

# PROJECT SUMMARY REPORT



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## Welfare assessments of analgesic options in female lambs for surgical mulesing and its alternatives



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# Executive Summary

Surgical mulesing is a painful husbandry procedure, carried out to minimise the risk of breech flystrike throughout life. The welfare benefits in terms of breech strike reduction are well documented, but in recent years there has been much discussion about the impact of the procedure itself on the individual animal. There is increasing pressure on the wool industry to end the practice of mulesing, but until the need to mules has been addressed through breeding, or a suitable alternative is developed, there remains a significant portion of the industry that requires mulesing in order to prevent severe welfare compromise and economic loss as a result of breech flystrike.

The Australian wool industry has investigated a number of potential alternatives to mulesing for the purpose of altering breech conformation, for example the application of plastic occlusive clips to the loose skin, or intradermal injection of necrotising agents such as cetrimide or sodium lauryl sulphate (SLS). Most recently, a cryosurgical alternative to mulesing (the Liquid Nitrogen Process, LNP) using liquid nitrogen to achieve a full-thickness freeze of excess skin has been under development.

Furthermore, new pain relief formulations are being made available for sheep. Current best practice for surgical mulesing includes the application of Tri-Solfen® to the mulesing wound. Tri-Solfen contains local anaesthetic agents to alleviate pain, and haemostatic and antiseptic agents to reduce bleeding and promote healing. In human medicine, a combination approach of a non-steroidal anti-inflammatory drug (NSAID) supplementary with the local anaesthetic is considered best practice for minor surgical procedures, as the NSAID will provide a longer duration of post-operative pain relief. Ilium® Buccalgesic® OTM contains the NSAID meloxicam, and has been shown to be effective in alleviating the pain of knife castration and tail docking in lambs and, within this project, mulesing. It is administered into the cheek cavity, and is very rapidly absorbed such that high plasma levels are present before the target receptor for the NSAID are fully expressed at the surgical site. This rapid absorption means that the agent can be administered at the time of the procedure and still be expected to provide pain relief.

This project consisted of a series of trials with the overall objectives:

- To assess the efficacy of Ilium Buccalgesic® OTM (meloxicam), alone, and in combination with Tri-Solfen®, in reducing the pain responses of female Merino lambs (aged 6 – 10 weeks) subjected to surgical mulesing or liquid nitrogen application (LNP);
- To assess the efficacy of Ilium Buccalgesic® OTM (meloxicam), in reducing the pain responses of older female Merino lambs (aged 8 – 10 month) subjected to surgical mulesing, or liquid nitrogen application (LNP);
- To assess the welfare outcomes of LNP as a means of breech modification in both young (aged 6-10 weeks) and older (aged 8-10 months) female Merino lambs.

To summarise the conclusions of the project, use of the analgesic agents Buccalgesic and Tri-Solfen, singly or in combination, provides benefits that persist for at least 6 h (based on behavioural observations), and up to 24 h (based on physiological parameters) post mulesing. Tri-Solfen provided rapid-onset analgesia, but the duration of analgesic effect of lignocaine and bupivacaine appeared to be shorter than that of meloxicam under the observation protocols used; Buccalgesic was slower to provide effective analgesia, but the duration of analgesic effect of meloxicam was longer than that of local anaesthetic agents; and the best outcome was seen where Tri-Solfen and Buccalgesic were used in combination, delivering the benefits of both local anaesthetic and non-steroidal anti-inflammatory agents.

In terms of LNP, a significant advantage over surgical mulesing (other than it is a bloodless method) was not identified, and analgesic administration did not appear to afford much benefit to weaner lambs undergoing LNP. The analgesic agents did provide some mitigation of the pain response in young lambs undergoing LNP, but it is unclear whether this was a result of mitigation of the pain of tail-docking, or mitigation of the discomfort associated with LNP.

# Introduction/Hypotheses

Surgical mulesing is a procedure that involves the removal of skin around the breech area and tail in order to reduce the risk of cutaneous myiasis of the breech (breech strike) and is most commonly carried out on Merino lambs. Its effectiveness in reducing breech strike is well documented, but in recent years there has been much discussion about the pain associated with surgical husbandry procedures, and there is increasing pressure on the wool industry to end the practice of mulesing. However, until a suitable alternative is developed, or selective breeding strategies have addressed the need to mules, there remains a significant portion of the industry that requires mulesing in order to prevent severe welfare compromise and economic loss as a result of breech flystrike.

There is an urgent need to make available a range of pain relief options to those producers that still carry out mulesing. Pain relief is also known as analgesia (the absence of pain or discomfort), and a component of analgesia is anaesthesia (the absence of sensation). The agents that provide pain relief can act through blocking nerve transmission (anaesthesia), or by reducing other physiological responses that lead to the experience of pain or discomfort (the broader scope of analgesia). At the time of study initiation there was only one product registered for alleviation of pain of mulesing. Tri-Solfen® is a topical product applied after surgical mulesing, which is being widely used (AWI survey data from 2011 and 12 indicated that 75 % of mulesed sheep received pain relief). However, the topical approach may not suit all operators, and furthermore, it is generally recognised that a combination drug approach to analgesia provides the greater benefit. This is why, in human and in companion animal medicine, patients are given a series of products including pre-medication; anaesthetic (local or general); and post-operative pain relief. This allows for prolonged pain relief, as one agent's efficacy wears off, another will be taking effect. The differing modes of action of drugs can also improve analgesic efficacy when applied in combination.

A buccal meloxicam formulation (Ilium® Buccalgescic® OTM), developed by Troy Laboratories has been demonstrated to reduce the pain of castration and tail docking in male lambs (Small et al., 2014), and, during the course of this project, mulesing (Small et al., 2018a, Small et al., 2018b). Surgical mulesing is a painful husbandry procedure, carried out on merino lambs, to reduce the risk of breech strike. Although alternative procedures are under development, and the potential to select genotypes that are resistant to breech strike has been demonstrated, the requirement to mules will still remain for some producers as a consequence of the particular genotype reared and environmental conditions on their property. Despite genetic selection criteria now being available to producers, it will take many generations of breeding to remove the need to mules across the Australian sheep industry.

At present, the vast majority of animals that require mulesing are treated at the time of marking (age range 6-10 weeks). Therefore, the target age group for the study is 6-10 weeks.

However, some animals are treated later, at around 9 months of age, in the situation where the producer has hoped to not mules in a particular year, but climatic conditions result in a high risk of fly strike, and so mulesing is re-instated on the property. It is thus desirable to also investigate analgesic efficacy at this older age, such that producer and customer concerns can be allayed.

The Liquid Nitrogen Process (LNP) is a cryosurgical alternative to surgical mulesing, which is expected to provide better welfare outcomes for the animals than surgical mulesing. However, it is likely that some pain or discomfort will still be experienced with this method, so it is desirable to assess the efficacy of the analgesic agents in providing relief. The optimal age of application for liquid nitrogen is not yet determined, so it would be desirable to investigate the welfare outcomes of liquid nitrogen application for mulesing within both young lambs (aged 6-10 weeks) and in weaners (aged 8-10 months).

The hypotheses tested during the project were as follows:

#### **Buccalgesic compared with surgical mulesing or LNP**

- Animals treated with Buccalgesic will show a reduction in the frequency of behaviours and postures classified as pain- or discomfort-related following surgical mulesing or LNP.
- The cortisol response of animals to surgical mulesing or LNP will be reduced by administration of Buccalgesic.
- The haptoglobin response of animals to surgical mulesing or LNP will be reduced by administration of Buccalgesic.
- The inflammatory response of animals to surgical mulesing or LNP will be reduced by administration of Buccalgesic.

#### **Tri-Solfen compared with surgical mulesing or LNP**

- Animals treated with Tri-Solfen will show a reduction in the frequency of behaviours and postures classified as pain- or discomfort-related following surgical mulesing or LNP.
- The cortisol response of animals to surgical mulesing or LNP will be reduced by administration of Tri-Solfen.
- The haptoglobin response of animals to surgical mulesing or LNP will be reduced by administration of Tri-Solfen.
- The inflammatory response of animals to surgical mulesing or LNP will be reduced by administration of Tri-Solfen.

#### **Both Buccalgesic and Tri-Solfen in combination compared with surgical mulesing or LNP**

- Animals treated with both Buccalgesic and Tri-Solfen in combination will show a reduction in the frequency of behaviours and postures classified as pain- or discomfort-related following surgical mulesing or LNP.
- The cortisol response of animals to surgical mulesing or LNP will be reduced by administration of both Buccalgesic and Tri-Solfen in combination.

- The haptoglobin response of animals to surgical mulesing or LNP will be reduced by administration of both Buccalgesic and Tri-Solfen in combination.
- The inflammatory response of animals to surgical mulesing or LNP will be reduced by administration of both Buccalgesic and Tri-Solfen in combination.

#### **Both Buccalgesic and Tri-Solfen in combination compared with administration of one agent alone**

- Animals treated with both Buccalgesic and Tri-Solfen in combination will show a reduction in the frequency of behaviours and postures classified as pain- or discomfort-related following surgical mulesing or LNP, as compared with a single agent administered individually.
- The cortisol response of animals to surgical mulesing or LNP will be reduced by administration of both Buccalgesic and Tri-Solfen in combination, as compared with a single agent administered individually.
- The haptoglobin response of animals to surgical mulesing or LNP will be reduced by administration of both Buccalgesic and Tri-Solfen in combination, as compared with a single agent administered individually.
- The inflammatory response of animals to surgical mulesing or LNP will be reduced by administration of both Buccalgesic and Tri-Solfen in combination, as compared with a single agent administered individually.

#### **LNP compared with surgical mulesing**

- Animals undergoing LNP will show a reduction in the frequency of behaviours and postures classified as pain- or discomfort-related following the procedure.
- Animals undergoing LNP will gain comparable breech conformation alterations to animals undergoing surgical mulesing.
- Animals undergoing LNP will show earlier onset wound healing, as compared with surgical mulesing.
- Animals undergoing LNP will show less negative impacts on growth, as compared with surgical mulesing.

In order to assess this list of hypotheses, the project was carried out in six distinct phases:

- Pen Trial 1: Analgesic options for surgical mulesing (assessing the efficacy of Ilium Buccalgesic® OTM, alone, and in combination with Tri-Solfen®, in reducing the pain responses of young female lambs, aged 6-10 weeks, subjected to surgical mulesing).
- Pen Trial 2: Analgesic options for young lambs undergoing the Liquid Nitrogen Process (assessing the efficacy of Ilium Buccalgesic® OTM, alone, and in combination with Tri-Solfen®, in reducing the pain responses of young female lambs, aged 6-10 weeks, subjected to surgical mulesing).
- Field Trial 1: A comparison of surgical mulesing and LNP in young lambs (aged 6-10 weeks).



- Field Trial 2: Analgesic options for surgical mulesing (assessing the efficacy of Ilium Buccalgesic® OTM, alone, and in combination with Tri-Solfen®, in reducing the pain responses of young female lambs, aged 6-10 weeks, subjected to surgical mulesing).
- Field Trial 3: Analgesic options for young lambs undergoing the Liquid Nitrogen Process (assessing the efficacy of Ilium Buccalgesic® OTM, alone, and in combination with Tri-Solfen®, in reducing the pain responses of young female lambs, aged 6-10 weeks, subjected to LNP).
- Field Studies: weaner lambs (a comparison of surgical mulesing and LNP in weaner lambs, aged 8-10 months).

## Project Objectives

- To assess the efficacy of Ilium Buccalgesic® OTM (meloxicam), alone, and in combination with Tri-Solfen®, in reducing the pain responses of female Merino lambs (aged 6 – 10 weeks) subjected to surgical mulesing or liquid nitrogen application (LNP);
- To assess the efficacy of Ilium Buccalgesic® OTM (meloxicam), in reducing the pain responses of older female Merino lambs (aged 8 – 10 month) subjected to surgical mulesing, or liquid nitrogen application (LNP);
- To assess the welfare outcomes of LNP as a means of breech modification in both young (aged 6-10 weeks) and older (aged 8-10 months) female Merino lambs.

## Success in Achieving Objectives

The project has demonstrated that analgesic benefits are provided for at least 6 h post mulesing (based on behavioural observations), and up to 24 h (based on physiological parameters). Based on the variables measured:

- Use of the analgesic agents Buccalgesic and Tri-Solfen singly or in combination improved the welfare of lambs undergoing surgical mulesing.
- Tri-Solfen provided rapid-onset analgesia, but the duration of analgesic effect was shorter than that of Buccalgesic.
- Buccalgesic was slower to provide effective analgesia, but the duration of analgesic effect was longer than that of Tri-Solfen.
- The best outcome was seen where Tri-Solfen and Buccalgesic were used in combination, delivering the benefits of both local anaesthetic and non-steroidal anti-inflammatory agents.

In terms of LNP, a significant advantage over surgical mulesing (other than it is a bloodless method) was not identified, and analgesic administration did not appear to afford much benefit to weaner lambs undergoing LNP. The analgesic agents did provide some mitigation of the pain response in young lambs undergoing LNP, but it is unclear whether this was a result of mitigation of the pain of tail-docking, or mitigation of the discomfort associated with LNP.

# Methodology

The project was composed of two distinct but complementary study formats: a pen study, carried out in an animal house, and a field study, carried out in a paddock situation. The pen study was a blinded controlled randomised block design, incorporating detailed individual behavioural and physiological pain indicators, and assessment of key haematological parameters; while the field study was a blinded controlled randomised block design, incorporating detailed individual behavioural pain indicators. An outline of the methodology for each study format is given below, but for specific detail of the methodology for each phase, refer to the individual phase reports.

## Pen Trials

### Animals and Housing

The study animals were unweaned female Merino lambs, aged 6-10 weeks at the time of the procedure. Ewe-lamb pairs were identified at birth, with only single-born lambs used in the study. The trials were undertaken at CSIRO's FD McMaster Laboratory, Armidale, New South Wales (NSW), Australia, and the protocol and conduct of the experiment was approved by the CSIRO Armidale Animal Ethics Committee under the NSW Animal Research Act, 1985 (Animal Research Authorities 15/12 and 16/29).

The lambs with their mothers were transferred to group pens in the Animal House 14 days before treatment. Four to eight days prior to treatment, each cohort of 24 ewe-lamb pairs was moved to their treatment pens (each pen containing four ewe-lamb pairs), where they remained until Day 2 (post procedure) when they were moved back into the larger group pens. The animals had fresh water available ad lib at all times and were fed sheep pellets at a rate of 0.8 kg/dry sheep equivalent/day and 100 g/d chaff, delivered each morning between 08:30 and 10:00 h, except for the day of treatment on which they were fed 1 h prior to mulesing so that feeding would not coincide with post procedure behavioural observations.

Lambs were handled twice daily during the two-week period before treatment to accustom them to human interaction. Each lamb was caught by an animal handler within the group pen and gently held for 2 min in a position that would be suitable for blood sampling.

### Experimental Procedures and Treatments

Testing occurred in cohorts of 24 lambs. Within each cohort, the lambs were weighed on Day -1, ranked according to weight, sequentially blocked into blocks of six and randomly allocated from within each block to six treatment groups (Tables 1 and 2). There were four lambs with

their ewes in each treatment pen. Each triad of pens contained two blocks of 6 lambs, two from each treatment group, such that no treatment was duplicated within a pen.

**Table 1: Pen Trial 1: Analgesic options for surgical mulesing**

Treatment code	Procedure	Therapeutic agent	No. lambs
1	Sham controls, handled	Placebo	20
2	Surgical mulesing and hot knife tail docking	Placebo	20
3	Surgical mulesing and hot knife tail docking	Buccalgesic	20
4	Surgical mulesing and hot knife tail docking	Tri-Solfen	20
5	Surgical mulesing and hot knife tail docking	Placebo + Tri-Solfen	20
6	Surgical mulesing and hot knife tail docking	Buccalgesic + Tri-Solfen	20

**Table 2: Pen Trial 2: Analgesic options for the liquid nitrogen process**

Treatment code	Procedure	Therapeutic agent	No. lambs
7	Sham controls, handled	Placebo	20
8	Liquid nitrogen process and hot knife tail docking	Placebo	20
9	Liquid nitrogen process and hot knife tail docking	Buccalgesic	20
10	Liquid nitrogen process and hot knife tail docking	Tri-Solfen (tail wound)	20
11	Liquid nitrogen process and hot knife tail docking	Buccalgesic + Tri-Solfen (tail wound)	20
12	Filler animal – no treatment	None	20

All treatments were applied pen side: the lambs were lifted out of the pen and restrained in either a marking cradle (Pen Trial 1) or a specially designed restraint sling (Pen Trial 2). Buccalgesic (Troy Laboratories Pty Ltd) and the buccal placebo (the base of Buccalgesic, minus the active agent, meloxicam, provided by Troy Laboratories Pty Ltd) were administered by the buccal route using a proprietary dosing gun with 0.5 mL increments supplied by the manufacturer. The dose was applied into the sulcus between the molar teeth and the inside of the cheek. Buccalgesic and the volumetric equivalent of buccal placebo were administered at a target dose rate of 1.0 mg/kg meloxicam. Individual dose volumes for Buccalgesic and the buccal placebo were calculated based on individual body weight and prepared to the dose

volume based on weight groups, 10.1 – 15.0, 15.1 – 20.0, 20.1 – 25.0 and 25.1 – 30.0 kg, allowing the target dose rate to be delivered at the maximum weight range for that group. Thus, lighter lambs within a weight bracket received a dose slightly above the 1.0 mg/kg target.

The lambs were tail-docked using a Primus BJ5000 gas-fired hot knife (Leader Agri-products, Australia). Surgical mulesing was carried out by an accredited commercial mulesing practitioner, and involved cutting off excess skin on the breech and tail, using mulesing shears as described by Lee and Fisher (2007). Removed tissue, including breech skin, tail skin, and the removed tail from each mulesed animal was collected and weighed. These data were compared across treatments to confirm that the outcomes of the mulesing procedure were similar across treatment groups. LNP was carried out by John Steinfort (John Steinfort Ag Vet). Excess skin on the breech and tail was clamped and liquid nitrogen applied until a full-thickness freeze was attained. Tri-Solfen (Lignocaine 40.6 g/L, bupivacaine 4.5 g/L, adrenaline 24.8 mg/L, cetrimide 5 g/L, Bayer Australia Ltd) was applied by spraying to cover the mulesed area and tail docking wound, using the commercial applicator, at dose rates of: lambs 5 - 10 kg 6 mL; 11 - 15 kg 8 mL; 16 - 20 kg 10 mL; > 20 kg 12 mL. Control lambs were placed on their backs in the lambing cradle, or suspended in the restraint sling and the breech skin and tail gently handled for a duration similar to that experienced by lambs that underwent the mulesing or LNP and tail docking procedure. Lambs were returned to their pen after treatment.

## **Behavioural Observation**

Video cameras were used to continuously record the behaviour of lambs in the study. For each pen, one camera was mounted on roofing rafters at each end of the pen. Each camera provided a view of the entire area available to the lambs. The cameras were connected to digital video recorders and captured by video management software. The behaviour of the lambs in their pens was collated from the digital video records by observation of a replay of the video record on the same software. The person performing the video observations post mulesing was blinded to treatment. Identification for observation of behaviour on video records was provided by coloured spray marks/symbols applied to the wool of lambs. Identification marks were randomised across treatments within a pen.

The assessment of behaviour post procedure was divided into pain avoidance behaviour and postural behaviour. The pain avoidance behaviour assessment took place every 5 minutes for the first 2 hours post procedure. The behaviours were: restlessness, kicking/foot stamping, rolling, jumping, licking/biting the wound site, and easing quarters. Observation times for each lamb were synchronised with its treatment time.

The postural behaviours were classified to pre-determined categories every 15 minutes for 6 hours post procedure. The postures classified were: normal ventral lying, abnormal ventral lying, lateral lying, abnormal lying, lying intention, lying other, normal standing, hunched

standing, statue standing, abnormal standing, standing other, normal walking, stiff walking, abnormal walking, walking other, feeding, and pawing. Total lying, total standing and total abnormal behaviours were calculated from these behaviours.

The postures and behaviours used in this study have been previously validated in a range of studies (Grant, 2004, Paull et al., 2008, Lester et al., 1996, Paull et al., 2012, Molony et al., 2002, Edwards et al., 2011).

### **Blood Sampling**

Blood samples were collected via 20 gauge needles into 4.5 mL vacutainers containing EDTA. Samples were collected just before treatment (0 h) and at 30 min, 6 h, 12 h, 24 h (= Day 2), Day 4, Day 7, and Day 10 post mulesing. Blood sampling time for each lamb was synchronised with its treatment time. General haematology, including total white cell count (TWCC), differential white cell count and haemoglobin, was analysed using an automated haematology analyser calibrated for sheep blood. Following haematology analysis, vacutainer tubes were spun at 1000 g for 12 min and plasma transferred to tubes for storage at -18 °C for later analysis of cortisol and haptoglobin. Plasma samples were analysed for cortisol and haptoglobin using previously validated techniques (Paull et al., 2007, Paull et al., 2008).

### **Clinical Observation**

The lambs were weighed at entry to the animal house (Week -3), on Day -7, Day -1, Day 4, Day 7 and Day 10. Removed tissue, including breech skin, tail skin, and the removed tail from each mulesed animal was collected and weighed. This measure was taken merely to confirm that there were no systematic differences between treatment groups related to the amount of tissue removed. Wounds on the tail and breech were scored for the presence of swelling and exudates; and sensitivity was assessed using a digital algometer applied to the wound edges on Days 4, 7 and 10. Wound appearance and swelling were scored on a 5-point scale from 0 (no visible wound or palpable swelling) to 4 (large area of wound or substantial pitting oedema). Wound sensitivity assessment consisted of an applied pressure reading at which a behavioural response of the hind quarters and the face was observed in the lamb and a nociceptive response characteristic scored. The response characteristics were scored by intensity on a 4-point scale from 0 (no response) to 3 (strong physical response, struggle or escape attempt). The applied pressure reading was divided by the intensity score, to derive a nociceptive threshold value that was utilised for further analysis, adapted from the methodology described by Espinoza et al. (2013) and Lomax et al (2008) using von Frey filaments. Where a zero response was recorded, the threshold value was given a default value of 2000 g, a figure that had been selected as the termination point for the test, based on prior evaluation of normal unmulesed lambs.

## Statistical Analysis

Data that were normally distributed or that could be transformed to satisfy normal distribution, were analysed with a repeated measures ANOVA model fitting pre-treatment values as a covariate when significant and the fixed effects of treatment, cohort and pen, and first order interactions. Active pain avoidance behaviour data were analysed using a linear model including fixed effects of cohort, pen and treatment. Postural data were analysed using a linear model including fixed effects of time, pen, cohort, treatment and first order interactions. Animal was included as a random effect to account for the repeated measures. Total time standing is the converse of total lying as the combined postures add to 100 % of time observed. There were insufficient data for analysis of some of the behaviours and postures and these were pooled for the sum of total abnormal behaviours or postures. Back transformed means are presented as all of the behaviours and postures required log transformations.  $P < 0.05$  was considered significant and  $0.1 > P > 0.05$  was considered to indicate a tendency (trend) towards statistical significance. When treatment or interactions between treatment and time were significant, post hoc comparisons between treatments were performed using a Bonferroni adjustment factor for multiple contrasts between least squares means to establish the significant differences between treatments. Data that were not able to be normalised by transformation (wound scores and nociceptive response) were analysed in R Statistical Software using the non-parametric Komolgorov-Smirnov comparison of distributions within time points.

## Field Trials

### Animals

The study animals were unweaned female Merino lambs, aged 6-10 weeks (young lamb trials) or 8-10 months (weaner trial) at the time of the procedure. For the young lamb trials, ewe-lamb pairs were identified at birth, with only single-born lambs used in the study. The trials were undertaken at CSIRO's FD McMaster Laboratory, Armidale, New South Wales (NSW), Australia. The protocol and conduct of the experiment was approved by the CSIRO Armidale Animal Ethics Committee under the NSW Animal Research Act, 1985 (Animal Research Authorities 15/15, 16/06 and 16/18).

### Experimental Procedures and Treatments

Testing occurred in cohorts of 30 lambs. Lambs were paint-marked with individual identification numbers and weighed 6 days prior to treatment. They were subsequently ranked by weight and within a block of six, randomly allocated to a treatment group (Tables 3-6). Note that the treatments for Field Trial 2 and 3 match those for Pen Trial 1 and 2 respectively, thus providing complementary data. A day prior to treatment, ewes and lambs



were acclimated to the observation paddock (0.34 ha), which had an observation hide located in its centre.

**Table 3: Field Trial 1: A comparison of surgical mulesing and the liquid nitrogen process**

Treatment code	Procedure	Therapeutic agent	No. lambs
A	Sham controls, handled as for surgical mulesing	None	20
B	Surgical mulesing and hot knife tail docking	None	20
C	Surgical mulesing and hot knife tail docking	Buccalgesic + Tri-Solfen	20
D	Sham controls, handled as for the liquid nitrogen process	None	20
E	Liquid nitrogen process and hot knife tail docking	None	20
F	Liquid nitrogen process and hot knife tail docking	Buccalgesic + Tri-Solfen (tail wound)	20

**Table 4: Field Trial 2: Analgesic options for surgical mulesing**

Treatment code	Procedure	Therapeutic agent	No. lambs
G	Sham controls, handled as for surgical mulesing	Placebo	20
H	Surgical mulesing and hot knife tail docking	Placebo	20
I	Surgical mulesing and hot knife tail docking	Buccalgesic	20
J	Surgical mulesing and hot knife tail docking	Tri-Solfen	20
K	Surgical mulesing and hot knife tail docking	Placebo + Tri-Solfen	20
L	Surgical mulesing and hot knife tail docking	Buccalgesic + Tri-Solfen	20

**Table 5: Field Trial 3: Analgesic options for liquid nitrogen process**

Treatment code	Procedure	Therapeutic agent	No. lambs
M	Sham controls, handled as for liquid nitrogen process	Placebo	20
N	Liquid nitrogen process and hot knife tail docking	Placebo	20
P	Liquid nitrogen process and hot knife tail docking	Buccalgesic	20
R	Liquid nitrogen process and hot knife tail docking	Tri-Solfen (tail wound)	20

<b>S</b>	Liquid nitrogen process and hot knife tail docking	Placebo + Tri-Solfen	20
<b>T</b>	Liquid nitrogen process and hot knife tail docking	Buccalgesic + Tri-Solfen (tail wound)	20

**Table 6: Weaner Field Trial Treatment groups**

<b>Treatment code</b>	<b>Procedure</b>	<b>Therapeutic agent</b>	<b>No. lambs</b>
<b>W1</b>	Sham controls, handled as for surgical mulesing or LNP	None	20 (10 surgical, 10 LNP)
<b>W2</b>	Surgical mulesing	None	20
<b>W3</b>	Surgical mulesing	Buccalgesic	20
<b>W4</b>	Surgical mulesing	Buccalgesic + Tri-Solfen	20
<b>W5</b>	Liquid nitrogen process	None	20
<b>W6</b>	Liquid nitrogen process	Buccalgesic	20

On the day of treatment (Day 0), ewes and lambs were separated, and the ewes returned to the observation paddock, while the lambs were held in a treatment pen adjacent to the paddock. Lambs were picked randomly from the pen and weighed for dose calculation, prior to treatment and surgery. Lambs were then restrained on their back in a marking cradle (Field trial 2 and 3 and Weaner trial) or a specially designed restraint sling (Field Trial 1). Buccalgesic and the volumetric equivalent of buccal placebo were administered by the buccal route using a proprietary dosing gun with 0.5 mL increments supplied by the manufacturer. The dose was applied into the sulcus between the molar teeth and the inside of the cheek. Buccalgesic and the buccal placebo were administered at a target dose rate of 1.0 mg /kg meloxicam. Individual dose volumes for Buccalgesic and the buccal placebo were calculated based on individual body weight and prepared to the dose volume based on weight groups, 10.1 – 15.0, 15.1 – 20.0, 20.1 – 25.0 kg and 25.1 – 30.0 kg, allowing the target dose rate to be delivered at the maximum weight range for that group. Thus lighter lambs within a weight bracket received a dose slightly above the 1 mg/kg target.

The lambs (other than Sham controls) were tail-docked using a Primus BJ5000 gas-fired hot knife. Surgical mulesing was carried out by an accredited commercial mulesing practitioner, and involved cutting off excess skin on the breech and tail, using mulesing shears as described by Lee and Fisher (2007). Removed tissue, including breech skin, tail skin, and the removed tail from each mulesed animal was collected and weighed. These data were compared across treatments to confirm that the outcomes of the mulesing procedure were similar across treatment groups. LNP was carried out by John Steinfort (John Steinfort Ag Vet). Excess skin on the breech and tail was clamped and liquid nitrogen applied until a full-thickness freeze was attained. Tri-Solfen was applied by spraying to cover the mulesed area and tail docking wound, using the commercial applicator, at dose rates of: lambs 5 - 10 kg 6 mL; 11 - 15 kg 8 mL; 16 - 20 kg 10 mL; > 20 kg 12 mL. Sham control lambs were placed on their backs in the

lambing cradle and the breech skin and tail gently handled for a duration similar to that experienced by lambs that underwent the mulesing and tail docking procedure. Following its treatment, each lamb was released into the observation paddock.

### **Behavioural Observation**

All observers were blinded to treatment. Three observers recorded lamb behaviour at 15 minute intervals by scan sampling for 6 h following treatments. At 24 h, eight scan samples at 15 min intervals were undertaken by two observers. The lambs and ewes were then moved to a larger paddock (1.0 ha) which did not contain an observation hide. On Days 2 – 10, two observers located in a vehicle to which the ewes and lambs were accustomed took five scan samples of lamb behaviours at 15 min intervals. Behavioural observations on Days 1 to 10 were conducted between 8:00 am and 12:00 noon on each day. The postures classified were: normal ventral lying, abnormal ventral lying, ventral lying other, lateral lying, lying intention abnormal lying, normal standing, hunched standing, abnormal standing, standing other, normal walking, abnormal walking, walking other, grazing, suckling, running, and jumping. Total lying, total standing, playing, and total abnormal behaviours were calculated from these behaviours.

### **Clinical Observation**

On Days 4, 7 and 10, lambs were mustered, weighed, restrained in the marking cradle and wounds on the tail and breech were scored for the presence of swelling and exudates; sensitivity was also assessed using a digital algometer applied to the wound edges at defined locations. Wound appearance and swelling were scored on a 5-point scale from 0 (no visible wound or palpable swelling) to 4 (large area of wound or substantial pitting oedema). Wound sensitivity assessment consisted of an applied pressure reading at which a behavioural response of the hind quarters and the face was observed in the lamb and a nociceptive response characteristic scored. The response characteristics were scored by intensity on a 4-point scale from 0 (no response) to 3 (strong physical response, struggle or escape attempt). The applied pressure reading was divided by the intensity score, to derive a nociceptive threshold value that was utilised for further analysis. Where a zero response was recorded, the threshold value was given a default value of 2000 g, a figure that had been selected as the termination point for the test, based on prior evaluation of normal unmulesed lambs (data not shown). During Field Trial 3, wound scoring and body weights were measured weekly from Day 14 to Day 28, and in the Weaner Trial, wound scoring and body weights were measured weekly from Day 14 to Day 35. These extensions to wound healing observations were made based on the findings in Field Trials 1 and 2, that the LNP insult had not fully healed by Day 10.

## Statistical Analysis

Data were analysed using a linear model including fixed effects of time, cohort, treatment and first order interactions. Animal was included as a random effect to account for the repeated measures. Behavioural data from Day 0 (treatment day) was analysed by splitting the 6 h of observation into three periods; period 1 from 0 – 2 h, period 2 from 2 – 4 h and period 3 from 4 – 6 h. Behavioural data from Day 0 were also collated with the subsequent observations from Day 1 to 10, and the entire time series analysed. There were insufficient data for analysis of some of the behaviours and postures so data were pooled for the sum of total abnormal behaviours or postures and total percentage of time spent lying. For body weight, weight from Day -1 was included as a covariate. Data for wound scores and wound sensitivity could not be normalised by mathematical transformation so were analysed in R Statistical Software using the Komolgorov-Smirnov non-parametric comparison of distributions.

# Results

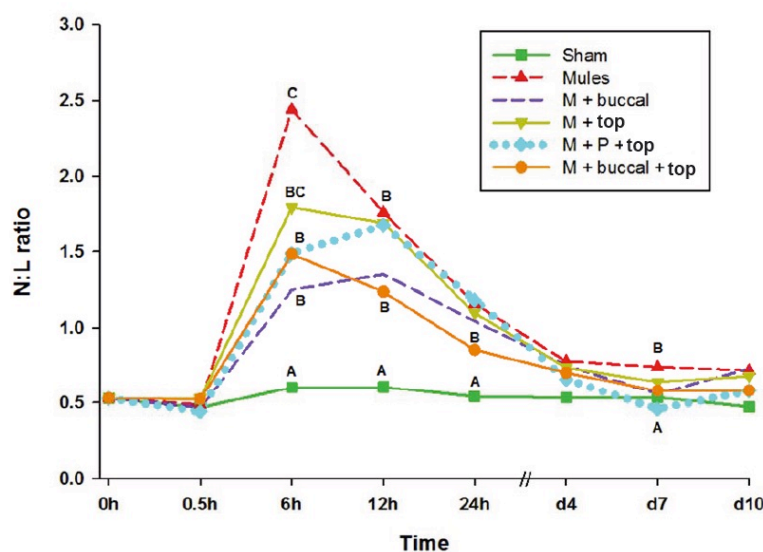
The project was carried out in six distinct phases, for each of which a detailed report was prepared. This section presents summary findings and key results from each phase.

## Physiological Measures

### Pen Trial 1: Analgesic Options for Surgical Mulesing

#### Neutrophil to Lymphocyte Ratio

Mulesing impacted profoundly on leucocyte (white cell) profile. The acute inflammatory response to the surgery lead to a marked neutrophilia and in consequence a marked increase in neutrophil:lymphocyte (N:L) ratio, evident from 6 h post procedure until Day 4. Administration of analgesic agents modulated the response, in particular Buccalgesic-only and the combination of Buccalgesic and Tri-Solfen, which significantly reduced N:L ratios at the 6 h time point ( $P < 0.05$ ) and modulation of the response continued (non-significant) at the 12 h time point.



**Figure 1: Neutrophil to Lymphocyte Ratio 0 Hours to Day 10 Post Procedure**

#### Cortisol

In mulesed lambs, not provided with analgesia, there was a marked cortisol response. This was observed in the initial 5-15 minutes post procedure, peaking within the first 30 min and remained elevated for 24 h post procedure. Administration of analgesic agents modulated the response. Tri-Solfen administration resulted in a significantly lower cortisol concentration at 30 min post treatment compared to the Mules and Buccalgesic-only groups ( $P < 0.05$ ). The

Buccalgesic-only group showed a lower cortisol concentration at 6 h post treatment, not significantly different from the Sham group.

## **Pen Trial 2: Analgesic Options for Young Lambs Undergoing the LNP**

### Neutrophil to Lymphocyte Ratio

As with surgical mulesing, LNP impacted profoundly on leucocyte (white cell) profile leading to a marked, persistent neutrophilia and in consequence a marked increase in neutrophil:lymphocyte (N:L) ratio, evident for at least 4 days post procedure. Administration of the analgesic agents modulated the response. The effects on the N:L ratio were evident at the 30 min time point (the Tri-Solfen-only and Buccalgesic+Tri-Solfen groups both had significantly lower N:L ratios than LNP without analgesia), and on Days 1 and 4 (the Buccalgesic+Tri-Solfen group had a significantly lower N:L ratio than the LNP+Placebo group).

### Cortisol

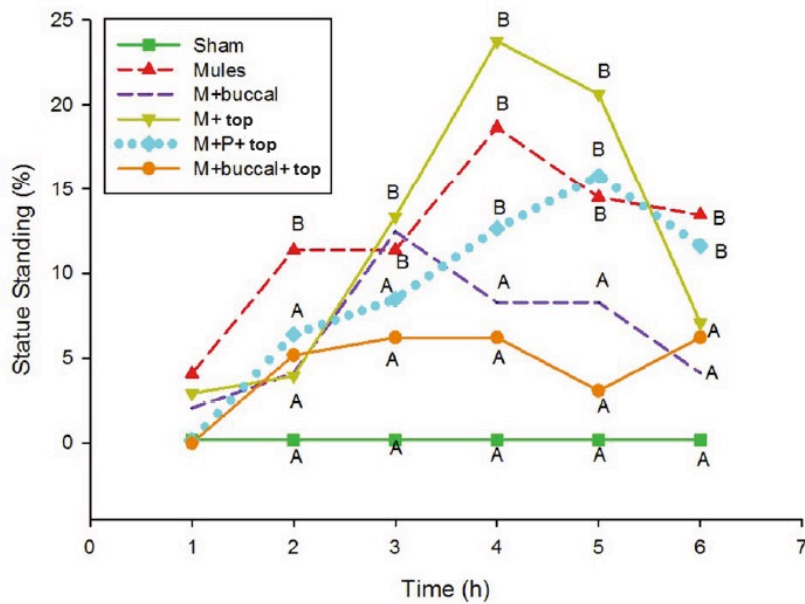
Elevations in plasma cortisol were seen in all LNP-treated lambs. Administration of analgesic agents did not modulate the response.

## **Behavioural Observations**

### **Pen Trial 1: Analgesic Options for Surgical Mulesing**

#### Statue Standing

The typical posture of 'statue standing' was observed in all mulesed lambs. There were no significant differences between groups in the first hour post mulesing. The Buccalgesic+Tri-Solfen group did not differ significantly from the Sham group at all time points, but did differ significantly from the Mules group at all time points. The Buccalgesic-only group did not differ significantly from the Sham group at all time points except at 3 h, and differed significantly from the Mules group at all time points except at 3 h.



**Figure 2: Percentage of Time Spent Statue Standing in the 6 Hours Post Mulesing**

## Pen Trial 2: Analgesic Options for Young Lambs Undergoing the LNP

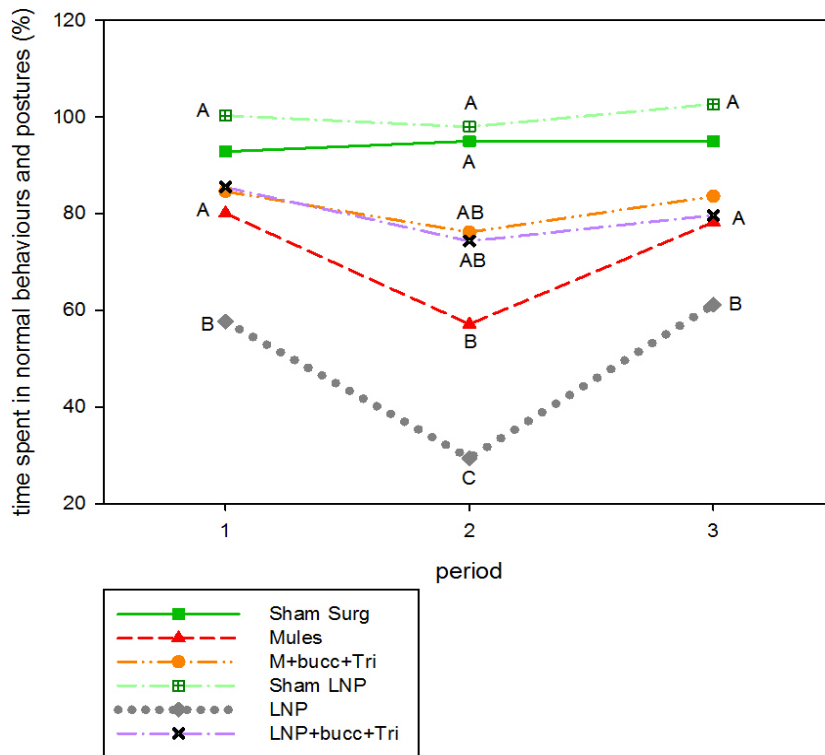
### Abnormal Postures

LNP resulted in the expression of abnormal behaviours and postures on the day of the procedure. Administration of the analgesic agents somewhat reduced the expression of abnormal behaviours and postures, particularly hunched standing. Buccalgesic reduced the total expression of abnormal postures in hours 5 and 6, reduced hunched standing in hours 5 and 6, and increased feeding behaviour in hours 2, 3 and 5 post procedure. Tri-Solfen reduced the total expression of abnormal postures in hour 4, reduced hunched standing in hours 1, 4, 5 and 6, and increased feeding behaviour in the first 5 hours post-procedure. The combination of Buccalgesic with Tri-Solfen reduced total expression of abnormal postures in hour 6, reduced hunched standing in hours 1, 2, 5 and 6, and increased feeding behaviour in hours 1, 2 and 3 post procedure.

## Field Trial 1: A Comparison of Surgical Mulesing and LNP in Young Lambs

### Normal Behaviours

In general, lambs that underwent a procedure (surgical mulesing or LNP) spent less time in normal behaviours and postures than Sham lambs. However, the difference was significant ( $P < 0.05$ ) for LNP in all periods, but significant for surgical mulesing (Mules) in period 2 (2-4 h post procedure) only. In period 2, time spent in normal behaviours and postures for LNP was significantly less than Mules, which was significantly less than Sham. Lambs that received the analgesic combination of Buccalgesic and Tri-Solfen were intermediate to, but not significantly different from Mules and Sham.



**Figure 3: Percentage of Time Spent in Normal Behaviours and Postures Post Procedure**

#### Time Spent Grazing

In period 1 (0-2 h post procedure), LNP lambs spent significantly less time grazing than LNP+Buccalgesic+Tri-Solfen lambs ( $P < 0.05$ ), which in turn spent significantly less time grazing than Sham lambs. Mules and Mules+Buccalgesic+Tri-Solfen lambs were intermediate between LNP and LNP+Buccalgesic+Tri-Solfen lambs, but not significantly different from either. In period 2, LNP lambs spent significantly less time grazing than Sham-LNP lambs, with all other treatment groups intermediate and not significantly different from LNP and Sham-LNP lambs. In period 3, LNP lambs spent significantly less time grazing than Mules and Sham-Surgical lambs (which did not differ significantly), with all other treatment groups intermediate to, but not significantly different from, LNP, Mules and Sham-Surgical.

#### Abnormal Behaviours and Postures

Surgical mulesing resulted in an increase in the expression of abnormal, pain-related behaviours as compared with sham handled animals on Day 0. This increase was only significant in the second period of observation (2 – 4 h post procedure). LNP resulted in a significant increase in the expression of abnormal, pain-related behaviours as compared with sham handled animals in all three periods on Day 0. The use of analgesic agents mitigated these effects, the expression of abnormal behaviours by lambs in the Mules+Buccalgesic+Tri-Solfen and LNP+Buccalgesic+Tri-Solfen groups was intermediate, and not significantly different to the Mules and Sham groups on Day 0. Interestingly, in the 10 day post-procedure observation period, LNP lambs demonstrated significantly less abnormal behaviours and



postures than Mules, Mules+Buccalgesic+Tri-Solfen or LNP+Buccalgesic+Tri-Solfen lambs, although still more than lambs in the Sham groups.

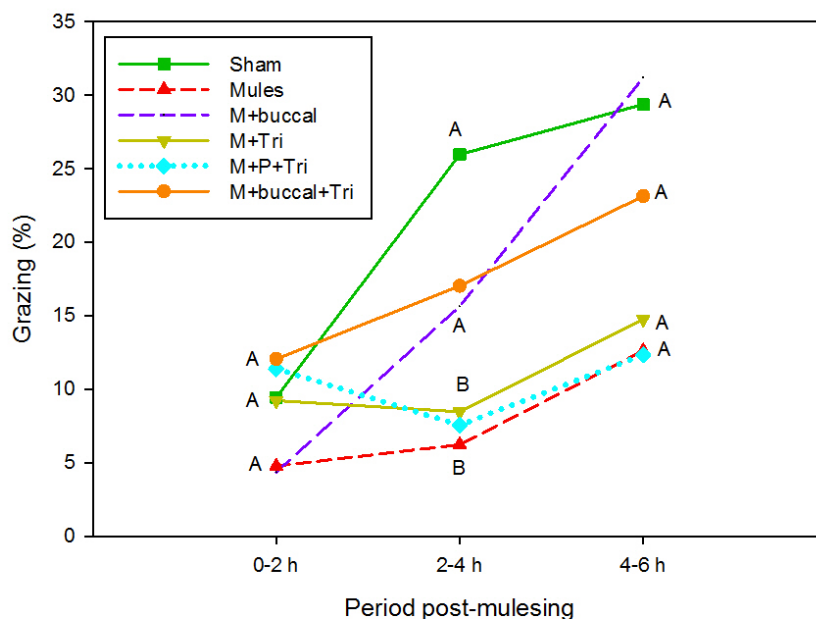
## Field Trial 2: Analgesic Options for Surgical Mulesing

### Abnormal Behaviours

All mulesed lambs exhibited more pain related abnormal behaviours on the day of mulesing than on subsequent days. Abnormal behaviours were reduced for all groups that received analgesia compared to the Mules group. The Buccalgesic-only group did not demonstrate an obvious reduction in abnormal behaviours until the second 2 h observation period, but the effect then persisted until the final observation at 6 hours. Pain related behaviours were not significantly different from surgical mulesing after 4 h for the Tri-Solfen-only and Tri-Solfen+Placebo groups. The combination of Buccalgesic+Tri-Solfen combined the benefits of both agents, resulting in reduced abnormal behaviours over the entire 6 hours of observation on Day 0.

### Time Spent Grazing

On Day 0, time spent grazing increased over the 6 hour observation period in the Sham, Buccalgesic-only and Buccalgesic+Tri-Solfen groups. In period 2 (2-4 h post-mulesing), time spent grazing was significantly greater ( $P < 0.05$ ) in the Sham, Buccalgesic-only and Buccalgesic+Tri-Solfen groups than in the Mules, Tri-Solfen-only and Tri-Solfen+Placebo groups. Although grazing behaviour in the Sham, Buccalgesic-only and Buccalgesic+Tri-Solfen groups in period 3 (4-6 h post-mulesing) remained almost double that demonstrated in the Mules, Tri-Solfen-only and Tri-Solfen+Placebo groups there were no statistically significant differences present.



**Figure 4: Percentage of Time Spent Grazing in the First 6 Hours Post Mulesing**

## **Field Trial 3: Analgesic Options for Young Lambs Undergoing the LNP**

### Abnormal Behaviours

In the first 6 hours post procedure, Sham lambs spent significantly less time in abnormal behaviours and postures than lambs in the LNP treatment groups. There were no significant effects of any analgesic or analgesic combination on time spent in abnormal behaviours and postures as compared with LNP. Lambs spent more time in abnormal behaviours and postures in period 3 (4-6 h post procedures) than in periods 1 (0-2 h) and 2 (2-4 h).

In the 10 days post procedure, Sham lambs spent the least amount of time in abnormal behaviours and postures, significantly less than LNP, LNP+Placebo+Tri-Solfen and LNP+Buccalgesic+Trisolfen lambs. Lambs that received Buccalgesic-only and Tri-Solfen-only displayed an intermediate incidence of abnormal behaviours and postures, not significantly different from any other treatment group.

## **Field Studies: Weaner Lambs**

### Abnormal Behaviours

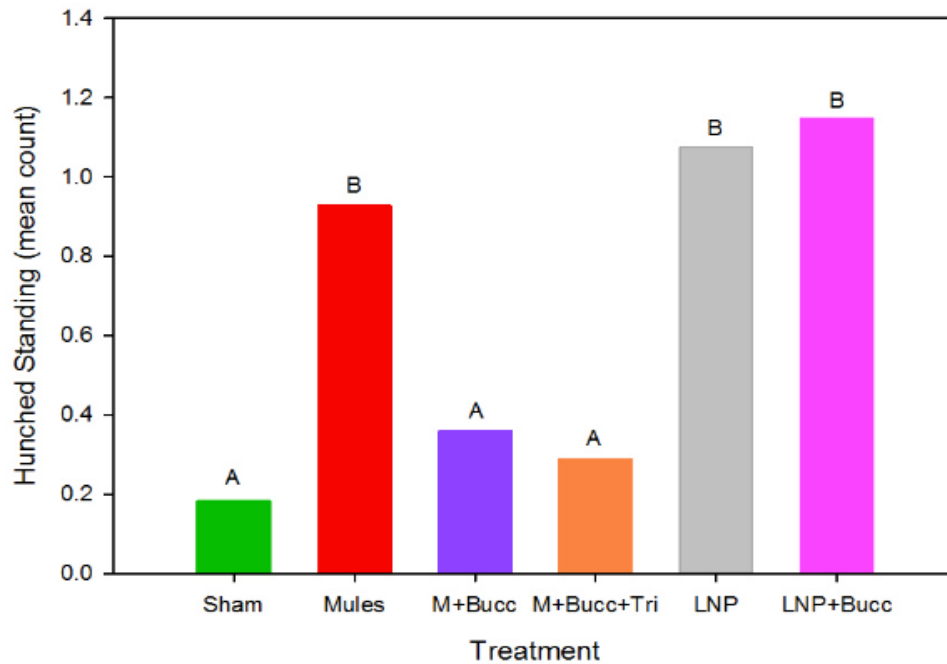
Surgical mulesing and LNP resulted in a significant increase in the expression of abnormal, pain-related behaviours. Both the Buccalgesic and Buccalgesic with Tri-Solfen combination significantly ( $P < 0.05$ ) reduced the behavioural response to surgical mulesing in the first 6 hours post-procedure, the benefits being particularly evident in terms of total abnormal behaviours expressed and hunched standing.

### Grazing

Over the 6 hour observation period on Day 0, the LNP and LNP+Buccalgesic groups spent a significantly smaller percentage of time grazing than all other groups.

### Hunched Standing

The surgically mulesed group (Mules) and both the LNP and LNP+Buccalgesic groups had significantly higher counts of hunched standing compared to the Sham, Mules+Buccalgesic and Mules+Buccalgesic+Tri-Solfen groups, which were not significantly different from each other.



**Figure 5: Hunched Standing in the First 6 Hours Post Procedure**

## Wound Assessments

Surgical mulesing results in an open wound, while LNP does not produce an open wound on the day of treatment. The area around the LNP treated area develops some swelling, leading to a wound score (the composite of appearance and swelling) greater than in sham lambs, but less than in surgically mulesed lambs. As the tissue necroses and begins to slough, a lesion becomes apparent, and subsequently resolves. In young lambs undergoing LNP, the tail is docked using a hot knife resulting in an open wound at the tip of the tail, and thus tail wound scores of LNP lambs were similar to those of surgically mulesed lambs. On the breech of LNP treated lambs, sloughing of tissue began between 10 and 14 days post procedure, and the subsequent wound resolved over the next 3-4 weeks. The pressure sensitivity of the edges of the treated area of the breech mimics the wound assessment scores, with LNP lambs intermediate between sham and surgically mulesed lambs. None of the trials clearly demonstrated analgesic effects on wound healing.

# Discussion

## Analgesic Options for Surgical Mulesing (Young Lambs)

Surgically mulesed lambs displayed characteristic pain-related behaviours and postures and a reduction in time spent lying. Abnormal postures increased in occurrence over the first 2 hours post-mulesing and reached a peak at 3 – 4 hours post-mulesing, which was sustained until 6 hours post-mulesing when observations were terminated.

Administration of analgesics did modulate lying behaviours with the combination of Buccalgesic and Tri-Solfen being the most effective. The typical postures of ‘hunched standing’ and ‘statue standing’ were observed in all mulesed lambs. Analgesic treatments did not significantly reduce the expression of hunched standing, but in terms of statue standing, the Buccalgesic+Tri-Solfen group did not differ significantly from the Sham group at any time point. Tri-Solfen-only provided a benefit with regard to statue standing in hours 2 and 6, while Buccalgesic-only provided a benefit in hours 2, 4, 5 and 6.

Elevations in plasma cortisol were seen in all mulesed lambs, but administration of analgesic agents modulated the response. The combination treatment of Buccalgesic and Tri-Solfen consolidated the benefits of both agents, resulting in serum cortisol levels significantly lower than surgical mulesing without analgesia at the 30 minute time point, and in levels not significantly different from the Sham group at the 30 minute and 6 h time points.

Surgical mulesing impacts profoundly on leucocyte (white cell) profile. The acute inflammatory response to the surgical insult leads to a marked, persistent neutrophilia, and in consequence a marked increase in neutrophil:lymphocyte (N:L) ratio. The analgesic treatments modulated the response, in particular the combination of Buccalgesic and Tri-Solfen, which resulted in a TWCC not significantly different from Sham at the 6 h and 24 h time points, and significantly lower than surgical mulesing without analgesia at 12 and 24 h post treatment.

## Analgesic Options for LNP (Young Lambs)

LNP resulted in the expression of abnormal behaviours and postures on the day of the procedure. Administration of Buccalgesic, Tri-Solfen or Buccalgesic with Tri-Solfen somewhat reduced the expression of abnormal behaviours and postures, particularly hunched standing. Buccalgesic reduced the total expression of abnormal postures in hours 5 and 6, reduced hunched standing in hours 5 and 6, and increased feeding behaviour in hours 2, 3 and 5 post procedure as compared to LNP with Placebo. Tri-Solfen reduced the total expression of abnormal postures in hour 4, reduced hunched standing in hours 1, 4, 5 and 6, and increased feeding behaviour in the first 5 hours post-procedure as compared to LNP with Placebo. The combination of Buccalgesic with Tri-Solfen reduced total expression of abnormal postures in

hour 6, reduced hunched standing in hours 1, 2, 5 and 6, and increased feeding behaviour in hours 1, 2 and 3 post procedure, as compared to LNP with Placebo.

As with surgical mulesing, elevations in plasma cortisol and a profound impact on leucocyte profile were seen in all lambs subjected to LNP. Administration of the analgesic agents did not modulate the cortisol response. The acute inflammatory response to LNP led to a marked, persistent neutrophilia, and in consequence a marked increase in neutrophil:lymphocyte (N:L) ratio, evident for at least 4 days post procedure. The analgesic treatments modulated the response in the first 24 hours post procedure, in particular the combination of Buccalgesic and Tri-Solfen, which resulted in a TWCC significantly lower than LNP without analgesia at 6 and 12 h post procedure ( $P < 0.05$ ). The effects on the N:L ratio were evident at the 30 min time point (the Tri-Solfen-only and Buccalgesic+Tri-Solfen groups both had significantly lower N:L ratios than LNP without analgesia), and on Days 1 and 4 (the Buccalgesic+TriSolfen group had significantly lower N:L ratio than the LNP+Placebo group). Interestingly, the Tri-Solfen-only group demonstrated a greater TWCC than the LNP+Placebo group throughout the sampling period post LNP, which is difficult to explain.

## LNP as an Alternative to Surgical Mulesing

LNP aims to alter breech conformation as an end point of the healing process, following deep freezing and subsequent sloughing of the loose skin.

The outcomes of both the pen study and field study made it difficult to compare between surgical mulesing and LNP due to the different expression of pain related behaviours, which may be a result of the treatments inducing different types of pain. As such a clear ranking between the two treatments could not be determined.

# Impact on Wool Industry – Now & In Five Years’ Time

The efficacy of Ilium Buccalgesic® OTM, alone and in combination with Tri-Solfen®, in reducing the pain responses of female lambs and female weaner lambs subjected to surgical mulesing has been demonstrated. Use of analgesia during mulesing provides better welfare outcomes.

In young lambs subjected to LNP, use of Ilium Buccalgesic® OTM, alone or in combination with Tri-Solfen® can provide some pain mitigation, but it is unclear whether this was a result of mitigation of the pain of tail-docking, or mitigation of the discomfort associated with LNP.

# Conclusions

Use of the analgesic agents Buccalgesic and Tri-Solfen singly or in combination provides benefits that persist for at least 6 h post mulesing (based on behavioural observations), and up to 24 h (based on physiological parameters). Based on the variables measured:

- Use of the analgesic agents Buccalgesic and Tri-Solfen singly or in combination improved the welfare of lambs undergoing surgical mulesing.
- Tri-Solfen provided rapid-onset analgesia, but the duration of analgesic effect was shorter than that of Buccalgesic.
- Buccalgesic was slower to provide effective analgesia, but the duration of analgesic effect was longer than that of Tri-Solfen.
- The best outcome was seen where Tri-Solfen and Buccalgesic were used in combination, delivering the benefits of both local anaesthetic and non-steroidal anti-inflammatory agents.

In terms of LNP, a significant advantage over surgical mulesing (other than it is a bloodless method) was not identified, and analgesic administration did not appear to afford much benefit to weaner lambs undergoing LNP. The analgesic agents did provide some mitigation of the pain response in young lambs undergoing LNP, but it is unclear whether this was a result of mitigation of the pain of tail-docking, or mitigation of the discomfort associated with LNP.

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