Castration methods and welfare of alpacas: Towards an appropriate solution

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Foreword

In the animal industry, castration is a valuable management tool, but it can be a source of concern from an animal welfare perspective. The existing standards for castration that have been developed for other livestock species, even the new standards, cannot be applied to alpacas because of the specific morphological and developmental characteristics of the alpaca. The best method to castrate alpacas is still being debated amongst animal protection groups, producers, animal scientists, and veterinarians. Each group has opinions about which method(s) could address their specific concerns, but a consensus is yet to be found.

The present project aimed to validate a standard method of castration for alpacas that could be recommended to either veterinarians or alpaca producers, and would be acceptable in welfare terms.

After an extensive consultation with industry stakeholders, a series of animal experimentations was conducted to test different methods of castration and pain management.

The project showed that two methods of castration could be recommended to the industry. One option offers the best standard of welfare, but this method would be costly. A second option can be used by certified producers, and is economically viable for large scale producers. Lastly, the project concluded that mechanical castration using rubber rings, even with some form of analgesia, is not acceptable.

This project was funded from industry revenue (Australian Alpaca Association) which was matched with funds provided by the Australian Government (AgriFutures Australia).

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About the Authors

Dr Dominique Blache was trained as a neuroscientist and has worked in the field of livestock reproduction for more than 25 years. Since 2007, Dr Blache has been working with the alpaca industry and in the field of animal welfare for close to two decades. Dr Blache has expertise in the field of the assessment of animal welfare using behavioural and physiological indicators, and is the first author on the definitive chapter on welfare assessment in the latest edition of “Animal Welfare” (3 Ed, edited by Appleby et al., CABI International 2018). Dr Blache has been teaching animal ethics and welfare since 2002. Dr Blache has been in charge of a unique laboratory dedicated to the measurement of over 25 reproductive, stress, pain and metabolic hormones in a large number of species including sheep, goat, cattle, alpaca, chicken, emu, fox, and duck. Dr Blache developed more than a dozen of these very specific hormone assays. Since joining UWA in 1993, Dr Blache has successfully supervised 21 PhD students, 6 Masters, and more than 40 Honours students. He has published more than 170 peer-reviewed articles and over a dozen of book chapters. In the field of animal welfare and stress physiology, Dr Blache has supervised more than 15 Honours students, 10 PhD students and published over 50 papers and book chapters.

Prof Shane Maloney has studied the physiology of alpacas since 2008 and has a strong interest in animal welfare, being a co-author on the chapter in “Animal Welfare” mentioned above. He has an extensive expertise in surgery in species such as alpacas, sheep, cattle, and African antelope such as wildebeest and gemsbok. He and Dr Blache have been collaborating, supervising students, and publishing together since 2000. Prof Maloney has published 160 papers and given more than 20 invited presentations at conferences and workshops on physiology and animal production, including an invited lecture at the International Union of Physiological Sciences Congress in Birmingham in 2013

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Abbreviations

AAA	Australian Alpaca Association
ANOVA	Analysis of variance
AVA	Australian Veterinary Association
OTM	Oral Trans-Mucosal

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Executive Summary

What the report is about

This report describes a R&D project on the most suitable castration method and pain management for alpacas that could be adopted by the Australian alpaca industry.

Who is the report targeted at?

The primary audience for this report comprises alpaca producers, and veterinarians serving the alpaca producers.

Where are the relevant industries located in Australia?

Alpaca producers and enthusiasts are located all around Australia, with a greater representation in Victoria, New South Wales, Queensland, South Australia, Tasmania, and Western Australia.

Background

Castration is an important component of the on-farm management of male alpacas in Australia, because it decreases aggression towards other alpacas and humans, and improves the economic value of the herd, since wethers can be sold as guard animals, for fleece production, or used in the evolving alpaca meat industry. However, there has been no standard method to castrate alpacas that could be widely adopted and recommended to either veterinarians or alpaca producers.

To produce a standard method of castration for alpacas there was a need to test the most promising methods derived from the knowledge of castration in other species, either close relatives such as the llamas, or those with morphological and developmental characteristics close to those of alpaca, such as dogs or horses.

Aims/objectives

To investigate and validate standard methods of castration for alpacas that would meet both industry constraints and future animal welfare standards.

Methods used

The approach taken to the project followed the 4th generation R&D approach, and included industry stakeholder engagement from the start to the finish of the project. An extensive consultation with stakeholders from the industry was conducted first, using an online survey and focussed discussions with a panel of experts. The survey results and the expert panel helped to identify that the experimental phase of the project should investigate both surgical, and mechanical, methods of castration, different pain management strategies, and the possibility for producers to perform castrations.

A series of animal experiments were conducted to test the efficiency of 1) meloxicam delivered via an oral trans-mucosal (OTM) route in combination with ketamine and xylazine during surgical castration, 2) meloxicam OTM alone or with addition of Tri-solfen® during surgical castration, 3) meloxicam OTM alone during mechanical castration, and 4) oral sedative/analgesic in combination with oral meloxicam. In all experiments the impact of welfare was measured using visual pain scoring, behavioural observations, balance testing, and cortisol secretion. All experiments followed the same sampling protocol over 2 weeks. The animals were monitored for up to 10 weeks following mechanical castration.

Results/key findings

The results were compared to the best strategy available in terms of welfare. The results showed that meloxicam OTM is a good analgesic in alpacas, and is not too expensive. Sedation in combination with meloxicam OTM gave the best pain management following surgical castration, and the combination of oral meloxicam and Tri-solfen® offers an economical and acceptable solution to trained lay operators. The limitations and advantages of both strategies are discussed and considered in the recommendations.

Implications for relevant stakeholders

This project provides evidence that could be used by the industry body to propose industry standards for the castration of alpacas that are acceptable in welfare terms, to engage policy makers and to answer community concerns.

Recommendations

Recommendations include 1) the description and scope of the best two methods of castration and pain management, 2) a strong case against the use of mechanical castration, 3) the creation of a training course to allow producers to perform castration in non-sedated but analgised animals and 4) the use of testicular size to decide which method of castration is to be used.

Introduction

In Australia, an industry has developed using alpacas farmed mainly for their fibre. In recent years, a market has also developed for their meat. As in most animal production systems, males that are not of high genetic merit will not be kept as breeding stock, and because of issues with the aggressive nature of males, need to be castrated. Castration is an important component of the on-farm management of male alpacas in Australia because it decreases aggression towards other alpacas and humans, and improves the economic value of the flock, since wethers can be sold as guard animals, for fleece production herds, or for meat. However, currently, there is no standard method to castrate alpacas that could be widely adopted and recommended to either veterinarians or producers. To produce a standard method of castration for male alpacas there is a need to test the most promising methods derived from the knowledge of castration in other species, either close relatives such as llamas, or those species with morphological and developmental characteristics close to those of alpaca, such as dogs or horses. Because of the limited number of scientific publications on castration of alpacas it is necessary to scientifically assess not only the efficiency of any method, but its impact on animal welfare, and the economic impact on production costs. This project aims to validate a standard method of castration for alpacas that meets both industry constraints and optimises animal welfare outcomes for alpacas undergoing castration using analgesics and/or anaesthetics that are currently commercially available in Australia.

Castration is a painful procedure (Mellor and Stafford, 2000) and other animal industries have been, and are still, developing guidelines and best practices to minimise the impact of castration on animals (Paull *et al.*, 2009, Coetzee, 2011, Nickell *et al.*, 2015). Meloxicam has been shown to decrease post-operative pain in several large animal species (Caulkett *et al.*, 2003, Bourque *et al.*, 2010, Ingvast-Larsson *et al.*, 2011, Goldschlager *et al.*, 2013, Repenning *et al.*, 2013, Tenbergen *et al.*, 2014), and recently, Ilium Buccalgesic, delivered via an oral trans-mucosal (OTM) route, an oral meloxicam preparation (see Appendix 1), has been shown to reduce the pain of surgical castration in bulls and pigs (Keita *et al.*, 2010, Repenning *et al.*, 2013). Unfortunately, pain management methods and castration techniques that have been developed in other production species may not be transferable as alpacas have quite a different anatomy of the external reproductive system compared to other mammalian species (see Appendix 2), and exhibit a slow and variable rate of maturation of their reproductive system (Tibary and Vaughan, 2006).

The project followed a 4th generation R&D approach, meaning that industry stakeholders were included from the start. We aimed to include all stakeholders, being those parties with any level of industry involvement, e.g. producers, veterinarians practising castration, animal welfare groups, and regulators (non-exhaustive list). The consultation phase allowed us to; 1) conduct a qualitative study on the needs, knowledge and perceptions about the methods of castration that are used, or could be used, by the alpaca industry, and 2) to survey the criteria that would contribute to make a castration strategy easily adoptable by the industry. Following this broad consultation, we organised contact by telephone with a small group (3-4) of selected representatives of each stakeholder group to define both the six most promising strategies to be tested, and the criteria to be used to assess those different strategies.

We expected that methods of castration to be discussed would include methods described in llamas, such as standing castration while restrained (Barrington *et al.*, 1993, Baird *et al.*, 1996), castration with the animal in a lateral recumbency (Fowler, 2010), the pre-scrotal castration technique (Pugh *et al.*, 1994), and less possibly the use of rings, a method that is popular in the farming community with respect to ease of ring application and cost, and not popular in the veterinary community with respect to welfare. Similarly, we expected that the use of ketamine and xylazine in combination with butorphanol, or butorphanol alone with a local analgesic would be discussed (Baird *et al.*, 1996 , Fowler, 2010). The combination(s) to be tested were directed by the survey and the panel of experts.

The project was divided into 2 phases

Phase 1: Survey of the stakeholders

A survey of stakeholders was conducted to establish the criteria that would contribute to a castration strategy that would be readily adoptable by the industry. A panel of expert stakeholders (about 20 people with a spectrum of experience in the industry) was presented with the results of the survey, and then the panel helped to define both the most promising castration strategies to be tested and the criteria to be used to assess those different strategies.

Phase 2: Animal experimentation

Phase 2.1: The use of meloxicam OTM in combination with ketamine and xylazine during surgical castration

As a first step to achieving the desired outcome, this first experiment was designed to identify a practical and affordable pain management protocol that would maximise animal welfare after surgical castration. A contemporary sedation and analgesia protocol that is commonly used in alpacas is a combination of xylazine, ketamine, and butorphanol (Snyder, 2007). However, it was proposed that the use of meloxicam may provide better pain management due its longer duration of action (Kreuder et al., 2012, Mosher et al., 2012), especially when delivered by an oral trans-mucosal (OTM) route using meloxicam incorporated into a gel that facilitates absorption (Patel et al., 2011). The efficacy of rubber rings to castrate alpacas had not been tested and had been questioned because, in alpacas, the testes are closely attached to the body (Appendix 2).

Aims and Hypotheses

The aims of this study were to 1) determine if meloxicam is more effective than the contemporary method of sedation at decreasing pain after surgical castration in alpacas; 2) to compare the efficacy of an oral versus injectable preparation of meloxicam on decreasing pain after surgical castration in alpacas; and 3) develop and validate physiological and behavioural indicators of pain in alpacas.

We hypothesised that 1) meloxicam would be more efficient than the contemporary method, 2) meloxican OTM would be as efficient as injectable meloxicam, and 3) a set of behavioural indicators can be used to assess small changes in pain in alpacas.

Phase 2.2: Use of meloxicam OTM alone or with addition of Tri-solfen® during surgical castration

In addition to the use of meloxicam in Phase 2.1, we tested the combination of meloxicam OTM with a recently commercialised local anaesthetic formulation (Tri-solfen®, Bayer Animal Health – Appendix 3). The new formulation has been shown to significantly reduce pain-related behaviours in lambs undergoing surgical castration (Lomax et al., 2010).

Aims and Hypotheses

The pain of surgical castration would be better managed using a combination of meloxican OTM and Tri-solfen® than it would using meloxican OTM alone.

Phase 2.3: Use of meloxicam OTM alone during mechanical castration

Veterinarians and the AAA have often questioned the efficacy of rubber rings to castrate alpacas because, in alpacas, the testes are closely attached to the body. The method has not yet been tested. The rubber ring method will be tested after administration of oral meloxicam.

Aims and Hypothesis

Mechanical castration with rubber rings will cause a long-lasting pain that can be alleviated using oral meloxicam.

Phase 2.4: Investigation of the use of an oral sedative/analgesic in combination with oral meloxicam.

In the previous experiment, in addition to meloxicam OTM that we were testing, we gave a combination of sedatives that are commonly used in alpacas (xylazine and ketamine). These scheduled drugs can be administered only by a registered veterinarian. In phase 2.4, we aimed to test the efficiency of an alternative sedative that can be dispensed over the counter to producers by veterinarians, detomidine (an alpha-2 adrenergic receptor agonist; Appendix 4). There was no scientific information on the dosage of oral detomidine in alpacas.

Aims and Hypotheses

Detomidine can be used orally in alpacas to induce sedation and some analgesia

Once we had demonstrated that oral detomidine was not effective in alpacas and based on veterinarian Jane Vaughan's suggestion to mix xylazine with meloxicam OTM, we aimed to test the efficiency of a mixture of xylazine and meloxicam for sedation and analgesia during alpaca castration. This alternative was promising since xylazine is extensively used in alpacas and it is a relatively safe sedative. The other advantage of using xylazine is that it would provide some analgesia that might not have been provided by detomidine.

Aims and Hypotheses

Xylazine mixed with meloxicam OTM can induce sedation and some analgesia in alpacas

Phase 2.5: Test electrocauterisation during surgery

The Australian Alpaca Veterinarians also raised the possibility of using electrocauterisation instead of ligature during the surgical procedure. Electrocauterisation is a very reliable method to avoid bleeding during surgery. This electrocauterisation technique was tested on animals receiving meloxicam OTM and xylazine

Aims and Hypotheses

Electrocauterisation is a safe and reliable alternative to ligature application during surgical castration

Objectives

The objective of this project was to identify and validate a standard method for the castration of alpacas that is based on scientific evidence and acceptable to both professionals involved in the alpaca industry and to welfare groups. To achieve this overarching objective, the project addressed the following objectives:

1. Consultation with professional groups and representatives of the industry and other interest groups involved in the debate about the castration of alpacas. This consultation had two objectives:

   a. Define what were the criteria for the potential most acceptable method of castration for alpacas

   b. Define the three most important experimental parameters to be tested. Six castration methods were targeted.

2. Assess the methods to castrate alpacas defined above

3. Validate and select the best castration method for alpacas

4. Produce materials, such as a factsheet, video material and training manual to demonstrate the technique and illustrate the low level of impact of the procedure on the welfare of the animals.

Methodology

Phase 1. Survey of the stakeholders

We had originally proposed a short survey of 6 questions. After discussion with the AAA, the survey was extended, resulting in a comprehensive survey of 31 questions (Appendix 5). Approval to conduct the survey was obtained from the Human Ethics Committee of the University (*www.qualtrics.com)*. The survey comprised of the collection of demographic data and then 3 different sections. Section 1 contained questions asking about current practices and knowledge about methods of castration in alpacas. Section 2 contained questions asking about preferred method of castration in alpacas. Section 3 contained questions asking about the criteria to be used to define the best methods of castration. The survey was advertised via email to groups such as the AAA, AVA, and RSPCA. The online survey was made available from late November 2014 to mid-January 2015.

We communicated the results of the survey to a panel of 12 experienced stakeholders. The panel was given time to read and digest the results of the survey, then we organised teleconferences with small groups of experts (2-3 experts at a time) to define both the six most promising castration strategies to be tested and the criteria to be used to assess these different strategies.

Phase 2. Animal experimentation

This section is divided into two sections. The first section describes the general methods and the second section describes the methods specific to each sub-phase. The overall design of each experiment is illustrated in Figure 1.

General materials and method

Animals

The animals were sourced by, and maintained at, Banksia Park alpaca stud. A total of 87 animals was used including 7 Suri alpacas and 80 Huacaya alpacas. The males were mainly of white colour, with 6 black and 6 fawn animals. The age ranged from 11 months to 3.5 years with a mean of 19 months. The animals were in good health and in good body condition, as confirmed by veterinarian George Jackson before they were included in the experiment.

Husbandry

The animals were kept in small paddocks at a stocking rate of 1 male/5 m^2 for a week prior and a week following castration. The animals were fed *at libitum* oaten hay supplemented with lucerne and alpaca chaff and grain mix, and had free access to fresh and clean water.

Castration

Surgical castration

Each alpaca was sedated and placed in dorsal recumbency on a low table. The scrotum and surrounding skin was disinfected with 1% chlorhexidine (Surgical Scrub, Vetasept) for surgery using the principles of aseptic surgical scrubbing. A scrotal incision was made over each testis 2 cm from and sagittal to the median raphe and then through the scrotal fat. Gentle pressure was applied to either side of the testicle to extrude it. The surrounding fat was separated away to allow visualisation of the spermatic cord. The spermatic cord was then clamped using a haemostat, and a ligature (Chromic gut, Ethicon) was placed and tied around the spermatic cord before it was transected. This procedure was then repeated for the remaining testicle. The scrotal incisions were left open to facilitate drainage.

Mechanical castration

Using a commercial ring applicator, two rubber Elastrator rings were placed, one at a time, behind the scrotal sac containing the two testes.

Sedation and recovery monitoring

The time between injection and full sedation was recorded. From the end of castration, the animals were monitored every 5 min for 2 h, and then every 30 min from 2h to 4h30 min post castration (Appendix 5). Body position, demeanour, drinking, vocalisations, gait, agitation, aggression, isolation, and social behaviours were recorded at each time point. Details are provided in Appendix 6.

Health and pain monitoring

Each castrated male was observed daily for any sign of post surgery complications such as bleeding, swelling, or infection using a set of indicators for one week after surgery. Pain was monitored daily for seven days using a standardised visual assessment (Appendix 7)

Behavioural activity

The behaviour of each animal was recording using 4 CCTV cameras mounted so that they captured the pens holding the alpacas post-surgery. The behaviour of each animal was analysed using Boris software (Friard and Gamba, 2016). The frequency and duration of behaviours that are known to be changed when an animal is in pain were calculated for each animal (Paull *et al.*, 2009). These behaviours included standing, eating, walking, laying-down, drinking, self-grooming, agonistic behaviour, and social behaviour. Individuals were identified using large numbers sprayed onto the side and rump. Individual behaviour was observed every hour for 5 min from 0600h to 1800h on the 3 days prior to castration to obtain a baseline for behaviour that was then compared to data collected on the first 2 days after castration. The same data were collected and analysed on Day 4 after castration.

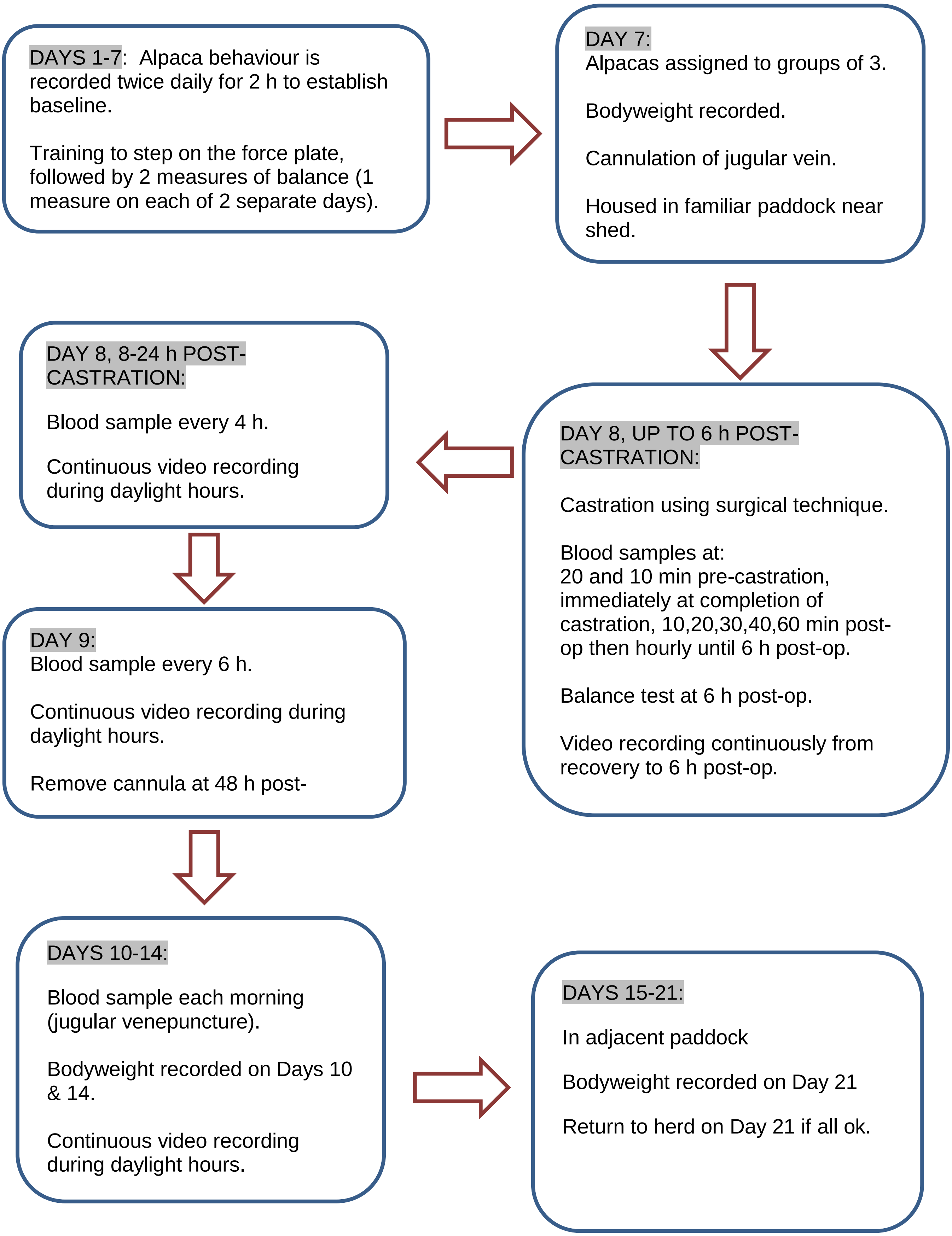


Figure 1. Flow chart for experimental protocol

Balance test

The technology for biomechanical measurements was developed originally for use in sports science and motor-developmental studies in humans, and has only recently been used in animals. The maintenance of postural balance is energetically costly and, therefore, a measure of balance and the efficiency of locomotion have been considered as indicators of sheep welfare. The most relevant technology to welfare is the use of 3D - accelerometers that produce data that can be translated into directional forces, directional workload, or total workload and therefore energy use (Gillette and Angle, 2008). These accelerometers can be attached to an animal's limbs and used to measure the gait. The same type of accelerometers can be inserted between two solid (non-deformable) plates to measure the ground reaction forces that are generated by a body standing on, or moving across, the upper plate (Figure 2). Specific algorithms can calculate the displacement of the centre of gravity during a walk or during a stationary period providing a measure of the balance and the total force required for maintaining balance. The data collected from force plates can also be used to determine the partitioning of weight between the forelimbs and hindlimbs, as well as the force and the work produced in each of the three dimensions (Figure 2). In dairy cattle and sows, it has been shown that force measurement can be used to predict lameness (Grégoire *et al.*, 2013, Pluym *et al.*, 2013, Conte *et al.*, 2014, Dunthorn *et al.*, 2015). Measuring the variation in ground reaction forces and the associated changes in weight distribution between the fore and back limbs, and between the right and left limbs, can represent a very useful indicator of pain as suggested from work done in pigs (Conte *et al.*, 2015, Pairis-Garcia *et al.*, 2015).

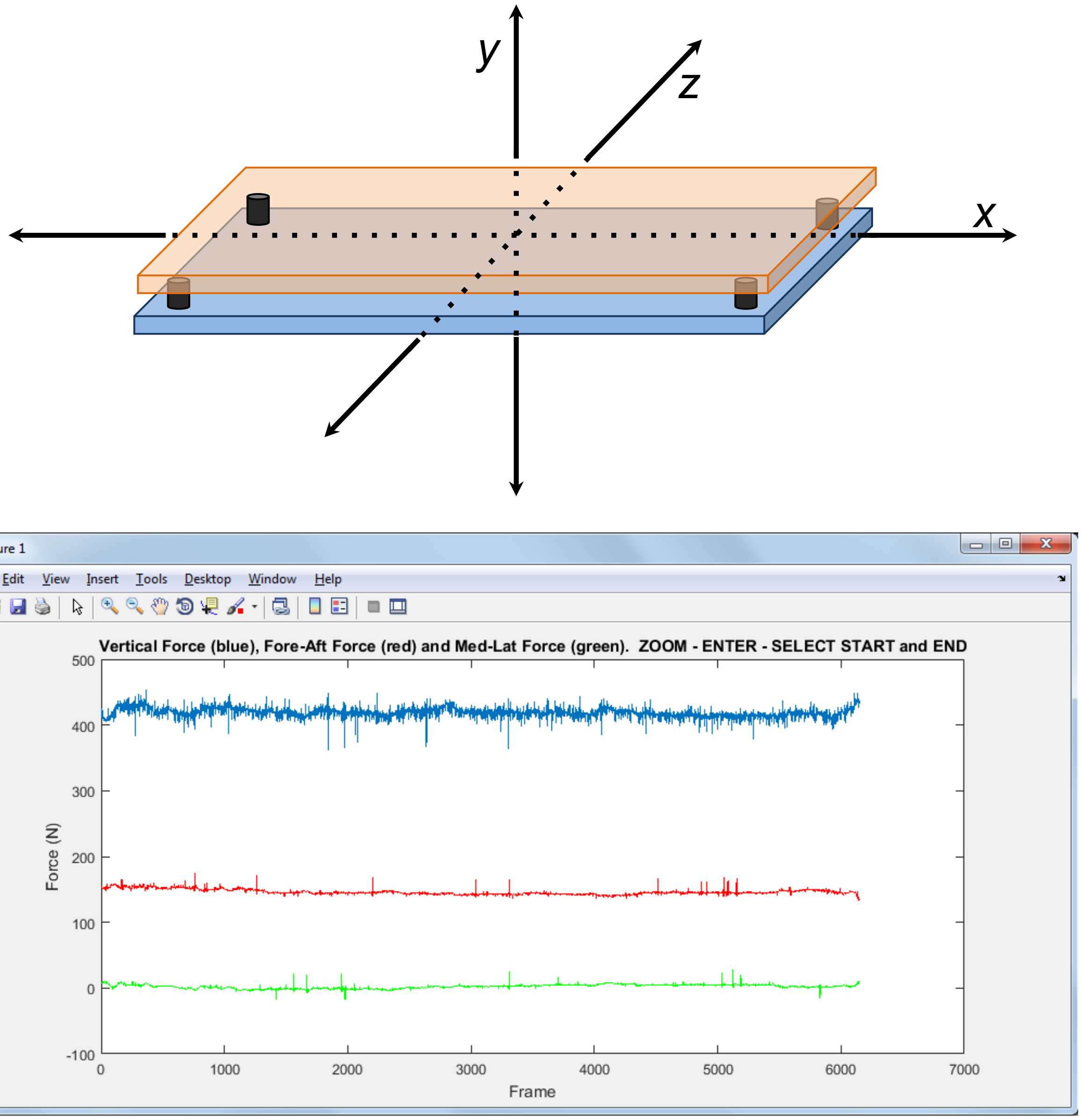


Figure 2. **Schematic representation of a force plate system comprising of two non-deformable plates (in blue and orange) with triaxial actometers or accelerometers (black cylinders) located between the two plates at the four corners of the plate.**

The accelerometers record acceleration along the three-dimensional axis. Specific algorithms can visualise the pattern of ground reaction forces produced by an alpaca standing on two force plates (bottom panel). The raw data were converted in vertical force (z; blue trace) and fore-aft force (x: red trace). The forces exerted and the work produced by the limbs can be calculated for each axis as well as their variation over time. The weight distribution between the fore and back limbs, and the jittering in the three dimensions can also be calculated.

In this project, we used two force plates. The alpacas were trained to stand still for at least 30 sec to a maximum of 60 sec with the forelimbs on one “front” force plate and the hind limbs on a different “back” force plate (Figure 3). On a few days before the start of the experiment the alpacas were trained to stand on all four legs on the force plates. Data from the force plate were collected on alternate days (Days -5, -3 and -1) prior to castration to obtain a baseline and then post castration at 6 hours post castration, and then on Day 1, 2, 4 and 6. The data were collected and digitised using Spike software (Heitler, 2007).

All data from the force plates during the motor balance tests were processed using MATLAB (Mathworks Inc., Novi, MI). From the data MATLAB calculates, for each set of limbs, the work in 3D (J/kg), the power in 3D (watts/kg), the rate of displacement (or jittering) in 3D (m/s), and the distribution of weight between the front and back limbs.

Figure 3. The setting and handling of alpacas on the force plates during a motor balance test (Photo K. Vadhanabhuti).

Plasma cortisol

Blood was sampled via a jugular cannula with an extension set fitted along the back of the animal, to allow sampling to come from the rear of the animal, to minimise interference with the animal (for details about the jugular cannulation procedure that occurred the day before castration, see Appendix 8). Samples were taken at regular intervals, from 10 min to 4 h, over the first 2 days after castration. However, it was not possible to maintain the jugular cannula for longer than 48 hours without increasing the risk of blockage and/or infection. Therefore, after 48 hours the cannula was removed and blood samples were then taken via jugular venepuncture to obtain a once daily sample on Day 3 to Day 7 (5 samples in total). Jugular venepuncture was minimised because blood sampling may stress animals as a result of physical restraint and/or insertion of the needle through the skin.

The animals were held in small groups and habituated to human contact. The blood sampling procedure was designed to accurately measure changes in cortisol over the first 24 hour period and then less frequently for the remaining seven days. The volume of blood taken was replaced by physiological saline at each sampling time.

After collection, the blood was centrifuged for 10 min and the plasma was collected and frozen at -18°C for later analysis. Plasma cortisol was measured using an Immunochem

Coated Tube Cortisol I125 RIA (MP Biomedicals, Belgium) validated for alpaca samples. The limit of detection was 1.9 ng/ml and the intra- and inter-assay coefficients of variation were less than 6%.

Liveweight

Liveweight was measured with each alpaca standing on a large wooden platform mounted on a set of Ruddweigh bars and reader.

Statistical analysis

The normality of data and the homogeneity of variance between each test group of alpacas was initially checked using the Shapiro-Wilk test (Shapiro and Wilk, 1965) and the Bartlett's test (Bartlett, 1937) and data were transformed accordingly. Using the statistical package R, comparisons were made between treatments using as a two-way analysis of variance (ANOVA) for repeated measures. Within group, the effect of treatment was analysed using ANOVA for repeated measures. Probability less than 0.05 was considered significant.

Specific material and methods

Phase 2.1 Use of meloxicam OTM in combination with ketamine and xylazine during surgical castration

We used a complete random block design that included two repeats (blocks) to compare the effect of butorphanol to that of injected meloxicam and to that of meloxicam administered via an oral trans-mucosal route. Fifteen different animals were allocated to each block: five animals to each of the three treatment protocols. With the two repeats there was a total of ten animals in each of the three treatments. The treatments are summarised in Table 1

Table 1. Pain management treatment

Group "But"	Butorphanol 42 µg/kg IM (injected 10 min before surgery)	Xylaxine 0.42 mg/kg IM (injected 10 min before surgery)	Ketamine 4.2 mg/kg IM (injected 10 min before surgery)
Group "iMel"	Meloxicam 1mg/kg IM (injected 10 min before surgery)	Same as above	Same as above
Group "oMel"	Ilium Buccalgesic Oral Trans Mucosal 1 mg/kg (applied 90 min before surgery)	Same as above	Same as above

The IM dose of meloxicam (1 mg/kg) was derived from the literature on goats, pigs, bulls, and llamas (Keita *et al.*, 2010, Ingvast-Larsson *et al.*, 2011, Kreuder *et al.*, 2012) and was administered 10 minutes before surgery.

The oral dose of meloxicam, administered as Ilium Buccalgesic OTM, was 1 mg/kg as used in cattle (Appendix 1, Repenning *et al.*, 2013) and was administrated 90 minutes before castration. The meloxicam preparation (Ilium Buccalgesic OTM) is formulated for oral trans-mucosal (OTM) absorption by adding the active ingredient to a gel that "sticks to the mouth soft tissue" (Appendix 1) and has been used successfully in sheep and cattle. The dose volume is applied into the sulcus between the molar teeth and the inside of the cheek. The Ilium Buccalgesic OTM preparation contains 10 mg meloxicam/ml and has been designed to provide pain relief in calves undergoing castration. Ilium Buccalgesic OTM was found to be bioequivalent to a meloxicam SC injection. The maximum concentration of meloxicam in plasma was reached within about 9 h after Ilium Buccalgesic OTM and 5 h after SC injection, however analgesia is achieved within 30 min after administration (Appendix 1). For clarity, hereafter “meloxicam OTM” refers to Ilium Buccalgesic OTM.

Phase 2.2: Use of meloxicam OTM alone or with addition of Tri-solfen® during surgical castration

Two groups of ten alpacas received either of meloxicam OTM (1mg/kg) alone or the combination of meloxicam OTM (1mg/kg) with a recently commercialised local anaesthetic formulation (Tri-solfen®, Bayer Animal Health). The meloxicam OTM was given 90 minutes before castration and 6 ml of Tri-solfen® was injected into the incision after castration using a 10 ml syringe (a drenching gun could also be used for a large number of animals).

Phase 2.3: Use of meloxicam OTM alone during mechanical castration

Twelve males were used and meloxicam OTM was given 90 minutes prior to application. Small, green Elastrator® rings (external diameter, 13.5 mm; internal diameter, 6.5 mm; Heiniger Australia) were applied to alpacas that had testes that measured between 1 and 3 cm long and were aligned close to the median raphe. That classification was used because about 10% of animals have testicles that sit too far laterally to be incorporated into a single rubber ring around the scrotal skin proximal to the testicles. The two testes were gently pulled together with one hand and the applicator was used to appose the rubber ring around the skin joining the scrotum to the body. Two rings were applied per animal using the elastrator pliers due to the wide-based nature of the scrotal attachment (see attachment 6). The efficiency of the rubber rings was monitored daily for the first 6 days post application, and then weekly until the animal had lost its avascular scrotum (9 weeks for the longest).

Phase 2.4: Investigation of the use of oral sedative/analgesic in combination with meloxicam OTM

Fifteen alpacas were used in this experiment to assess the quality and kinetics of sedation and pain reduction of four different dose rates (40, 60, 80, 120 µg/kg) of detomidine (Dormosedan® gel, Zoetis). The range of doses tested was based on previous publications in horses, dogs, and felids (Kaukinen *et al.*, 2011, Hopfensperger *et al.*, 2013, Hokkanen *et al.*, 2014, Phillips *et al.*, 2015).

To test the efficiency of a mixture of xylazine and meloxicam, we mixed an injectable solution of xylazine (Ilium Xylazil-100, Troy Laboratories) with the Ilium Buccalgesic OTM gel. We tested three doses of the gel compound (2.5, 5 and 10 ml, providing doses of 1, 2 and 4 mg of xylazine on 5 animals/dose.

The sedation quality and the response to a painful stimulus were measured using the standardised protocol described in Appendix 10

Phase 2.5: Test of electrocauterisation during surgery

Ten male alpacas were used and sedated using the combination of xylazine and ketamine and meloxicam OTM as described in phase 2.1. During surgical castration, once the testicle was extruded and the surrounding fat was separated, the spermatic cord was clamped and sectioned and sealed using electrocauterisation.

1. Survey of the stakeholders

A total of 381 people logged on to the online survey (Appendix 5), 259 participants completed the survey and 67 participants partially completed the survey, giving a participation rate of 85% of those that showed an interest by starting the survey.

Personal information

The participants who completed the survey (Table 2) were mostly alpaca producers (54%), with a good representation from alpaca enthusiasts (hobby farmers, 32%), and veterinarians (11%).

Most of the participants (91%) owned alpacas with 11% owning less than 10 alpacas, 45% between 11 to 50 alpacas, 22% between 51 and 100, and 13% more than 100.

Participants were from all the states with 37% from NSW, 27% from Victoria, 12% from Queensland, 12% from South Australia, 9% from Western Australia, and 5% from Tasmania.

The majority of respondents were female, except for veterinarians, aged mainly between 45 to 65 years old, and lived mostly in New South Wales and Victoria. A large proportion of all groups had a Bachelor degree level, except for Hobby farmers who had a lower level of education.

Information about the industry

Most of the respondents owned more than 10 alpacas, except veterinarians who had only 1 to 10 alpacas (67%). However veterinarians cared for from none (30%) to more than 300 animals (27%). Producers (primary and secondary activity) owned between 10 and 100 alpacas and cared for them by themselves (around 70%). Similarly, hobby farmers owned alpacas (1 to 50) and seemed to care only for their own animals (70%) (See Table 3).

Most male alpacas are castrated due to behaviour or market reasons (pet, herd guard, control breeding). All of the respondents reported that they did not castrate males if the males had good qualities for fleece and conformation to be used for breeding.

The majority of stakeholders preferred to castrate alpacas at between 12 to 18 months of age (40%), after that the same proportion of owners castrate their males between 6 to 12 months or 18 to 24 months (around 20% each period of time – Table 4).

Table 2. Personal characteristics of respondent groups

Personal information	Producers (primary)	Producers (secondary)	Hobby farmers	Veterinarians	Other Stakeholders
Gender					
Female/Male	62/38	68/32	72/28	37/63	71/29
Age					
>65	37%	23%	23%	10%	28%
Between 55 and 65	29%	40%	39%	27%	43%
Between 45 and 55	29%	27%	21%	37%	29%
Between 35 and 45	3%	6%	15%	20%	
<35	3%	3%	2%	7%	
States					
New South Wales	44%	37%	30%	33%	29%
Australian Capital Territory			1%		14%
Victoria	27%	22%	26%	43%	29%
Queensland	13%	10%	15%	3%	14%
South Australia	4%	16%	12%	10%	29%
Western Australia	7%	9%	11%	10%	14%
Tasmania	4%	6%	5%		
Owner of pet	87% of persons have a pet	91% of persons have a pet	93% of persons have a pet	93% of persons have a pet	100% of persons have a pet
Level of education	Mainly Bachelor degree level (31%) and Graduate diploma (20%)	Mainly Bachelor degree level (27%), Postgraduate degree level (19%) and Diploma level from TAFE (16%)	Diploma level from TAFE (20%), Graduate Diploma (18%), Secondary education (17%), Certificate level from TAFE (16%) or Bachelor degree level (16%)	Mainly Bachelor degree level (70%) or Postgraduate degree level (27%)	Mainly Bachelor degree level (29%)

Table 3. Breeding characteristics in function of respondent groups

Caring information	Producers (primary)	Producers (secondary)	Hobby farmers	Veterinarians	Other Stakeholders
Owner	100% owners	100% owners	99% owners	30% owners	71% owners
Alpacas owned					
1 to 10	2%	5%	21%	67%	-
11 to 50	27%	45%	68%	22%	60%
51 to 100	31%	36%	7%	-	40%
101 to 200	18%	12%	2%	-	-
201 to 300	11%	1%	-	-	-
Over 300	11%	1%	-	11%	-
Alpacas cared					
None	2%	-	6%	30%	29%
1 to 10	2%	4%	17%	13%	14%
11 to 50	22%	48%	70%	17%	43%
51 to 100	29%	32%	5%	3%	14%
101 to 200	18%	13%	2%	3%	-
201 to 300	13%	1%	-	7%	-
Over 300	13%	2%	-	27%	-
Males castrated					
Yes always	53%	40%	49%	37%	29%
No never	2%	5%	4%	7%	14%
Sometimes	44%	54%	48%	57%	57%
Reasons to not castrate	Males are not castrated systematically: No males owned; good qualities for fleece and conformation; used as stud; for genetic selection; sell too young to be castrated; no market for pet or herd guard; depends on owners request.				

NB: if respondents did not answer all questions then the percentage is a function of the number of answers

Table 4. Period of age preferred by all respondents for alpaca castration

Age of castration	All of respondents
<6 months	3%
6 to 12 months	20%
12 to 18 months	40%
18 to 24 months	21%
24 to 30 months	3%
Over 30 months	2%
6 to 24 months	4%
12 to 24 months	3%
Other period of age	4%

NB: if respondents did not answer all questions then the percentage is a function of the number of answers

Table 5. Technique and management used for the surgical castration across stakeholder groups

Surgical Castration (81%)	Producers (primary)	Producers (secondary)	Hobby farmers	Veterinarians	Other Stakeholders
Technique					
Double incision	71%	59%	37%	87%	67%
Single incision	17%	24%	37%	3%	17%
Other	3%	4%	1%	10%	
Don’t know	9%	13%	25%	-	17%
Management					
Local anaesthesia used					
All times	40%	63%	68%	57%	71%
Sometimes	11%	4%	2%	17%	14%
Not use	49%	26%	17%	26%	14%
Don’t know	-	7%	13%	-	-
Reasons for not using	Use other drugs for sedation, general anaesthesia. Not need, risk of infection, no availability,	Use other drugs for sedation, general anaesthesia, pain relief) No need, too risky, don’t access to drug	Use other drugs (sedation, general anaesthesia), not necessary (quicker, easier or less stress)	General anaesthesia Only if owner requests	Animal recovers more quickly
Don’t know the drug	70%	71%	88%	-	67%
Drug used	lignocaine	lignocaine, xylocaine, ketamine, lidocaine	lignocaine, prilocaine, bupivacaine, emla	xylazine, butorphanol, ketamine, lignocaine, lidocaine or prilocaine	xylocaine, lignocaine
Timing	5-15 min before 79%	5-15 min before (71%) or 15-30 min before (24%)	5-15 min before 65%	5-15 min before (55%) At the start (32%)	5-15 min before (50%), at the start (33%)

Sedation used					
All times	38%	28%	34%	57%	29%
Sometimes	14%	19%	7%	20%	14%
Not used	44%	44%	39%	23%	43%
Don’t know	4%	10%	20%	-	14%
Reason for not using	use other drugs (sedation, general anaesthesia), Not need, illegal, potential risk	not need, not use by the operator too risky	Unnecessary (effect blood pressure, quick surgery), no access to drug, other drugs sufficient	Depends on the owner, on the animal behaviour. Preference for general anaesthesia or any anaesthesia	No answer
Don’t know the drug	78%	80%	89%	-	67%
Drug used	acepril, Ketamine, herbal "valerian" concentrate	midazolam, ketamine, flunixil, buprenorphanol	ketamine, propofol	triple mix IM, xylazine, ketamine, butorphanol	ketamine, xylazine
General anaesthesia used					
All times	14%	10%	22%	30%,	14%
Sometimes	9%	14%	4%	33%	-
Never use	75%	64%	56%	37%	86%
Don’t know	2%	12%	18%	-	-
Reason for not using	too dangerous for the animal	too dangerous for the animal, not necessary, recovery long time or too expensive	No answer	owners request other method, conditions of the animals, sedation and local anaesthesia are sufficient	too risky for animals use other method
Don’t know the drug	50%	90%	100%	-	-

Drug used	ketamine+/- xylazine	ketamine+/- xylazine	-	xylazine, ketamine, butorphanol, isolfurane	ketamine, xylazine
Pain killer used					
All times	42%	31%	40%	50%	43%
Sometimes	5%	12%	3%	13%	-
Not use	47%	40%	35%	37%	43%
Don't know	7%	17%	22%	-	14%
Reason for not using	Operation is quick, analgesia is contained in sedation	Other drugs are sufficient, no access to pain relief, no drugs registered for alpaca use	Use other drugs, animals don't appear in pain, other animal species are not under pain relief	It is a function of the surgical operation done, the budget of the client and age and testicles size. Useless because other drug used is enough, expensive, animals don't appear in pain or discomfort after castration	Animals don't need it, No sign of pain after castration
Don't know the drug	42%	50%	88%	-	67%
Drug used	flunixin, lignocaine	flunixin, xylocaine, lidocaine, buprenorphine	butylscopolamine flunixin, meloxicam Emla cream	Triple Mix, flunixin, butorphanol, xylazine, meloxicam	finadyne
Timing	1 to 20 min before (40%) and immediately to 2 h after (45%)	1 to 20 min before (40%) and immediately to 2 h after (45%)	5 to 30 min before (29%) and immediately to 48 h after (54%)	5 to 30 min before (68%) and immediately to 2 mins after (16%)	During (67%) Immediately after (33%).

NB: if respondents did not answer all questions then the percentage is a function of the number of answers

Methods of castration used

Surgical castration (Table 5)

The surgical approach was the most common choice among the respondents for the castration of alpacas (81% of respondents) and the technique of double incision (each side of scrotum) was the most used technique (more than 60% of respondents). The second most used technique was the single incision. Only hobby farmers seem divided between these two approaches (37% each) and a high proportion didn't know which technique was used to castrate alpacas (25%).

Most respondents had a similar pain management strategy toward alpaca castration. Most respondents used local anaesthesia and painkillers all of the time. The use of general anaesthesia was in reverse proportional to the use of local anaesthesia. Reasons for not using anaesthesia were often because anaesthesia is too risky for the animal, drugs are not available to farmers, or that other drugs are sufficient. Sedation was reported only by around 30% of respondents because most of them applied other pain management or found sedation unnecessary.

A large majority of stakeholders did not know the name of drugs used for castration, except for veterinarians (from 40 to 80% for every kind of pain management). Hobby farmers had less knowledge about drugs (nearly 90% did not know the drugs). In general veterinarians used mainly a combination of different drugs to manage pain, while producers (primary and secondary activity) used fewer drugs. The principal drugs reported were, ketamine, xylazine, lignocaine, flunixin, meloxicam and butorphanol. The timing of drug provision depended on respondents and pain management. For local anaesthesia most respondents said that they gave drugs 1 to 15 min before surgery (50 to 80%). Painkillers were given 1 to 30 min before surgery (50%) or immediately or until 2h after surgery (50%).

Rubber ring method (Table 6)

The rubber ring method is not used often for the castration of alpacas (17%) because a lot of respondents don’t like this practice and say that it is not really appropriate for alpacas (sheep’s rubber rings). When rubber rings were used, stakeholders chose mainly professional operators to do the castration (around 70%) except for primary activity producers who preferred to do it themselves (93%). All groups thought that the castration operator must be trained by a professional (more than 50%) and some of them recognised the value of previous experience with other livestock.

With regard to pain management, only 13% of veterinarians and primary activity producers gave pain killers all of the time, while the majority of respondents didn't report using any drugs at all (50% to 70%). Reasons around the non-use of painkillers were mainly because drugs are too risky, unnecessary, or because operators have no access to drugs. The other respondents didn't know what drug was given for pain management because they had never used rubber rings. Few of them knew the name of a pain killer given: flunixin, homeopathic pain reliever, or meloxicam. They gave these drugs at different times: immediately to 60 min before (50% for veterinarians) or immediately to 20 min after (57% for Hobby farmers) surgery.

Table 6. Techniques and management used for the rubber ring method across the stakeholder groups

Rubber ring (17%)	Producers (primary)	Producers (secondary)	Hobby farmers	Veterinarians	Other Stakeholders
Technique					
Only use	11%	10%	10%	-	-
sometimes	7%	4%	2%	7%	14%
Never used	81%	86%	88%	93%	86%
Reasons for not using	it's too cruel and too risky for infection, not adapted for Alpaca, vet prefers using the surgical method	Yes if access to someone with equipment and expertise. Not use because the anatomy is not adapted, not always successful, long term pain, too cruel, no training, veterinarians don't approve it	prefers surgical method, too cruel and painful, not allowed by the industry, not adapted for Alpacas, too difficult to fit safely, too invasive	cause reactions, problem of correct applying, too much pain and risk of infection, too long, Alpacas anatomy not reliable, illegal post 6 months, preference for surgery	preference for the surgical method, not ethical, lack of knowledge about the impact on the animal, too much problem of infection, not appropriate
Don't know brand	80%	44%	100%	100%	100%
Brand	Leader products, Small Expander rings	Te Pari Triband castrator rings, Triband bands, sheep green rubber, Elastrator	No answer	No answer	No answer
Operator					
Main operator	Themselves 93%	Themselves 23% or other professional 67%	Other professional 62%	Other professional 73%	Themselves (14%) or other professional (71%)
Training	By other professionals (67%)	By other professionals (63%)	By other professionals (57%)	By other professionals (47%) or no training (27%)	By other professionals (57%)

Comment on training	Qualification is important to avoid complications and limit pain, previous experience with cattle can be useful	Experience using rubber rings, previous experience with other livestock, need 3 persons to proceed or done by a vet with special techniques (wash with betadine solution, pull the testes down, spray the ring with chloromide)	Experience using rubber rings on other species is important, qualification, some rings are more effective than others, depend to the development of the animal	Need to know the anatomy of Alpaca, veterinarian who accept to do it	No answer
Management					
Local anaesthesia used					
All times	4%	5%	6%	7%	14%
Sometimes	2%	5%	2%	3%	0%
Not used	70%	54%	46%	40%	43%
Don't know	24%	35%	46%	50%	43%
Reason for not using	Not qualified to give anaesthetics and animals recover pain-free from the banding within 24 hours	No access to drug, animals never exhibit pain or great discomfort	No access to drugs or not necessary	The pain persist longer	Most respondents do not know
Don't know the drug	No answer	No answer	No answer	33%	No answer
Drug used	No answer	No answer	No answer	lignocaine	No answer
Timing	15-30 min before (33%)	At the time of application (37%) or after (56%)	15-30 min before (17%) or 5-15 min before (33%)	5-15 min before (33%), at the time of application (33%) or other (34%)	At the time of application (100%)
Sedation used					
All times	2%	1%	2%	3%	0%

Sometimes	4%	3%	2%	3%	0%
Not used	73%	59%	47%	47%	57%
Don’t know	20%	37%	48%	47%	43%
Reason for not using	Not qualified to give anaesthetic drugs or it is too risky	Effect of sedation on the animal, procedure is quick, no access to drug, expensive	No access to drugs, too intrusive for the procedure, no need it	Not applicable, illegal, unless do by a vet, no access to drugs for farmers	Most respondents do not know
Pain killer used					
All times	13%	3%	5%	13%	14%
Sometimes	9%	5%	3%	0%	0%
Not used	58%	52%	43%	40%	43%
Don’t know	20%	40%	49%	47%	43%
Reason for not using	Pain relief is not required and expensive	Big risk is the infection, no access to the drugs, not require and expensive	No answer	Not applicable, illegal unless done by a vet, pain persists longer than medication effects	Most respondents do not know
Don’t know the drug	70%	100%	43%	0%	No answer
Drug used	fluxinil	No answer	Homeopathic pain reliever	flunixin, meloxicam	No answer
Timing	15 min before (30%) or few sec to 20 min after (30%)	5 min before (13%) or 5 min after (25%)	Immediately before (14%) or immediately after (57%)	10 to 60 min before (50%)	No answer

NB: if respondents don’t answer all questions then the percentage is in function of the number of answers

Strategies and criteria choice for castration method

Preferred strategies - Tables 7, 8 and 9

Taking a global view, the majority of respondents preferred to use the surgical approach to castrate alpacas (70 to 100% for veterinarians). However they managed pain in various ways. Producers (both primary and secondary activity) preferred not to use drugs (more 20%), while others used local anaesthesia or pain killers, or a combination of both drugs. They preferred to do it by themselves or engaged veterinarians (40% for each operator).

Table 7. First preferred method (surgical strategy (80%) according to stakeholders

Methods	Producers (primary)	Producers (secondary)	Hobby farmers	Veterinarians	Others
Surgery	77%	78%	81%	100%	71%
Rubber rings	23%	20%	17%	-	14%
None		2%	2%	-	14%
Pain management					
Local anaesthesia	16%	23%	31%	7%	29%
General anaesthesia	9%	4%	5%	17%	-
Sedation	4%	9%	3%	7%	-
Pain relief	20%	15%	9%	-	-
Combination sedation-pain killer	4%	1%	6%	3%	14%
Combination local anaesthesia-pain killer	18%	12%	3%	17%	-
Combination general anaesthesia-pain killer	2%	1%	5%	13%	-
Combination local anaesthesia-sedation	-	5%	5%	17%	14%
Combination sedation-local anaesthesia-pain killer	-	7%	6%	13%	14%
Other combination	-	-	4%	7%	14%
No use of drug	27%	22%	18%	-	14%
Preferred Operator					
Owners	44%	41%	16%	3%	14%
Staff	9%	5%	-	3%	-
Veterinarian	38%	41%	60%	93%	71%
Another producer	2%	5%	1%	-	-
Shearer	4%	5%	15%	-	-
Contractor	2%	1%	7%	-	14%

NB: if respondents did not answer all questions then the percentage is a function of the number of answers

Hobby farmers followed the same techniques as producers, but preferred using more local anaesthesia (31%) and engaging veterinarians (60%).

Veterinarians used at least one drug when castrating alpacas. They had a diversity of approaches, but mostly they preferred local and general anaesthesia (24%) or a combination based on anaesthesia: local anaesthesia/pain killer (17%); local anaesthesia/sedation (17%); general anaesthesia/pain killer (13%); sedation/local anaesthesia/pain killer (13%).

Table 8. Second preferred method (rubber rings in total 17%) according to stakeholders

Methods	Producers (primary)	Producers (secondary)	Hobby farmers	Veterinarians	Others
Surgery	56%	56%	61%	51%	-
Rubber rings	24%	35%	25%	19%	75%
none	13%	10%	15%	30%	25%
Pain management					
Local anaesthesia	17%	28%	25%	10%	-
General anaesthesia	7%	4%	1%	5%	-
Sedation	7%	4%	9%	5%	-
Pain relief	12%	17%	29%	24%	-
Combination sedation-pain killer	7%	4%	6%	-	-
Combination local anaesthesia-pain killer	17%	12%	3%	14%	-
Combination general anaesthesia-pain killer	2%	-	1%	-	-
Combination local anaesthesia-sedation	2%	11%	3%	5%	-
Combination sedation- local anaesthesia-pain killer	2%	2%	4%	29%	-
Other combination	-	-	2%	5%	50%
No use of drug	26%	19%	13%	5%	50%
Preferred Operator					
Owners	36%	44%	20%	10%	33%
Staff	13%	1%	3%	5%	-
Veterinarian	31%	36%	45%	67%	33%
Another producer	3%	6%	5%	5%	-
Shearer	3%	6%	15%	-	-
Contractor	15%	5%	12%	15%	33%

NB: if respondents did not answer all questions then the percentage is a function of the number of answers

Other professionals preferred local anaesthesia (30%) but a few of them reported using a combination based on local anaesthesia or sedation.

For the second and the third preferred strategies all respondents chose mostly the surgical method (more 50%) than rubber rings. A high proportion preferred local anaesthesia, but more respondents preferred to use painkiller or not use drugs.

Table 9. Third preferred method (surgical strategy) according to stakeholders

Methods	Producers (primary)	Producers (secondary)	Hobby farmers	Veterinarians	Others
Surgery	51%	58%	49%	50%	50%
Rubber rings	7%	12%	15%	8%	-
None	18%	30%	36%	42%	50%
Pain management					
Local anaesthesia	9%	17%	27%	22%	-
General anaesthesia	0%	8%	6%	-	-
Sedation	12%	9%	8%	6%	-
Pain relief	15%	24%	31%	-	-
Combination sedation-pain killer	15%	5%	2%	-	-
Combination local anaesthesia-pain killer	21%	9%	2%	28%	-
Combination general anaesthesia-pain killer	3%	-	2%	-	-
Combination local anaesthesia-sedation	6%	5%	-	6%	-
Combination sedation-local anaesthesia-pain killer	3%	2%	6%	17%	-
Other combination	-	-	2%	-	100%
No use of drug	18%	23%	17%	22%	-
Preferred Operator					
Owners	23%	37%	24%	6%	-
Staff	17%	2%	7%	6%	-
Veterinarian	43%	41%	44%	64%	-
Another producer	7%	4%	9%	12%	-
Shearer	7%	8%	9%	6%	-
Contractor	3%	8%	9%	6%	100%

NB: if respondents did not answer all questions then the percentage is a function of the number of answers

Criteria for best methods (Table 10)

Stakeholders preferred to choose a castration method as a function of welfare (43%), followed by efficiency (22%). Then there was a similar percentage for other criteria, such as the age of the animal, the cost of the castration, and the animal's health (less than 10%).

Table 10. First 3 criteria identified by the respondents to define the most acceptable/desirable method of castration

Criterion	First	Second	Third
Welfare of the animal (minimise pain, stress and discomfort)	43%	32%	22%
Efficiency of the method (ease, quick, efficient castration)	22%	15%	22%
Age of the alpacas (age or testis size)	7.5%	-	-
Cost of the procedure	5%	11%	15%
Animal health (infection, quick recovery)	2%	16%	17%

NB: The combination of the 2 criteria "animal welfare" and "efficiency of the method" was selected by 40% of the respondents.

The outcomes of the survey were analysed and a summary was sent to a panel of stakeholders (producers, veterinarians, and APOs). We conducted phone interviews with 4 veterinarians, 6 producers, and 2 representatives of APOs. The survey results and the discussions with the panel of experts indicated that we should test:

1. surgical castration with different combinations of long acting painkillers (oral or injectable meloxicam) and sedation and local analgesic or Tri-solfen® and

2. rubber ring with oral meloxicam.

An unexpected outcome was that the impact of testis size rather than age should be investigated.

2. Animal experimentation

Phase 2.1: Use of meloxicam OTM in combination with ketamine and xylazine during surgical castration

Sedation, surgery and recovery

The duration of sedation was significantly longer in the males receiving meloxicam OTM in combination with ketamine and xylazine than in males receiving either injectable meloxicam or butorphanol (Table 11). The quality of sedation was comparable between the three pain management methods (Table 11). The respiratory rate was not different between the treatments and the pupillary reflex was suppressed for at least 10 minutes in all animals (Table 11).

The duration of the surgical castration was 4.4 minutes and was not different between the pain management methods (Table 12). The recovery was smooth for all animals but the males receiving meloxicam OTM took longer to stand after the procedure (Table 12). The time taken for the males to be bright, alert and responsive (BAR) was similar between the three groups. After receiving meloxicam OTM the males started eating 60 minutes after the procedure, while the males that received butorphanol or injectable meloxicam took significantly longer to initiate eating (Table 12).

Pain assessment

The indicators that were used during the visual assessment of pain (Description in methods and in Appendix 7) were all negative for all animals at all times (data not show) on each of the seven days post castration.

Behaviour

None of the behaviours that are thought to be relevant indicators of discomfort such walking, standing, laying, eating, or investigatory behaviour, were affected by castration or by treatment (Table 13). It has to be noted that the animals spent about 25% of their time standing during the day and that the second most frequent activity was spending time at the feed trough and eating (~20%; Table 13).

Motor balance

None of the indicators of balance that could be linked to pain in the groin changed from before to after castration, and there was no difference between the pain management strategies (Table 14).

Cortisol

The plasma concentrations of cortisol were similar between the three groups from before the castration and up to the first hour following surgery (Figure 4 and Table 15). Following surgery, the plasma concentration of cortisol increased within 5 minutes in all three groups, reaching a plateau at 20 min post castration. The amplitude was similar between the three groups but the duration was longer for the group receiving injectable meloxicam than the groups receiving butorphanol or meloxicam OTM (Table 15). After 100 minutes, the plasma concentration of cortisol increased again in all groups, but the increase in the butorphanol group was higher than in the two groups receiving meloxicam (Table 15). The timing of the second increase (surge) was identical in all three groups (Table 15). By 10 hours post-surgery, the cortisol levels in all three groups were not different to pre-surgery.

Table 11. Quality and duration of sedation following different pain management treatment in male alpacas surgically castrated.

Ket: Ketamine, Xyl: Xylazine, But: Butorphanol, Mel: Meloxicam (i: injectable, o: oral). Different superscripts in the same column indicate significant difference at P < 0.05

N	Treat	Body weight (kg)	Respiration rate (breathe/15 sec) Time relative to start of surgery (min)						% of animals without pupillary reflex Time relative to start of surgery (min)						Quality of sedation during surgery	Duration of sedation (min)
			0	5	10	15	20	25	0	5	10	15	20	25		
10	Ket + Xyl +But	43.0 ± 3.9	28 ± 1	28± 1.5	30 ± 2	31 ± 3	29± 2	29 ± 2	100	100	70	60	20	10	1.4 ± 0.2	29 ± 2.6[a]
10	Ket + Xyl + iMel	41.3 ± 3.2	29 ± 1	29± 1.2	33± 1	29 ± 1	26 ± 1	27 ± 1	100	100	100	90	70	10	1.1 ± 0.1±	26 ± 3.0[a]
10	Ket + Xyl + oMel	43.2 ± 3.9	30 ± 2	28 ± 1	28 ± 1.6	28 ± 1	25 ± 1	26 ± 1	100	100	80	50	20	10	1.3 ± 0.2	38 ± 6.5[b]

Table 12. Surgery and quality of the recovery following different pain management treatment in male alpacas castrated surgically or using mechanical castration.

Ket: Ketamine, Xyl: Xylazine, But: Butorphanol Mel: Meloxicam (i: injectable, o: oral), Surg: Surgical castration, BAR: Bright, Alert and Responsive.
NB: *: see text for more details in the change in body position during the course of the recovery.

N	Treat	Castration method	Bodyweight (kg)	Length of procedure (min)	Time (min) when % male standing equals		Time (min) when % male BAR equals		Time (min) when % male eating equals	
					50%	100%	50%	100%	50%	100%
10	Ket + Xyl +But	Surg	43.0 ± 3.9	4.4 ± 0.3	25	35	45	120	180	240
10	Ket + Xyl + iMel	Surg	41.3 ± 3.2	4.4 ± 0.2	15	35	45	150	210	240
10	Ket + Xyl + oMel	Surg	43.2 ± 3.9	4.3 ± 0.2	35	90	60	120	150	180

Table 13. Behaviours of male alpacas castrated surgically and receiving Ketamine and Xylazine, different pain management treatment in male alpacas in combination with Butorphanol (But), injectable Meloxicam (iMel) or meloxicam OTM (oMel).

#: Drinking was not always observed. *:On castration day (Day 0), the alpacas were observed only for 3-4 hours.

Behaviour item	Group	Time relative to castration						Effect of		
		Pre castration	Day 0*	Day 1	Day 2	Day 3	Day 5	Time	Treat	Time x Treat
Walking	But	7.2 ± 4.5	4.5 ± 1.4	7.1 ± 2.3	6.8 ± 2.7	6.4 ± 2.1	5.9± 1.5	NS	NS	NS
	iMel	8.5 ± 2.7	4.8 ± 2.0	7.0 ± 2.4	7.2 ± 1.8	7.0 ± 1.9	4.8 ± 2.2			
	oMel	7.8 ± 4.1	5.1 ± 2.4	7.8 ± 3.7	6.5 ± 2.1	6.8 ± 2.3	6.1 ± 1.8			
Standing	But	24 ± 4.8	27 ± 4.6	24 ± 5.4	28 ± 6.1	30 ± 4.3	26 ± 3.8	NS	NS	NS
	iMel	26 ± 6.8	23 ± 4.5	29 ± 6.2	26 ± 5.2	27 ± 6.0	28 ± 5.1			
	oMel	28 ± 5.2	27 ± 5.5	30 ± 7.2	28 ± 4.9	29 ± 4.8	25 ± 4.6			
Laying	But	21 ± 6.1	24 ± 5.8	25 ± 6.7	25 ± 5.8	28 ± 6.8	22 ± 5.4	NS	NS	NS
	iMel	18 ± 7.5	20 ± 7.0	19 ± 5.9	23 ± 6.4	25 ± 6.7	27± 6.3			
	oMel	20 ± 6.7	22 ± 6.5	24 ± 6.8	21 ± 7.2	23 ± 6.1	24 ± 7.1			
Eating	But	21 ± 8.2	25 ± 7.5	29 ± 6.5	27 ± 7.6	30 ± 8.6	23 ± 7.2	NS	NS	NS
	iMel	19 ± 7.4	23 ± 6.9	24 ± 6.5	25 ± 8.5	22 ± 7.2	21 ± 6.1			
	oMel	22 ± 6.9	27 ± 8.6	28 ± 7.4	24 ± 6.9	25 ± 7.4	20 ± 6.7			
Drinking#	But	0.5 ± 0.8	0.1 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	NA	0.4 ± 0.2	NS	NS	NS
	iMel	0.3 ± 0.3	0.2 ± 0.0	0.5 ± 0.3	0.2 ± 0.1	NA	0.2 ± 0.1			
	oMel	0.2 ± 0.2	0.3 ± 0.1	0.2 ± 0.2	0.4 ± 0.2	NA	0.3 ± 0.2			
Sniffing	But	6.1 ± 1.1	1.2 ± 0.9	3.8 ± 2.3	4.1 ± 1.4	5.2 ± 1.5	5.8 ± 1.5	NS	NS	NS
	iMel	7.2 ± 1.5	1.3 ± 0.8	4.6 ± 1.9	5.0 ± 1.8	4.9 ± 1.3	6.1 ± 1.8			
	oMel	5.9 ± 1.4	1.4 ± 1.0	5.1 ± 1.6	4.6 ± 1.5	5.0 ± 1.8	6.4 ± 2.0			
Grooming	But	1.1 ± 0.8	2.3 ± 0.8	1.8 ± 0.5	0.9 ± 0.7	1.4 ± 1.0	1.6 ± 0.2	NS	NS	NS
	iMel	0.8 ± 0.7	2.0 ± 0.7	1.7 ± 0.9	1.2 ± 0.6	1.3 ± 0.9	1.4 ± 0.6			
	oMel	1.2 ± 0.6	1.9 ± 1.0	2.1 ± 1.1	1.5 ± 1.1	1.1 ± 0.5	1.2 ± 0.5			

Table 14. Parameters obtained from the balance test in alpacas surgically castrated and receiving a combination (IM) of ketamine and xylazine in combination with butorphanol (IM: But) or meloxicam (IM: iMel) or meloxicam OTM (oMel).

Parameters	Group	Time relative to castration					Effect of		
		Pre castration	6h	Day 1	Day 2	Day 3	Time	Treat	Time x Treat
Front-to-Back weight ratio (%)	But	101 ± 10	102 ± 9	99 ± 6	104 ± 9	98 ± 10	NS	NS	NS
	iMel	110 ± 9	94 ± 12	108 ± 10	102 ± 9	104 ± 11			
	oMel	105 ± 8	100 ± 8	110 ± 7	105 ± 9	103 ± 8			
Forelimb force (N)	But	172 ± 13	170 ± 19	168 ± 13	176 ± 14	168 ± 16	NS	NS	NS
	iMel	195 ± 16	183 ± 23	187 ± 14	190 ± 18	189 ± 15			
	oMel	183 ± 21	171 ± 13	194 ± 16	182 ± 20	178 ± 13			
Forelimb vertical jittering	But	2185 ± 145	2144 ± 198	1972 ± 237	2048 ± 182	2149 ± 168	NS	NS	NS
	iMel	2457 ± 159	2082 ± 288	2184 ± 202	2215 ± 231	2351 ± 249			
	oMel	2220 ± 302	1832 ± 278	2390 ± 214	2249 ± 224	1998 ± 281			
Forelimb horizontal jittering	But	178 ± 24	194 ± 29	179 ± 23	174 ± 24	181 ± 30	NS	NS	NS
	iMel	235 ± 31	186 ± 29	175 ± 26	193 ± 28	204 ± 24			
	oMel	228 ± 34	167 ± 22	214 ± 24	208 ± 21	216 ± 28			
Forelimb Fore-Aft jittering	But	1303 ± 190	1252 ± 181	1355 ± 104	1287 ± 176	1323 ± 143	NS	NS	NS
	iMel	1180 ± 125	943 ± 116	1132 ± 119	1125 ± 138	1167 ± 119			
	oMel	1194 ± 111	1096 ± 142	1174 ± 92	1147 ± 134	1185 ± 127			
Back limb force (N)	But	174 ± 13	165 ± 29	170 ± 12	169 ± 15	172 ± 17	NS	NS	NS
	iMel	167 ± 14	186 ± 15	173 ± 9	185 ± 20	176 ± 14			
	oMel	171 ± 18	156 ± 11	174 ± 14	172 ± 15	168 ± 19			
Back limb vertical jittering	But	2121 ± 149	2021 ± 118	2083 ± 144	2193 ±150	2058 ± 136			
	iMel	2150 ± 182	2202 ± 163	2091 ± 96	2067 ±135	2188 ± 152			
	oMel	2035 ± 242	2087 ± 232	2239 ±166	2125 ± 212	2212 ± 184			
Back limb horizontal jittering	But	215 ± 18	286 ± 47	203 ± 35	224 ± 27	278 ± 34	NS	NS	NS
	iMel	223 ± 32	326 ± 75	257± 61	234 ± 54	245 ± 48			
	oMel	295 ± 64	397 ± 71	329 ± 50	309 ±62	346 ± 57			
Back limb Fore-Aft jittering	But	312 ± 26	294 ± 49	281 ± 37	299 ±38	309 ± 32			
	iMel	366 ± 33	358 ± 53	272 ± 36	334 ± 46	381 ± 37	NS	NS	NS
	oMel	371 ± 53	348 ± 84	356 ± 25	362 ± 52	358 ± 61			

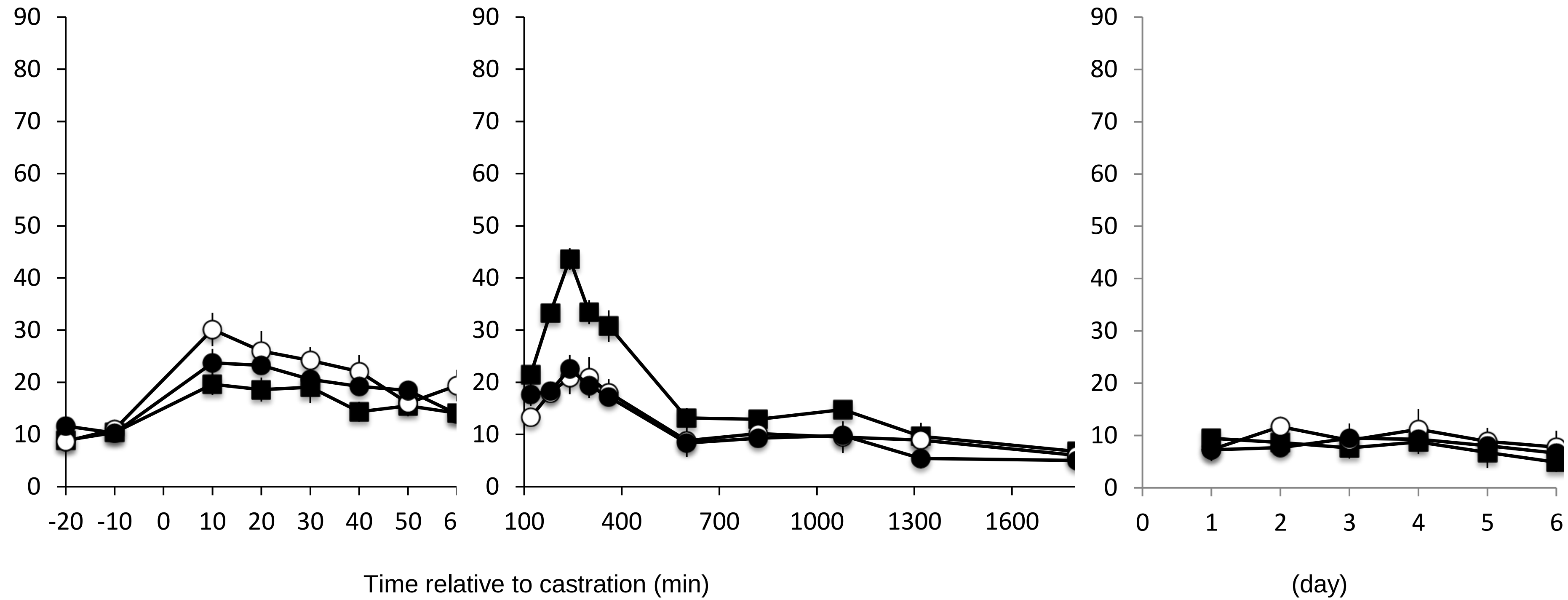


Figure 4. **Plasma concentrations of cortisol in alpacas surgically castrated at t= 0 treated with xylazine and ketamine (t = -10) and meloxicam OTM (closed circles) at t = - 90, or IM meloxican at t= -10 (open circles) or IM butorphanol at t = -10 (closed squares).**

Table 15. Descriptive parameters obtained from the profiles of plasma cortisol in male alpacas castrated surgically and receiving different pain management treatment in male alpacas.

Ket: Ketamine, Xyl: Xylazine, But: Butorphanol Mel: Meloxicam (i: injectable, o: oral), Surg: Surgical castration, BAR: Bright, Alert and Responsive. NB: *: see text for more details in the change in body position during the course of the recovery. Time is given relative to the end of castration. # The profile of cortisol for the mechanically castrated alpacas is rather a large surge than a plateau. Different superscripts indicate difference within a column.

				Plateau			Second increase (surge)		
N	Treat	Castration method	Baseline concentration (ng/ml)	Onset (min)	Duration (min)	Mean amplitude (ng/ml)	Presence (% of animals)	Max	Time of max (min)
10	Ket + Xyl +But	Surg	10.1 ± 1.3 [a]	5.0 ± 0.0[a]	38.0 ± 3.3[a]	7.8 ± 0.9[a]	100	33.5 ± 4.1[a]	240 ± 0
10	Ket + Xyl + iMel	Surg	9.9 ± 1.3 [a]	5.0 ± 0.0[a]	51.0 ± 8.6[b]	15.7 ± 2.5[b]	90	18.4 ± 3.2[b]	228 ± 32
10	Ket + Xyl + oMel	Surg	10.9 ± 2.1 [a]	5.0 ± 0.0[a]	35.6 ± 3.8[a]	9.1 ± 2.2[a]	80	17.4 ± 5.8[b]	247 ± 22

Live weight

The animals gained an average +0.75 ± 0.21 kg between before castration to 12 days after castration. There was no effect of treatment, or time, or interaction between time and treatment, on the change in live weight.

Phase 2.2: Use of meloxicam OTM alone or with addition of Tri-Solfen during surgical castration

Surgery and recovery

The application of Tri-solfen® added about 1 minute to the duration of the surgical castration compared to meloxicam OTM alone (Table 16).

The recovery was smooth for all animals and was not affected by the addition of Tri-solfen®. Within 5 minutes after these two treatments, the males were standing and were scored as bright, alert and responsive (BAR - Table 16). All the males had eaten by 4h30 minutes after castration. Bleeding was observed in 4 animals in the group receiving meloxicam OTM only and in 2 animals in the group receiving both meloxicam and Tri-solfen®. One animal from the Tri-solfen® group needed to be ligatured to stop the bleeding. The bleeding was observed in larger animals that had large spermatic cords.

Pain assessment

The indicators that were used during the visual assessment of pain (Description in methods and in Appendix 7) were all negative for all animals at all times (data not show) on each of the seven days post castration.

Behaviour

There was no effect of treatment, or interaction between treatment and time, on any of the behaviour items recorded (Table 17). There was an effect of time on the percentage of time spent standing and lying, with an increase in both behaviours on Day 1. However, it has to be noted that eating behaviour also increased on Day 1 (Table 17), which could explain the increase in time lying down, because alpacas tend to lye down when they ruminate.

Motor balance

The parameters obtained from the balance test were not affected by treatment or time. There were some changes in jittering in the forelimb and the back limb on Day 1 (Table 18). The fore-aft jittering score decreased in the forelimb in both groups while horizontal jittering increased in the back limb. These changes in jittering do not seem to be representative of any change in balance due to discomfort since they can’t be related from a biomechanical viewpoint

Cortisol

The plasma concentrations of cortisol were similar between the two groups before the castration and during the first hour following surgery (Figure 5 and Table 19). Following surgery, the plasma concentration increased within 5 minutes in these two treatment groups reaching a plateau at 20 min post castration. The amplitude and the duration of the plateau were similar between the two groups (Table 19). After 100 minutes, the plasma concentration of cortisol started to decrease but increased again in both groups to concentrations similar to that observed during the plateau at 20 min (Figure 5 and Table 19). The timing of the second increase (surge) was similar in both groups (Table 19).

Table 16. Surgery and quality of the recovery following different pain management treatment in male alpacas castrated surgically or using mechanical castration.

Mel: Meloxicam (o: oral), TRiS: Tri-solfen®, Surg: Surgical castration, Mech: Mechanical castration using elastrator®, BAR: Bright, Alert and Responsive. NB: *: see text for more details on the change in body position during the course of the recovery.

N	Treat	Castration method	Bodyweight (kg)	Length of procedure (min)	Time (min) when % male standing equals		Time (min) when % male BAR equals		Time (min) when % male eating equals	
					50%	100%	50%	100%	50%	100%
10	oMel	Surg	39.1 ± 5.2	2.3 ± 0.3	5	5	5	5	150	270
10	oMel + TriS	Surg	39.0 ± 6.7	3.2 ± 0.4	5	5	5	5	150	270
12	oMel	Mech	39.6 ± 5.1	2.7 ± 1.3	5*	5*	5	5	120	>270

Table 17. Behaviours of male alpacas castrated surgically and receiving meloxicam OTM (oMel) alone or in combination with Tri-solfen®. #: Drinking was not always observed. *:On castration day (Day 0), the alpacas were observed only for 3-4 hours.

Behaviour item	Group	Time relative to castration						Probability		
		Pre castration	Day 0*	Day 1	Day 2	Day 3	Day 5	Time	Treat	Time x Treat
Walking	oM	9.1 ±1.6	11± 1.4	7.8 ± 1.5	7.2 ±1.5	5.5 ± 2.1	5.4 ± 1.2	NS	NS	NS
	oM+T	8.4 ± 1.7	4.8 ± 2.0	14 ± 4.0	6.7 ± 1.3	4.2 ± 0.5	6.7 ± 1.6			
Standing	oM	26 ± 3.9	29 ± 7.0	42 ± 7.8	24 ± 4.9	12 ± 2.7	43 ± 4.5	0.05	NS	NS
	oM+T	28 ± 3.5	28 ± 8.3	50 ± 8.9	20 ± 4.2	17 ± 3.6	51 ± 4.8			
Laying	oM	16 ± 3.4	32 ± 15	37 ± 7.6^{b}	27 ± 5.4^{b}	30 ± 4.6^{b}	32 ± 5.1^{b}	0.05	NS	NS
	oM+T	17 ± 5.2	37 ± 17	33 ± 8.1^{b}	30 ± 3.9^{b}	38 ± 5.5^{b}	34 ± 5.8^{b}			
Eating	oM	19 ± 5.5	18 ± 7.0	37 ± 7.1	21 ± 1.2	27 ± 4.1	30 ± 7.8	NS	NS	NS
	oM+T	17 ± 3.4	14 ± 4.8	37 ± 7.2	19 ± 2.9	29 ± 5.3	24 ± 3.1			
Drinking#	oM	0.1 ± 0.05	0.1 ± 0.1	3.5 ± 1.7	0.4 ± 0.2	NA	0.9 ± 0.7	NS	NS	NS
	oM+T	0.2 ± 0.07	0.2 ± 0.0	2.8 ± 1.8	0.2 ± 0.1	NA	0.3 ± 0.1			
Sniffing	oM	7.4 ± 1.1	4.1 ± 1.2	1.0 ± 0.5	5.8 ± 1.6	3.1 ± 1.3	0.2 ± 0.2	NS	NS	NS
	oM+T	9.7 ± 1.3	3.0 ± 1.4	0.9 ± 0.4	5.7 ± 1.3	1.7 ± 0.9	0.4 ± 0.1			
Grooming	oM	0.9 ± 0.2	1.6 ± 0.6	1.1 ± 0.2	1.1 ± 0.5	1.0 ± 0.3	1.4 ± 0.8	NS	NS	NS
	oM+T	1.3 ± 0.5	3.4 ± 1.8	1.5 ± 0.3	1.2 ± 0.4	0.7 ± 0.4	0.6 ± 0.1			

Table 18. Parameters obtained from the balance test in alpacas surgically castrated and receiving meloxicam OTM (oMel) alone or in combination with Tri-solfen®.

NB: Different superscripts indicate difference from pre-castration level within a row.

Parameters	Group	Time relative to castration					Effect of		
		Pre castration	6h	Day 1	Day 2	Day 3	Time	Treat	Time x Treat
Front-to-Back weight ratio	oM	1.6 ± 0.2	1.7 ± 0.2	1.5 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	NS	NS	NS
	oM+T	1.7 ± 0.3	1.5 ± 0.1	1.3 ± 0.1	1.5 ± 0.3	1.6 ± 0.2			
Forelimb force (N)	oM	174 ± 13	169 ± 12	168 ± 9	173 ± 11	171 ± 12	NS	NS	NS
	oM+T	165 ± 16	169 ± 8	128 ± 12	158 ± 9	162 ± 10			
Forelimb vertical jittering	oM	2069 ± 238	2157 ± 143	2287 ± 77	2201 ± 85	2184 ± 72	NS	NS	NS
	oM+T	2178 ± 166	2245 ± 134	1932 ± 162	2143 ± 132	2146 ± 128			
Forelimb horizontal jittering	oM	1052 ± 153	1064 ± 134	1705 ± 129	1486 ± 143	1154 ± 124	NS	NS	NS
	oM+T	1161 ± 148	1264 ± 264	1432 ± 168	1187 ± 167	1164 ± 162			
Forelimb Fore-Aft jittering	oM	512 ± 36	378 ± 43^{b}	273 ± 30	489 ± 42	524 ± 37	NS	NS	NS
	oM+T	416 ± 52	493 ± 52	339 ± 55^{b}	387 ± 50	427 ± 47			
Back limb force (N)	oM	110 ± 14	108 ± 14	119 ± 16	111 ± 12	115 ± 14	NS	NS	NS
	oM+T	96 ± 13	119 ± 9	109 ± 9	107 ± 9	103 ± 8			
Back limb vertical jittering	oM	1570 ± 180	1418 ± 137	1662 ± 136	1576 ± 143	1607 ± 128	NS	NS	NS
	oM+T	1349 ± 157	1545 ± 109	1462 ± 109	1408 ± 117	1385 ± 124			
Back limb horizontal jittering	oM	539 ± 128	522 ± 120	607 ± 93	581 ± 72	586 ± 92	NS	NS	NS
	oM+T	384 ± 83	416 ± 74	553 ± 88^{b}	421 ± 67	408 ± 76			
Back limb Fore-Aft jittering	oM	309 ± 54	387 ± 48	337 ± 53	317 ± 54	352 ± 47	NS	NS	NS
	oM+T	307 ± 48	289 ± 42	436 ± 59	327± 51	318 ± 45			

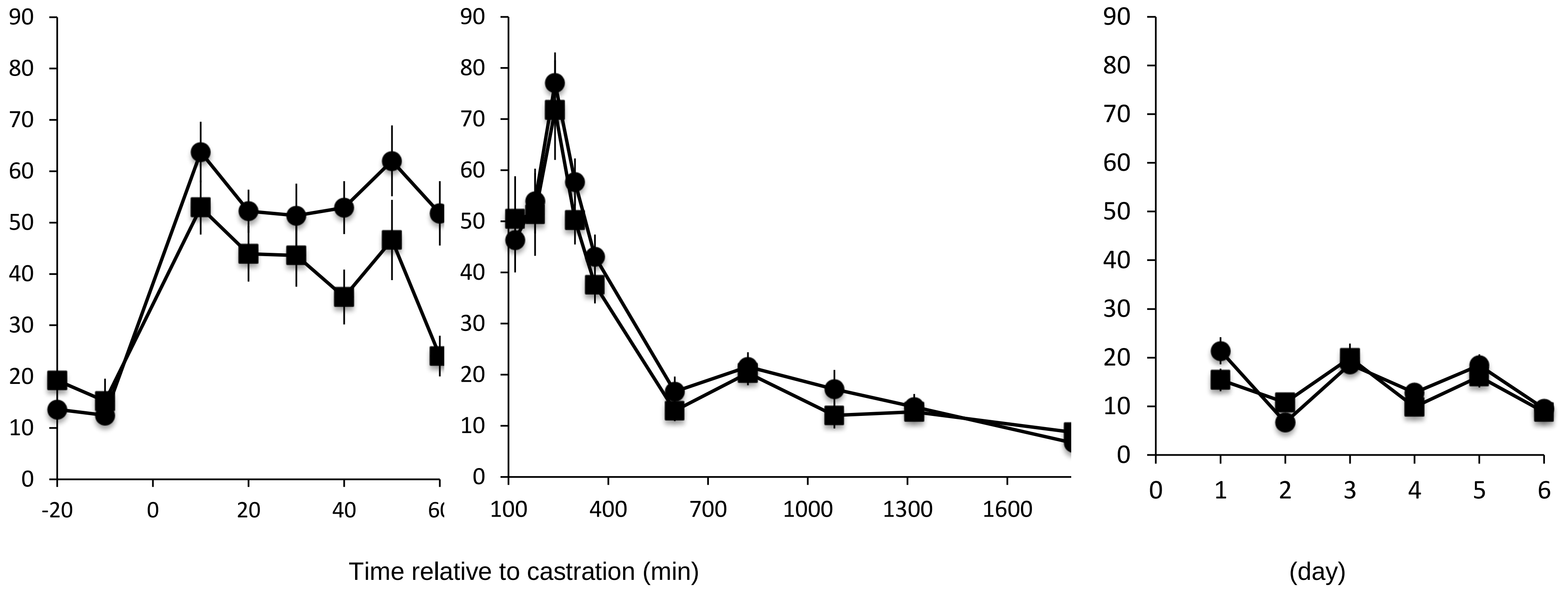


Figure 5. **Plasma concentrations of cortisol in alpacas surgically castrated at t= 0 treated with meloxicam OTM (closed circles) at t = - 90 alone or receiving 6 ml of Tri-solfen® at the end of surgery (closed squares).**

Table 19. Descriptive parameters obtained from the profiles of plasma cortisol in male alpacas castrated surgically and using mechanical castration and receiving different pain management treatment in male alpacas.

Mel: Meloxicam (o: oral), TRiS: Tri-solfen®, Surg: Surgical castration, Mech: Mechanical castration using elastrator®, BAR: Bright, Alert and Responsive. NB: *: see text for more details on the change in body position during the course of the recovery. Time is given relative to the end of castration. # The profile of cortisol for the mechanically castrated alpacas is rather a large surge than a plateau. Different superscripts indicate difference within a column.

				Plateau			Second increase (surge)		
N	Treat	Castration method	Baseline concentration (ng/ml)	Onset (min)	Duration (min)	Mean amplitude (ng/ml)	Presence (% of animals)	Max	Time of max (min)
10	oMel	Surg	12.9 ± 2.8^{a}	5.0 ± 0.0^{a}	65.0 ± 8.0^{b}	42.9 ± 5.2^{c}	100	59.2 ± 8.8^{a}	248 ± 28
10	oMel + TriS	Surg	14.3 ± 5.8^{a}	5.0 ± 0.0^{a}	57.8 ± 7.8^{b}	31.7 ± 4.7^{d}	100	68.8 ± 12.6^{a}	233 ± 16
12	oMel	Mech	24.0 ± 2.6^{b}	13 ± 3.2^{b}	$200 \pm 17.3^{c\#}$	28.2 ± 2.9^{d}	30*	51.0 ± 4.3^{a}	233 ± 15

Liveweight

The animals gained +1.62 ± 0.55 kg from before castration to 12 days after castration. There was no effect of treatment, or time, or interaction between time and treatment, on the change in live weight.

Phase 2.3: Use of meloxicam OTM alone during mechanical castration

From the twelve animals scheduled for treatment using meloxicam OTM and mechanical castration, the elastrator rings could not be placed on two of the animals because one animal had one small testis and the other had testis that were two large to pass through the extended ring (Figure 6).

Figure 6. Male alpacas that could not be mechanically castrated using elastrator rings.

Male with one small testis (left – testis retracted) and male with large testis (right).

Figure 7. Alpacas lying down with their legs extended after the application of two elastrators.

The behaviour was observed from 25 minutes after application and for the next 60 to 100 minutes. NB: The two alpacas standing were not mechanically castrated.

Castration and recovery

The application of two elastrators to a standing male took around 3 min. The males were walking as soon as the procedure was done. For the next 20-25 minutes all of them were standing, then they all started lying down with their legs extended (Figure 7). During this time all of the animals were less responsive and had obvious difficulty standing-up when they were disturbed.

Oedema and redness of the skin was observed in all of the alpacas 24 h after the application of the rings, and that continued after 48 hours (Figure 8). The skin of the scrotum began to be compromised (as indicated by black spots and wetness) on Day 3. Then, necrosis was observed on Day 4 most often accompanied with an unpleasant smell. Continued necrosis was observed for 2 weeks and then the scrotum became dried. By the third week there were signs of separation. The time taken for the scrotum to detach from the body was very variable with the first alpaca losing the dried scrotum by week 4, while the last one detached only 9 weeks after the application of the rings. No sign of infection was seen during the 9 weeks of observation. No rings were lost during the same period

At the checks that occurred seven weeks after the application of the rings, it was observed that one of the two testes had gone back into the body cavity in two of the alpacas. Subsequently, those animals were surgically castrated. The efficiency of the technique was thus 80% after application of the rings, but only 66% when considered for the group of animals that was used.

Pain assessment

After the 4 h recovery period, the indicators that were used during the visual assessment of pain (Description in methods and in Appendix 7) were all negative for all animals at all times (data not show) on each of the seven days post castration.

Behaviour

The percentage of time spent standing increased on the two days after castration, while the percentage of time spent lying down decreased on those same days (Table 20). The time spent at the feed trough increased on Days 1, 3, and 4. The time spent sniffing decreased on Day 0, indicating a change in investigatory behaviour.

Table 20. Behaviours of male alpacas in alpacas mechanically castrated using elastrators and receiving meloxicam OTM.

#: Drinking was not always observed. *:On castration day (Day 0), the alpacas were observed for only 3 hours. NB: Different superscripts indicate difference from pre-castration level within a row.

Behaviour item	Time relative to castration					
	Pre castration	Day0*	Day 1	Day2	Day 3	Day5
Walking	6.4 ± 0.8	7.1 ± 1.4	5.2 ± 1.7	4.2 ± 0.8	3.6 ± 0.7	3.6 ± 0.7
Standing	20 ± 2.9	32 ± 7.5^{a}	36 ± 4.1^{a}	42 ± 3.8^{a}	26 ± 5.3	35 ± 2.8^{a}
Laying	29 ± 4.1	34 ± 17	10 ± 2.3^{a}	16 ± 4.1^{a}	31 ± 3.5	32 ± 1.7
Eating	21 ± 3.0	26 ± 8.2	32 ± 2.4^{a}	22 ± 6.3	49 ± 5.6^{a}	41 ± 4.7^{a}
Drinking$^{\#}$	0.5 ± 0.8	NA	1.1 ± 0.9	0.2 ± 0.1	NA	0.1 ± 0.1
Sniffing	6.0 ± 3.0	0.3 ± 0.2^{a}	1.2 ± 0.2	2.3 ± 0.6	1.7 ± 0.1	0.2 ± 0.1
Grooming	1.1 ± 0.8	0.2 ± 0.1	0.6 ± 0.2	2.5 ± 0.7	0.2 ± 0.2	0.6 ± 0.1

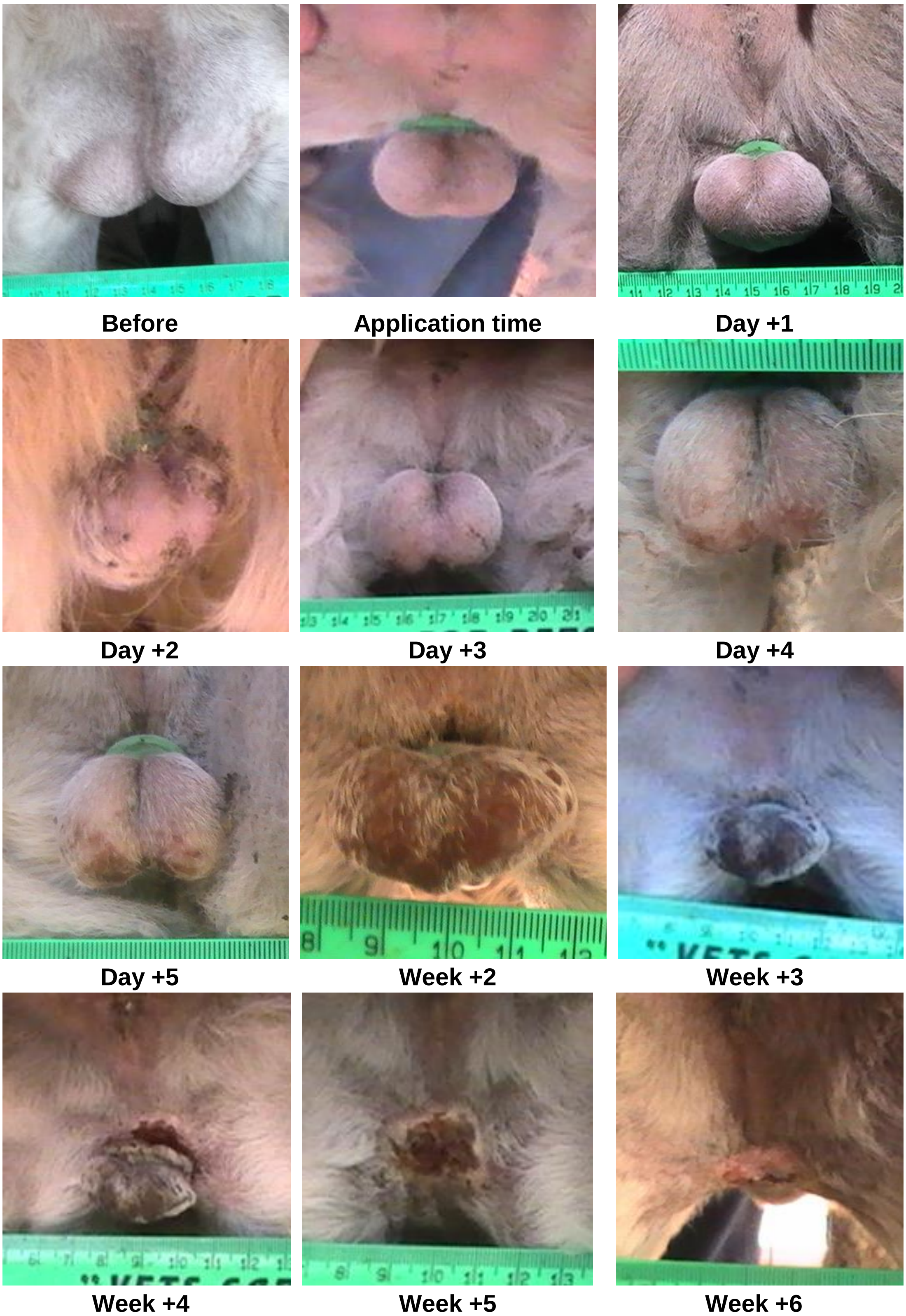


Figure 8. Photos of the testis of one alpaca at time points relative to the application of two elastrators.

Motor balance

The distribution of weight between the fore- and back limbs was affected by mechanical castration. The alpacas reduced the force applied from their back limbs from 6 h and up to 48 h after ring application. Similarly, the amount of jittering decreased in all three dimensions and on both fore- and back limbs from 6 h and up to 48 h after ring application (Table 21).

Cortisol

The plasma concentrations of cortisol before the castration were slightly higher in this group compared to the other experiments. The profile of secretion of cortisol after application of the rings followed a very different pattern compared to that observed in the other experimental phases after surgical castration. Following the application of the two elastrators, the plasma concentrations of cortisol started to increase within about 15 minutes (Figure 9 and Table 21) to reach a maximum at 120 minutes after application of the elastrators (Figure 9). The total duration (~200 minutes) of the surge of cortisol was longer than that observed in all of the other experimental groups (Table 21). The amplitude of the surge was similar to that measured following castration after treatment with meloxicam alone or with Tri-solfen®, but the amplitude was much higher than that observed when ketamine and xylazine was used (Table 21).

Liveweight

The animals gained +1.62 ± 0.92 kg between before castration and 12 days after castration.

Table 21. Parameters obtained from the balance test in alpacas mechanically castrated using elastrators and receiving meloxicam OTM. NB: Different superscripts indicate difference from pre-castration level within a row.

Parameters	Time relative to castration					Effect of time
	Pre castration	6h	Day 1	Day 2	Day 3	
Front-to-Back weight ratio	1.4 ± 0.1	2.4 ± 0.3[a]	2.4 ± 0.3[a]	1.6 ± 0.2	1.2 ± 0.1	0.05
Forelimb force (N)	176 ± 9	178 ± 14	169 ± 9	168 ± 11	165 ± 10	NS
Forelimb vertical jittering	2230 ± 100	2201 ± 140	2066 ± 85[a]	1985 ± 82[a]	2300 ± 124	0.05
Forelimb horizontal jittering	458 ± 55	396 ± 57[a]	341 ± 41[a]	338 ± 39[a]	396 ± 44	0.05
Forelimb Fore-Aft jittering	617 ± 35	522 ± 31[a]	568 ± 23[a]	559 ± 28[a]	625 ± 36	0.05
Back limb force (N)	129 ± 8	84 ± 10[a]	108 ± 8[a]	108 ± 9[a]	141 ± 9	0.05
Back limb vertical jittering	1747 ± 170	1110 ± 114[a]	1254 ± 120[a]	1346 ± 112[a]	1587 ± 185	0.05
Back limb horizontal jittering	487 ± 58	341 ± 27[a]	390 ± 35[a]	385 ± 25[a]	512 ± 62	0.05
Back limb Fore-Aft jittering	333 ± 55	487 ± 18[a]	396 ± 18	425 ± 21[a]	347 ± 38	0.07

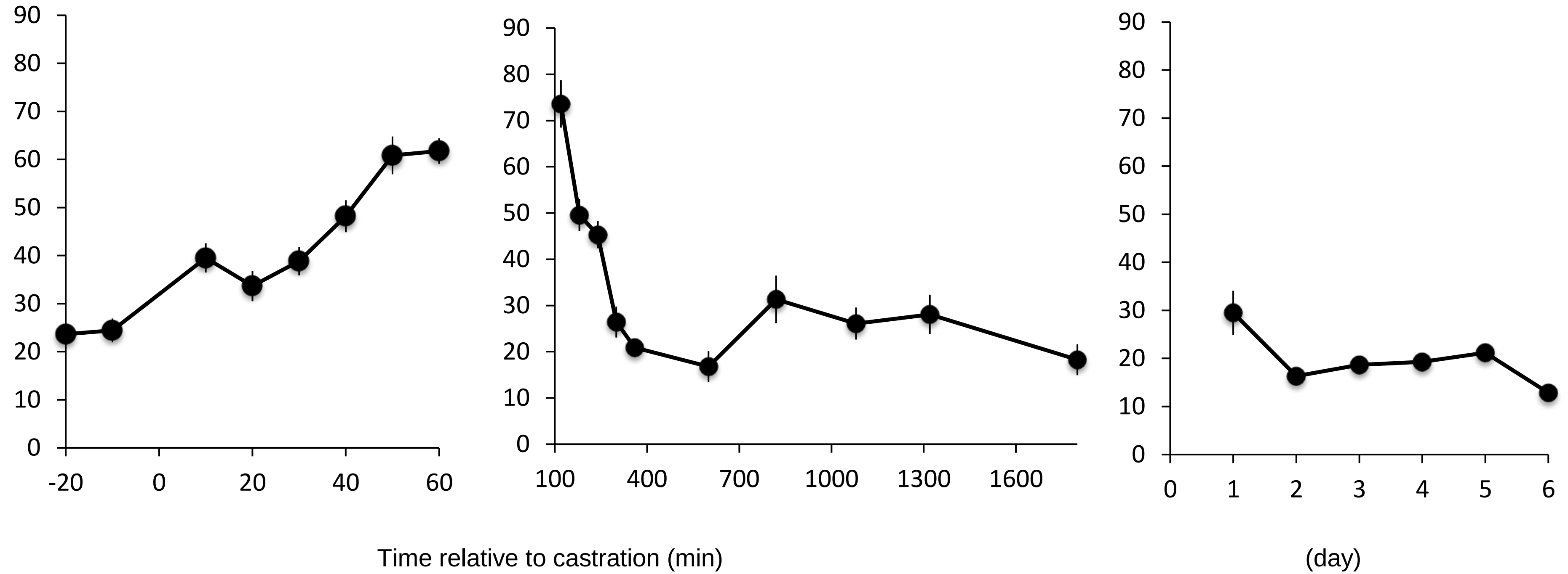


Figure 9. Plasma cortisol in alpacas after application of two elastrators (t = 0) and treated with meloxicam OTM (closed circles) at t = - 90.

Phase 2.4: Investigation of the use of oral sedative/analgesic in combination with oral meloxicam

We didn't observe any of the animals spitting out the detomidine gel or the meloxicam OTM with xylazine. Some increases in salivation were observed after the administration of volumes of detomidine greater than 5 ml.

No sedation and very little change in behaviour or reaction to testis palpation was observed following the oral administration of detomidine gel (Dormosedan® Gel; 60 mg/kg to 180 mg/kg).

Similarly, oral administration of a mixture of meloxicam OTM and xylazine did not induce sedation or decrease the response to testis palpation.

Phase 2.5: Test of electrocauterisation during surgery

Surgery and recovery

The surgery with electrocauterisation took 3.1 ± 0.2 minutes. All of the animals had recovered within 5 min and were all standing and were bright alert and responsive straight after surgery (data not show). Light bleeding was observed in 5 animals for 15 to 20 minutes following surgery. One animal required manual clamping to stop the bleeding.

Pain assessment

The indicators that were used during the visual assessment of pain (Description in methods and in Appendix 7) were all negative for all animals at all times (data not show) on each of the seven days post castration.

Behaviour and motor balance

Neither the behaviour nor the parameters obtained from the balance test were affected by the use of electrocauterisation (data not show)

Cortisol

The profile of cortisol following electrocauterisation was similar to that observed following surgical castration using meloxicam OTM with or without the addition of Tri-solfen® (data not show)

Live weight

The animals gained +1.0 ± 0.55 kg between before castration and 12 days after castration.

Correlation between age of castration and indicators

The average age of the alpacas that were used in this project was 19 ± 6 months (mean ± SD). The baseline concentration of cortisol was the only parameter that was correlated with age at the time of castration (Coefficient of regression = +0.35). The correlation between age at castration and the other parameters that were derived from the cortisol profile had a coefficient of regression ranging from +0.26 to 0.62.

Cost comparison between methods and pain management

Table 22 provides a cost comparison between methods and pain management.

The drugs were priced as “sold” by a veterinarian and valid as at May 2017. A wastage of 10% was included in the drugs used by veterinarians, and 15% when used by producers.

The costs were calculated for the castration of 12 males with an average live weight of 35 kg.

The veterinarian time includes preparation of the surgical set, injection of the animals, as well as surgery and consumables. From our experimental work, it was estimated that the total time spent on site to castrate 12 alpacas and monitor them up to end of recovery would be 90 min. The veterinarian time was costed at $AU240/h.

The consumable cost was estimated at $AU10 when the castration was performed by a producer and included the cost of antiseptic solution, scalpel blade and handle, swabs, and ligature material. The consumable cost was increase by $AU2 for electrocauterisation to include the cost of the electrocauterisation unit.

No cost for a veterinary nurse or animal handlers was included.

Travel time was calculated for a round trip of 100 Km @ $AU1.91/km.

Table 22. Items and cost of the different methods of castration and pain managements used in the present project (as at May 2017).

Pain management	Castration method	Ketamine (4.2 mg/kg)	Xylazine (0.42 mg/kg)	Butorphanol (42µg/kg)	IM Meloxicam (1mg/kg)	Meloxicam OTM (1 mg/kg)	Tri-solfen (6ml/head)	Rubber Ring	Consumable	Veterinarian Time	Travel Time	Total cost	Cost per male
Ketamine Xylazine Butorphanol	Surgical + ligature	21.07	3.77	23.71						360	191	599.55	49.96
Ketamine Xylazine IM Meloxicam	Surgical + ligature	21.07	3.77		55.40					360	191	631.24	52.60
Ketamine Xylazine Oral Meloxicam	Surgical + ligature	21.07	3.77			24.49				360	191	600.33	50.03
Oral Meloxicam	Surgical No ligature					24.49			10			34.49	2.87
Oral Meloxicam Tri-Sulfen	Surgical No ligature					24.49	11.08		10			45.57	3.80
Oral Meloxicam	Mechanical					24.49		1.20				25.69	2.14
Oral Meloxicam	Surgical + Electrocaut					24.49			12			36.49	3.04

Discussion of Results

The project aimed to investigate several methods of castration for male alpacas that would be suitable for the industry and acceptable in terms of the welfare of the animals. The project was designed following a 4th generation Research and Development model. This model allows the inclusion of the stakeholders of the industry, mainly producers and veterinarians, in all of the main steps of the project.

Objective 1. Consult with professional groups and representatives of the industry and other interest groups involved in the debates about the castration of alpacas.

The consultation with the industry started when a survey was developed. From a small paper survey, an online comprehensive survey was developed and conducted over a three-month period. The survey was extremely informative and demonstrated the willingness of alpaca producers and stakeholders of the industry to be progressive and interactive in the decision making process. The willingness to engage was illustrated by the good number of respondents taking the survey and the high rate of survey completion.

The consultation was set with two objectives:

a. Define what are the criteria for the potential most acceptable method of castration for alpacas

The survey results showed that Australian alpaca producers and industry stakeholders agreed that the first criteria should be the welfare of the animals and, as illustrated by the findings of this project, wanted to adopt the best method to castrate alpacas. The practicality of the method and the cost were the second and third criteria listed by producers.

When similar surveys have been undertaken for other livestock industries, the same three criteria have been rated the most important; *welfare, practicality and cost.* However, in the alpaca survey it was remarkable that the welfare of the animals was the highest priority whereas in other livestock industries practicality and cost generally rate higher than welfare. A large number of the respondents had a small number of animals, and that might have skewed the response towards welfare. Small producers are usually closer to their animals than large producers.

b. Define the 3 most important experimental parameters to be tested.

The survey revealed that a large number of pain management strategies were in use, but no consensus, even for sedation, was reached between producers or veterinarians. After discussion with the expert panel it was concluded that the combination of injectable butorphanol, ketamine, and xylazine should be considered as the reference practice for this project. From the national survey, it was found that both surgical castration and mechanical castration were to be investigated as they were frequently used methods, but neither method was universally accepted by the industry stakeholders.

The practicality of the technique directed the choice for suitable pain management methods and castration techniques towards ones that could be applied / used by the producers without the assistance of a veterinarian. With the recent release of meloxicam OTM, and its successful application in other species such as cattle, it was decided that this new meloxicam formulation should be tested. We tested the efficiency of meloxicam OTM (used with ketamine and xylazine) and the route of administration by comparing this combination to both the most contemporary drug mix (ketamine, xylazine, and butorphanol) and the mix of ketamine, xylazine, and injectable meloxicam. That way we had good control testing whether the drug itself made a difference, or if the route of administration made a difference.

Castration using rubber rings proved to be very controversial and was advocated by some while denounced by others. Mechanical castration has been controversial in other livestock industries, such as sheep and cattle, and welfare lobbying has required that the animals receive some form of analgesia. It was decided to test the efficiency of mechanical castration using the traditional rubber ring. A report published in a newsletter of the AAA (LAA September 2005 page 26-28) detailed the technique of rubber ring castration in alpacas and concluded that the technique was reliable and painless. The present results contrast with those results, showing that the method is not animal friendly because all of the behavioural and physiological indicators that were measured were affected for at least 24 h, and up to 48 h, after application. Also the method proved unreliable, since in 2 out of 10 alpacas the testis regressed back into the body cavity through the rubber rings 6 weeks after application.

Surgical castration in sheep or cattle has similarly been controversial. In sheep and cattle, castration is always performed by producers or contractors. The use of an analgesic, such as a local analgesic or Tri-solfen®, after castration or taildocking has proven effective (Lomax *et al.*, 2010) and it was deemed to test the efficiency of Tri-solfen® in alpacas.

In an attempt to further improve the practicality of the technique of castration, we tested oral detomidine gel and oral xylazine (mixed with meloxicam OTM gel) as sedatives and analgesics. In addition, after further discussion with the stakeholders and after our experience with the procedures, it became necessary to test different methods to prevent bleeding from the spermatic cord after castration. We tested methods that were deemed acceptable from a welfare viewpoint and also recommended by veterinarians, with the potential that the methods could be performed by trained producers: no ligature, ligature, or electrocauterisation.

We conducted 7 different animal experimentations with at least 10 males in each experimental group to compare methods of pain management (sedation or not, as well as different pain management drugs), castration technique (mechanical *vs* surgical) and the use of ligature and electrocauterisation (see summary Table 23).

Table 23. Summary of the results observed after different methods of castration and pain management.

Ket: ketamine, Xyl: xylazine, But: butorphanol Mel: Meloxicam (i: injectable, o: oral), BAR: Bright, Alert and Responsive. Coding 1: poor to 5: very good.

Castration method	Treatment	Sedation quality	Recovery	Balance	Behaviour	Cortisol
Surgical + ligature	Ket Xyl But	**3**	**5**	**5**	**5**	**3**
Surgical + ligature	Ket Xyl iMel	**3**	**5+**	**5**	**5**	**4**
Surgical + ligature	Ket Xyl oMel	**3**	**5**	**5**	**5**	**5**
Surgical no ligature	oMel	NA Restraint	**4**	**4**	**5**	**3**
Surgical no ligature	oMel + Tri-solfen®	NA Restraint	**4**	**4**	**5**	**3**
Ring	oMel	NA Restraint	**2**	**1**	**2**	**3**
Surgical + electrocauterisation	oMel	NA Restraint	**4**	**4**	**5**	**3**

Objective 2. Assess the strategies to castrate alpacas defined above

Method of castration and welfare

Each of the treatment methods was assessed using the same experimental protocols that allowed us to collect behavioural data, classical pain assessment data, physiological indicators such as cortisol, and parameters derived from the motor balance of the animals. It is essential to consider all of the indicators together to assess the welfare impact, especially the pain, of husbandry practices (Coetzee, 2011).

Behavioural indicators, either specific to pain, or describing the general behaviour of the alpacas, are not necessarily the most sensitive indicators of discomfort and pain. The present project has demonstrated that measurement of the parameters of balance and equilibrium represent very promising indicators of discomfort in animals after castration. The measurement of jittering in both the fore and back limbs can provide a very sensitive measure of discomfort and the method could be applied to other species. In fact, data from accelerometers attached to the limbs has been used in pigs to detect leg problems (Grégoire *et al.*, 2013) and force plate technology similar to that used in the present experiments has been used to detect lameness in pigs (Conte *et al.*, 2014) and the efficiency of pain management treating lameness

(Conte *et al.*, 2015). It has to be noted that the balance test detected differences between treatments only after the application of rings.

It is important to note that very little change in behaviour was observed after any of the castration methods used, except for the application of the rubber rings, after which the daily activity of the alpacas was disturbed for 24 to 48 h. This lack of behavioural expression would generally be explained by the fact that alpacas, as prey animals, have a natural capacity to mask behavioural signs of discomfort or weakness. However, the castration with rubber rings did affect the behaviour of the alpacas suggesting that discomfort can still affect behaviour. Given that we detected changes in the behavioural and balance data after ring application, but not after surgical castration while under the action of meloxicam OTM, we conclude that castration while under the action of meloxicam OTM is less painful than ring application.

The cortisol results followed a similar pattern in all of the experiments following surgical castration, with first a rapid increase immediately after the procedure and then a plateau that lasted one to one and a half hours, followed by a short decrease, and then a second increase that peaked around 6 hours after surgery. Comparison between the profiles suggests that the first increase in cortisol (in the non-sedated animals) was possibly due to the handling and restraint of the animals. In our experiments, for safety and experimental reasons, the animals were restrained in a shearing apparatus. The first increase from baseline took levels to less than double the baseline level in sedated animals, and to 2 to 3 times the baseline level in restrained animals that were surgically castrated with a duration of less than 60 min. In other species, such as sheep, short-term restraint induces an increase of cortisol of similar magnitude and of similar duration (Niezgoda *et al.*, 1993). Our results show that sedation alleviates the stress of restraint while physical restraint using the shearing restraining apparatus was likely to induce an increase in cortisol. If time and budget had allowed it, it would have been beneficial to have a group of animals that was only restrained. As suggested by previous authors (Baird *et al.*, 1996), experienced operators could perform the castration while the animals is standing, as long as it could be safely restrained.

The second surge of cortisol levels at about 2 hours after surgery to around 6 h after surgery was likely due to animal movement and the interaction with the animals that was necessary to conduct the blood sampling and other measurements. It is highly probable that this second increase would not have occurred if the animals were not moved to conduct the experiment. This suggestion is supported by the fact that, as discussed above, no change in the general behaviour of the animals or the parameters obtained from the balance test, were affected further than 6 h after castration. Unfortunately, due to time and budget constraints, it was not possible to test every permutation of treatments to address the possible impact of other parameters, such as handling, on the welfare indicators.

In conclusion the cortisol data suggest that 1) the non-sedated animals responded to handing stress and surgery during the first hour following the procedure, a response that was reduced by sedation, and 2) all of the animals presented a second cortisol increase at 6 hours post castration likely due to discomfort and pain experienced when the animals were moved for experimental purposes. The second surge of cortisol was blunted by the use of meloxicam (either injectable or OTM).

Pain management

Our data suggest that meloxicam OTM is an effective method of pain management in alpacas. Meloxicam provides better pain management due its longer duration of action (Kreuder *et al.*, 2012, Mosher *et al.*, 2012, Goldschlager *et al.*, 2013) when delivered by an oral trans-mucosal route because meloxicam is mixed with a gel that facilitates absorption (Patel *et al.*, 2011). The duration of action in alpacas seems to be very comparable to previous pharmacokinetic studies in llamas (Kreuder *et al.*, 2012) and dogs, where it was reported that a dose of meloxicam (1 mg/kg) could be effective up to 72 h after administration.

An advantage of oral meloxicam, which is a Schedule 4 drug, is the flexibility in the timing of administration. In this project we administered the meloxicam 90 minutes before the start of the procedure to allow maximum analgesic effect, as suggested by previous studies in llamas (Kreuder *et al.*, 2012) and cattle (Allen *et al.*, 2013) and the recommendation of the manufacturer.

While meloxicam gave better pain management than butorphanol (based on the second peak of the cortisol response), a result that concurs with previous reports in rabbits (Bourque *et al.*, 2010), butorphanol, in combination with ketamine and xylazine gave very good results and could be used if the animals were not to be moved during the 24 h after castration.

The quality of sedation induced by the administration of ketamine and xylazine was comparable between the groups that received in addition either butorphanol, injectable meloxicam, or meloxicam OTM. All of the animals, except one that took 50 minutes to stand, recovered within 30 minutes after the administration of the mixture of ketamine and xylazine, without any negative signs. These results illustrate that sedation using the dosage described in this report is safe for alpacas that are healthy and handled gently at the time of injection.

It was very disappointing and surprising that neither oral detominide (Dormosedan – Schedule 4) nor the oral mixture of xylazine in meloxicam, at both of the doses tested, induced any sedation or analgesia in male alpacas. The injectable formulations of both of these alpha-2 adrenoreceptor agonists are very effective at providing sedation and analgesia and are currently used in veterinary practice and in livestock (for example: (Carroll *et al.*, 1998, Moens *et al.*, 2003, Khan *et al.*, 2004, Messenger *et al.*, 2016). In addition, oral detomidine has been shown to be an effective sedative in others species, including ferrets, horses, cattle, and dogs (Kaukinen *et al.*, 2011, Hopfensperger *et al.*, 2013, Hokkanen *et al.*, 2014, Phillips *et al.*, 2015, Lizarraga *et al.*, 2016). Detomidine has also been shown to have some somatic analgesic potential in horses (Moens *et al.*, 2003). There is no information on the absorption or sensitivity to alpha-2 adrenoreceptor agonists in camelids, but it is likely that both transmucosal absorption and dose might be low in alpacas and other camelids. It would be beneficial to the alpaca industry to further investigate these drugs or similar oral sedatives and analgesics.

Objective 3. Validate and select the best castration method for alpacas

After discussion with the panel of veterinarians and producers and in the light of the results, two methods were selected for recommendation. Each method has some degree of flexibility, a flexibility that is driven by the anatomy of the scrotum in the alpaca rather than the age and the temperament of the animals. While it was deemed that the best method was surgical castration under sedation, a limitation is that the method can be practiced only by veterinarians because of the use of a Schedule 8 drug (ketamine). The second best method (physical restraint, with pain management using meloxicam OTM) could be performed by producers after training by a veterinarian. The validity of the methods has been discussed in the previous section (Objective 2).

The best two methods are:

1. Surgical castration under sedation using ketamine (4.2 mg/kg) and xylazine (0.42 mg/kg) in addition to meloxicam OTM (1 mg/kg given 90 minutes prior to procedure). Of the methods tested in this research, this method is the best method in terms of welfare. Meloxicam OTM is a cheaper option than butorphanol, but butorphanol (42 mg/kg) is still a very acceptable option. This method would be recommended for those owners who wish to use a veterinarian to conduct the procedure under sedation.

2. Surgical castration with meloxicam OTM (1 mg/kg given 90 minutes prior to procedure) and Tri-solfen® (2 x 3ml in the empty scrotum) is the second best method. This method offers an acceptable level of welfare as long as the animals are not moved for the first 24 h after castration. The use of Tri-solfen® is recommended to help reduce bleeding. Ligature of the spermatic cord is recommended when the diameter of the spermatic cord is large. This second method could be performed by a veterinarian or by producers once they have been trained to the satisfaction of a veterinarian.

We found no evidence that the age of the animal had an effect on any of the measured parameters. However, for safety reasons, surgical castration, especially performed without sedation, would be suitable only in small animals or animals with small testes.

Objective 4. Produce training materials to demonstrate the technique and illustrate the low level of impact of the procedure on the welfare of the animals.

A short training video has been produced to illustrate the different steps in the castration process and to familiarise users to the pain management products. These videos describe:

- the application of meloxicam OTM to alpacas
- the surgical castration of a male using the double incision technique
- the ligature technique
- the application of Tri-solfen®

These videos could be available on the AAA website for viewing or download by members.

Implications

For the time being, the alpaca industry has two strategies to castrate alpacas at its disposition that respect the welfare of the animals while allowing the industry to cater for industry needs.

The first strategy is surgical castration performed by a veterinarian using meloxicam OTM. The method offers the best solution for a lower cost than using opiates such as butorphanol. In addition, the producers can prepare the animals before the veterinarians start the surgical castration, therefore providing a cost saving.

Outside of the problem of castration, meloxicam OTM represents a cheap and versatile analgesic for alpacas that can be used by any producer who can obtain the product from a veterinarian. It has to be noted that this product has a limited shelf life, like any other drug.

The second strategy is also a surgical strategy that can be performed either while the animals are standing, if their temperament allows such a restraining method, or restraint in a shearing harness if the animal is agitated.

The AAA now has the data necessary to suggest that rings should not be used to castrate alpacas. The ring method, that is also controversial in other livestock industries, has the potential to create a negative public attitude towards the alpaca industry. The behaviour of the animals exhibiting discomfort over the first 2 hours after castration, the change in balance observed following ring castration (but none of the other methods), the lack of efficiency of the method, and the limitation of using the method in animals with only the appropriate testis size and scrotal confirmation made the method very unreliable and not appropriate for the level of welfare sought by the industry and required by the general public.

Recommendations

The recommendations have been developed in discussion with a panel of experts including producers and veterinarians with experience at alpaca castration.

Recommendation 1

Ring castration using rubber rings, such as elastrator, should not be used since behavioural and physiological measurements showed evidence of decreased welfare after the application of rings. Moreover, the method is not suitable for males with small testes or with very large testes and is reliable in only 80% of cases.

Recommendation 2

The use of a combination of ketamine and xylazine and a long acting analgesic before surgical castration represents the best method in terms of welfare. Meloxicam OTM (1 mg/kg administered 90 min before procedure) as an analgesic is a cheaper option than IM meloxicam and provides a longer lasting analgesia than butorphanol. However, using butorphanol is still a very acceptable option if the animals are not moved for 24 h after the procedure. This combination (ketamine, xylazine, and analgesic) would be recommended for those owners who wish to use a veterinarian to conduct the procedure under sedation.

Recommendation 3

The combination of meloxicam and Tri-solfen® and surgical castration under restraint is the second best method. This method offers an acceptable level of welfare as long as the animals are not moved for the first 24 h after castration. The use of Tri-solfen® is recommended because it helps reduce bleeding as it contains a vasoconstrictor agent. However, ligature of the spermatic cord is recommended when the diameter of the spermatic cord is large. The method could be performed by veterinarians or by producers once they have been trained to the satisfaction of a veterinarian. Training videos to assist with the training of producers have been developed.

Recommendation 4

A training certification could be developed by the AAA in collaboration with the AVA so that producers could be certified to castrate their alpacas. It would be preferable to limit the ability of a producer to perform castration to the animals owned by the certified producer to prevent the emergence of a non-veterinarian castration business that could jeopardise the safety and welfare of the animals. As certified trainees, the producers should keep good records of their castration activities and should undergo a refresher course if not practicing for more than a year.

Recommendation 5

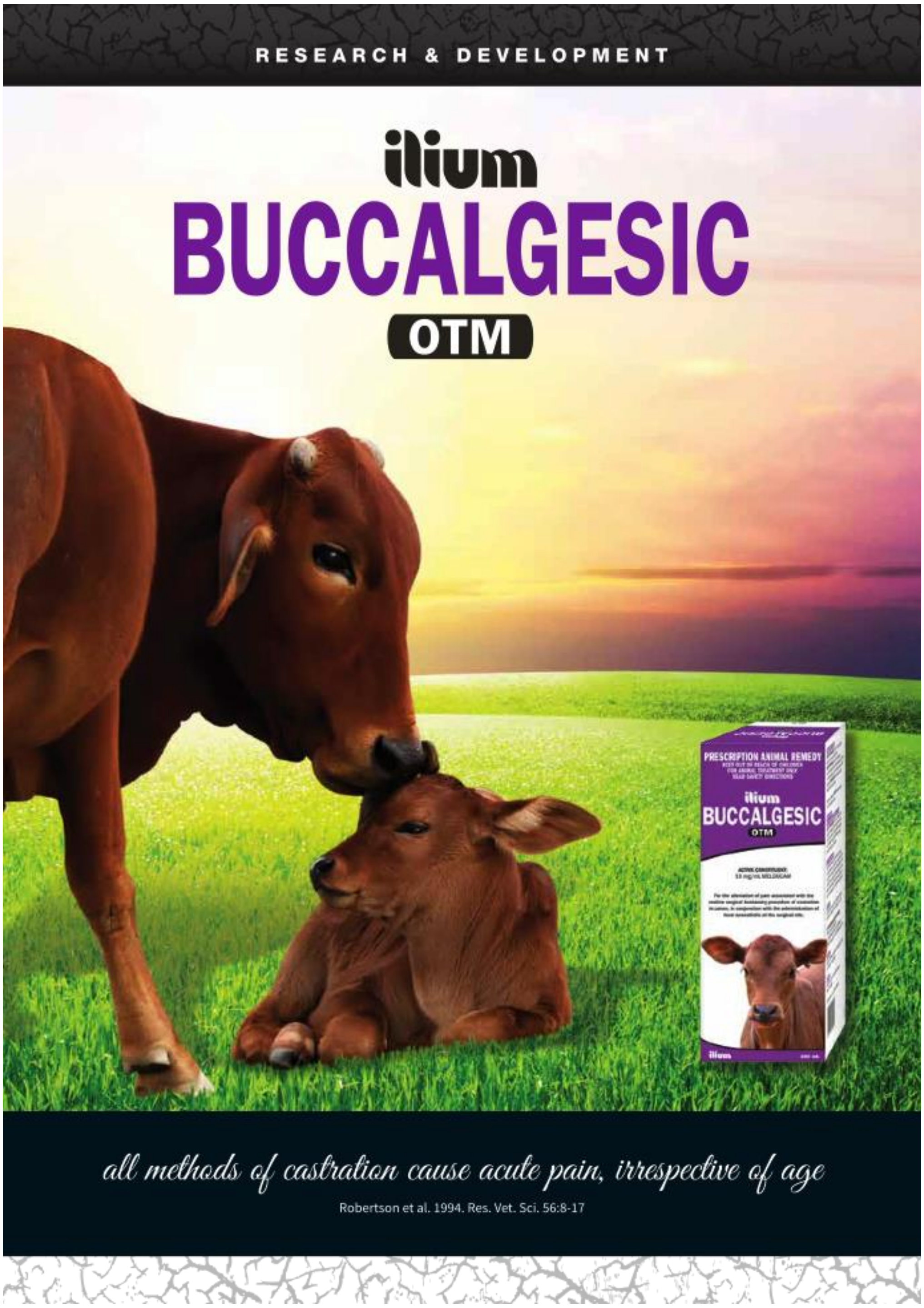
The arbitrary age limit of castration (6 months) should be removed and it be recommended that older animals with large testis (greater than 2.5 cm long) be castrated under sedation (see recommendation 2) for the safety of the operator and the animals. Other animals, either younger or those with small testes, could be castrated using the strategy described in Recommendation 3.

Recommendation 6

The AAA and possibly the whole livestock industry should encourage pharmaceutical companies to develop mild sedatives, and possibly analgesics that could be administered orally, in a similar way to meloxicam. These oral gels are safe, efficient, and easy to use, and do not require the use of needles and syringes, limiting both pollution and accidents.

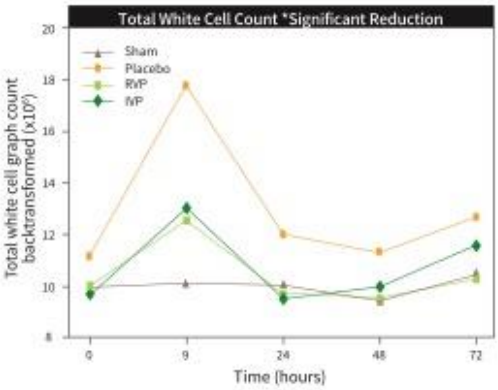
Appendices

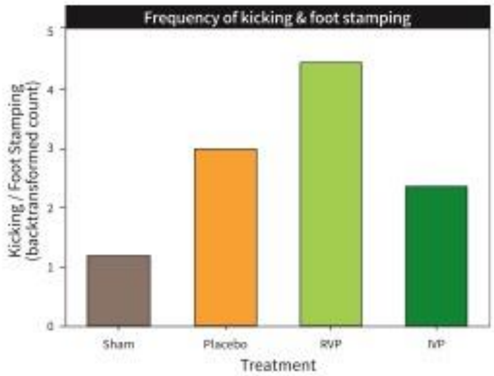
Appendix 1. Excerpts of Buccalgesic OTM® pamphlet

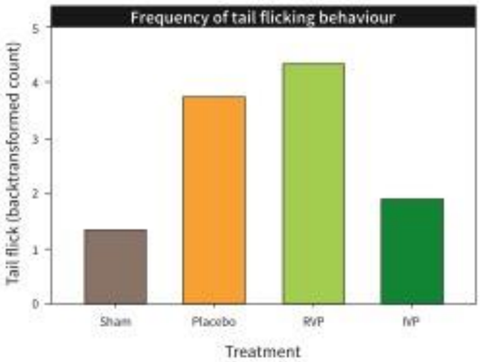


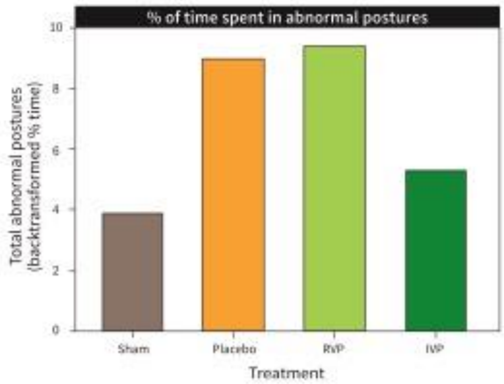
BUCCALGESIC

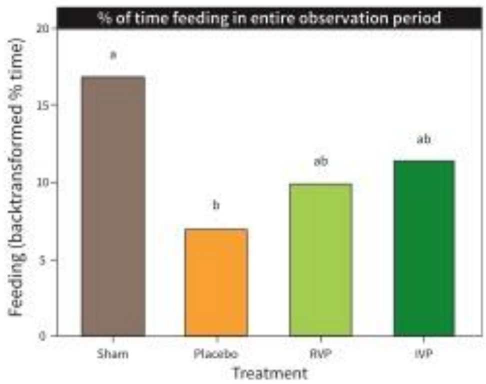
Knife castration significantly increased cortisol, WBC (particularly neutrophils), acute pain avoidance behaviours, pain related postural behaviours, and decreased normal postural behaviours. Pre-treatment with Buccalgesic OTM significantly reduced WBC, mitigated the acute pain avoidance behaviours, especially tail flicking, kicking/foot stamping and overall sum of pain avoidance behaviours.











Legend

- **Sham** — buccal placebo formulation plus sham handling of the scrotum
- **Placebo** — buccal placebo formulation plus surgical castration
- **RVP** — meloxicam injection (0.5 mg/kg BW, subcutaneous (SC)) plus surgical castration
- **IVP** — buccal meloxicam formulation (0.5 mg/kg BW) plus surgical castration

Although statistically significant differences were not achieved in all measures there was an overall trend that the analgesic pre-surgical administration of Ilium Buccalgesic OTM resulted in the mitigation of both physiological and behavioural pain responses.

Appendix 2. Illustration of the anatomy of male alpacas

AUSTRALIAN ALPACA

ALPACA NOTE > 1

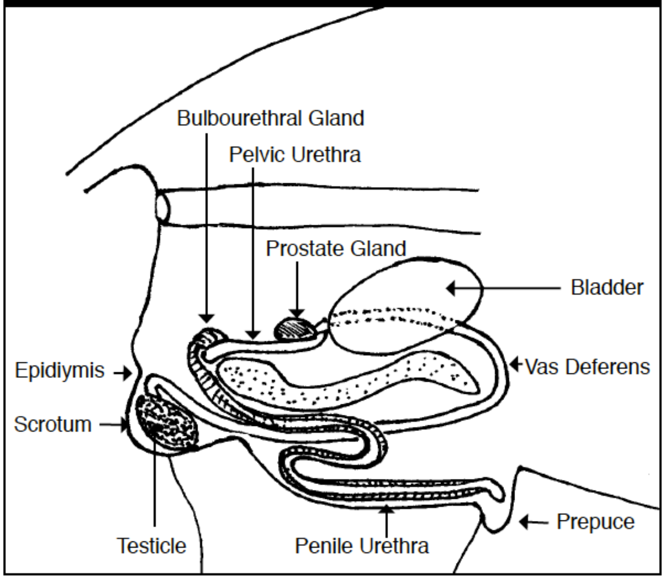
Prepared by AAA Inc. Education & Training Sub-committee
Reviewed 2008

KEY REPRODUCTIVE FEATURES

Male Anatomy

The male has two testicles in a non-pendulous scrotum situated below the anus. The function of the scrotum is to maintain the testicles, which are the source of sperm, at a slightly lower temperature than the rest of the body. The prepuce is the sac holding the penis. This normally points backwards during urination, but when the male is sexually aroused the prepuce points forward and the penis is extruded for mating. The tip of the penis has a slightly clockwise curvature. In young males the penis is adherent to the prepuce and they are usually unable to extrude the penis sufficiently for mating until they are about 2.5-3 years old. Eruption of fighting teeth appears to correlate with approximate time of sexual maturity.

Figure 1 : Reproductive anatomy of a male alpaca



Appendix 3. Tri-Solfen® information sheet (Bayer)

Australian Government
Australian Pesticides and Veterinary Medicines Authority

Product: TRI-SOLFEN TOPICAL ANAESTHETIC & ANTISEPTIC SOLUTION FOR PAIN RELIEF IN LAMBS FOLLOWING MULESING
Status: Registered (2015-07-01) **Product number:** 60099

General details

Registrant: BAYER AUSTRALIA LTD (ANIMAL HEALTH)
Category: VetChem **Poison schedule:** 5
Product type: ANAESTHETICS/ANALGESICS **Registration date:** 2011-12-14
Formulation type: TOPICAL CREAM, OINTMENT, PASTE, GEL, LOTION **Expiry date:** 2016-06-30

Constituents details

Constituent name	Chemical group	Type	Amount	Units
BUPIVACAINE HYDROCHLORIDE	ANAESTHETIC	Active	4.2	g/L
LIGNOCAINE AS LIGNOCAINE HYDROCHLORIDE	CAINE	Active	40.6	g/L
ADRENALINE-L-ACID TARTRATE-D	HORMONE	Active	24.8	mg/L
CETRIMIDE	AMMONIUM-QUATERNARY	Active	5.0	g/L

Pack size details

Pack size information	10L 15L 1L 2.5L 20L 22L 5L

State registration details

State	Status	First registered date
ACT	Registered	2011-12-14
NSW	Registered	2011-12-14
NT	Registered	2011-12-14
QLD	Registered	2011-12-14
SA	Registered	2011-12-14
TAS	Registered	2011-12-14
VIC	Registered	2011-12-14
WA	Registered	2011-12-14

Label details

Label no	Date
37420	2011-12-14
60816	2014-03-07

Protected data details

Appl no	Data no	Ref. product	Author	Title	Study date	Data type	End date	Auth party
37420	1660	0	S Thompson	Dose determination study on a topical local anaesthetic preparation for sheep	Jun-05	Efficacy and Safety	2019-12-14	Animal Ethics Pty Ltd/ PO Box 363 Yarra Glen, VIC, 3775
37420	1661	0	S Thompson	A field study to evaluate the safety and efficacy of a topical local anaesthetic spray for use in sheep	Jun-05	Efficacy and Safety	2019-12-14	Animal Ethics Pty Ltd/ PO Box 363 Yarra Glen, VIC, 3775
37420	1662	0	S Thompson	Safety evaluation on a topical local anaesthetic preparation for use on sheep	Jun-05	Efficacy and Safety	2019-12-14	Animal Ethics Pty Ltd/ PO Box 363 Yarra Glen, VIC, 3775
37420	8158	0	Various	Chemistry data received in conjunction with an application made between 10/01/2005 and 31/08/2005.	Various	Chemistry and Manufacture	2019-12-14	Animal Ethics Pty Ltd/ PO Box 363 Yarra Glen, VIC, 3775

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Appendix 4. Dormosedan Gel® (detominitide) Information sheet from Zoetis

Section 1 - Identification of The Material and Supplier

zoetis

Zoetis Australia Pty Ltd
38-42 Wharf Road
West Ryde NSW 2114
Tel: 1800 814 883
Fax: (02) 8876 0444

Zoetis Australia Pty Ltd
ABN 94 156 476 425

Chemical nature:	Detomidine is an imidazole derivative; an alpha-2-adrenergic receptor agonist.
Trade Name:	**Dormosedan Gel**
APVMA Code:	65579
Product Use:	Veterinary product used as analgesic, sedative. For veterinary use only as described on the product label.
Creation Date:	**June, 2013**
This version issued:	**June, 2013** and is valid for 5 years from this date.

Section 2 - Hazards Identification

Statement of Hazardous Nature
This product is classified as: Not classified as hazardous according to the criteria of SWA.
Not a Dangerous Good according to the Australian Dangerous Goods (ADG) Code.

Risk Phrases: Not Hazardous - No criteria found.
Safety Phrases: S23, S36, S24/25. Do not breathe vapours. Wear suitable protective clothing. Avoid contact with skin and eyes.
SUSMP Classification: S4
ADG Classification: None allocated. Not a Dangerous Good under the ADG Code.
UN Number: None allocated

GHS Signal word: NONE. Not hazardous.

HAZARD STATEMENT:
PREVENTION
P102: Keep out of reach of children.
RESPONSE
P353: Rinse skin or shower with water.
P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P332+P313: If skin irritation occurs: Get medical advice.
P337+P313: If eye irritation persists: Get medical advice.
P370+P378: Not combustible. Use extinguishing media suited to burning materials.
STORAGE
P402+P404: Store in a dry place. Store in a closed container.
P403+P235: Store in a well-ventilated place. Keep cool.
DISPOSAL
P501: Dispose of small quantities and empty containers by wrapping with paper and putting in garbage. For larger quantities, if recycling or reclaiming is not possible, use a commercial waste disposal service.

Emergency Overview

Physical Description & colour: Blue gel.
Odour: No data re odour.
Major Health Hazards: no significant risk factors have been found for this product. This is a physiologically active product and so contact should be minimised, especially if the user is taking a form of medication, as interactions can sometimes give unexpected and undesired results.

Appendix 5. Online survey

Default Question Block

.

We seek your assistance in responding to the questionnaire "Method of castration used in alpacas". Your cooperation is vital for the completion of this project. We anticipate that the questionnaire should take no longer than 20 minutes to complete.

Your participation in this research is strictly voluntary and you are under no obligation to complete the questionnaire. Completion of the questionnaire will be taken as evidence of consent to participate. Your answers will remain anonymous. Thank you for your consideration of this important research project.

The research is being conducted on the behalf of Rural Industries Research and Development Corporation RIRDC (#PRJ-009469), supervised by Assoc/Prof Dominique Blache, School of animal Biology, The University of Western Australia. Should you have any questions relating to this study Assoc/Prof Dominique Blache may be contacted on 08 6488 6763 or at dominique.blache@uwa.edu.au

Approval to conduct this research has been provided by the University of Western Australia, in accordance with its ethics review and approval procedures. Any person considering participation in this research project, or agreeing to participate, may raise any questions or issues with the researchers at any time.

In addition, any person not satisfied with the response of researchers may raise ethics issues or concerns, and may make any complaints about this research project by contacting the Human Research Ethics Office at the University of Western Australia on (08) 6488 3703 or by emailing to hreo-research@uwa.edu.au

All research participants are entitled to retain a copy of any Participant Information Form and/or Participant Consent Form relating to this research project.

.

If needed, you can quit the survey at anytime, your responses will be saved

You can return to the survey as long as you use the same Internet browser and computer you started the survey on.

29/01/2015 6:45 am

Q1.
Which of the following categories describes you best (Pick one)?

- ○ Alpaca producer (primary activity)
- ○ Alpaca producer (secondary activity)
- ○ Alpaca enthusiast/Hobby farmer
- ○ Shearer
- ○ AI/ET Contractor
- ○ Veterinarian
- ○ Other, please specify: ____________________

.

The first questions are about your involvement with alpacas.

Please choose the answer that best applies to you.

Q2. Do you own alpacas?

- ○ Yes
- ○ No

Q3. How many alpacas do you currently own?

- ○ 1 - 10
- ○ 11 - 50
- ○ 51 - 100
- ○ 101 - 200
- ○ 201 - 300
- ○ 301 - 500
- ○ over 500

29/01/2015 6:45 am

Q4. How many alpacas are directly under your care?

- none
- 1 - 10
- 11 - 50
- 51 - 100
- 101 - 200
- 201 - 300
- 301 - 500
- over 500

Q5.

The following questions are about castration of male alpacas. For each question, please choose the answer that best applies to you.

Do some of your males get castrated?

- Yes, always
- No, never, explain why
- Sometimes – please briefly explain when and why you would castrate males

.

We would like all participants to answer all the following questions

If you answered NO to question 4 (you do not castrate male alpacas), please answer the following questions thinking about how you would castrate male alpacas if you were ever to do so.

Q6. How many males you castrate/get castrated per year (average over the last 3 years)

- 0
- 1 - 5
- 6 - 10
- 11 - 20
- 21 - 30
- 31 - 40
- 41 - 50
- over 50

Q7. Insert any additional comments about the number of males you castrate/get castrated

29/01/2015 6:45 am

Q8. At what age do you **generally** castrate your males?

- ☐ Less than 6 months
- ☐ 6 to 12 months
- ☐ 12 to 18 months
- ☐ 18 to 24 months
- ☐ 24 to 30 months
- ☐ 30 to 36 months
- ☐ over 36 months

Q8a. Please, add any comments about the age of castration of alpacas

.

The following questions are about the techniques used to castrate alpacas

Q9. Do you currently use surgical castration?

- ○ Yes
- ○ No, please explain why
- ○ Sometimes, please explain why and when you use surgical castration

.

For the following questions, participants who do not own, care for or castrate alpacas should answer thinking about what should be done (see examples)

29/01/2015 6:45 am

Q10. Who performs most of the surgical castrations?

NB: For participants who do not own or care for alpacas the questions is:
Who should perform most of the surgical castrations?

- Yourself
- Staff member
- Shearer
- AI/ET Contractor
- Another producer
- Veterinarian
- Other, please specify:

Q10a. Add any additional comments about the person performing the surgical castration

Q11.

For the following questions, participants who do not own, care for or castrate alpacas should answer thinking about what should be done (see examples)

How was the person performing most of the surgical castration trained?

NB: For participants who do not own or care for alpacas the questions is:
How should the person performing most of the surgical castration trained?

- No training
- By a fellow producer
- By a contractor
- By a veterinarian
- Specific professional training
- Other, please specify

29/01/2015 6:45 am

Q11a. Add any additional comments about the training of the person performing the surgical castrations

Q12. Which surgical approach is most used?

- Double incision, one each side of the scrotum
- Single incision into the scrotum
- Cut away part of the scrotum
- Cut away the entire scrotum
- Don't know
- Other, please specify:

Q13.

For the following questions, participants who do not own, care for or castrate alpacas should answer thinking about what should be done

Do the animals receive local anaesthesia with surgical castration?

- Yes, always
- No, never, please explain why
- Sometimes, please explain why
- Don't know

Q13a. Which drug do you use for local anaesthesia with surgical castration?

Q13b.

When is the local anaesthetic given?

- 15-30 min before surgical castration
- 5-15 min before surgical castration
- At the start of surgical castration
- During surgical castration
- Other, please specify:

29/01/2015 6:45 am

Q14.

For the following questions, participants who do not own, care for or castrate alpacas should answer thinking about what should be done

Are the animals sedated when alpacas are surgically castrated?

- Yes, always
- No, never, please explain why
- Sometimes, please explain why
- Don't know

Q14a. Which drug do you use for sedation when alpacas are surgically castrated?

Q15.

For the following questions, participants who do not own, care for or castrate alpacas should answer thinking about what should be done

Are the animals placed under general anaesthesia when alpacas are surgically castrated?

- Yes, always
- No, never, please explain why
- Sometimes, please explain why
- Don't know

Q15a. What drugs/ agents are used for general anaesthesia when alpacas are surgically castrated?

Q16.

For the following questions, participants who do not own, care for or castrate alpacas should answer thinking about what should be done

Are the animals given any pain relief when alpacas are surgically castrated?

- Yes, always
- No, never, please explain why
- Sometimes, explain why
- Don't know

Q16a. Which drug do you use for pain relief when alpacas are surgically castrated?

Q16b. When is the pain relief given when alpacas are surgically castrated?

- Before castration
- During castration
- After castration

Q16b1. How long before the surgical castration?

Q16b2. How long after the surgical castration?

Q17.

For the following questions, participants who do not own, care for or castrate alpacas should answer thinking about what should be done

Do you ever use rubber rings?

- Yes
- No, please explain why
- Sometimes - Please, explain why and when you use rubber rings

Q17a.

Which brand of rubber rings do you use? (if you do not know, please type "DK")

29/01/2015 6:45 am

Q18.

For the following questions, participants who do not own, care for or castrate alpacas should answer thinking about what should be done

Who applies the rubber rings?

- Yourself
- Staff member
- Shearer
- AI/ET Contractor
- Another producer
- Veterinarian
- Other, please specify

Q18a. Add any additional comments about the person applying the rubber rings

Q19. How was the person applying the rubber rings trained?

- No training
- By a fellow producer
- By a contractor
- By a veterinarian
- Specific professional training
- Other, please specify

Q19a. Add any additional comments about the raining of the person applying the rubber rings

Q20.

For the following questions, participants who do not own, care for or castrate alpacas should answer thinking about what should be done

Do the animals receive local anaesthesia when using rubber rings?

- Yes, always
- No, never, please explain why
- Sometimes, please explain why
- Don't know

Q20a. Which drug do you use for local anaesthesia when using rubber rings?

Q20b.

When is the local anaesthetic given?

- 15-30 min before the application of the rubber ring
- 5-15 min before the application of the rubber ring
- At the time of application of the rubber ring
- After the application of the rubber ring
- Other, please specify:

Q21.

Are the animals sedated when using rubber rings?

- Yes, always
- No, never, please explain why
- Sometimes, explain why
- Don't know

Q21a. Which drug do you use for sedation when using rubber rings?

29/01/2015 6:45 am

Q22.

For the following questions, participants who do not own, care for or castrate alpacas should answer thinking about what should be done

Are the animals given any pain relief when using rubber rings?

- Yes, always
- No, never, please explain why
- Sometimes, explain why
- Don't know

Q22a. Which drug do you use for pain relief when using rubber rings?

Q22b. When is the pain relief given when using rubber rings?

- Before castration
- During castration
- After castration

Q22c. How long before the application of the rubber rings (in minutes)?

Q22d. How long after the application of the rubber rings (in minutes)?

Q23.

Any additional comments on technique(s) of castration in alpacas

.

In the next section, we would like to have your opinion about what **should be the best method(s) of castration** for alpacas.

29/01/2015 6:45 am

Q24. What criteria should be considered to define the best castration method in alpacas - **Indicate at least 3**?

1 ______

2 ______

3 ______

4 ______

5 ______

6 ______

Q25.
In the next 3 screens, you are ask to **rank 3 strategies** to castrate alpacas that could fit these criteria

A strategy to castrate alpacas is a combination of a castration method, a method of pain management, who is performing it, the age of the animals at castration, etc

Q25a1. **First preferred strategy**

○ Surgical castration ○ Rubber ring ○ Other ______

Q25a2. **Pain management**

☐ with pain relief ☐ with sedation ☐ with local anaesthesia

☐ under general anesthesia ☐ none

Q25a3. **Done by**

○ my staff ○ myself ○ a veterinarian ○ another producer ○ a shearer

○ a contractor

Q25a4. Age at castration (in months)

______

Q25a5. Comments

______

29/01/2015 6:45 am

Q25b1. **Second preferred strategy**

○ Surgical castration ○ Rubber ring ○ Other ______________________

Q25b2. **Pain management**

☐ with pain relief ☐ with sedation ☐ with local anaesthesia

☐ under general anesthesia ☐ none

Q25b3. **Done by**

○ my staff ○ myself ○ a veterinarian ○ another producer ○ a shearer

○ a contractor

Q25b4. Age at castration (in months)

____

Q25b5. Comments

____

Q25c1. **Third preferred strategy**

○ Surgical castration ○ Rubber ring ○ Other ______________________

Q25c2. **Pain management**

☐ with pain relief ☐ with sedation ☐ with local anaesthesia

☐ under general anesthesia ☐ none

Q25c3. **Done by**

○ my staff ○ myself ○ a veterinarian ○ another producer ○ a shearer

○ a contractor

Q25c4. Age at castration (in months)

____

Q25c5. Comments

Q26.
Please, provide any additional comments you have about castration in alpacas

.
The last few questions collect some basic information about you. Your answers to these questions are confidential, and cannot be used to identify you personally.

Q27.
Are you male or female?

- ○ Female
- ○ Male

Q28. In what year were you born?

Q29.
What is your postcode?

Q30. Do you currently own a pet?

- ○ Yes
- ○ No

29/01/2015 6:45 am

Q31.
What is the highest level of education you have completed?

- Primary education
- Secondary education
- Certificated level from TAFE/ other training institution
- Diploma level from TAFE/ other training institution
- Bachelor degree level
- Graduate diploma or graduate certificate level
- Postgraduate degree level

Q32.

You have answered all the questions.

You can use the "back" button to correct your answers

Please, insert any additional comments about the survey or the castration of alpacas

29/01/2015 6:45 am

Appendix 6. Sedation and recovery monitoring

Sedation Record

Protocol Title:
Protocol Number:
Chief Investigator:

Date:		**Animal ID**		**Procedure**	
Surgeon(s):					
Anaesthetist:					
Physical Exam:				**Weight**	
Premedication:			**Effect:**	**TIMES:**	**Fluids given:**
Drug	*Dose*	*Route*	*Time*	**Start sedation:**	
				Start surgery:	
				End surgery:	
Induction			**Quality:**	**End sedation:**	
Drug	*Dose*	*Route*	*Time*	**Extubated:**	

Time	00	05	10	15	20	25	30	35	40	45	50	55	00	05	10	15	20	25	30	35	40	45	50	55	00
Pupillary reflex $																									
Quality of sedation																									
Body Position*																									
Eating$																									
Drinking$																									
Urine/Faeces $																									
Demeanour**																									

*lying, sitting or standing; **S= sleepy, QAR = quiet but awake and responsive, BAR = bright, alert, responsive

#Temperature not required if awake and responsive, $ Yes or No

Quality of sedation scale:

1 – No reaction to restrain and procedure (limb movement, increase respiration or/and heart rate)

2 – Minimal reaction to restrain and procedure

3 – Moderate reaction to restrain and procedure

Time (post surgery)	**2h30**	**3h00**	**3h30**	**4h00**	**4h30**
Temperature#					
Heart Rate					
Respiration Rate					
Body Position*					
Eating$					
Drinking$					
Urine/Faeces$					
Demeanour**					

Appendix 7. Routine monitoring for health and welfare

Standard AEC recommended criteria	**0**	**1**	**2**
Demeanour	Alpaca is bright, alert, responsive	Alpaca is quieter, slower to respond, generally remains with group	Alpaca is dull, reluctant to move, may have isolated self from group.
Project specific criteria	**0**	**1**	**2**
Feeding	Seen feeding or ruminating	Not seen feeding or ruminating for 24 hours	Not seen feeding or ruminating for more than 24 hours
Drinking	Seen drinking or hydration status good	Not seen drinking within 24 hours, or mild dehydration evident	Not seen drinking for more than 24 hours, moderate dehydration.
Gait	Normal, even weight bearing on all legs	Mild lameness or slight incoordination	Moderate lameness or incoordination
Injuries	No injuries to body	Presence of small superficial scratches/scabs, skin only.	Presence of small superficial scratches/scabs deeper than skin thickness or large skin scratches/tears.
Coat condition	Normal, reasonably clean but expect some dirt	Somewhat ruffled, dirtier than normal	Moderately ruffled and dirty
Eyes/nostrils	Clean or minimal accumulation of dirt	Slight accumulation of dirt	Moderate dirt accumulation
Foot condition	Healthy hooves	Mild superficial abrasions of, mild inflammation, no infection.	Moderate injury, moderate inflammation, infection present
Head position	Animal moved is head up and down in slow movement	Head is kept down and occasional raised	Head is down at all time and no respond to auditory stimulation
Surgical site	Clean, +/-mild inflammation in days immediately post-op	Slight inflammation +/- minor cleaning of serous discharge required.	Purulent discharge +/- moderate inflammation of site.
Other presenting signs/symptoms Although the most relevant monitoring criteria have been selected, if a circumstance arises where there are additional presenting signs or behaviours, these must be acknowledged, scored, recorded and reported to the AWO as an adverse event.		Slight or intermittent or possible deviation from normal for this sign.	Moderate or consistent or definite deviation from normal but not marked, for this sign.

Appendix 8. Visual pain assessment sheet and criteria

Check

Time:

Initials:

<u>Circle each score</u>

Total Score:

- **>6 requires intervention**
- **Contact CI for analgesia plan**

CRITERIA	SCOR	DESCRIPTOR	CRITERIA	SCOR	DESCRIPTOR
Gait	1	Normal gait	*Aggression*	1	Quite friendly
	2	Slight change with slow movements		2	Tendency to move away
	3	Moderate change with small		3	Biting and screaming when touched
	4	Immobile or severe reluctance to		4	Biting and screaming without being
Restlessness	1	Cannot sleep restfully	*Posture*	1	Slightly hunched back
	2	Poor wake/sleep times, moving		2	Protecting surgical site
	3	Frequent pacing, momentary		3	Kneeling and hardly moving
	4	Continuous pacing		4	Immobile/hunched
Vocalisation	1	Low volume, occasional cries	*Isolation*	1	Positions away from mates
	2	Low volume, continuous cry		2	Occasionally moves away from mates
	3	High volume, continuous cry		3	Keeps moving away from mates
	4	Regular screaming		4	Screams and runs away from mates
Appearance	1	Slightly dilated pupils	*Social*	1	Interacts with mates or personnel
	2	Dilated pupils		2	Interacts with toys only
Agitation	1	Slightly moves away when		3	No interest in mates
	2	Jumps away when approached		4	Does not interact or move

Comments

Appendix 9. Procedure for jugular cannulation in alpaca

Animal Preparation (2 person operation)

1. Clip an area of wool around the jugular vein that is big enough to allow easy visualisation of and access to the jugular vein.
2. Using gloves, apply local anaesthetic cream (Emla®; lignocaine 25mg/g, prilocaine 25mg/kg) to the area – note the time of application.
3. Inject the animal with sedative (ilium xylazil; 0.1mg/kg i.m.)
4. Approximately 30 minutes after application of the local anaesthetic cream, the designated animal holder should restrain the animal by straddling their back and gently supporting the head.

Site Preparation (2 person operation)

1. Set up the cannulation trolley for the required number of animals. Ensure that all needles, tubing and equipment are kept in 70% ethanol.
2. Spray the shaved window with betadine surgical scrub
3. The vein must be clearly located by pressing down on the bottom clipped area and watching the vein rise and fall with pressure and release. Ensure that you are confident in where the vein is located before moving on to step 4.
4. Make a small incision in the skin above the jugular vein using a scalpel.
5. Insert the 13G luer lock needle into the jugular vein and quickly feed the tubing through the needle into the vein.
6. Slide the needle off the tubing and quickly attach the tap to the end of the tubing.
7. Check that blood is flowing freely through the tubing using a syringe filled with sterile, heparinised (40 iu[1]) 0.9% saline. Rinse the tubing by pushing 2-3mls of heparinised saline back into the tubing.
8. Wrap a piece of fabric tape (Elastoplast®) around the tubing at the point where it enters the neck, leaving two ‘wings’ on either side. Stitch the wings to the skin on the neck to secure the cannula in place.
9. Wrap a piece of fabric tape around the junction between the tap and tubing to reduce the risk of kinking or holes forming in the tubing.
10. Lie the cannula and tap on the neck of the alpaca and wrap 2-3 layers of tape around the neck of the alpaca. Ensure that the tape covers the cannula but is not too tight to restrict the movement of the alpaca.

[1] Note this concentration of heparin (40 iu) is only used during cannulation – a lower concentration (10iu) is used during the frequent bleeding period.

11. Check the animals daily while the cannula is in place for any sign of ill health (withdrawn from the group, not eating, dull eyes and ears carried low), record any observations on the cannula insertion monitoring sheet and take the appropriate action (see step 14).

12. At the end of the experiment, remove the cannula and check the cannulation site – record your observations on the cannula Insertion/removal monitoring sheet. If there is any sign of swelling or infection, take the temperature of the animal and treat with antibiotics as outlined in step 14.

13. Continue to check the cannulation site twice daily for 2 days after cannula removal to check for any residual effects of the cannulation procedure - record your observations on the cannula Insertion/removal monitoring sheet

14. **If any animal appears unwell after cannula insertion or removal, (i.e. withdrawn from the group and off food or water)** - check their rectal temperature and inform the AWO. If the animal has an elevated temperature indicative of infection, give a dose of antibiotics (Noracillin 10mg/kg i.m.) and check the animal 24 hours later. If there is no improvement (i.e. the temperature is still elevated) seek veterinary advice. If the animal does not have a temperature give it electrolytes and vitamin B complex to encourage their appetite. If they do not respond within 24 hours with an improved appetite seek veterinary advice. If you are in doubt of the health and welfare of any animal seek veterinary advice immediately.

Appendix 10. Monitoring sheet after administration of oral detomidine or xylazine

Sedation Record

Protocol Title:

Protocol Number:

Chief Investigator:

Date:		**Animal ID**	**Procedure**
Surgeon(s):			
Anaesthetist:			
Physical Exam:			**Weight**

Premedication:				**Effect:**	**TIMES:**	**Fluids given:**
Drug	*Dose*	*Route*	*Time*		**Start sedation:**	
					Start surgery:	
					End surgery:	
Induction				**Quality:**	**End sedation**	
Drug	*Dose*	*Route*	*Time*		**Extubated:**	

Time	00	10	20	30	40	50	00	10	20	30	40	50	00	10	20	30	40	50	00	10	20	30	40	50	00
Heart Rate																									
Respiration rate																									
Pupillary reflex																									
Body posture																									
Jaw resistance																									
Reaction to palpation of the scrotum																									
Reaction to a prick to the scrotum																									

NOTES:

Description of the criteria (adapted from Carrol et al., 1998 and Phillips et al., 2015)

Body posture:

0 – Ambulates normally

1 – Mild to moderate ataxia, can stand but do not walk more than 3 steps

2 – Recumbent but head still up and react to stimulation

3 – Recumbent, head lying on the floor

Jaw resistance (ability to open the mouth, tested using 2 fingers to open the lower jaw)

0 – Notable resistance, cannot not open the mouth

1 – Mild resistance, can open the mouth with two fingers

2 – No resistance, can easily open the jaw with one finger

Reaction to palpation of the scrotum (The scrotum is gently squizzed between two fingers)

0 – Impossible to palpate the scrotum

1 – Marked reaction to the palpation: vocalisation, leg and head movements. Moderate restraint necessary

2 – Mild reaction to the palpation. Mild restraint necessary

3 – No reaction to the palpation. No restraint necessary

Reaction to a prick of the scrotum (A 21 G needle is gently applied to the skin with very little force)

NB: This test will be only performed when the animal scores 3 to the scrotal palpation test.

0 – Impossible to do the test prick

1 – Marked reaction to the prick: vocalisation, leg and head movements. Moderate restraint necessary

2 – Mild reaction to the prick. Mild restraint necessary

3 – No reaction to the prick. No restraint necessary

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