an accelerated path to adoption of improved pain relief for lambs at marking.

Objectives

The project met the following objectives:

- To collect additional quantifiable evidence of the pain mitigating impacts that may result from the commercial use of the NUMNUTS® device.
- To improve understanding of the animal safety implications of using the NUMNUTS® device in a commercial setting.
- To gather robust evidence of the efficacy of the NUMNUTS® device under controlled conditions, specifically for castration.

These objectives were met under two separate trials. Trial A addressed the first two dot points. Trial B addressed the last dot point.

Methodology

Trial A comprised two parts: an on-farm trial conducted on commercial properties around the Southern states of Australia, and a producer survey on attitudes to pain relief for lamb marking. 52 farms were represented in the trial*. On farm, producers scored groups of lambs in terms of level of rolling/fidgeting, level of escape attempts and level of discomfort. At each farm three groups of lambs were ring castrated and tail docked without pain relief, while 3 groups were ring castrated and tail docked using the NUMNUTS® device, delivering 1.5 mL lignocaine local anaesthetic (Numocaine®) at the site of ring application.*
Trial B was a controlled study evaluating the effect of NUMNUTS® castration. It included 60 male Merino lambs tested across 5 cohorts of 12 lambs. Lambs were assigned to 1 of 3 treatment groups 1) NUMNUTS - ring castrated with 1.5 mL lignocaine 20mg/mL administered, using the NUMNUTS® tool 2) SHAM - the scrotum manipulated but no ring applied 3) RING - ring castration performed using an elastrator, no pain relief provided. Acute pain related behaviours and postural behaviours associated with ring castration were assessed in the 2 hours post castration, through video recordings.

Results/key findings

Trial A

All observers identified a benefit of NUMNUTS over RING based on assigning a lower score for rolling/fidgeting at one or more timepoint in one or more replicates, while 47 of 52 (90 %) of observers identified a benefit of NUMNUTS over RING based on assigning a lower score for discomfort at one or more timepoint in one or more replicates.

A benefit of NUMNUTS was most clearly evident in the first 40-60 minutes post marking, with 45-55 % of replicates yielding scores indicative of reductions in pain-related behaviours; while during the 60-120 minute time period a benefit was recorded in 10-30 % of replicates.

Despite the confounding factors inherent in conducting a study across multiple locations with multiple operators; and despite inter-observer variability, NUMNUTS provided a significant reduction in rolling/fidgeting scores ($P < 0.001$) and a significant reduction in discomfort scores ($P < 0.01$) as compared to RING.

Survey Findings

In general, participants' attitudes towards the use of pain relief during painful husbandry procedures were positive.

On-farm differences observed between control and treatment groups does not necessarily reflect producers' willingness to keep using NUMNUTS.

Attitudes seem to be more important factors in the decision. Participants that had more positive attitudes towards the use of pain relief and towards NUMNUTS were more likely to state that they will keep using it and recommend it to others.

A small percentage of participants felt dissatisfied with the levels of pain relief provided by NUMNUTS and may not keep using it in the future. Main barriers to adoption related to issues with practicality (e.g., size of the prototype, issues with leaking product and overall malfunctioning) and/or low perceived effectiveness and/or the costs associated with its implementation.

There were some issues with leaking or malfunctioning equipment – these have been corrected by the NUMNUTS company (Senesino Pty Ltd) for the 2022 marking season.

Trial B

A single central injection of local anaesthetic, using the NUMNUTS® tool can alleviate the behavioural responses to ring castration in the immediate post-procedure period.

The duration of effect is limited, which may be a result of the agent used (lignocaine 20 mg/mL).

Development of a longer-lasting local anaesthetic formulation is imperative to optimise pain mitigation for ring castration.

Benefits to industry

This project has provided evidence that reductions in pain-related behaviours in lambs are observed in a commercial setting, provided the opportunity for a group of producers to try out the NUMNUTS® device with support from research staff, and identified some of the challenges to wider adoption of pain relief at marking.

Issues relating to equipment malfunction were reported to the NUMNUTS® company, and these have been addressed for the 2022 marking season.

Adverse effects were limited to one incident of hindlimb ataxia, across 52 farms and 1470 lambs that received NUMNUTS® application.

Future research and recommendations

Further research is required to understand the hidden costs of pain in lambs and develop a bio-economic model to demonstrate cost/benefit rations to producers.
The duration of effect of lignocaine in lambs is disappointing. Further research to develop a longer-lasting local anaesthetic agent or formulation is warranted.

Table of contents

1. Background

Many husbandry procedures that are necessarily carried out to improve animal and herd wellbeing and productivity, can be painful. Changing community expectation and market access requirements are driving improvements in how the pain caused by these procedures is managed. MLA continues to invest in the development of new products and techniques to facilitate these improvements. One such product is the NUMNUTS® device, which has been designed to deliver a measured dose of local anaesthetic at the time of application of an elastrator ring to the tail for tail docking and/or scrotum for castration (4cDesign Ltd., 2015, Smith et al., 2017, Smith, 2019). The device thus provides dual functionality, both numbing the area to mitigate the immediate intense pain caused and facilitating application of the ring.

While research has shown that the administration of a local anaesthetic is the most effective way to address the intense immediate pain caused by procedures such as tail docking and castration (Mellor and Stafford, 2000, Kent et al., 2000, Kent et al., 2001, Molony et al., 2012), in the past it has required significant skill to achieve reliable consistent injection at production speeds, which in turn presents a barrier to its use. The NUMNUTS® device has the potential to overcome this as it has been engineered to absorb this skill requirement, such that the tool accurately and repeatedly delivers the dose to the target tissues.

Over five years, during the development of the NUMNUTS® device, pen and field trials were carried out in multiple locations on more than 15,000 animals. These trials consistently demonstrated reduced pain behaviours at a flock level during marking (Small et al., 2020, Small et al., 2021a, Small et al., 2021b). These positive welfare results, plus ease of use of the applicator prototype on farm, formed the basis for commercialisation.

An independent peer review of the animal testing done on the NUMNUTS® device during its development, concluded that significant benefits could be derived by collecting additional quantifiable evidence of the pain mitigating impacts that may result from its use, particularly for castration (Peer review of research undertaken in the support of the development of the NUMNUTS® device | Meat & Livestock Australia (mla.com.au)). Feedback gathered from early adopters regarding unexpected side effects has also provided motive to further test the device in a commercial setting to improve understanding of the safety implications.

By validating the commercial application and animal safety of the NUMNUTS® device and confirming its efficacy for castration, this project provides livestock producers with the evidence needed to build trust in the product and confidence in its use. In so doing, this project aims to ultimately provide an accelerated path to adoption of improved pain relief for lambs at marking.

2. Objectives

The project met the following objectives:

- To collect additional quantifiable evidence of the pain mitigating impacts that may result from the commercial use of the NUMNUTS® device.
- To improve understanding of the animal safety implications of using the NUMNUTS® device in a commercial setting.

- To gather robust evidence of the efficacy of the NUMNUTS® device under controlled conditions, specifically for castration.

These objectives were met under two separate trials. Trial A addressed the first two dot points. Trial B addressed the last dot point.

3. Methodology

3.1 Trial A

Trial A comprised two parts: an on-farm trial conducted on commercial properties around the Southern states of Australia, and a producer survey on attitudes to pain relief for lamb marking.

3.1.1 On-farm trials

3.1.1.1 Outline methodology

The on-farm trials were conducted under the authority of the CSIRO 'Wildlife and Large Animal' Animal Ethics Committee, reference 2020-15.

52 farms were involved in the trial, representing NSW (10), VIC (13), SA (10), WA (8) and TAS (11). Producers were recruited through the local co-ordinators' networks and a CSIRO media release (Appendix 1). Due to COVID-19 travel restrictions, CSIRO staff were unable to travel to all producer sites and the role of the local co-ordinators (of which there were nine) was pivotal in completing the trial.

To enable the producers and local co-ordinators to conduct the on-farm trials in a standardised manner, a detailed protocol was drawn up (Appendix 2) and video training materials were prepared.

The video training materials were password-protected for security and hosted by CSIRO. Producers were asked to conduct three replicates of a behavioural comparison between two groups of 10 male lambs, one group being marked (castrated and tail docked) using the standard elastrator rubber ring applicator (RING) and the other group being marked using the NUMNUTS® device (NN), delivering a measured 1.5 mL dose of 2% Lignocaine (NumOcaine, Mavlab, Brisbane Australia) at each ring application site. To achieve this, producers constructed two matched pens, approximately 4 m by 4 m, and suspended a clock on the rear wall of the pens, clearly visible to an observer standing in front of the pens. One group of 10 lambs was to be placed into each pen, immediately after marking, such that all lambs in a pen had received the same treatment. Producers could prepare up to three pairs of pens, so that replicates could be conducted concurrently, or replicates could be conducted sequentially, depending on the available space and resources on each property. Producers were also asked to mount GoPro (Hero5, GoPro Inc, USA) video cameras so that all lambs in the pair of pens and the clock were clearly visible on the footage. This footage was to be archived for future use e.g., for student training. For the purposes of individual identification of lambs from video footage, each lamb had a number spray-marked onto each flank. These numbers were not relevant to the live observation protocol used by the observers on each property.

To conduct the study, lambs were marked, alternating the treatment (RING or NN), sprayed with a sequential number (1 – 10) on both flanks and placed into the respective treatment pen. Lambs were male and were both castrated and tail-docked. Routine ear tagging, anthelmintic, vaccination

and fly-strike prevention were applied according to the producers' normal practice. No other procedures were conducted on these lambs nor were other analgesic treatments given.

An observer (not involved in the marking process, so that there was a degree of blinding to treatment) then scored each pen of lambs every 10 minutes from the point at which the pen was full till 2 hours had elapsed, on a 0 – 3-point scale for:

- Amount of rolling and fidgeting
    - 0: no lambs rolling or fidgeting
    - 1: a quarter of the lambs (1-3) rolling or fidgeting
    - 2: half of the lambs (4-6) rolling or fidgeting
    - 3: most or all lambs (7-10) rolling or fidgeting
- Interest in escape
    - 0: no lambs trying to escape and mother up
    - 1: a quarter of the lambs (1-3) trying to escape and mother up
    - 2: half of the lambs (4-6) trying to escape and mother up
    - 3: most or all lambs (7-10) trying to escape and mother up
- Overall demeanour
    - 0: most or all lambs looking comfortable/OK
    - 1: a quarter of the lambs (1-3) looking uncomfortable/unhappy
    - 2: half of the lambs (4-6) looking uncomfortable/unhappy
    - 3: most or all lambs (7-10) looking uncomfortable/unhappy

And, once the observations were complete:

- Ease of emptying the pen
    - 0: no lambs needing assistance
    - 1: a quarter of the lambs (1-3) needing assistance
    - 2: half of the lambs (4-6) needing assistance
    - 3: most or all lambs (7-10) needing assistance

After the first on-farm trial, a set of four 'calibration' video clips were extracted from the footage generated, representing 10-second blocks of time at approximately 10, 30, 80 and 110 minutes after marking. These were arranged in random order (by selecting a numbered chip from a bag) and produced as a single 'calibration' video which was password-protected for security and hosted by CSIRO. The 'calibration' clips were scored by the observer from each farm to allow an assessment of inter-observer reliability of the scoring system.

3.1.1.2 Success in completing the planned methodology

52 producers were involved in the trial, representing NSW (10), VIC (13), SA (10), WA (8) and TAS (11). 45 producers each conducted 3 replicates of the study. Two completed 1 replicate only. One of these did not follow the scoring procedure so that data were excluded from the data set. Four completed 2 replicates; and one completed 4 replicates, 2 replicates being scored by each of 2 observers. Thus, data from 148 replicates, scored by 52 observers across 51 producers were included in the data set.

In two replicates, one or three RING lambs physically escaped from the pen during the observation period; while in two other replicates, limited lamb numbers meant that there were only 4 or 6 lambs in each treatment group. The data from these replicates was visually inspected for coherence with

other replicates scored by the same observer, and being coherent, were included in the data set. Overall, 2940 lambs were included in the study.

Two observers did not score timepoint 0 (immediately after the pens were filled) but began scoring at the 10-minute timepoint. 21 observers scored timepoint 120 minutes, immediately before pen emptying (51 replicates), the remaining 31 observers did not score timepoint 120 minutes (89 replicates). Three producers terminated observations early due to logistical issues (e.g., inclement weather looming, loss of daylight), thus data were missing for the 100-minute timepoint in 4 replicates and for the 110-minute timepoint in 6 replicates.

Two observers did not score ease of pen emptying.

When the study was planned, it was envisaged that a CSIRO or University of Melbourne research technician would attend each property to assist in set-up and data collection, particularly with regards to collection of video footage. However, travel restrictions relating to the concurrent COVID-19 pandemic prevented this, and the on-farm trials were conducted by the producers, with assistance where possible form the local co-ordinators. Animal work and live behavioural observations were conducted admirably, but collection of video footage was restricted, either due to inability to supply equipment in a timely manner or to errors in camera placement or focusing, such that the time displayed by the clock or the numbers on the sides of the lambs were not clearly visible in all cases. The video footage was to be archived for future use (e.g., for student training), so it was not essential to the conduct of the current study, and although sufficient high-quality footage for use in research was not collected, sufficient suitable footage to train students in lamb behaviour observation was collected.

A range of breeds were represented in the study: Merino (17 producers) Dohne (1); First-cross Merino (8); Australian milking sheep (1); Australian white (1); Dorper (2); CoopworthX Charrolais (1); Romney/ Dorset (1); Wiltipoll (1); DorsetX (1); BLMxPD & BLMxWS (1); Composite (5); Other cross-bred (12). These were clustered into two types: ‘wool’ (Merino and Dohne) and ‘other’ for analysis. These types were represented in all participating States (Table 1).

Table 1. Sheep types represented in each State

State	Wool flocks	Other flocks
NSW	3	7
SA	6	4
VIC	2	11
TAS	2	9
WA	5	3

3.1.1.3 Analysis

Data pertaining to the number of observers identifying a benefit of NUMNUTS, and number of replicates in which NUMNUTS was scored as beneficial (based on scoring lower than RING for rolling/fidgeting or discomfort) did not undergo statistical analysis and are presented in a descriptive format.

The scores assigned were analysed using a general linear model in R (R Core Team, 2018) under a pseudoreplication (repeated measures) structure to account for the fact that time series observations are non-independent. Three separate models were generated for each of the response

variables (rolling/fidgeting; escape attempts; and comfort). The models fitted treatment (NUMNUTS or RING), timepoint (0 to 120 minutes at 10-minute intervals), State (NSW, SA, VIC, TAS, WA) and sheep type (wool or other) as fixed effects. First second and third order interactions were fitted initially, and non-significant interactions were sequentially removed to lead to the final model for each case. For rolling/fidgeting and comfort, there was an interaction between State and sheep type; while for escape attempts there was an interaction between State and sheep type and an interaction between treatment and timepoint.

3.1.2 Producer survey

Surveys are considered an appropriate research approach to gathering quantitative and qualitative information on the opinions of people (Ajzen and Fishbein, 1980). This specific component of the project will be used to further understand farmer attitudes towards the use of pain relief at lamb marking and main barrier to adoption of Numnuts®. The objectives of the survey were:

a. To assess farmer attitudes towards pain relief and their opinion on the efficacy and usefulness of NUMNUTS®
b. To assess farmer experience with the trial

The key questions that were addressed with this component included:

- Do farmer attitudes influence perceptions of the effectiveness of the NUMNUTS® device?
- What are the general opinions about the use of pain relief at lamb marking?
- What are the perceived barriers to using pain relief at lamb marking?

Farmers needed to complete a short pre- and post-trial survey (Appendix 3). Questions in the survey were in relation to general attitudes towards the provision of pain relief at lamb marking, knowledge about Numnuts® and satisfaction with the Numnuts® prototype. Time commitment was 15-20 minutes approximately. Participants had the option to complete the survey using a secured link that was sent via email. Paper versions of the survey and pre-paid envelopes were also offered.

A total of n=20 participants completed the pre-trial survey. Based on this response rate, the pre- and post- surveys were merged to increase producer engagement. The delivery of the survey also changed from a secured online link to a telephone survey. This change meant that participants now had to only complete one survey after the on-farm trial. A total of 41 producers competed the attitudinal survey after the trial using either a secure email link or over the phone with someone from the research team.

3.1.2.1 Analysis

The survey was analysed quantitatively and qualitatively. Quantitative data was analysed using a combination of descriptive statistics, correlations and chi-squared analyses. Qualitative data were analysed according to themes.

3.2 Trial B

The on-farm trials were conducted under the authority of the CSIRO Armidale Animal Ethics Committee, reference ARA 20-16.

Five cohorts of 12 single-born male lambs, aged between 6 and 8 weeks, were treated between 23rd November and 16th December 2020. On entry to the animal house, each lamb was de-wormed, weighed (Figure 1) and health checked, including rectal temperature, heart rate, respiratory rate and thoracic auscultation (Figure 2), mucous membranes, body condition, gait and musculoskeletal assessment and appetite (interest in feed offered).

Figure 1: A lamb being weighed. The Trutest readout unit had previously been tared to the weight of the platform and handler.

Figure 2: Thoracic auscultation.

Within each cohort, the lambs were ranked according to weight, sequentially blocked into blocks of 3 and randomly allocated from within each block, by picking coloured marbles from a bag, to 3 treatment groups (Table 2).

Table 2: Treatment groups in trial B

Treatment	Description
NUMNUTS	Lamb placed in a marking cradle and ring castrated with 1.5 mL local anaesthetic administered, all using the NUMNUTS tool
SHAM	Lamb placed in marking cradle and the scrotum manipulated as though an elastrator castration ring were applied
RING	Lamb placed in the marking cradle, ring castration performed using an elastrator ring, without administration of local anaesthetic

In the animal house, there were three lambs with their ewes in each pen (Figure 3). Each pen contained one block of 3 lambs, one from each treatment group, such that no treatment was duplicated within a pen. There were 20 replicates per treatment (four per cohort). The order of pens treated was altered for each cohort, and the order of treatment of lambs within pens was conducted according to the order in which lambs were picked up by the handler.

Figure 3: One block of three lambs and their ewes

Lambs were individually captured and placed on their back in a lamb marking cradle (Figure 4) within the animal house for up to 60 s for treatment.

- NUMNUTS lambs had an elastrator ring applied at the scrotal neck and an injection of 1.5 mL NumOcaine administered, using the NUMNUTS® tool, according to the manufacturer's instructions (www.numnuts.store).
- SHAM lambs had the scrotum manipulated as though an elastrator castration ring were applied.
- RING lambs had the elastrator castration ring applied according to standard industry procedures (Lloyd and Playford, 2013). No local anaesthetic was applied.

The lamb was then returned to its trial pen.

Figure 4: A lamb restrained in the marking cradle.

Figure 5: NUMNUTS treatment being carried out.

GoPro HERO5 video cameras were used to continuously record the behaviour of lambs for a two-hour period after treatment of the final lamb in the cohort. For each pen, one camera was mounted on roofing rafters at one corner of the pen, such that each camera provided a view of the entire area available to the lambs in one pen.

After the 2-hour observation period had elapsed, castrated lambs were tail docked using NUMNUTS, sham lambs were castrated and tail docked using NUMNUTS, and all lambs were given Ilium Buccalgesic OTM for ongoing pain relief. Fly-strike prevention was applied and the ewes and lambs returned to the holding paddock, where they remained for at least 24 hours prior to return to the CSIRO Chiswick commercial flock.

Behavioural annotation of the video footage was conducted according to the agreed protocol. The assessment of the behaviour post-treatment focused on the acute pain related behaviours and postural behaviours associated with ring castration. The pain related behaviour assessment took place for one full minute at five-minute intervals for the first 2 hours post-procedure. The ethogram used in this study (Table 3) was based on behaviour patterns described in previous studies of behavioural responses of lambs following ring castration (Dinniss et al., 1999, Thornton and Waterman-Pearson, 2002, Paull et al., 2012, Small et al., 2020, Small et al., 2021a). All behaviour assessments were conducted from the video recordings and carried out by staff trained in categorizing behaviours. The person performing the video observations post-treatment was blinded to treatment.

Statistical analysis was conducted using R and R studio software (R Core Team, 2018). Data were tested for normality through visual inspection of residual plots and the Shapiro-Wilks test. Starting with the maximal model that included all predictors and interactions, the most appropriate model that fit the data was selected based on information criterion (AIC and BIC). The hypothesis tested was that lambs treated with NUMNUTS® and Numocaine have reduced counts of pain related behaviours as compared with lambs not receiving local anaesthetic.

Acute pain behaviour counts were analyzed using a Generalized linear mixed model with Poisson distribution and Quasi-Poisson distribution when there was over dispersal. Factors included Treatment, Time, Cohort and Pen as well as the interaction of Treatment x Time.

Postural behaviours were collated for a total count this included all upright behaviours (Nu, SS, Au), lying behaviours (Nl, Ll, Al) and all abnormal behaviours (Au, Al, SS) analyzed as a group. Postures were analyzed using a non-linear mixed effects model, fixed factors included Treatment, Cohort, Pen, and their interactions where appropriate, lamb was included as a random effect.

A P-value of < 0.05 was considered statistically significant and $0.1 > P > 0.05$ was considered a statistical tendency. Data are presented as means ± standard error.

Table 3: Ethogram for behaviour annotation

Behavior	Abbreviation	Description
Postural behaviors		
Normal upright	Nu	Standing, walking or playing while exhibiting a usual posture or gait; smooth movements
Standing stretched	SS	Lamb stands with its hind legs extended backwards, and the pelvis and lumbar areas dipped towards the pen floor
Abnormal upright	Au	Standing exhibiting unusual posture e.g. Rounded, hunched appearance; ataxia; jerky movements; walking unsteadily, backwards, on knees.
Normal lying	Nl	Ventral recumbency, all legs tucked under body or very close to body
Lateral lying	Ll	Lamb is lying flat on one side, with head and shoulders in contact with the pen floor
Abnormal lying	Al	Twisted lying; ventral recumbency with forelimbs tucked under body, one or both hind limbs partially or fully extended; including dog sitting and lateral lying (lateral recumbency with one shoulder on ground, hind limbs and/or forelimbs fully extended)
Active pain related behaviours		
Restlessness	Rst	Number of times lamb stood up and laid down. Instances of lamb rising as far as its knees included in the one count.
Kicking/foot stamping	Fsk	Either a front or hind limb (usually hind limb) was lifted and forcefully placed on the ground while standing or was used to kick while standing or lying.
Rolling	Rl	Rolled from lying on one side to the other without getting up. Half rolls where the lamb rolled on its back and then returned to lying on the same side included.
Jumping	Jmp	All four feet off ground simultaneously
Licking/biting wound site	Lbw	Movement of the head beyond the shoulder, including both looking and touching at the source of pain and grooming.
Head shake	Sh	Forceful voluntary shake of the head
Easing quarters	Eq	Abnormally lowers rear quarters (standing) or attempts to keep quarters off the ground (lying).
Sum of Pain Related Behaviours	Sum of: **Rst + Fsk + Rl + Jmp + Lbw + Sh + Eq**	All pain related behaviours pooled.

4. Results

4.1 Trial A

4.1.1 On-farm trials

4.1.1.1 Inter-observer reliability

The calibration clips represented approximately 5 (Clip 4), 30 (Clip 1), 80 (Clip 2) and 110 (Clip 3) minutes after marking. They were scored by 51 observers. Mean score, standard deviation and coefficient of variation for each clip and behavioural parameter are shown in Table 4.

For clip 4 (5 minutes after marking), 45 observers (88 %) reported a difference in rolling/fidgeting between the two pens shown; 42 (82 %) detected a difference in interest in escape; and 46 (90 %) detected a difference in overall demeanour. For Clip 1 (30 minutes after marking), 20 observers (39 %) detected a difference in rolling/fidgeting between the two pens shown; 13 (25 %) detected a difference in interest in escape; and 16 (31 %) detected a difference in overall demeanour. For Clip 2 (80 minutes after marking), 14 observers (27 %) detected a difference in rolling/fidgeting between the two pens shown; 9 (18 %) detected a difference in interest in escape; and 21 (41 %) detected a difference in overall demeanour. For Clip 3 (110 minutes after marking), 14 (27 %) observers detected a difference in rolling/fidgeting between the two pens shown; 15 (29 %) detected a difference in interest in escape; and 12 (24 %) detected a difference in overall demeanour.

Table 4: Inter-observer reliability. Mean score, standard deviation (Sd) and Coefficient of variation (Cv) assigned by 50 observers for each calibration clip.

Time post marking		Rolling/fidgeting		Interest in escape		Overall demeanour	
		RING	NN	RING	NN	RING	NN
5 min (Clip 4)	Mean	2.53	0.62	1.00	2.53	2.46	0.72
	Sd	0.69	0.95	0.90	0.79	0.76	0.93
	Cv	0.24	1.52	0.90	0.31	0.31	1.29
30 min (Clip 1)	Mean	2.29	2.10	0.39	0.29	2.23	2.14
	Sd	0.72	0.77	0.58	0.67	0.90	0.92
	Cv	0.31	0.37	1.96	1.70	0.41	0.43
80 min (Clip 2)	Mean	0.61	0.49	0.47	0.37	0.87	0.69
	Sd	0.70	0.70	0.54	0.53	0.87	0.89
	Cv	1.14	1.44	1.15	1.42	0.99	1.28
110 min (Clip 3)	Mean	0.34	0.37	0.19	0.51	0.78	0.60
	Sd	0.55	0.66	0.48	0.64	0.79	0.83
	Cv	1.06	1.79	2.54	1.26	1.01	1.39

4.1.1.2 Producer observations

4.1.1.2.1 Observed performance of NUMNUTS across all replicates

A benefit of NUMNUTS, based on having a lower rolling/fidgeting score assigned, was observed in over 53% of replicates at timepoints 0, 10 and 20 minutes; in over 35% of replicates at timepoints

30, 40 and 50 minutes; and in over 10% of replicates at timepoints 60, 70, 80, 90 and 100 minutes (Figure 6). At all timepoints, RING scored lower for rolling/fidgeting in less than 10% of replicates.

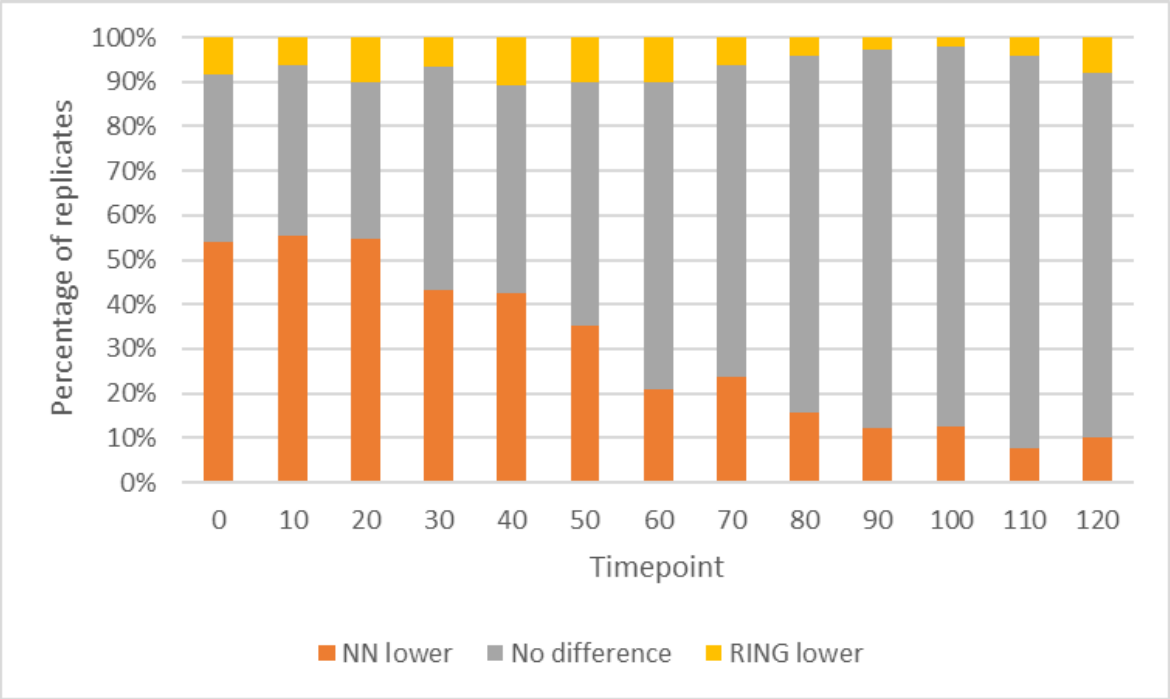

Figure 6: Percentage of replicates showing differences between treatment in terms of rolling/fidgeting score. NN: 1.5 mL of 2% lignocaine delivered at each ring application site for both tail docking and castration. RING: elastrator ring applied without local anaesthetic administration for both tail docking and castration.

A benefit of NUMNUTS, based on having a lower discomfort score assigned, was observed in over 42% of replicates at timepoints 0, 10, 20, 30, 40, 50, 60 and 70 minutes; in over 32% of replicates at timepoints 80 and 90 minutes; and in over 21% of replicates at timepoints 100 and 110 minutes (Figure 7). At all timepoints, RING scored lower for discomfort in less than 10% of replicates.

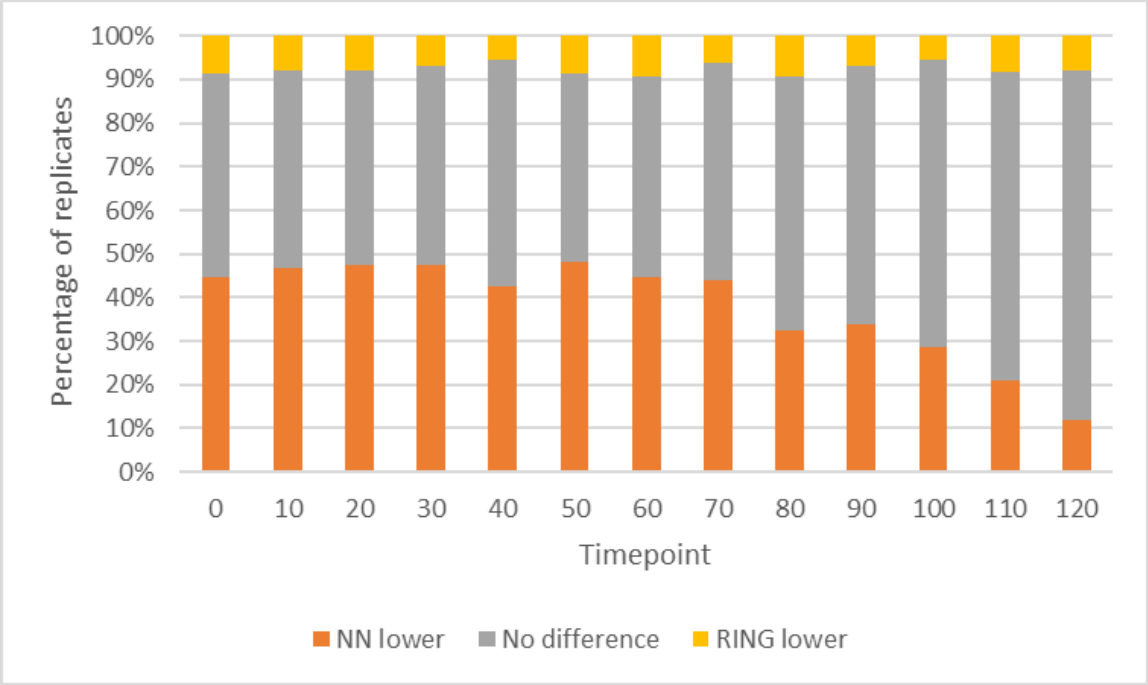

Figure 7: Percentage of replicates showing differences between treatment in terms of discomfort score. NN: 1.5 mL of 2% lignocaine delivered at each ring application site for both tail docking and castration. RING: elastrator ring applied without local anaesthetic administration for both tail docking and castration.

In terms of the proportion of replicates in which escape attempts were recorded, there were no clear differences between treatments (Figure 8).

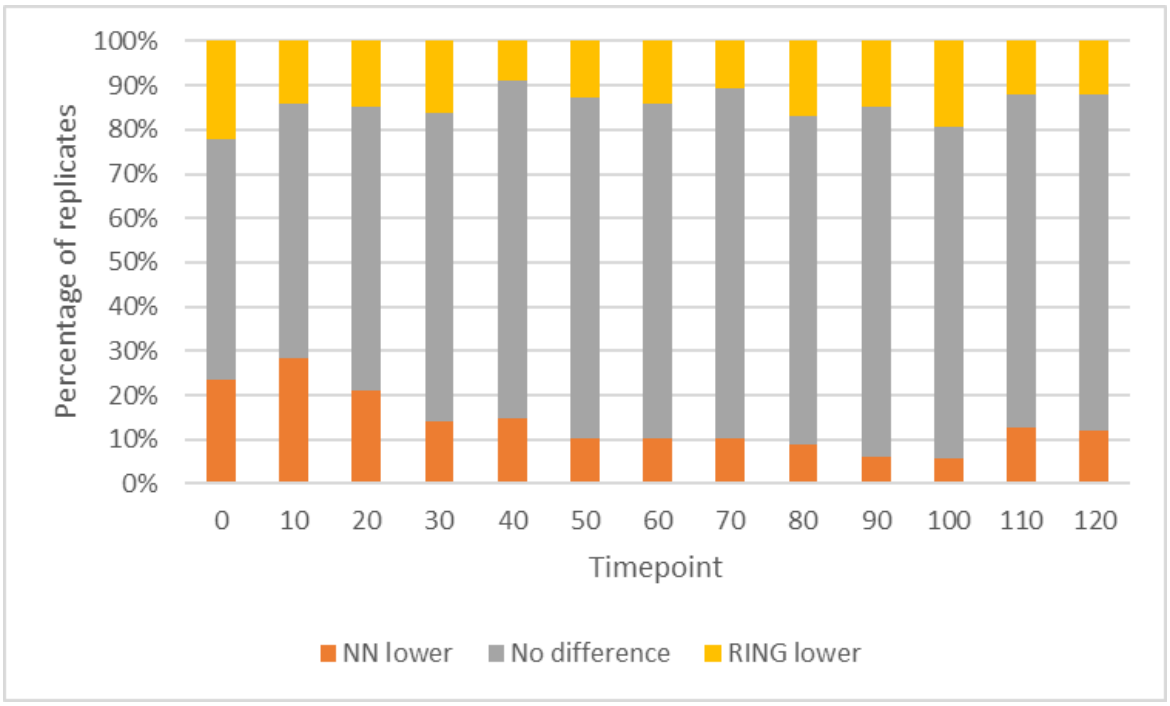

Figure 8: Percentage of replicates showing differences between treatment in terms of escape attempts score. NN: 1.5 mL of 2% lignocaine delivered at each ring application site for both tail docking and castration. RING: elastrator ring applied without local anaesthetic administration for both tail docking and castration.

4.1.1.2.2 Observed performance of NUMNUTS across observers

All observers identified a benefit of NUMNUTS over RING based on assigning a lower score for rolling/fidgeting at one or more timepoint in one or more replicates. At timepoint 0 minutes, 23 out of 49 observers (47%) identified a benefit in two or more replicates and 42 out of 49 observers (86%) identified a benefit in one or more replicates (Figure 9). Over 50% of observers identified a benefit in two or more replicates and over 85% identified a benefit in one or more replicates at timepoints 10 and 20 minutes. Between timepoints 30 and 50 minutes, 30-40% of observers identified a benefit in two or more replicates and 67-77 % identified a benefit in one or more replicates. From 60 to 100 minutes, a benefit was observed in one or more replicates by 25-40% of observers, and in 110 and 120 minutes, a benefit was observed in one or more replicates by 14-17% of observers.

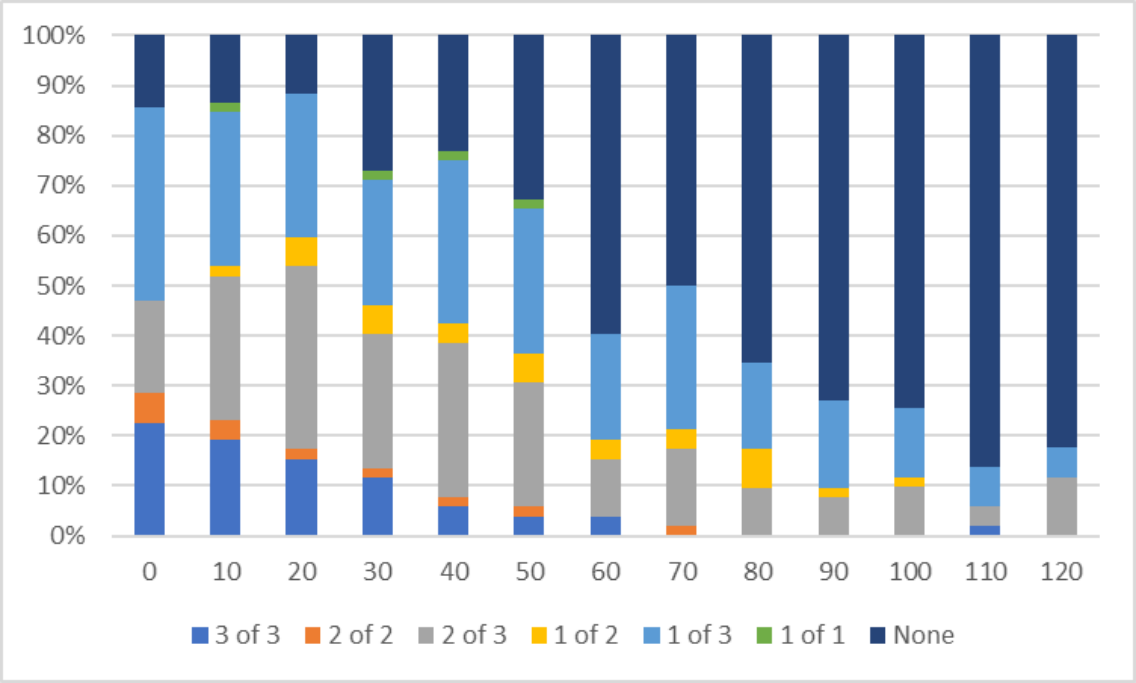

Figure 9: Percentage of observers identifying a benefit of NUMNUTS (based on assigning a lower score for rolling/fidgeting) in one or more replicate. Segments indicate the number of replicates in which a benefit was identified out of the number of replicates observed by the individual.

NUMNUTS was scored lower than RING for escape attempts in one or more replicates by over 46% of observers in timepoints 0 to 20 minutes; by 32-35% of observers in timepoints 30 and 40 minutes; and by 15-25% of observers between timepoints 50 and 100 minutes (Figure 10). At timepoints 110 and 120 minutes, NUMNUTS was scored lower than RING for escape attempts in one or more replicates by 27-29% of observers.

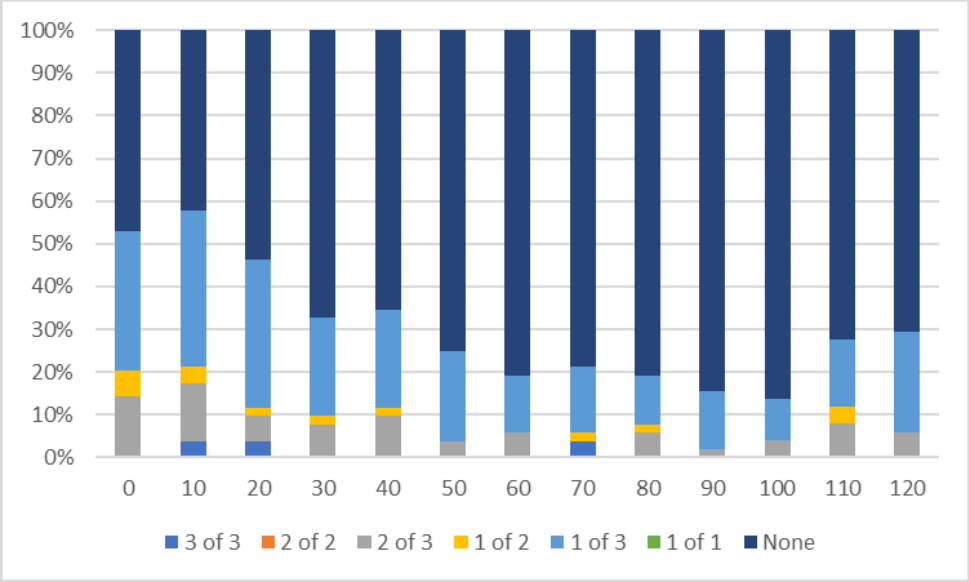

Figure 10: Percentage of observers assigning a lower score to the NUMNUTS group for escape attempts in one or more replicate. Segments indicate the number of replicates in which NUMNUTS scored lower than RING out of the number of replicates observed by the individual.

47 of 52 (90%) of observers identified a benefit of NUMNUTS over RING based on assigning a lower score for discomfort at one or more timepoint in one or more replicates. 35-40% of observers observed a benefit of NUMNUTS in two or more replicates, and over 74% observed a benefit in one or more replicates for up to 70 minutes post pen filling (Figure 11). From 80 to 100 minutes, 30-31% of observers observed a benefit of NUMNUTS in two or more replicates, and 50-60% observed a benefit in one or more replicates. At the 110-minute timepoint, 43% of observers noted a benefit of NUMNUTS in one or more replicate, while at 120 minutes, this had reduced to 18%.

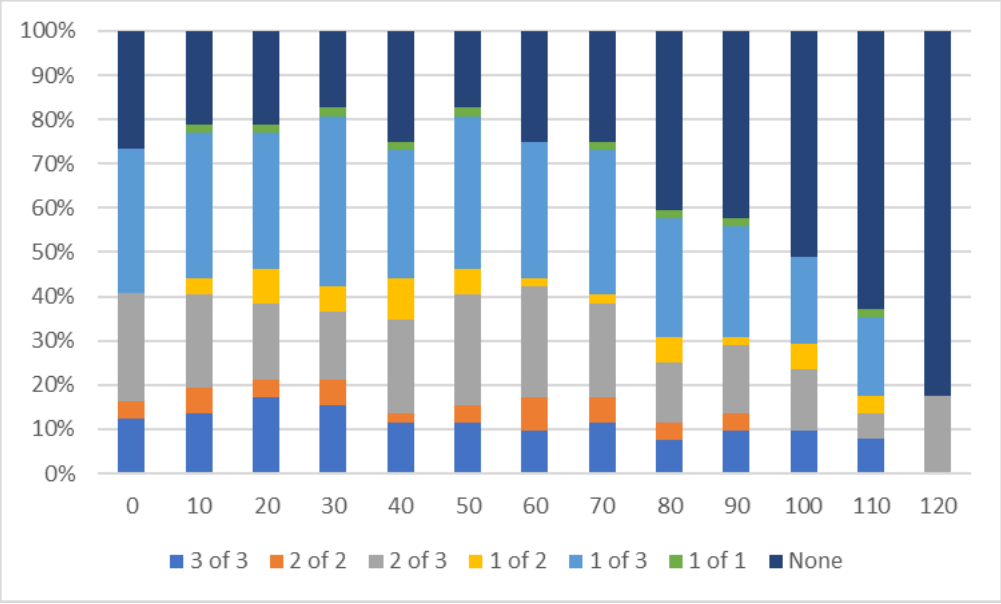

Figure 11: Percentage of observers identifying a benefit of NUMNUTS (based on assigning a lower score for discomfort) in one or more replicate. Segments indicate the number of replicates in which a benefit was identified out of the number of replicates observed by the individual.

4.1.1.2.3 Analysis of producer assigned scores

For rolling/fidgeting, NUMNUTS scored significantly lower than RING ($P < 0.001$); there was a significant effect of timepoint ($P < 0.001$; Figure 12) and a significant State:type interaction ($P < 0.01$; Figure 13).

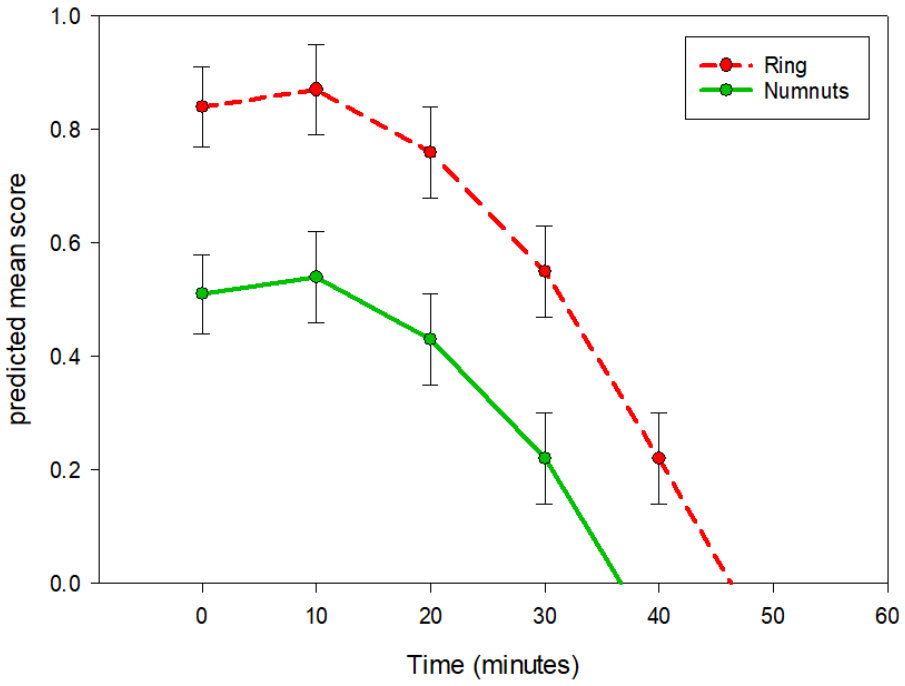

Figure 12: Illustration of predicted mean scores (Adjusted to sit on the positive scale for ease of interpretation) for rolling/fidgeting in NUMNUTS (NN) and RING lambs over the time series. State NSW and Sheep Type 'other'. A higher score indicates more rolling or fidgeting is observed in the pen.

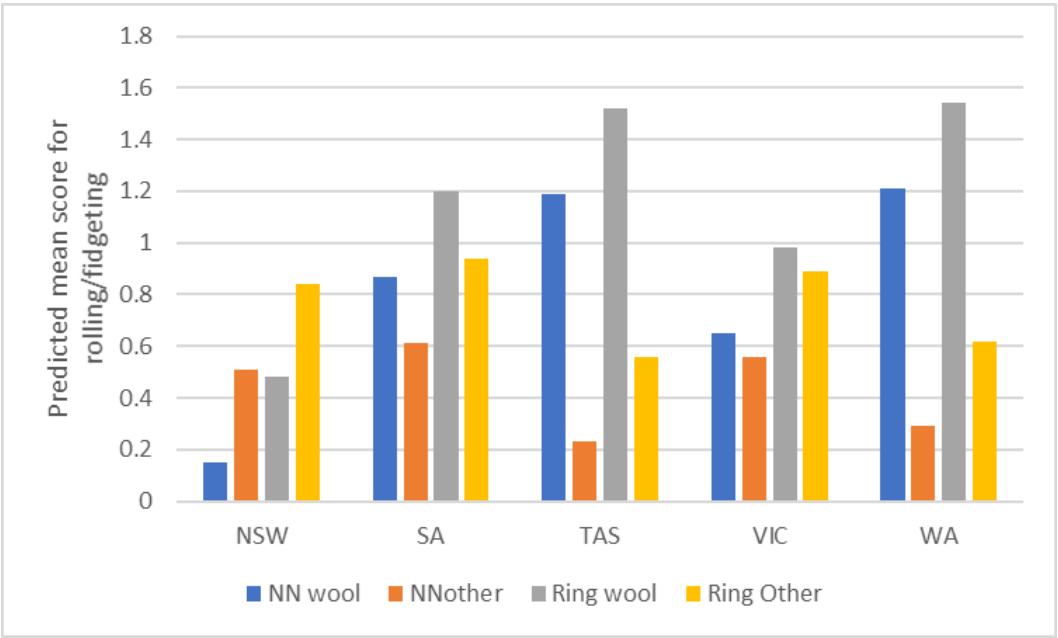

Figure 13: Illustration of the interactions between sheep type and State, showing predicted mean scores for rolling/fidgeting at timepoint 0.

For escape attempts, there was a significant effect of timepoint ($P < 0.001$) and a significant State:type interaction ($P < 0.01$). There was no significant effect of treatment on escape attempts.

NUMNUTS scored significantly lower than RING in terms of levels of discomfort ($P < 0.01$), there was a significant effect of timepoint ($P < 0.001$; Figure 14) and a significant State:type interaction ($P < 0.01$; Figure 15).

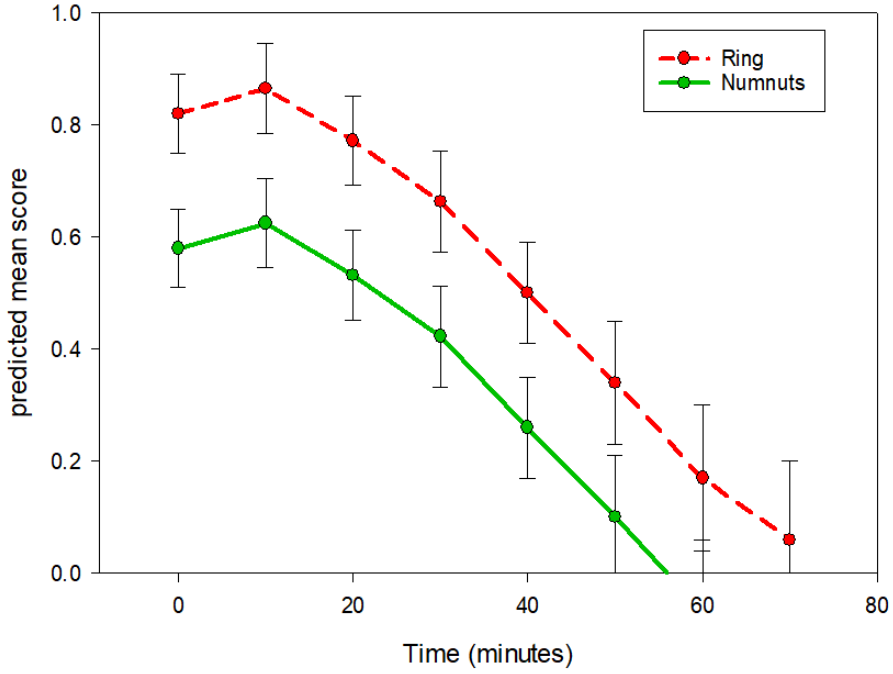

Figure 14: Illustration of predicted mean scores for discomfort in NUMNUTS (NN) and RING lambs over the time series. State NSW and Sheep Type 'other'. A higher score indicated greater discomfort.

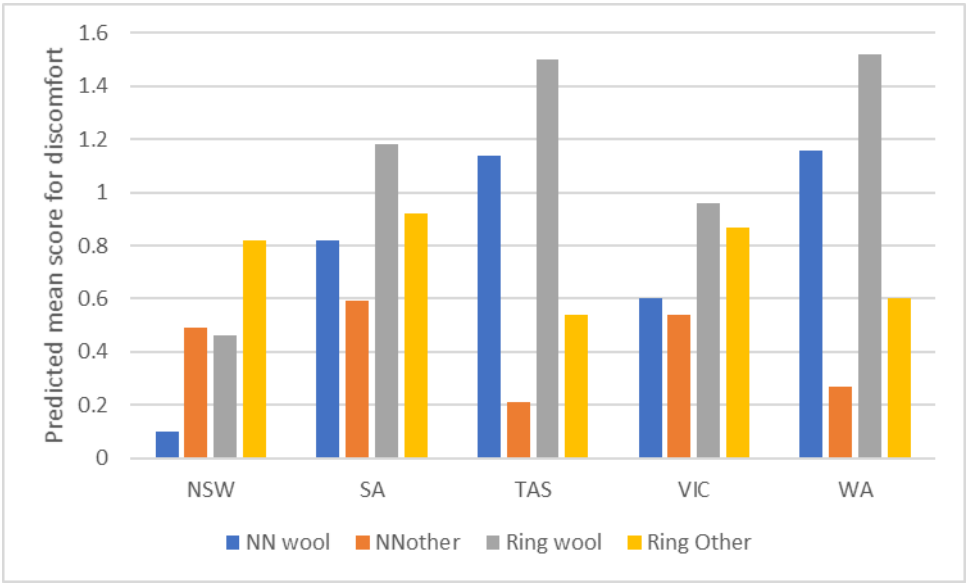

Figure 15: Illustration of the interactions between sheep type and State, showing predicted mean scores for discomfort at timepoint 0.

4.1.1.2.4 Observed adverse effects

One observer noted ataxia in a lamb: the observation was noted as 'one lamb wobbly on back legs' twice for the same pen, so it is unclear if one or two lambs were affected.

4.1.2 Producer survey

4.1.2.1 Characteristics of survey respondents

A total of n=41 surveys were completed; thus, the total response rate was 82.4%. Most survey respondents were from meat-wool enterprises, followed by meat-focused enterprises and mixed productions* (Figure 16). Flock sizes ranged from 300 to 6500 breeding ewes and the average years of experience with working sheep was 26 years (the minimum was 3 years, and the maximum was 75). Most survey respondents reported to use some form of pain relief during lamb marking before participating in this study (Figure 17). The most common pain relief reported was Tri-Solfen (48.6%) and NUMNUTS® (34.3%). The age of survey respondents ranged from 25 years to more than 65 years. Most respondents felt in the category of 35-44 years (31.8%) followed by 55-64 years (29.5%, Figure 18).

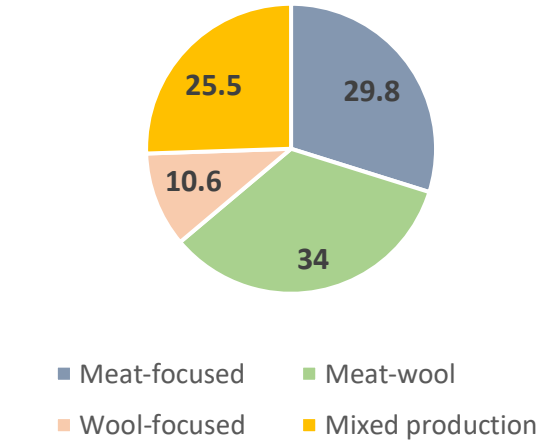

Figure 16: Main farming enterprise of survey respondents

*Producers were categorised as running mixed enterprises if sheep was not their main farming enterprise.

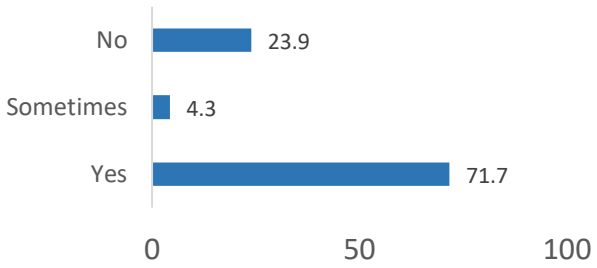

Figure 17: Percentage of participants that used pain relief before participating in the study (n=41)

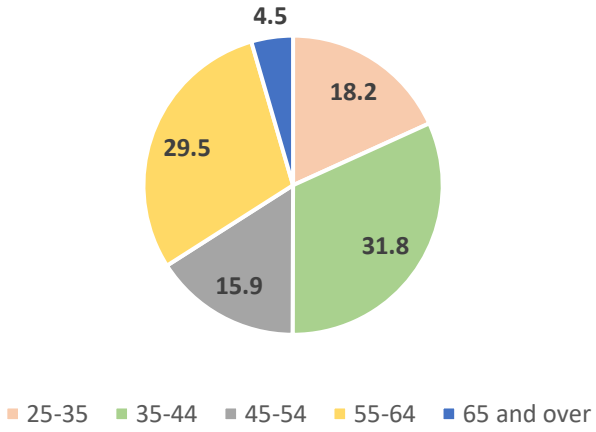

Figure 18: Age of participants

4.1.2.2 General opinions about pain relief

Participants were asked about their general opinions about the provision of pain relief at lamb marking. Overall, provision of pain relief was considered important by the participants, but the majority of survey respondents believed that the use of pain relief at lamb marking is not at all common (31.1%) or only slightly common (31.1%) among producers (Figure 19).

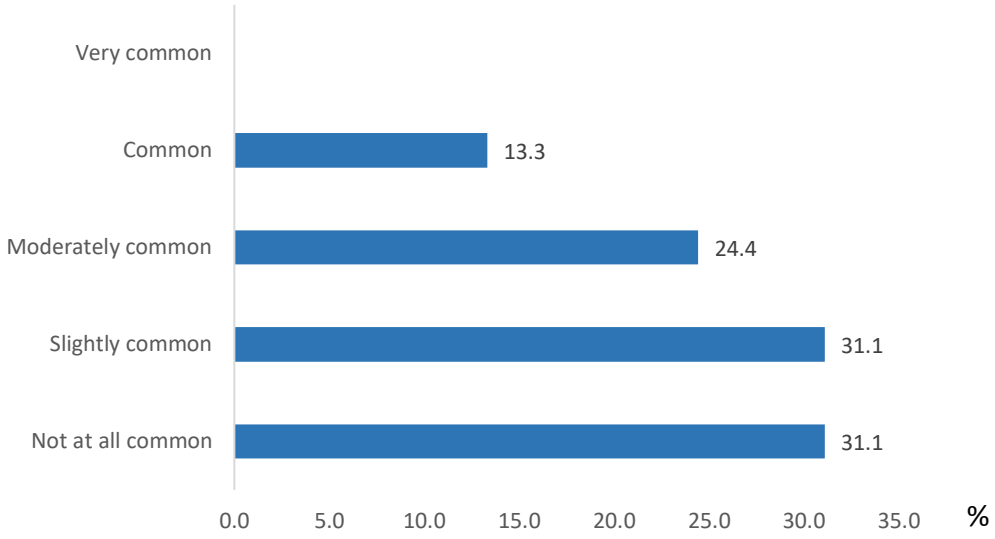

Figure 19: How common do you think the use of pain relief at lamb marking is?

When asked about why the provision of pain relief at lamb marking may not be common among producers, the most common reasons given by the participants were the costs associated (56%) and 'not enough information' (40%). Other reasons given were related to culture, poor education, and ignorance.

Participants were asked to rate the level of importance of providing sheep with pain relief for different husbandry procedures (they had to use a scale from 1 to 5, 1 meaning not at all important, and 5 meaning very important). Mulesing and tail stripping were considered the most important

procedures in relation to provision of pain relief (average of 4.8 and 4.3 out of 5, respectively), followed by breech freeze branding (average 3.8), castration and tail docking (both 3.5) and ear tagging (1.6).

In general, survey participants expressed positive views in relation to the provision of pain relief at lamb marking (Figure 20). Most survey respondents agreed/strongly agreed that the public supports the use of pain relief, agreed that there are effective pain relief options, and that the industry is somewhat at risk if pain relief is not mandatory. However, they also believed that current pain relief options are expensive and that most producers do not use pain relief at lamb marking.

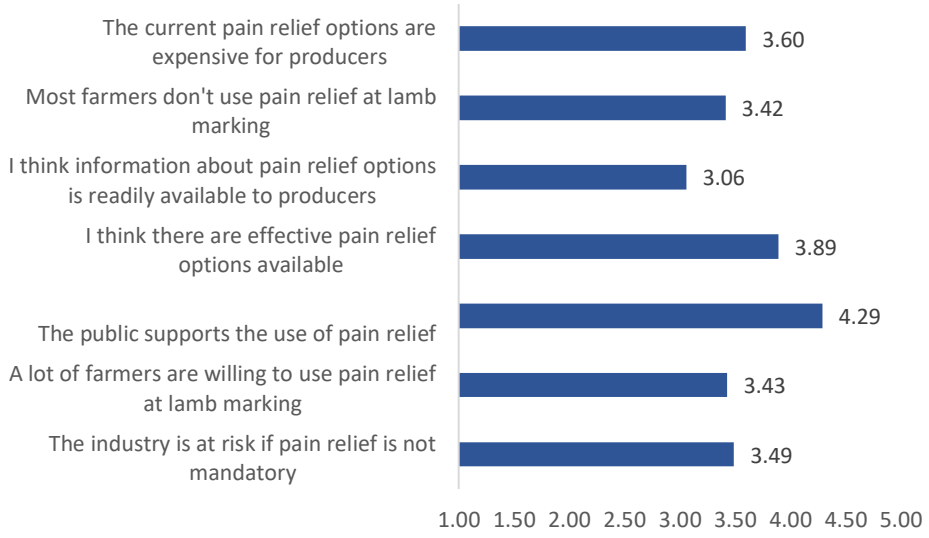

Figure 20: Mean score of general statements about pain relief at lamb marking using a Likert scale from 1 to 5. Mean scores closer to 5 reflect an agreement to the statement, mean scores closer to 1 reflect a disagreement to the statement.

4.1.2.3 General opinions about NUMNUTS®

When specifically asked about NUMNUTS®, participants' opinions where somewhat positive. In general, most participants believed NUMNUTS® is safe to apply, and somewhat believe that NUMNUTS® is effective in reducing pain and will recommend it to other producers (Figure 21).

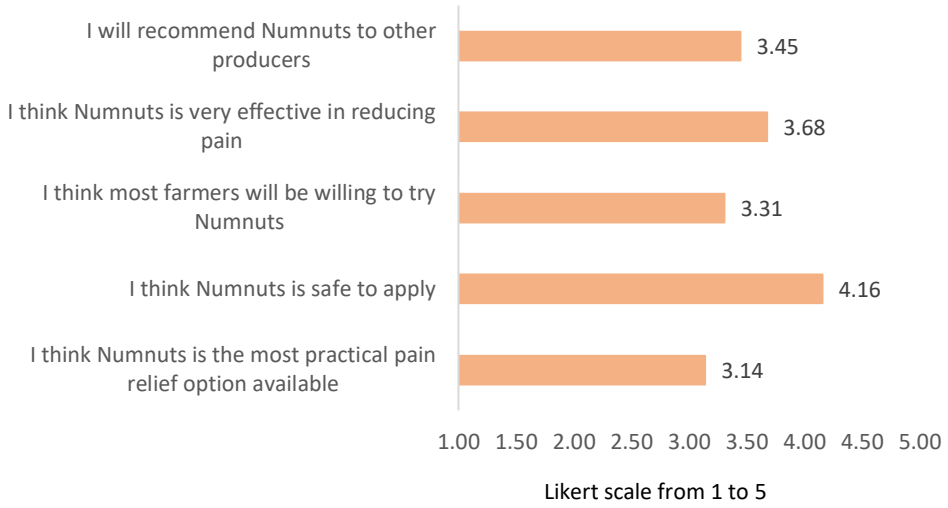

Figure 21: Mean score of general statements about NUMNUTS®

Most producers that participated in this study were aware of how NUMNUTS® works. A total of 77.3% of participants responded correctly to the question *'What is your understanding of how NUMNUTS® works?'* while 20.5% of the respondents believed NUMNUTS® is an anti-inflammatory, combination of quick acting and long-lasting, and 2.3% of the respondents believed it is an anti-inflammatory, quick acting and not long-lasting (Figure 22).

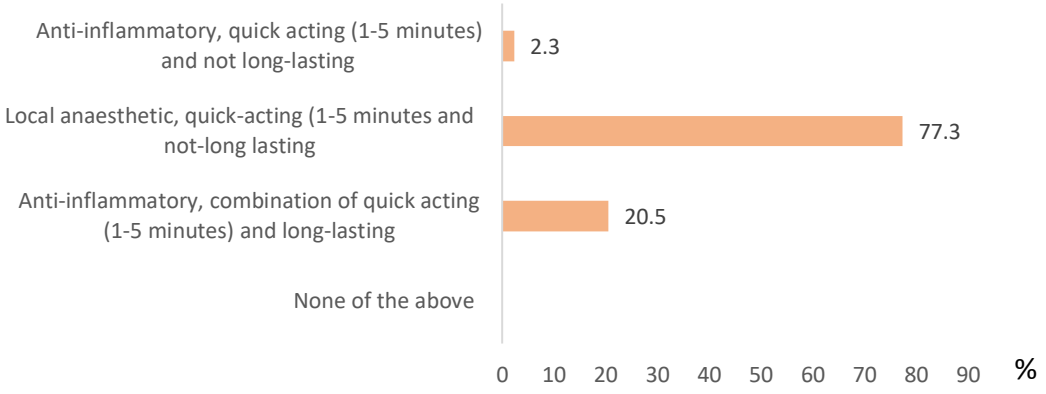

Figure 22: Percentage of responses to the question 'What is your understanding of how NUMNUTS® works?'

Satisfaction with the levels of pain relief provided by NUMNUTS® varied among the participants. Overall, a total of 45.5% of participants felt very satisfied/somewhat satisfied with the level of pain relief provided by NUMNUTS®, while 34% felt somewhat dissatisfied/very dissatisfied and 20.5% felt neutral (neither satisfied nor dissatisfied) (Figure 23). In terms of perceived effectiveness, survey respondents rated the effectiveness of NUMNUTS® in reducing pain relief as a 3.5 out of 5 for lamb castration and a 3.3 for tail docking.

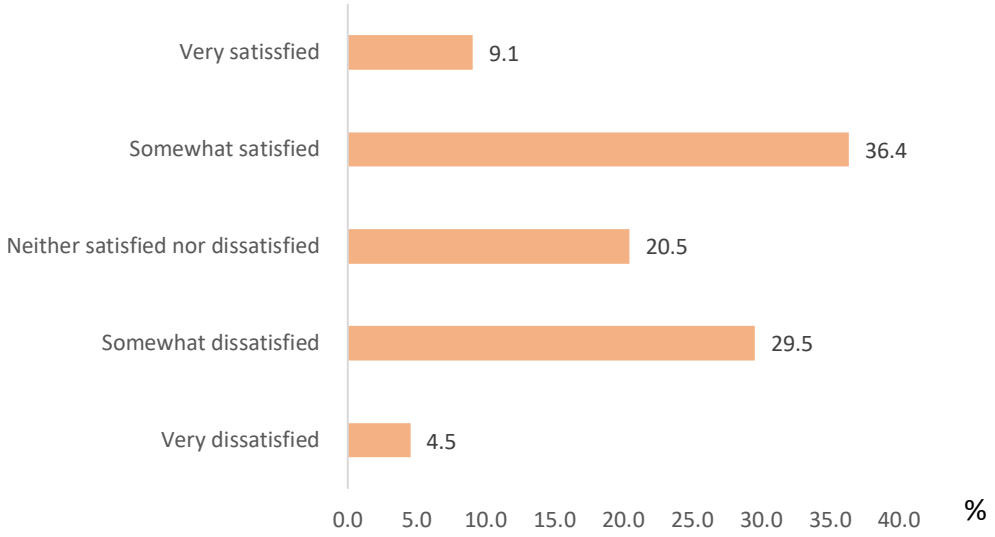

Figure 23: Percentage of responses to the question 'Overall, how satisfied were you with the level of pain relief provided by Numnuts?'

When asked about the likelihood of keep using NUMNUTS® for lamb marking, most participants (47.7%) stated that they are very likely/likely to keep using it, while 34% stated that they will not keep using it, and 18% felt neutral, or undecided (Figure 24).

Main comments given by the participants that will keep using NUMNUTS® included:

- 'Animal welfare reasons, [we] believe it is the best option for the animal'
- 'I can bring lamb marking forward probably 3-4 weeks this year meaning get them marked with minimal disturbance and weight loss'
- 'Like the overall response from our lambs - less stress and pain exhibited'
- 'We are an education facility, and we want to spread the message that pain relief should be of a prime concern for all producers as this aid's animal welfare issues'

Main comments given by the participants that will not keep using NUMNUTS® or felt undecided included:

- 'Costly, took time and didn't see enough effectiveness'
- 'It was difficult to use the applicator and very time-consuming. I felt that they worked better for castrating than tail docking. Not sure if it was me, but had the feeling that the needle hit bone or gristle in the tail too often'
- 'Not as effective as we'd like'
- 'We won't use it for tail docking as the rubber rings is not the preferred method for tail docking. We may consider it for castration'.

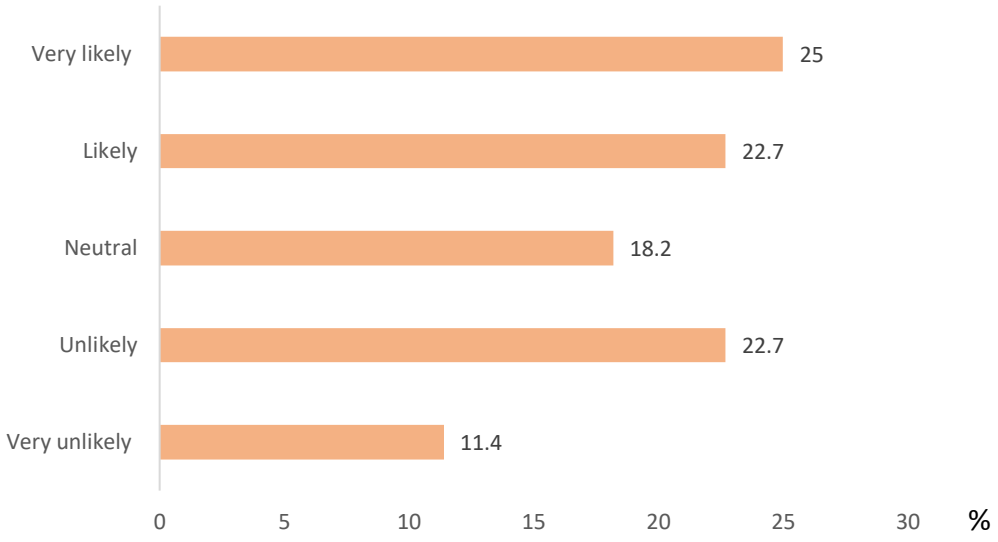

Figure 24: Percentage of responses to the question 'How likely are you to keep using NUMNUTS® for lamb marking?'

Most farmers that participated in the trial are likely to recommend NUMNUTS® to other producers (45.4%), while 38.6% of participants are neutral/undecided and 15.9% are unlikely to recommend it (Figure 25).

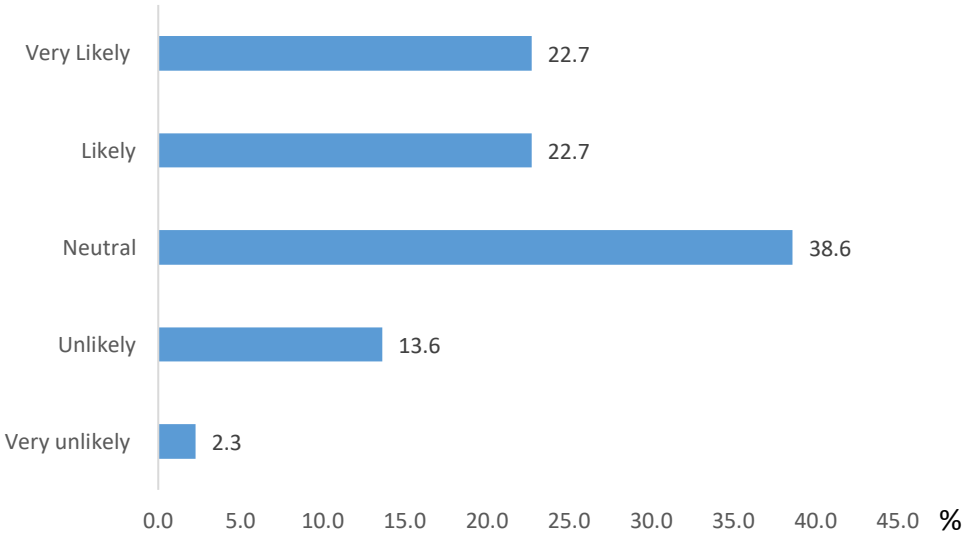

Figure 25: Percentage of responses to the question 'How likely are you to recommend NUMNUTS® to other producers?'

To further explore these results, the on-farm behavioural observations conducted during Trial A were analysed against attitude data obtained from the survey. Spearman correlations and chi-squared analyses revealed no significant relationships between behavioural observations

(differences observed between control and treatment groups) and participants' likelihood of/willingness to keep using NUMNUTS® or their likelihood to recommend it to other producers.

When attitude data was compared against participants' likelihood of keep using NUMNUTS®, moderate positive correlations were disclosed. Overall, results showed positive correlations between the likelihood of keep using NUMNUTS® at lamb marking and participants' beliefs that *'Numnuts is very effective in reducing pain' (r=0.60, p=<0,001)* that *'Numnuts is the most practical pain relief option available'* (r=0.46, p=0.02)*, 'I think most farmers will be willing to try Numnuts' (r=0.31, p=0.02* and that '*Numnuts is safe to apply' (r=0.41, p=0.007)*. Similarly, moderate positive correlations were disclosed between positive attitudes towards pain relief in general (r=0.34, p=0.02) and the likelihood of recommending Numnuts to other producers. Participants were also asked to provide feedback or recommendations to NUMNUTS®. Main comments/concerns were in relation to the practicality of the applicator, and issues with leaking product. Specific comments and concerns from the participants included:

- *'Applicator needs to be improved to prevent drug loss and suitable for smaller hands'*
- *'Numnuts applicator was leaking and fiddly'*
- *'The idea seems to be very good, however, the applicator would need to be more user-friendly, and the cost reduced'*
- *'The chance of temporal paralysis if not applied correctly at tail'*
- *'Would be easier if the device was smaller, easier to use'*
- *'Our applicator did give us trouble on a regular basis'.*

4.1.2.4 Experience with the trial

Most participants felt very satisfied/satisfied with how the trial was conducted (81.8%, Figure 26), and most participants (65.9%) felt supported by the research team during the trials. General comments from the participants around this topic included:

- *Having Jim come and be there on the day was very beneficial, and it was interesting to take part in the study.*
- *It takes a considerable amount of time and effort to conduct it properly. We needed an extra person to help.*
- *I think we were the first or one of the first to do the trial in South Australia.*
  Colin Trengove was instrumental in getting us to do the trial and helped with its implementation. There was quite a bit of work involved in setting up extra pens in the yards to run the trial and extra manpower involved. I think we had to observe the lambs for 3 hours. 2 hours would probably be long enough.
- *Thanks for your support and encouragement to join in this trial*

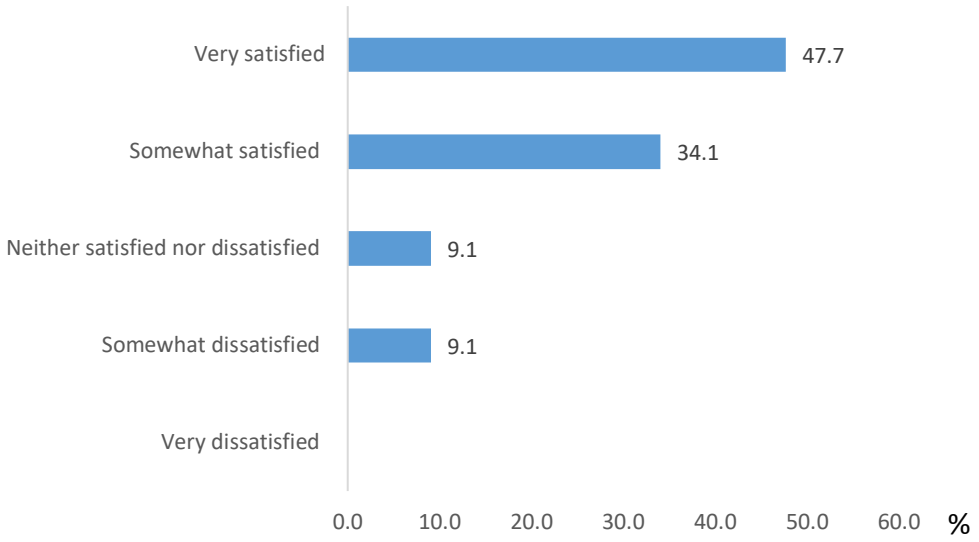

Figure 26: Percentage of responses to the question How satisfied were you with the experiment?

According to participants, main challenges with the trial included issues with the GoPro cameras, issues with the applicator (e.g., malfunction or leaking) and the time commitment involved during lamb marking and the extra handling of animals.

Further comments raised to the research team were in general positive (Figure 27). Producers in general thank the team for the opportunity to participate in the trial with comments including:

- ‘ Very well run!’
- ‘Thanks for letting us be involved! Always keen to try new things’
- ‘It was very well done, efficient, effective and hopefully the results pave the way forward’.
- ‘It was very effective comparing the pens side by side. I don’t think our business would have so easily taken it onboard without our own visual witness to the event on our own property and own animals’.

Other comments for the research team to considered included:

- ‘Short term relief was reasonable but no real long-term difference. I'd need to see a better-quality lamb at market time or a higher sale price to make me use this product in the future. It just adds cost to production and extended time to use’
- ‘ Trials are always a bit of a fiddle because usually they involve separate mobs and specific treatments etc. The $1,000 was not expected (when it was first mentioned I got confused and thought I was expected to pay $1,000 to participate in the trial, I wasn't too keen on that idea) and I felt very fairly covered the cost of our participation in the trial.

Figure 27: Word cloud to the question 'Any further comments to the research team?'.

4.2 Trial B

4.2.1 Acute pain related behaviours

There was no time by treatment interaction effect for Restlessness (RST), however there was a treatment ($\chi 2$, 1437 = 810.3, $P < 0.001$), time ($\chi 23$, 1414 = 506.1, $P < 0.001$), and pen effect ($\chi 3$, 1407= 491.9, $P = 0.007$). At 10 minutes post castration the RING group displayed significantly more RST behaviour than NUMNUTS lambs (mean = 1.0 ± 0.4 vs 0.2 ± 0.4 respectively, $z = 2.5$, $P = 0.01$). There was no difference between NUMNUTS and SHAM lambs at 10 minutes ($z = -0.008$, $P = 0.99$) (Figure 28).

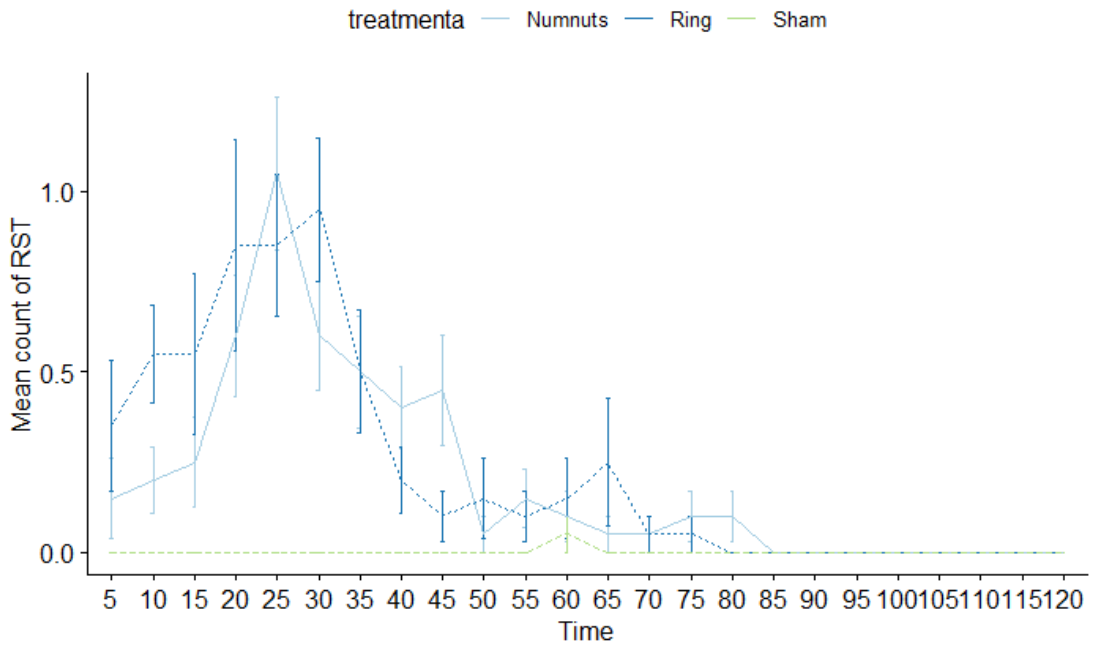

Figure 28: Mean count of restless behaviour (RST) by time point post castration.

For Kicking/foot stamping behaviour (FSK), there was a time by treatment effect (χ46, 1361= 717.2, P < 0.001). As well as an effect of cohort (χ4, 1410= 893.8, P < 0.001) and pen (χ3, 1407= 820.3, P < 0.001). At 5 minutes post castration lambs in the RING group displayed significantly more FSK behaviours (mean = 1.5 ± 0.4, z = 3.93 P < 0.001) compared to lambs in the NUMNUTS group (mean = 0.4 ± 0.4). There was no difference between the NUMNUTS and SHAM lambs at 5 minutes (z = -0.01, P = 0.99) (Figure 29).

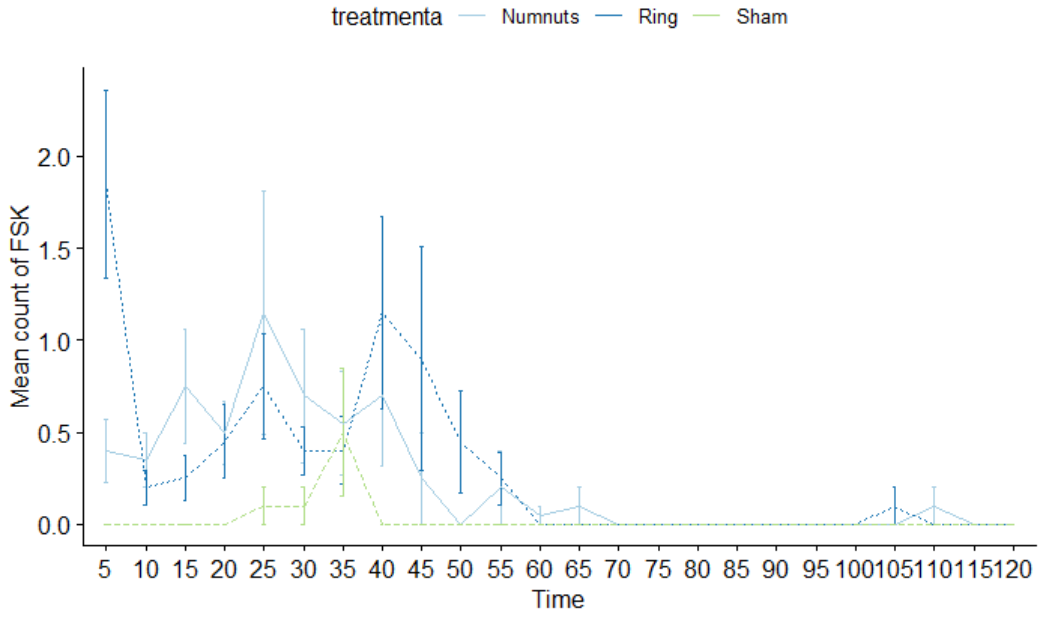

Figure 29: Mean count of foot stamping and kicking (FSK) behaviours at each time point post castration.

For sum of all acute behaviours there was a time by treatment (χ46, 1361= 979.9, P < 0.001), cohort (χ4, 1410 = 1123.9, P < 0.001) and pen (χ3, 1407 = 1073.5, P < 0.001) effect. There was a significant difference between NUMNUTS, RING (z = 4.36, P < 0.001) and SHAM (z = -2.35, P = 0.01) lambs at 5 minutes post castration (figure 30).

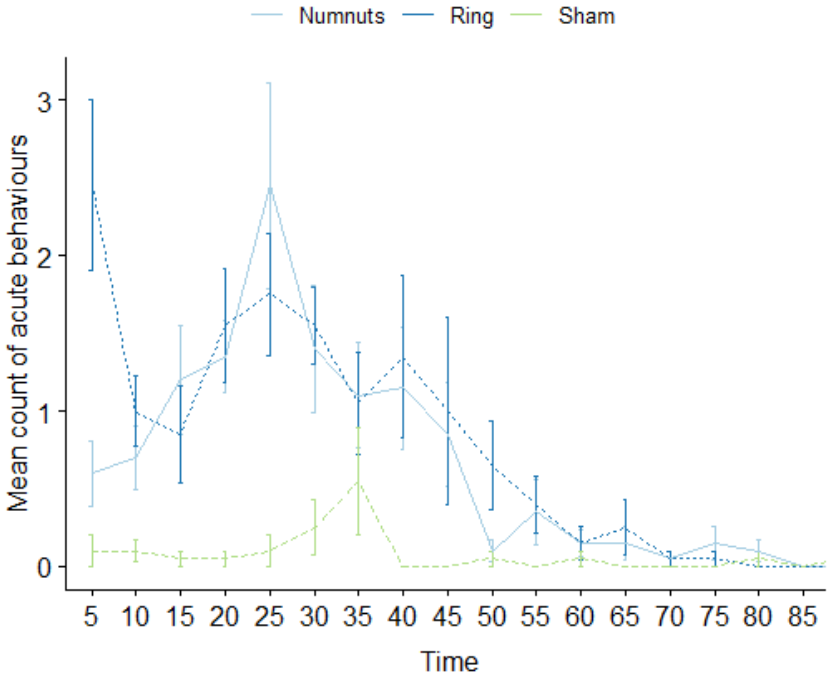

Figure 30: Mean count of sum of all acute pain behaviours every 5 minutes post castration in the first 85 minutes after castration.

4.2.2 Postural behaviours

There was no difference in the mean sum of upright postures between the NUMNUTS (mean = 6.3 ± 0.6) and RING group (mean = 5.1 ± 0.6, t_{50} = -1.4, P = 0.17) or NUMNUTS and SHAM group (mean = 7.25 ± 0.8, t_{50} = 1.1, P = 0.29). There was no effect of cohort (F_4 = 1.6, P = 0.17) or pen (F_3 = 0.9, P = 0.45)

For lying postures there was a treatment effect (F_2 = 5.7, P = 0.006). Lambs in the RING group tended to display more lying behaviors than the NUMNUTS lambs (t_{50} = 1.9, P = 0.06, Figure 31). There was no difference between NUMNUTS and SHAM lambs (t_{50} = -1.4, P = 0.16). There was no effect of cohort (F_4 = 1.4, P = 0.35) or pen (F_3 = 1.1, P = 0.37).

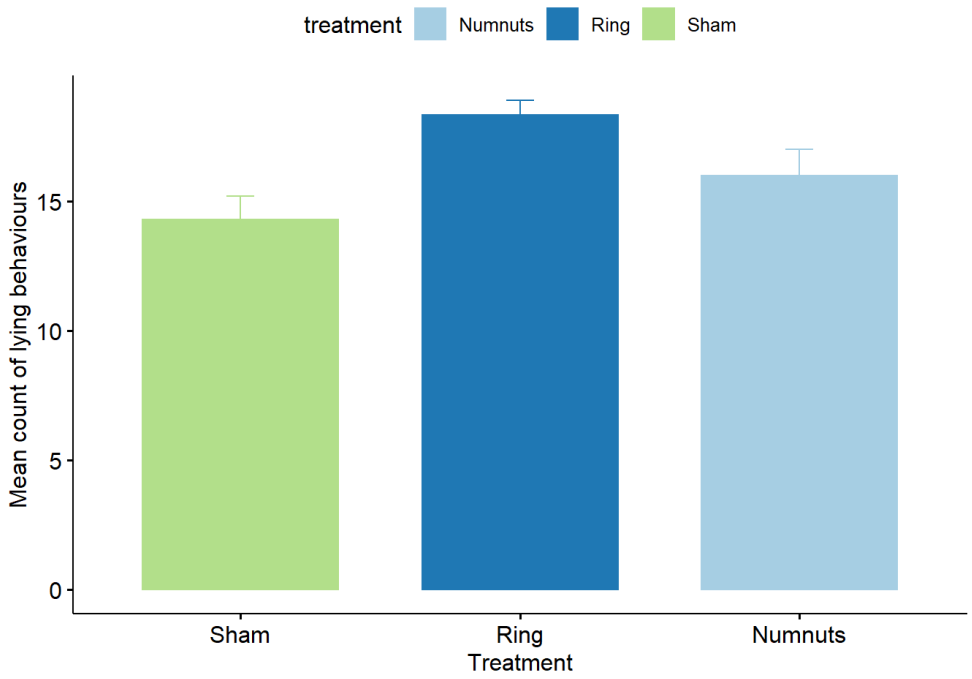

Figure 31: Mean count of all lying behaviors (Nl, Ll, Al) with standard error bars, for SHAM, RING and NUMNUTS lambs (n = 20 each group) in the 2 hours following treatment.

There was a significant treatment effect for abnormal postures (F_2 = 42.91, P < 0.001). There was no difference observed between RING lambs and NUMNUTS lambs in the display of abnormal postures over the 2 hours post castration (t_{50} = 0.62, P = 0.53). SHAM lambs displayed significantly less abnormal postures (mean = 6.1 ± 1.1) compared to RING (mean = 17.4 ± 1.0, t_{50} = 7.9, P = < 0.001) and NUMNUTS lambs (mean = 16.6 ± 1.0, t_{50} = 7.3, P = < 0.001). There was no effect of cohort (F_4 = 1.9, P = 0.11), but pen tended to affect the display of abnormal postures (F_3 = 2.4, P = 0.08): animals in Pens 3 and 4 (on the northern side of the animal house) showed an increased mean display of 3.3 ± 1.6 (P = 0.04) and 3.06 ± 1.6 (P = 0.06) of abnormal postures, as compared to Pens 1 and 2 (on the southern side of the animal house).

5. Discussion

5.1 Trial A

5.1.1 On-farm trials

Observations of lamb behaviour were carried out by individuals who, although familiar with sheep, were untrained in the nuances of detailed behavioural observation. This is in contrast to controlled studies in a research context, where a defined ethogram is prepared and structured observations conducted (Mellor et al., 1991, Molony et al., 1993, Paull et al., 2012, Small et al., 2020). In the current study, the observation protocol used was more qualitative and subjective, which would be expected to lead to a greater likelihood of inter-observer differences than a structured count of specific behaviours. Furthermore, active pain-related behaviours are performed intermittently

rather than continuously, such that at any particular observation point, some lambs may behave normally, despite experiencing pain. Many factors can influence the expression of active pain-related behaviours, such as individual stoicism, the presence of conspecifics, presence of feed and other distractions (Small et al., 2014, Small et al., 2018). As prey species, there is an evolutionary imperative for lambs to return to near-normal behaviours as soon as possible after an insult, to reduce the risk of predation. Thus, it is expected that the scores for rolling/fidgeting or for discomfort assigned to the lambs in the study will reduce over time.

The observers were asked to score a series of calibration video clips prior to conducting the on-farm trials. Tellingly, during this exercise, 10-20 % of observers failed to identify a difference between the two pens in terms of rolling/fidgeting, escape attempts or comfort/demeanour at the 10-minutres post marking time point, when the difference between the two groups is expected to be at its most pronounced. At later time points, when the difference between the two groups is expected to diminish, and/or lamb behaviours to return towards normal (Small et al., 2020), the ability of the untrained observers to detect differences between the two pens was reduced to 40 % or less.

Nevertheless, the outcomes of this study reflect the findings of controlled studies under research conditions, namely that a benefit of delivering lignocaine using the NUMNUTS® device can be observed for the first 40-60 minutes post marking. This period is slightly longer than that identified in some of the controlled studies (Small et al., 2020, Small et al., 2021b, Small et al., 2021a), and may be a reflection of the different research methodologies used. In the controlled studies, experienced, trained observers count incidences of specific behaviours performed by individual lambs; while in the current study, untrained observers perform a more qualitative mob-based assessment of the overall activity levels of groups of lambs. In context of the outcomes of the 'calibration' exercise, it is interesting to note that every observer identified a benefit of NUMNUTS over RING based on assigning a lower score for rolling/fidgeting at one or more timepoint in one or more replicates, while 47 of 52 (90%) of observers identified a benefit of NUMNUTS over RING based on assigning a lower score for discomfort at one or more timepoint in one or more replicates.

The observers were fully blinded to treatment when scoring the calibration video clips, but during the on-farm trials, although the observers were not to be part of the team conducting lamb marking, true blinding cannot be guaranteed, and a degree of bias cannot be ruled out. This may account for the observed benefit of NUMNUTS over RING by some observers for some replicates in the period of 60-120 minutes post marking, which is not evident in previous controlled trials.

Nevertheless, despite the confounding inherent in conducting a study across multiple locations with multiple operators; and despite inter-observer variability, NUMNUTS provided a significant reduction in rolling/fidgeting scores ($P < 0.001$) and a significant reduction in discomfort scores ($P < 0.01$) as compared to RING.

5.1.2 Producer survey

Survey results showed that participants had overall positive attitudes to the provision of pain relief at lamb marking and positive attitudes towards NUMNUTS. Most participants believed NUMNUTS is safe to apply and were satisfied with the level of pain relief provided. Overall, participants perceived that NUMNUTS was more effective for lamb castration than tail docking. After conducting the trial, a small majority of producers stated that they will keep using NUMNUTS and they will recommend it to producers.

Main results suggests that attitudes are important factors influencing the decision to use NUMNUTS. Survey data indicated that participants that had more positive attitudes towards the use of pain relief in general, and more positive attitudes towards the effectiveness of NUMNUTS are more likely to keep using it or recommend it in future. These results are in agreement with a vast amount of research demonstrating that attitudes are important drivers of behaviour in the livestock sector (Coleman and Hemsworth, 2014, Hemsworth and Coleman, 2011, Waiblinger et al., 2002). When on-farm data (differences between control and treatment groups) were assessed against survey data, no relationship was observed with participants' intention to keep using NUMNUTS. This means, that even if low differences in behaviours were observed on-farm, between control and treatment groups, that was not necessarily a significant factor to influence participants' intention to keep using NUMNUTS. It needs to be considered, however, that the person assessing lamb behaviour on-farm may not have been the same person that completed the attitudinal survey, but the observed was meant to be blind to the treatments. While attitudes seem to be important factors, it should be considered that the decision on using pain relief is based on more than perceived effectiveness. It is a business decision that includes cost/benefit considerations and effects that extend beyond animal welfare and direct costs per se such as social license, positive effect on work force and competitive advantage (Fernandes et al., 2021).

It is also worth noting, that after the trial, a small percentage of participants felt dissatisfied by the level of pain relief provided by NUMNUTS and started that may not keep using it in future lamb markings and may not recommend it to others. Main barriers to adoption identified in this study related to issues with practicality (e.g., size of the prototype, issues with leaking product, etc) and/or low perceived effectiveness and/or the costs associated with its implementation. Important aspects to consider to increase the adoption rate included improvements with size (smaller will make it more user-friendly) and the applicator (to prevent leakage and drug loss). A deeper understanding of the true cost/benefit picture associated with pain in lambs is also required to support adoption of pain relief for marking across industry. At present, 'benefit' is considered in terms of the visible behaviour changes observed; and is therefore considered to be 'limited' due to the fact that the observed behavioural differences between treated and untreated lambs are not large after about 30 minutes post marking. However, there are a number of hidden costs to the lamb as a result of unmitigated pain, that provision of adequate pain relief is likely to ameliorate. For example: In the lamb itself, there may be impacts on feed conversion efficiency. Although previous studies have failed to show a significant difference in weight gains over a 4-week period between marked and unmarked lambs, let alone showing a difference between marked lambs that have or have not received pain relief, these studies are conducted in the context of *ad libitum* feed provision and/or the presence of a lactating ewe. Thus, the feed intake required to achieve the observed compensatory growth following marking is not measured, and the feed conversion efficiency not calculated. In human medicine, it is well known that provision of adequate pain relief following surgery leads to more rapid return to function. In lambs this can be partially indicated through improvements in mothering-up (Small et al., 2020), and also from anecdotal feedback from producers that they find it easier and faster to return lambs to paddocks following marking when pain relief has been provided (Senesino, personal communication). Better return to function can also improve lamb survival, as they will be better able to stay with the flock, reducing the risk of abandonment and starvation. Again, much of the information on mortality rates in lambs post marking with or without pain relief is anecdotal, some farmers reporting an increase in lamb survival to weaning of 2% or more (Senesino, personal communication). However, there is a single publication supporting the potential of pain relief (Meloxicam) to improve lambs survival rates

(Small et al., 2021c), and further work is warranted to assess the production effects of local anaesthetic and multimodal pain relief approaches on farm.

Other hidden costs of pain in marking include effects on the dam. If the lamb is requiring additional nourishment to heal and grow, there may be increased demand on her lactation, which in turn affects her nutritional balance, and the potential to lose body weight as a result. Loss of body condition can in turn influence her ability to conceive in a future season. When multiple ewes in a flock are affected, this reduces the overall conception rate of the flock, which in turn reduces the number of lambs weaned in subsequent years, can increase ewe culling rates and affect the overall profitability of the farm. There are also labour/staffing aspects: lambs that are in pain are more difficult to move and may require more intensive monitoring over the first few days post marking to ensure that mismothering and abandonment does not occur. This leads to additional strain on labour resources, can lead to frustration in handlers, and an overall decline in job satisfaction and increased staff turnover.

5.2 Trial B

The current study found that a 1.5 ml delivery of lignocaine to the site of ring castration using the Numnuts® device led to a reduction in acute pain related behaviours in the first 10 minutes following castration. However, the lambs in the NUMNUTS group still displayed similar amounts of pain related behaviours and pain related postures as RING lambs for the remaining duration of the study. This result is similar to previous studies that have found that lignocaine decreases acute behavioural responses but not postural behaviours of pain, however the effects of lignocaine in this study did not last as long as previously reported (Kent et al., 1998, Small et al., 2020).

Pain caused by ring castration has previously been reported to last over an hour with acute behaviours such as restlessness and kicking/foot stamping being displayed by lambs undergoing the procedure (Kent et al., 1998, Grant, 2004). These behaviours were observed in the lambs in the current study with acute pain behaviours being at its peak between 5- and 20-minutes and reducing by 60 minutes post castration. These results are supported by previous research in which lambs that are ring castrated and tail-docked reported to display increased active pain avoidance behaviours in the first 20 minutes post treatment and dramatically reducing over the hour (Mellor et al., 1991, Molony et al., 1993, Small et al., 2020). It has also been previously reported that lambs undergoing ring castration and tail docking spend more time lying compared to lambs that do not undergo treatment (Molony et al., 1993). This was also the case in the current study. Lambs in the RING group were observed lying down more often than SHAM lambs, and the occurrence of the behaviour being slightly reduced in the NUMNUTS group.

When looking at cortisol response alone during ring castration, lambs administered with lignocaine can have a cortisol increase of 30% in some cases, but it can be as high as 200% (Mellor and Stafford, 2000). The cortisol response following castration is highly variable depending on the age of the lambs, the injection site of lignocaine and the additional use of other equipment such as clamps (Mellor and Stafford, 2000).

Provision of lignocaine to lambs undergoing ring procedures has previously been shown to ameliorate the pain response in lambs undergoing ring castration as well as ring tail docking for up to 8 hours (Kent et al., 1998, Thornton and Waterman-Pearson, 1999, Mellema et al., 2006, Stewart

et al., 2014). Lignocaine is a fast onset short-acting local anaesthetic with its peak plasma concentration time occurring between 5 - 10 minutes when injected subcutaneously. The peak concentration of lignocaine varies depending on the location injected and the level of blood flow in the region, for example a 1 mL solution of 10% lignocaine reaches 1.15 mg/l when injected in the forelimb vs 1.96 mg/l in the hindlimb (Karatassas, 1992). The plasma half-life of lignocaine has been reported to be between 30 and 60 minutes (Santos et al., 1988, Karatassas, 1992). When administered with adrenaline the time to peak plasma increases as does the half-life (Karatassas, 1992). Due to the short duration of action of lignocaine other studies have opted to use alternative local anaesthetics such as bupivacaine which are longer acting, in order to ameliorate the pain response associated with castration (Graham et al., 1997, Molony et al., 1997).

The amelioration of some pain related behaviour in lambs in the NUMNUTS group compared to RING lambs, indicates the effectiveness of the NUMNUTS® device at delivering a measured dose of local anaesthetic to the site of ring castration. The effects of pain relief only lasting for the first 10 minutes following treatment is most likely due to the fact lignocaine was used as the analgesic for this trial. Due to the high vascularity of the area where the ring is applied and the fast onset and short duration of lignocaine it is not surprising that pain relief effects were not observed beyond 10 minutes.

6. Conclusion

6.1 Key findings

Trial A

All observers identified a benefit of NUMNUTS over RING based on assigning a lower score for rolling/fidgeting at one or more timepoint in one or more replicates, while 47 of 52 (90 %) of observers identified a benefit of NUMNUTS over RING based on assigning a lower score for discomfort at one or more timepoint in one or more replicates.

A benefit of NUMNUTS was most clearly evident in the first 40-60 minutes post marking, with 45-55 % of replicates yielding scores indicative of reductions in pain-related behaviours; while during the 60-120 minute time period a benefit was recorded in 10-30 % of replicates.

Despite the confounding factors inherent in conducting a study across multiple locations with multiple operators; and despite inter-observer variability, NUMNUTS provided a significant reduction in rolling/fidgeting scores ($P < 0.001$) and a significant reduction in discomfort scores ($P < 0.01$) as compared to RING.

Survey Findings

In general, participants' attitudes towards the use of pain relief during painful husbandry procedures were positive.

On-farm differences observed between control and treatment groups does not necessarily reflect producers' willingness to keep using NUMNUTS.

Attitudes seem to be more important factors in the decision. Participants that had more positive attitudes towards the use of pain relief and towards NUMNUTS were more likely to state that they will keep using it and recommend it to others.

A small percentage of participants felt dissatisfied with the levels of pain relief provided by NUMNUTS and may not keep using it in the future. Main barriers to adoption related to issues with practicality (e.g., size of the prototype, issues with leaking product and overall malfunctioning) and/or low perceived effectiveness and/or the costs associated with its implementation.

There were some issues with leaking or malfunctioning equipment – these have been corrected by the NUMNUTS company (Senesino Pty Ltd) for the 2022 marking season.

Trial B

A single central injection of local anaesthetic, using the NUMNUTS® tool can alleviate the behavioural responses to ring castration in the immediate post-procedure period.

The duration of effect is limited, which may be a result of the agent used (lignocaine 20 mg/mL).

Development of a longer-lasting local anaesthetic formulation is imperative to optimise pain mitigation for ring castration.

6.2 Benefits to industry

This project has provided evidence that reductions in pain-related behaviours in lambs are observed in a commercial setting, provided the opportunity for a group of producers to try out the NUMNUTS® device with support from research staff, and identified some of the challenges to wider adoption of pain relief at marking.

Issues relating to equipment malfunction were reported to the NUMNUTS® company, and these have been addressed for the 2022 marking season.

Adverse effects were limited to one incident of hindlimb ataxia, across 52 farms and 1470 lambs that received NUMNUTS® application.

7. Future research and recommendations

A key challenge in Trial A was achieving consistent conduct of the trial protocol across farms. This was facilitated on those farms on which a research technician or trained local co-ordinator could be present. We would recommend that future on-farm studies always involve a research technician.

A small percentage of participants felt dissatisfied with the levels of pain relief provided by NUMNUTS and may not keep using it in the future. The decision on using pain relief is based on more than perceived effectiveness. It is a business decision that includes cost/benefit considerations and effects that extend beyond animal welfare and direct costs per se such as social license, positive effect on work force and competitive advantage. Further research is required to understand the hidden costs of pain in lambs and develop a bio-economic model to demonstrate cost/benefit rations to producers.

There were some issues with leaking or malfunctioning equipment – these have been corrected by the NUMNUTS company (Senesino Pty Ltd) for the 2022 marking season.

The duration of effect of lignocaine in lambs is disappointing. Further research to develop a longer-lasting local anaesthetic agent or formulation is warranted.

8. References

4CDESIGN LTD. 2015. Numnuts Phase 2: Humane, Low Pain Method for 'marking' Young Ruminants. Sydney: Meat & Livestock Australia.

AJZEN, I. & FISHBEIN, M. 1980. *Understanding attitudes and predicting social behavior.,* Englewood Cliffs, N.J., Prentice-Hall.

COLEMAN, G. J. & HEMSWORTH, P. H. 2014. Training to improve stockperson beliefs and behaviour towards livestock enhances welfare and productivity. *Revue Scientifique Et Technique-Office International Des Epizooties,* 33**,** 131-137.

DINNISS, A. S., STAFFORD, K. J., MELLOR, D. J., BRUCE, R. A. & WARD, R. N. 1999. The behaviour pattern of lambs after castration using a rubber ring and/or castrating clamp with or without local anaesthetic. *New Zealand Veterinary Journal,* 47**,** 198-203.

FERNANDES, J. N., HEMSWORTH, P. H., COLEMAN, G. J. & TILBROOK, A. J. 2021. Costs and Benefits of Improving Farm Animal Welfare. *Agriculture-Basel,* 11.

GRAHAM, M. J., KENT, J. E. & MOLONY, V. 1997. Effects of four analgesic treatments on the behaviouraland cortisol responses of 3-week-old lambs to tail docking. *The Veterinary Journal,* 153**,** 87-97.

GRANT, C. 2004. Behavioural responses of lambs to common painful husbandry procedures. *Applied Animal Behaviour Science,* 87**,** 255-273.

HEMSWORTH, P. H. & COLEMAN, G. J. 2011. *Human-Livestock Interactions, 2nd Edition: The Stockperson and the Productivity and Welfare of Intensively Farmed Animals*.

KARATASSAS, A. 1992. *The effect of regional blood flow on the pharmacokinetics of infiltrated doses of lignocaine.* Master of Surgery Thesis, University of Adelaide.

KENT, J. E., JACKSON, R. E., MOLONY, V. & HOSIE, B. D. 2000. Effects of acute pain reduction methods on the chronic inflammatory lesions and behaviour of lambs castrated and tail docked with rubber rings at less than two days of age. *Veterinary Journal,* 160**,** 33-41.

KENT, J. E., MOLONY, V. & GRAHAM, M. J. 1998. Comparison of methods for the reduction of acute pain produced by rubber ring castration or tail docking of week-old lambs. *Veterinary Journal,* 155**,** 39-51.

KENT, J. E., MOLONY, V. & GRAHAM, M. J. 2001. The effect of different bloodless castrators and different tail docking methods on the responses of lambs to the combined Burdizzo rubber ring method of castration. *Veterinary Journal,* 162**,** 250-254.

LLOYD, J. & PLAYFORD, M. 2013. *A producer's guide to sheep husbandry practices,* Sydney, Meat & Livestock Australian (MLA).

MELLEMA, S. C., DOHERR, M. G., WECHSLER, B., THUEER, S. & STEINER, A. 2006. Influence of local anaesthesia on pain and distress induced by two bloodless castration methods in young lambs. *Veterinary Journal,* 172**,** 274-283.

MELLOR, D. J., MOLONY, V. & ROBERTSON, I. S. 1991. Effects of castration on behavior and plasma-cortisol concentrations in young lambs, kids and calves. *Research in Veterinary Science,* 51**,** 149-154.

MELLOR, D. J. & STAFFORD, K. J. 2000. Acute castration and/or tailing distress and its alleviation in lambs. *New Zealand Veterinary Journal,* 48**,** 33-43.

MOLONY, V., KENT, J. E., HOSIE, B. D. & GRAHAM, M. J. 1997. Reduction in pain suffered by lambs at castration. *Veterinary Journal,* 153**,** 205-213.

MOLONY, V., KENT, J. E. & ROBERTSON, I. S. 1993. Behavioral-responses of lambs of 3 ages in the 1st 3 hours after 3 methods of castration and tail docking. *Research in Veterinary Science,* 55**,** 236-245.

MOLONY, V., KENT, J. E., VINUELA-FERNANDEZ, I., ANDERSON, C. & DWYER, C. M. 2012. Pain in lambs castrated at 2 days using novel smaller and tighter rubber rings without and with local anaesthetic. *Veterinary Journal,* 193**,** 81-86.

PAULL, D. R., SMALL, A. H., LEE, C., PALLADIN, P. & COLDITZ, I. G. 2012. Evaluating a novel analgesic strategy for ring castration of ram lambs. *Veterinary Anaesthesia and Analgesia,* 39**,** 539-549.

R CORE TEAM 2018. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

SANTOS, A. C., PEDERSEN, H., MORISHIMA, H. O., FINSTER, M., ARTHUR, G. R. & COVINO, B. G. 1988. Pharmacokinetics of lidocaine in nonpregnant and pregnant ewes. *Anesthesia and Analgesia,* 67**,** 1154-1158.

SMALL, A., FETIVEAU, M., SMITH, R. & COLDITZ, I. 2021a. Three Studies Evaluating the Potential for Lidocaine, Bupivacaine or Procaine to Reduce Pain-Related Behaviors following Ring Castration and/or Tail Docking in Lambs. *Animals,* 11**,** 3583.

SMALL, A., MARINI, D. & COLDITZ, I. 2021b. Local Anesthetic Delivered with a Dual Action Ring and Injection Applicator Reduces the Acute Pain Response of Lambs during Tail Docking. *Animals,* 11**,** 2242.

SMALL, A. H., BELSON, S., BREWER, H. & SCHMOELZL, S. M. 2021c. Marking to weaning production aspects of lambs provided with NSAID analgesia compared with lambs receiving no analgesia at the time of elastrator ring marking. *Australian Veterinary Journal,* 99**,** 40-43.

SMALL, A. H., BELSON, S., HOLM, M. & COLDITZ, I. G. 2014. Efficacy of a buccal meloxicam formulation for pain relief in Merino lambs undergoing knife castration and tail docking in a randomised field trial. *Australian Veterinary Journal,* 92**,** 381-388.

SMALL, A. H., JONGMAN, E. C., NIEMEYER, D., LEE, C. & COLDITZ, I. G. 2020. Efficacy of precisely injected single local bolus of lignocaine for alleviation of behavioural responses to pain during tail docking and castration of lambs with rubber rings. *Research in Veterinary Science,* 133**,** 210-218.

SMALL, A. H., MARINI, D., DYALL, T., PAULL, D. & LEE, C. 2018. A randomised field study evaluating the effectiveness of buccal meloxicam and topical local anaesthetic formulations administered singly or in combination at improving welfare of female Merino lambs undergoing surgical mulesing and hot knife tail docking. *Research in Veterinary Science,* 118**,** 305-311.

SMITH, R. 2019. Numnuts Phase 4: Late Stage R&D - Numnuts Low Pain Marking. Sydney: Meat & Livestock Australia.

SMITH, R., GRAHAM, M., COLDITZ, I. & SMALL, A. 2017. Numnuts® – consistent, safe delivery of local anaesthetic for lamb castration and tail docking. *In:* SARGISON, N. (ed.) *International Sheep Veterinary Conference.* Harrogate, UK: International Sheep Veterinary Association.

STEWART, M., BEAUSOLEIL, N. J., JOHNSON, C. B., WEBSTER, J. R., SCHUTZ, K. E., COX, N. & STAFFORD, K. J. 2014. Do rubber rings coated with lignocaine reduce the pain associated with ring castration of lambs? *Applied Animal Behaviour Science,* 160**,** 56-63.

THORNTON, P. D. & WATERMAN-PEARSON, A. E. 1999. Quantification of the pain and distress responses to castration in young lambs. *Research in Veterinary Science,* 66**,** 107-118.

THORNTON, P. D. & WATERMAN-PEARSON, A. E. 2002. Behavioural responses to castration in lambs. *Animal Welfare,* 11**,** 203-212.

WAIBLINGER, S., MENKE, C. & COLEMAN, G. 2002. The relationship between attitudes, personal characteristics and behaviour of stockpeople and subsequent behaviour and production of dairy cows. *Applied Animal Behaviour Science,* 79**,** 195-219.

9. Appendix

9.1Appendix 1: Flyer and media release content

Are you:

- Interested in trying NUMNUTS for ring castration and tail docking in lambs?
- Willing to run a research study on your property (with our support)?
- Able to set up 2 pens, to place 10 lambs in each pen and hold them there for 2 hours – and do this 3 times?
- Willing to score lamb behaviours during this 2-hour period (3 times)?
- Able to source NumOcaine from your veterinarian?
- Willing to answer a few questions about lamb marking and pain relief?

If so, we'd love to hear from you.

Contact:

or CSIRO:

Jim Lea: Jim.Lea@csiro.au, 0407137466

Alison Small: Alison.small@csiro.au

Media release as displayed on the CSIRO website:

Help us trial the Numnuts® targeted pain relief system for marking lambs – Livestock (csiro.au)

Help us trial the Numnuts® targeted pain relief system for marking lambs

CSIRO, in conjunction with Meat & Livestock Australia and Australian Wool Innovation, have tested and validated a targeted pain relief system for farmers to use when tail docking and castrating their lambs using the ring method.

Called Numnuts®, the system is now commercially available to the Australian sheep sector to improve the welfare of lambs at marking time.

In trials we conducted with the University of Melbourne, Numnuts® reduced the number of lambs displaying pain behaviours immediately after marking by up to 68%.

Take part in the Numnuts® extended commercial trial

We need farmers to try the Numnuts® targeted pain relief system to help trial it in real life on-farm settings.

It's a single day's commitment and there's an incentive payment of $1000 to cover costs. We provide all instructions and, where possible, arrange for a sheep vet or research technical support to be present all day.

The Numnuts anaesthetic, Numocaine. Image supplied.

Are you

- interested in trying Numnuts® for ring castration and tail docking in lambs?
- willing to run a research study on your property (with our support)?
- able to set up 2 pens, to place 10 lambs in each pen and hold them there for 2 hours – and do this 3 times?
- willing to score lamb behaviours during this 2-hour period (3 times)?
- able to source NumOcaine® from your veterinarian?
- willing to answer a few questions about lamb marking and pain relief?

Animal Behaviour and Welfare

Rearing enrichment for laying hens
Social facilitation of virtual fencing
Measuring how farm animals 'feel'
Is virtual fencing welfare-friendly?
Reviewing impacts of housing systems for cattle and poultry welfare
Precision Technology to Understand Animal Behaviour
Benchmarking beef cattle welfare
Understanding and relieving pain
Help us trial the Numnuts® targeted pain relief system for marking lambs

Share this

The Numnuts on-farm commercial trial set-up

For more information

Visit the Numnuts® store.

Contact:

Dr Ali Small →

Ali is a principal research scientist at CSIRO's McMaster Laboratory in Armidale, NSW, leading livestock welfare research in a number of areas including neonatal development and survival, alternatives to painful husbandry procedures, pain mitigation for livestock and humane slaughter.

Ali.Small@csiro.au +61 2 6776 1435

Jim Lea →

Jim has worked in large animal research for over 30 years researching immunology, parasitology, livestock transport on land and sea, pain alleviation in husbandry procedures, feed choice in feed lots and virtual fencing. Jim is also based at CSIRO's McMaster Laboratory in Armidale, NSW.

Jim.Lea@csiro.au 0407 137 466

9.2Appendix 2: Producer protocol

NN-ECT Producer Protocol

Extended Commercial Trials of NUMNUTS – On-Farm Study Protocol

Background

Controlled trials have demonstrated the efficacy of the NUMNUTS® device in providing effective pain relief for ring castration and tail-docking. This project aims to collect additional information on using the NUMNUTS® device in a commercial setting.

Before you start

All research studies are governed by formal animal ethics approvals and contracts – make sure you have read this protocol and the Animal Ethics Application and completed the following forms:

- CSIRO contract
- AEC privately owned animals agreement
- Participant engagement form for every participant involved in the study.

If you have any questions, please discuss with your local co-ordinator and/or Jim Lea (CSIRO – jim.lea@csiro.au; 02 6776 1419).

Make sure you have a Numnuts® tool, and a supply of NumOcaine® for the study. the Numnuts® tool and spares can be sourced from www.numnuts.store; while the NumOcaine® is obtained under prescription from your registered veterinarian.

You will probably need an extra person or two on the day of the trial, so that marking can carry on as normal in the background. Please discuss your required support with your local co-ordinator.

For each replicate of the study (and there is to be three replicates on each farm), the aim is to place 10 lambs in each pen, one pen containing 10 lambs that have received NUMNUTS with Local Anaesthetic (NN+LA) and the other pen containing 10 lambs that have been ringed using the elastrator tool, or using the NUMNUTS tool without injecting the Local Anaesthetic (RING).

Lambs are all to be male, and in the age range 4-8 weeks. Lambs are fully marked (scrotum and tail). The assigned treatment for each lamb occurs to both the scrotum and tail, so if they get LA in the scrotum, they also get LA in the tail.

There are 3 'calibration videos' to score – this gives an opportunity to try out the scoring system before you go live, and also provides us with a baseline for each farm so we can align the data across all participants.

Preparing the study pens

Set up two pens, reasonably close to where you will be lamb marking.

The pens should be approximately 4 m by 4 m, with easy open gates and a straight run from the pens back to the ewe holding area. Attach a clock above the pens, and label the pens (A, B or 1,2) on the far side of the pens. Mount a video camera so that you capture the entirety of both pens with no blind spots (you may need a couple of cameras).

The idea is that lambs will be taken from the marking cradle, sprayed with a number (you could use a different colour of spray mark for each pen instead of pen labels), and placed in the pens for observation. When observations are finished, the pen can be easily opened and the lambs run out to their ewes.

NN-ECT Producer Protocol

Figure 1: diagram of pen set-up

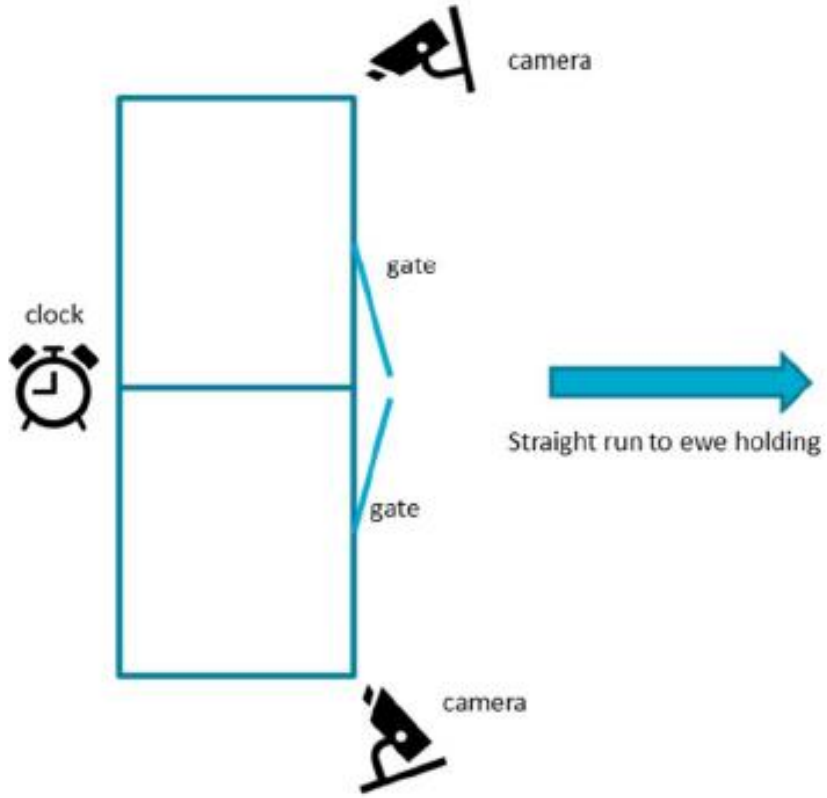

Figure 2: example of pen set-up

NN-ECT Producer Protocol

Carrying out the study

Before you start – ensure all cameras are recording.
For each lamb:

1. Apply the assigned treatment (try to alternate RING and NN+LA)
2. Spray a number on each lamb (e.g. 1-20, or 1-10 for each pen, but using different colours), once on each flank, so that regardless which side of the lamb is facing the camera, it's identification number can be seen.
3. Record the lamb identification number and the treatment it received
4. Place the lamb into the appropriate pen – separate pens for RING and NN-LA lambs. Record on the treatment sheet which pen contains which group of lambs (e.g. have a pen number displayed close to the clock so that it too is visible on the camera, or identify pen by spray mark colour)
5. Do not disturb the pens for the next 2 hours, but every 10 minutes, visually score the activity level in each pen on a 0-3 scale according to the scoring protocol attached. Ideally, the person doing the scoring is not one of the team filling the pens, so that the person scoring is not aware of which pen holds RING and which holds NN-LA lambs.
6. When 2 hours have elapsed, open the pen gates and allow the lambs to move at their own pace to the ewes. Allow 15 minutes for all lambs to exit the pen, then if there are any remaining in the pen, assist them to return to the ewe. Score 'ease of emptying pen' on a 0-3 scale according to the scoring protocol attached.

Stop the video recording, later download the video, and also scan/photo the treatment and observations record sheets. Provide these to your local co-ordinator or CSIRO.

DO:

- Vaccinate, drench and ear-mark/tag the lambs as normal;
- Minimise disturbance of the test pens (e.g. keep farm dogs or personnel away from the pens): activity in and next to the pens will alter the behaviour pattern of the lambs).

DO NOT:

- Carry out an additional procedure (e.g. mulesing, tail-stripping, hot/cold knife tail removal): these will all alter the behaviour pattern of the lambs;
- Provide additional pain relief until after the observations are complete: these will alter the behaviour pattern of the lambs.

NN-ECT Producer Protocol

Scoring protocol

From the time the pens are full, and every 10 minutes thereafter, score each pen in terms of:

Amount of rolling and fidgeting

0: no lambs rolling or fidgeting

1: a quarter of the lambs (1-3) rolling or fidgeting

2: half of the lambs (4-6) rolling or fidgeting

3: most or all lambs (7-10) rolling or fidgeting

Interest in escape

0: no lambs trying to escape and mother up

1: a quarter of the lambs (1-3) trying to escape and mother up

2: half of the lambs (4-6) trying to escape and mother up

3: most or all lambs (7-10) trying to escape and mother up

Overall demeanour

0: most or all lambs looking comfortable/OK

1: a quarter of the lambs (1-3) looking uncomfortable/unhappy

2: half of the lambs (4-6) looking uncomfortable/unhappy

3: most or all lambs (7-10) looking uncomfortable/unhappy

Ease of emptying the pen

0: no lambs needing assistance

1: a quarter of the lambs (1-3) needing assistance

2: half of the lambs (4-6) needing assistance

3: most or all lambs (7-10) needing assistance

Numnuts Extended Commercial Trials

Observation sheet

Date: Location: Name of observer:

Time pen filling started: Time pen filling ended (time 0):

Timepoint (min)	time	Pen A			Pen B			Comment
		Rolling/fidgeting	Escape	Comfort	Rolling/fidgeting	Escape	Comfort	
0								
10								
20								
30								
40								
50								
60								
70								
80								
90								
110								
120								
End		Pen Emptying			Pen Emptying			

9.3Appendix 3: Producer Survey – pre-trial

Pain Relief at lamb marking

-Questionnaire-

This 25-min survey contains questions about your opinions about **Numnuts®** and pain relief options during lamb marking. This MLA funded study is being conducted by Dr Alison Small (CSIRO) and Dr Ellen Jongman and Dr Carolina Munoz (University of Melbourne).

All electronic data will be kept securely for five years from the date of publication before being destroyed. Participation in this study is completely voluntary.

Please access the PLS **Here**

If you have any questions, please contact:

Carolina Munoz

Faculty of Veterinary and Agricultural Sciences

Email: munoz.c@unimelb.edu.au

This project has been approved by the University of Melbourne Ethics Committee. Should you have any concerns about the conduct of the project, you are welcome to contact the Executive Officer, Human Research Ethics, The University of Melbourne, on telephone: 03 8344 2073 or fax: 03 9347 6739

Instructions

Please answer all questions to the best of your ability. If you do not know the answer to a question, please give us your best estimate or leave them blank. There are no "right" or "wrong" answers to any of the questions, just answer what is true for you. **Your responses will remain strictly confidential. Only the summary results for the entire sample will be used.**

Please enter your assigned unique identifier

This next section contains questions about your farm.

1. What is the address postcode of your farm? (If you have more than one property, please complete one survey per property).

2. What is your main farming enterprise?

- ☐ Meat-focused enterprise
- ☐ Meat-wool enterprise
- ☐ Wool-focused enterprise
- ☐ Mixed production, please specify

3. How long have you farmed sheep? (Years)

4. How many heads of livestock do you **currently** own?

	Ewes	Wethers	Weaners	Rams
1st cross ewes				
2nd cross ewes				
Prime lambs				
Merino				

5. Including leased land, what is your total grazing area allocated to sheep production? (approximation in hectares)

6. How many people work on the farm? (Including yourself, family members and employees)

FTE (Full Time Employees)

7. In your opinion, how painful are the following procedures?

Procedures	Not at all painful (1)	Slightly painful (2)	Moderate (3)	Painful (4)	Very painful (5)
Ear tagging					
Mulesing					
Castration (Rubber rings)					
Castration (Knife)					
Tail docking (Rubber rings)					
Tail docking (Hot knife)					
Clips					
Tail-striping					
Breech freeze branding					

8. For each of the husbandry practices listed below, please **rate** the importance of providing sheep with pain relief during the procedure. *For each item, **select** the option on the scale that most closely represents your answer.*

Procedures	Not at all important (1)	Slightly painful (2)	Moderate (3)	Painful (4)	Very important (5)
Ear tagging					
Mulesing					
Castration					
Tail docking					
Clips					
Tail-striping					
Breech freeze branding					

9.Have you used pain relief at lamb marking before?

☐ Yes, always

☐ Sometimes

☐ No

If yes or sometimes,

9.a What type of pain relief?

If yes or sometimes,

9.b Were you happy with the effectiveness of the pain relief?

☐ Yes

☐ Sometimes

☐ No

If no, why not?

10. How common do you think the use of pain relief at lamb marking is?

☐ Not at all common

☐ Slightly common

☐ Moderately common

☐ Common

☐ Very common

11. What do you think is the main reason why some farmers do not use pain relief at lamb marking?

☐ Labour

☐ Cost

☐ Lack of time

☐ Not enough information

☐ Other, please specify

12. For each statement below, please **select** the option on the scale that most closely represents your level of **agreement** or **disagreement** with each statement.

	N/A	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
The industry is at risk if pain relief is not mandatory						
A lot of farmers are willing to use pain relief at lamb marking						

The public supports the use of pain relief						
I'm responsible for the welfare of my animals						
Farm animals experience physical pain as humans do						
Widespread adoption of pain relief at lamb marking is not practical						
I think there are effective pain relief options available						
I think information about pain relief options is readily available to producers						
Most farmers don't use pain relief at lamb marking						
The current pain relief options are expensive for producers						
I think **Numnuts®** is the most practical pain relief option available						
I think **Numnuts®** is safe to apply						
I think most farmers will be willing to try **Numnuts®**						
I think **Numnuts®** is very effective in reducing pain						
I will recommend **Numnuts®** to other producers						

13. What is your understanding of how does **Numnuts®** work?

a) Anti-inflammatory, combination of quick-acting (1-5 minutes) and long-lasting
b) Local Anaesthetic, quick-acting (1-5 minutes) and not long-lasting
c) Anti-inflammatory, quick-acting (1-5 minutes) and not long-lasting
d) None of the above

14. How effective do you think **Numnuts®** is in reducing pain relief for lamb castration?

☐ Not at all effective

- ☐ Slightly effective
- ☐ Moderately effective
- ☐ Effective
- ☐ Very effective
- ☐ N/A

15. How effective do you think **Numnuts®** is in reducing pain relief for tail docking?

- ☐ Not at all effective
- ☐ Slightly effective
- ☐ Moderately effective
- ☐ Effective
- ☐ Very effective

16. Who would you seek advice from about pain relief options for lamb marking? (tick all that apply)

	Never	Rarely	Occasionally	Frequently	Very Frequent
Veterinarians					
Other farmers					
Closest family members (e.g. spouse, parents, siblings, etc.)					
Friends					
Farm consultant					
Stock agent					
Shearers					
Other (please specify)					

17. To what extent do you **approve or disapprove** of the following procedures/practices carried out on sheep?

	Strongly disapprove (1)	**2**	**3**	**4**	**Strongly approve (5)**
Mulesing					
Tail docking					
Clips					
Tail-strip					
Breech freeze branding					
Chemical use					
Use of pain relief					

18. What was the main reason why you decided to participate in this project?

- ☐ I'm interested in using pain relief in the future
- ☐ I believe in the effectiveness of **Numnuts®**
- ☐ I'm only interested in the results
- ☐ Because of animal welfare concerns
- ☐ Due to monetary incentive
- ☐ Other, please specify

This section contains questions about you.

19. Gender

- ☐ Male
- ☐ Female
- ☐ Other/Prefer not to say

20. What is your age?

- ☐ 18 - 24
- ☐ 25 - 34
- ☐ 35 - 44
- ☐ 45 - 54
- ☐ 55 - 64
- ☐ 65 and over

21. What is your highest level of education?

- ☐ No Formal Schooling
- ☐ Primary School
- ☐ Secondary School
- ☐ Technical or further educational institution (including TAFE College)
- ☐ University or other higher educational institution
- ☐ Other educational institution, please specify _____
- ☐ Don't wish to answer

Thank you for completing this questionnaire!

22. Any additional comments?

23. Please provide your contact details if you wish to get a summary of the main findings of this study.

24. Would you like to be contacted for a follow-up?