



UNIVERSIDADE DE LISBOA
Faculdade de Medicina Veterinária

PAIN REDUCTION IN DAIRY GOAT KIDS' DISBUDDING

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CONSTITUIÇÃO DO JURI

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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“If we acknowledge that animals have the capacity to experience pain, then we are obligated to minimize its occurrence, through both prevention and treatment”

Weary et al., 2006

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ABSTRACT

Cautery disbudding is a routinely done practice in intensive dairy goat farms as hornless animals are easier to handle, cause less damage and have lower space requirements. It is usually carried out within the kids' first two weeks of life and, in spite of being extremely painful, no anaesthesia or analgesia is generally used. With this work we aimed to assess the efficacy of ketamine, either when used alone or in combination with an NSAID, in reducing pain related behaviours in disbudded kids. The effect of an anaesthetic and antiseptic gel effect on preventing immediate wound inflammation and infection after the procedure was also evaluated.

The behavioural response to ketamine at three different dosages was observed and registered for 1 h. 10mg/kg was considered to be the safest dose after a higher one resulting in a prolonged anaesthetic period and lethargic kids afterwards. Nine healthy goat kids were randomly allocated to one of three treatment groups: K – given ketamine; K+M – given ketamine and meloxicam; S – sham disbudded after being given ketamine. Ketamine was administered intramuscularly to all kids and meloxicam (0.5 mg/kg) subcutaneously to K+M, 2 to 5 minutes and 5 minutes prior to disbudding, respectively. Vocalizations, tail flicking and leg movements were registered during disbudding. After the procedure, the frequency of pain-related behaviours was recorded for 3 h based on a previously developed ethogram. Sixteen healthy goat kids were randomly assigned to one of two groups after disbudding: D – application of an antibacterial spray on each cauterized horn bud site; TS – application of an anaesthetic and antiseptic gel. The same behaviours of the previous trial were registered for 3 h with D group results also being compared with K, K+M and S ones in the statistical analysis. Three days after the procedure, TS and D kids wounds were evaluated based on a 3-point healing scale.

K and K+M kids showed a lower frequency of leg movements during disbudding. Following the procedure, K kids had a higher frequency ($p<0.05$) of head shaking and head scratching than other groups in the second half-hour and of body shaking in the third hour. No significant differences were found between TS and D groups on wound infection but the use of the gel in detriment of the antibacterial spray may be beneficial in preventing antimicrobial resistance.

The use of ketamine either alone or in combination showed to be unsafe, unpractical and not cost-effective in spite of having some effect in reducing pain-related behaviours during disbudding.

Keywords: cautery disbudding, goat kids, pain reduction, ketamine

RESUMO

REDUÇÃO DA DOR NA DESCORNA DE CABRITOS DE EXPLORAÇÕES INTENSIVAS DE LEITE

A descorna de cabritos por termocautério é um procedimento de rotina realizado em explorações intensivas de leite devido à maior facilidade de manipulação dos animais, redução dos danos e menores exigências de espaço. É geralmente efetuada nas duas primeiras semanas de vida do animal e, apesar de extremamente dolorosa, sem recurso anestésicos ou analgésicos. A eficácia da quetamina na redução de comportamentos de dor na descorna de cabritos, quer quando usada isoladamente ou em combinação com um AINE foi avaliada neste estudo, bem como o efeito de um gel anestésico e antisséptico na prevenção de infeções após a descorna.

A resposta comportamental à administração três doses diferentes de quetamina foi observada e registada durante 1 h. 10 mg/kg foi considerada a dose mais segura e utilizada nos ensaios subsequentes, após uma dose mais elevada ter resultado num período anestésico prolongado e em letargia. Nove cabritos saudáveis foram aleatoriamente atribuídos a um de três grupos de tratamento: K – administração de quetamina; K+M – administração de quetamina e meloxicam; S – simulação de descorna após administração de quetamina. Foi administrada quetamina a todos os cabritos e meloxicam (0.5 mg/kg) ao grupo K+M, 2 a 5 e 5 minutos antes do procedimento, respetivamente. Vocalizações, movimentos dos membros e abanar da cauda foram comportamentos registados durante a descorna. Após a mesma, a frequência de comportamentos de dor foi registada durante 3 h com base num etograma previamente desenvolvido. Dezas seis cabritos saudáveis foram aleatoriamente atribuídos a um de dois grupos: D – aplicação de um spray antibacteriano no local do botão cornual cauterizado; TS – aplicação de um gel anestésico e antisséptico. Os mesmos comportamentos do ensaio anterior foram registados durante 3 horas e os resultados do grupo D foram comparados com os dos grupos K, K+M e S. Três dias após o procedimento, as feridas resultantes da cauterização foram avaliadas com base numa escala de cicatrização de 3 pontos.

Os grupos K e K+M apresentaram uma menor frequência de movimentos dos membros durante a descorna. Após a mesma, o grupo K demonstrou uma maior frequência de abanar e coçar a cabeça na segunda meia-hora e de abanar o corpo na terceira hora. Não existiram diferenças significativas entre TS e D quanto à presença de infeção, mas o uso do gel em detrimento do spray antibacteriano poderá ser benéfico na prevenção de resistências a antibióticos.

A administração de quetamina demonstrou-se pouco segura ou prática com baixo custo-benefício apesar de algum efeito na redução de comportamentos de dor durante a descorna.

Palavras-chave: descorna por termocautério, cabritos, redução de dor, quetamina.

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LIST OF ABBREVIATIONS, INITIALS AND ACRONYMS

NMDA - N-methyl-D-aspartate
NSAID – Non-steroid anti-inflammatory
TS – Tri-Solfen
h – hour
d – day(s)

PART I - DESCRIPTION OF THE TRAINING PERIOD

As part of the 6th year of the Masters in Veterinary Medicine I did my training period in the area of farm animal clinics at *Faculdade de Medicina Veterinária, Universidade de Lisboa*. From September 2017 to April 2018, under the supervision of Professor George Stilwell, I had the opportunity of accompanying the Farm Animal Clinics ambulatory work which consisted of visits to different farms in the Center-South region of Portugal.

The several farms we visited allowed me to come in contact with an interesting caseload of beef and dairy cattle, goats, sheep and swine. About 70% of the consultations fell into the category of Internal Medicine. For each clinical case, a thorough physical exam was performed and the most likely differential diagnosis and therapeutics were discussed. In a majority of cases, samples were collected for further analysis allowing an accurate diagnosis and follow up of the patients. One particular case resulted in two scientific posters I co-authored, “*Caso clínico atípico de besnoitiose (Besnoitia besnoiti) num touro limousine*”, which was presented at *XIX Jornadas da Associação Portuguesa de Buiatria*, in Azores (Appendix I) and “*Besnoitiosis as an emerging and exotic disease – new clinical features in breeding bulls*”, presented at the 30th World Buiatric Congress in Japan.

The areas of preventive medicine, surgery, reproduction and obstetrics were covered as well in this period in which I was able to develop my skills in pregnancy testing in cattle through rectal palpation and ultrasound and to assist on a few deliveries and two cesarean-sections. In addition to these, I had the opportunity of participating in two different surgical treatments for left displaced abomasum in cows – right flank omentopexy and left flank abomasopexy. There was also the chance to do field necropsies and simulate surgeries on cadavers, both of which played a crucial role in the learning process. Animal welfare was always a present and valued matter. This training period allowed me to reinforce what I had learned in previous curriculum subjects, develop clinical reasoning and apply it on a practical context, communicate with farmers and better understand the farming system, all of which contributed to my improvement as a future veterinarian and human being.

Continuous learning plays a fundamental part in the veterinary medicine profession and for that matter I attended the *XIX Jornadas da Associação Portuguesa de Buiatria, V Jornadas Técnico-Veterinárias do Campo Branco* and the 8th EFOMV in which a poster I co-authored was presented on “*Aplicação tópica de anestésicos locais para controlo da dor durante a aparagem curativa de lesões podais de vacas leiteiras – dados preliminares*” (Appendix II). I also got the opportunity of doing an oral presentation about dehorning and disbudding in small ruminants at a *Workshop de Descorna em bovinos* and of collaborating in Professor George Stilwell’s

book “*Bem-estar dos Ruminantes*” (2017) and in an oral communication on “Topical application of local anaesthesia for pain control during the curative trimming of podal lesions of dairy cows” (Appendix III) at the World Buiatric Congress in Japan.

As an extra training period, I did an externship at the University of Tennessee, College of Veterinary Medicine (UTCVM) from April to July 2018. I rotated through Farm Animal Medicine and Surgery, Theriogenology, Anaesthesiology and a couple of small animal services. The Medicine and Surgery service provides a great amount of advanced patient care, 60% of it being smallholders (goats, sheep, camelids and pet pigs). I participated in all aspects of case management, including receiving, examination, diagnosis, daily management, client communication, discharging and follow-up communication. I was also responsible for daily problem oriented medical records of the cases I was assigned to. Daily rounds were held on inpatient cases and medicine and surgery topics were discussed. This rotation allowed me to improve my diagnostic skills such as abdominal ultrasound and thorough neurological examination, become familiar with a farm animal hospital in an English-speaking setting and, through a hands-on approach of all cases, gain confidence and self-sufficiency. The other rotations allowed me to become comfortable with breeding soundness exam in bulls, breeding soundness exam in mares and cows, artificial insemination in mares, having also mild exposure to cows AI, and anaesthesia mainly focused on pigs and equine.

PART II – EXPERIMENTAL STUDY: PAIN REDUCTION IN DAIRY GOAT KIDS’ DISBUDDING

1. LITERATURE REVIEW

1.1. DISBUDDING

1.1.1. Context

With the increase of intensive dairy farms with group housing systems, feed barriers and milking parlours, the prevention of horn growth has become a routine practice. Under the same space conditions, when in comparison to hornless goats, horned animals, especially when low in the hierarchy, have a higher necessity of sharing feeding places which can lead to a reduction in body weight and milk production if they do not have sufficient access to feed (Loretz et al., 2004). Consequently, more space is required at the feed barrier for horned goats in order to prevent high competition for feed and ultimately welfare is compromised. Dehorned animals are considered to be safer to handle, cause less damage to other animals and have less negative impact on their housing environment and social interactions (Alvarez & Gutiérrez, 2010). For all these reasons, disbudding constitutes, at the present moment, an unavoidable management tool for dairy goat farmers.

1.1.2. Methods

Disbudding is achieved by thermal cautery, cutting or chemical methods, most of which have been adopted from cattle. In order to remove the germinal tissue of the horn, disbudding is usually carried out within the goat kids’ first two weeks of life, although further investigation should be done to determine the optimal age for disbudding (Alvarez et al., 2009; Smith & Sherman, 2009).

An online survey (Valdmanis et al., 2008) stated that cauterization is the method of choice for disbudding for 97% of veterinarians and 95% of producers. It consists of an electric dehorning device with a top ring that heats the bud and the surrounding tissue for some seconds until the full thickness of the skin has been destroyed (Stafford & Mellor, 2011). All of the horn corium must be within the ring and the isolated central circle of skin that contains the horn bud can be lifted off (Smith & Sherman, 2009). This type of disbudding causes third degree burns, which can result in a bacterial infection and varying amounts of subcutaneous damage. Because the skull of goat kids is much thinner than calves’ cranium, the safety margin for thermal injury is noticeably reduced, resulting in a higher risk of damage when this method is not used correctly. There have been reports of meningoencephalitis and cerebral infarction following hot-iron

disbudding and it is believed that far more kids either die or suffer from these consequences than are brought to the attention of veterinarians (Thompson et al., 2005).

The injection of clove oil essence in the horn bud region has been studied (Molaei et al., 2015) as a new chemical technique for disbudding. It is suggested that the main component of clove oil, eugenol, can inhibit proliferation of the horn germination cells and induce their apoptosis, as well as inducing cell necrosis through its cytotoxic effect on horn bud cells. Because of its anti-inflammatory and analgesic effects, no local anaesthetics and/or sedative drugs were necessary before the injection. A complete necrosis of epidermis and underlying dermis with collagenolysis in horn bud tissues occurred within one week after treatment and stopped horn growth. Hempstead et al. (2018b) suggested that animals treated with clove oil had less tissue damage around the horn buds and appeared to lead to earlier tissue repair with some animals, however, developing scurs. Further studies should be done to assess horn regrowth and stress response to this method.

Chemical burn of underlying tissue is most commonly achieved by caustic chemicals such as sodium hydroxide and potassium hydroxide that burn the underlying tissues. This method should be avoided as it can run to the face and eyes and create skin wounds elsewhere on the kid and other animals in contact with it, as for example the udder of the doe (Smith & Sherman, 2009; Thompson et al., 2005).

Because the hot-iron is the most commonly used technique, it will be this dissertation's main focus.

1.1.3. Improper disbudding

When goats that have been disbudded still show visible horn tissue (scurs) (Figure 1), they are considered to have been improperly disbudded. Incorrect disbudding can result in scurs pressed against the head or eye and consequently cause lesions and pain (Smith & Sherman, 2009). Scurs getting caught in fences and pen partitions have also been reported (Waiblinger et al., 2011, cited by Battini et al., 2014).

Figure 1 – Goat with horn development after having been disbudded.



1.1.4. Alternatives

It is possible to genetically select for the absence of horns, i.e. polled goats, and this would be the ideal choice for animal welfare. In spite of the polled trait being dominant it is linked to a serious genetic reproductive disorder caused by a recessive gene for infertility. Being homozygous for the polled gene results in a sterile intersex in the female and in a bigger risk for development of sperm granulomas in the head of the epididymis in the male. Nevertheless, breeding pairs with one naturally horned member and another one polled can obtain polled goats with normal reproductive potential and the kid does not require disbudding but, in order to maintain good fertility, horned goats cannot be totally eliminated (Smith & Sherman, 2009). It is simple to identify polled kids as a single central twirl of hair on top of the head is usually presented (Figure 2) instead of one around each horn bud and the part of the skin where the horn buds would be is tightly attached to the underlying bone. However, some producers disbud these animals anyway, cauterizing tissue in the area where horn buds would normally be (Mackenzie, 1975, cited by Smith & Sherman, 2009).

Figure 2 – Polled kid with a single central twirl of hair. (Adapted from Smith & Sherman, 2009)



1.2. HORN ANATOMY

Horns are of continuous and permanent growth; each horn is composed of closely packed tubules produced by corium and germinal epithelium. Its osseous base is provided by the frontal bone's cornual process and the dermis is firmly adherent to it. The cornual diverticulum of the frontal sinus forms a cavity within the horn, which is consequently opened when an adult goat is dehorned (Dyce, 2010; Smith & Sherman, 2009). The blood to the horn is supplied by the ventral and dorsal cornual arteries, branches of the superficial temporal artery, and blood returns from the horn by two orbital veins, the superficial temporal vein and the angular tributary of the facial vein (Taylor, 2015).

1.2.1. Innervation and nerve blocking

Goats differ from cattle in the sense that each horn bud is innervated by the cornual branches of two nerves; lacrimal nerve and infratrochlear nerve. In cattle, horn innervation is achieved only by the lacrimal nerve, which makes nerve blocking and pain mitigation much easier and effective (Hempstead et al., 2017). Because of the dual branching in goats, the cornual nerve blocking requires two injection sites per horn (Figure 3): one being midway between the lateral base of the horn and the lateral canthus of the eye and the other one halfway between the medial base horn and the medial canthus of the eye (Plummer & Schleining, 2013), making the administration of local anaesthesia a challenge.

Figure 3 – Needle placement for desensitizing the cornual branch of the lacrimal nerve (A) and cornual branch of the infratrochlear nerve (B) in the goat (Adapted from Skarda, 1986).



1.3. PAIN

The absence of pain in routine procedures is one of the criteria for good health and animal welfare (Stilwell, 2017). Disbudding methods are invasive and painful and compromise animal welfare, not only during the procedure but also postoperative. In spite of cautery disbudding being less painful than other methods (Stafford & Mellor, 2011), it has been associated with acute pain and stress in goat kids with high-intensity behaviours and acute cortisol increase. Destruction of the surrounding tissue of the horn bud causes fluid movement from the vascular compartment into that region resulting in inflammation and oedema. The influx of inflammatory mediators and their local actions from the adjacent blood vessels justify the pain experienced during and after disbudding (Hempstead et al., 2017).

Pain has negative ethical, biological and economic effects; animals that experience pain show changes in ruminal function with atony or hypotony, decreased immunity as prolonged stress hormonal changes lead to a reduction of the phagocytic capacity, neutrophil mobilization, lymphocytic differentiation and production of antibodies, as well as anorexia and consequentially an increased susceptibility to infectious diseases. Also, pain in mature animals can affect fertility due to the inhibition of reproductive hormones' production and release and to a nutritional deficit with reduction of normal heat behaviour (Stilwell, 2017).

It is possible to identify and evaluate pain through morphological, physiological, and behavioural measures (Stilwell, 2017).

1.3.1. Pain physiology

Various stimuli are capable of originating a complex pain response. It is important to differentiate between physiological and pathological pain. The first type is part of a normal and useful defence mechanism to noxious stimuli resulting in strategies of avoidance facilitated by a specialized network of primary sensory neurons and nociceptors. Pathological pain is a result of damage to tissue and to nerves (Ellison, 2017) and its prolonged duration has a negative impact on the animal's homeostasis (Stilwell, 2017).

1.3.1.1. Nociception

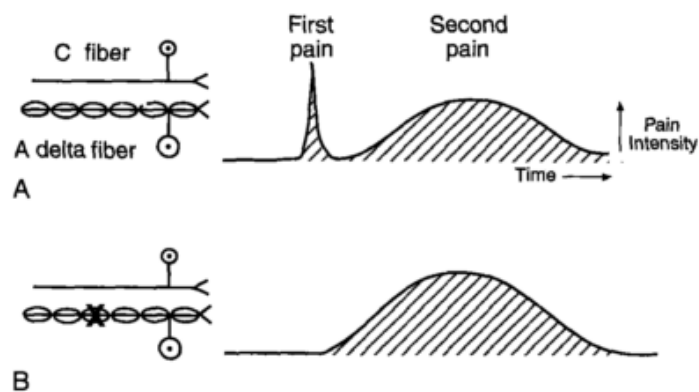
Nociception is the physiological element of pain and involves the components of transduction, transmission and modulation of neural signals created by external noxious stimuli (Ellison, 2017). Primary afferent neurons free endings connected to the central nervous system (CNS) intended to detect tissue damage are called nociceptors and are triggered by mechanical, chemical and thermal stimuli (Bourne et al., 2014).

Transduction consists on the nociceptors' capability of transforming external noxious stimuli into electric activity, generating nerve impulses. These are then transmitted by membrane depolarization of afferent specialized fibers, the primary afferent nociceptive fibers, primarily to the dorsal horn of the medulla and then to the supraspinal structures (Stilwell, 2017). This is achieved through an increase of ion permeability when facing a mechanical stimulus or of membrane permeability due to a chemical one. There are some different types of fibers; the "first pain" is signalized by A-delta fibers (Figure 4). These are lightly myelinated neurons of fast conduction (5 to 30 meters per second) responsible for reflex behaviour related to acute, fast and momentary pain that only lasts as long as the painful stimulus is triggering the nociceptor and is generally associated with mechanical and thermal stimuli. They are easily blocked by local anaesthetics. C fibers are suggested to have a role in monitoring the overall

tissue condition as they can be associated with polymodal receptors. They are responsible for the “second” or “slow pain” which is diffuse and prolonged and are of slow conduction (0.5 to 2 meters per second). Inflammation mediators such as substance P, serotonin, potassium, hydrogen, histamine and prostaglandins lead to hyperalgesia with an exaggerated response from these fibers. When tissues are already damaged, further nociceptors are activated (silent fibers). A-beta fibers are responsible for the conduction of non-noxious stimuli, such as tact (Bourne et al., 2014; Stilwell, 2017), but can be involved in pain transmission in some cases.

Modulation consists of pain moderation or inhibition mechanisms, through endogenous opioids and other substances, that act at the level of the CNS and peripherally.

Figure 4 – Primary afferent pain transmission. First pain and second pain sensations after a noxious stimulus (A). The first pain sensation is abolished when the A fibers are blocked (B). (Adapted from Ellison, 2017)



1.3.1.2. Central Nervous System

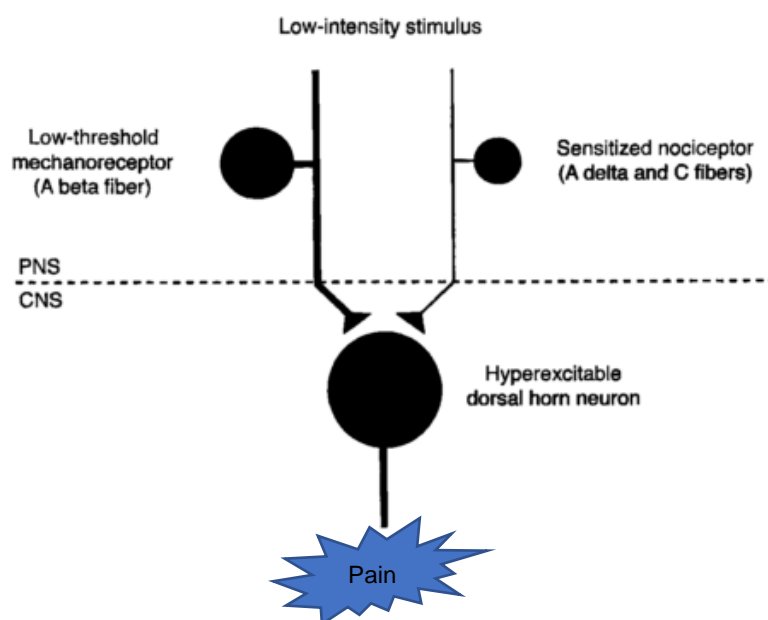
The first synapses for the nociceptive fibers are found in the dorsal root ganglia where sensory neuron cell bodies are located and neurotransmitters act. Glutamate is the most common neurotransmitter and induces fast synaptic potentials in the superficial horn neurons, participating in the modulation of noxious stimuli conduction (Ellison, 2017; Stilwell, 2017). Synapses at the dorsal horn of the medulla bind the nociceptors to ascendant neurons that conduct the stimulus to the brain stem. These can be interneurons that produce an instantaneous flight response (e.g. withdrawal reflex) or reduce the conduction of noxious stimuli resulting in an endogenous analgesia and increased pain conduction threshold, propriospinal neurons or projection neurons. The latter are excited only by noxious thermal or mechanical stimuli from C and A-delta fibers and conduct the nerve impulse to supraspinal centres. The fibers ascend through the spinothalamic tract that terminates in the thalamus which is the first part of the brain to receive and redirect noxious stimuli through synapses of these fibers. The impulse is then transmitted to the cerebral cortex where perception of pain occurs (Ellison, 2017)

1.3.1.3. Hypersensitivity or hyperalgesia

Due to the accumulation of inflammation mediators and to tissue and nerve damage, the nociceptors' excitability threshold is decreased, silent fibers are activated and C fibers become more responsive to any stimulus, resulting in peripheral hyperalgesia. It can be primary or secondary. In the first, only the aggression site becomes more sensitive, whereas in the latter it also happens in the surrounding undamaged tissues (Stilwell, 2017).

Central hyperalgesia occurs at the level of the medulla with a reduction of the neuron activation threshold so that they fire at the minimal provocation. After a period of constant C fiber activation in pathological pain, neurotransmitters that are hyperalgesic such as glutamate, substance P, prostaglandins and interleukins accumulate in the spinal cord, an extra number of channels and receptors are created and opened and nociceptive silent fibers and A-beta fibers, which start to act as pain conductors, are activated (Figure 5), resulting in allodynia with any stimuli being felt as painful. Animals with hypersensitivity respond poorly to analgesia or anaesthetic nerve blocks thus prevention plays a major role in pain management (Bourne et al., 2014; Ellison, 2017; Stilwell, 2017).

Figure 5 – Pathological pain results from abnormal excitability in the nervous system. This involves both central and peripheral changes and the net result is a low-intensity stimulus can elicit pain. PNS = peripheral nervous system; CNS = central nervous system



1.3.2. Pain evaluation – indicators

1.3.2.1. Behaviour

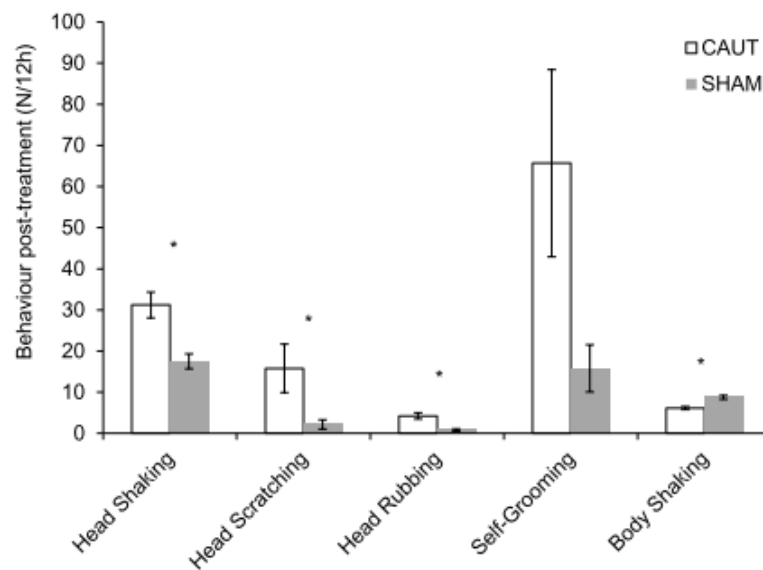
Little research exists on the evaluation of pain related behaviours in disbudded kids. Nevertheless, behavioural response constitutes an important and non-invasive measure of pain. The awareness of pain-specific behaviours allows for the study of strategies of pain relief and subsequently improvement of goats' welfare. Three classes of behaviours can be useful in pain evaluation such as pain-specific behaviours manifested at a higher frequency, a decline in the magnitude or frequency of a certain behaviour and measures of preference based on how the animals perceive different treatments (Weary et al., 2006).

Cautery disbudding increases the presentation of behaviours potentially associated with pain and discomfort. During the procedure, the frequency of high vocalizations, leg movements (kicks, attempts to escape) and tail shaking has been found to be higher in kids that were cautery disbudded in comparison with others in which the procedure was simulated with a cold iron and with other groups given pain relief (Alvarez et al., 2009; Alvarez & Gutiérrez, 2010; Chandrahas et al., 2013). While vocalizations, which are defined as “emission of bleats with open or closed mouth” (Alvarez et al., 2015), are suggested to be a sign of pain in cattle, its evaluation becomes a challenge in goats due a frequent expression of this behaviour in ordinary circumstances and therefore the correlation between the sound's frequency or amplitude and pain is difficult (Stilwell, 2017). In several studies, a digital sound meter has been used and only vocalisations of >90 dB have been considered as high intensity and indicative of pain (Alvarez et al., 2009; Alvarez & Gutiérrez, 2010; Alvarez et al., 2015).

Behaviours considered to be associated with pain were described for up to 3 to 6 hours after disbudding, indicating stressful and prolonged experience of pain (Chandrahas et al., 2013). It is not unexpected that behaviours focussed in the top of the head such as head scratching, shaking and rubbing have been found to be the most frequent after disbudding (Figure 6) as a result of the inflammation and damaged tissue on the horn buds (Hempstead et al., 2017). At 5 minutes after disbudding, the frequency of head shakes in disbudded animals is double the one by kids with disbudding simulation (Hempstead et al., 2018a). Scratching and rubbing the burned area may stand as the kids' effort to relieve the pain and pressure caused by cauterization. Self-grooming was also shown to be performed at higher frequencies following disbudding although individual differences among kids were found. Body shaking was less frequent and the animal's attention being all diverted to the head was suggested as the main cause for the reduced occurrence of this behaviour. Regardless of being disbudded or not, all kids exhibited these behaviours before and after the procedure indicating that none of the above

are pain specific and their simple manifestation is not reliable for measuring pain associated with thermal disbudding in goat kids. Although a few studies on pain mitigation techniques have shown a reduction of some of these behaviours (Chandrabhas et al., 2013; Nfor et al., 2016; Hempstead et al., 2018a), further research is required in order to potentially consider them as valid pain indicators.

Figure 6 – Back-transformed mean (\pm exact 95% confidence intervals) frequencies of behaviour post-treatment frequencies. CAUT kids (n=5) were disbudded with a cauterizing iron and SHAM kids (n=5) were sham handled but not disbudded. * indicates the behaviour that differ between treatments at the 5% significance level. (Adapted from Hempstead et al., 2017)



1.3.2.1.1. Assessment of behaviour

In order to assess behaviour either a visual analogue scale or a numerical scale are generally used. The first method scores pain in a subjective manner and normally relies on the presence or absence of several significant behaviours whereas the latter records the frequency of certain pain-related behaviours (Weary et al., 2006). Both have been used for assessing severe pain after caustic paste disbudding in calves and similar results were achieved but, in regards to prolonged pain, the objective method, i.e. numerical scale, was found to be more accurate (Stilwell, 2009). The value of the two methods will depend on the quality of the measures, the pain experienced by the animal and, for the subjective approach, on the observer's training (Weary et al., 2006); the use of an ethogram that records several behaviours will make pain less likely to be underestimated. A few ethograms have been developed on behaviour patterns of dairy goat kids and used for behaviour analysis following disbudding (Heinrich et al., 2010; Hempstead et al., 2017; Hempstead et al., 2018).

1.3.2.2. Cortisol

Plasma cortisol level is usually used as a physiological pain indicator in research with livestock. Stress, be it traumatic, inflammatory or psychological, includes the release of cortisol due to the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis followed by stimulation of the adrenocorticotrophic hormone's (ACTH) release (Smith, 2009). Cortisol acts as a moderator of the inflammatory response and consequently of pain although its levels are not specific for pain and can increase with stress, anxiety or excitement (Stilwell, 2017; Tsigos & Chrousos, 2002). Thus, when using cortisol levels for pain evaluation, the stress involved with handling of the animals for sample collection should be considered so that results are not misperceived. In calves, as long as blood sampling is carried out within one minute of restraining, cortisol levels have been suggested to not be evident (Lane, 2006; Stilwell et al., 2008). As an alternative to plasma cortisol, non-invasive sampling methods such as determination of corticoid in urine, saliva or milk have been explored, as well as faecal samples in chronic stress situations in which cortisol metabolites can be detected through high performance liquid chromatography (HPLC) (Möstl & Palme, 2002). Only about 10% of circulating plasma cortisol is in the free form with biological activity. The level of cortisol in saliva has been confirmed to be a direct reflection of the biologically active cortisol in plasma, with the advantage of less negative impact on animal welfare (Lane, 2006).

Glucocorticoids are known to be produced on a circadian rhythm with physiological variations throughout the day, namely peak levels at pre and post wakening. However, a study on this matter conducted with goats at six different times of the year (Alila-Johansson et al., 2003) showed that no significant daily rhythm in plasma cortisol levels existed at any time of the year, whereas a significant seasonal variance was observed; cortisol concentration was higher in the winter than in any other season, in contrast to low concentrations from early spring to summer. Plasmatic cortisol levels have been determined immediately after kids' disbudding and shown to significantly increase and last for 2 to 3 hours (Alvarez & Gutiérrez, 2010; Hempstead et al., 2018a) or up to 5 hours (Nfor et al., 2016) with a concentration peak at 15 minutes after the procedure. This shows that not only it is a painful procedure, but that the pain also persists. In calves, elevated cortisol concentrations were found 24 hours after disbudding (Morisse et al., 1995). This variance in time corroborates the suggestion that because the amount of time needed to disbud calves is superior to that of kids and due to the kids' horn buds being less developed at the time of the procedure, the pain kids experience during disbudding may be lower and relatively short-lived when in comparison with calves (Hempstead et al., 2018a).

1.3.2.3. Technological methods

Inflamed lesions and exposure of nerve endings allow to test for the pain tolerance threshold when pressure is applied to an affected area or its surroundings due to the hyperalgesia. Algometers are instruments that measure the sensibility to touch or pressure applied to a certain region (Stilwell, 2017). When the animal tries to move away (withdrawal reflex), a value is registered and it is considered as the first point at which pressure is sensed as pain. Comparing it to healthy regions values aids in quantifying and evaluating pain and possibly tracking healing. To achieve great reliability, the manual force applied should be consistent and perpendicular to the body surface (Kinser et al., 2009). This method has been used to determine pain sensitivity in disbudding wounds in calves (Heinrich et al., 2010; Espinoza et al., 2016). The degree of inflammation can also be assessed by determining the temperature of a specific area and therefore confirm a suspicion of pain (Stilwell, 2017). In this matter, a thermography camera has been used recently to measure and compare skin temperature at the level of the horn buds pre and post different methods of disbudding, including several weeks after treatment to evaluate wound healing (Hempstead et al., 2018b).

1.3.3. Pain management

Disbudding is routinely performed without any anaesthesia or analgesia. A majority (69%) of veterinarians that participated in an online survey (Valdmanis et al., 2008) reported that they include analgesia as part of the disbudding procedures (Table 1) which, along with the reduction of pain related behaviours and physiological parameters modifications in kids given anaesthetics, strongly supports the necessity for pain relief. On the other side, 75% of farmers who do not use analgesic drugs consider it unnecessary, whereas of the 33% of producers that provide analgesia, 91% reported welfare benefits as a reason to do so.

Table 1 – Proportion of veterinarians and goat producers that provide analgesia for any goats during disbudding and dehorning. (Adapted from Valdmanis et al., 2008)

	Disbudding		Dehorning	
	Vets	Farmers	Vets	Farmers
Total analgesic use	69	33	100	23
Sedatives	68	7	91	14
Local nerve blocks	60	17	59	11
NSAIDs	36	14	44	6

1.3.3.1. Local anaesthesia

The ionic fluxes generation and conduction that are required for the conduction of nerve impulses are inhibited by local anaesthetics. They interfere with membrane depolarization by blocking Na⁺ channels and act primarily on mechanical pain, followed by cold, heat and profound pressure. Their action is decreased when necrotic tissue, pus or acid settings are present as they ionize in acid environments because local anaesthetics pKa is normally slightly alkaline (Lomax et al., 2013; Stilwell, 2017).

Local anaesthesia has been shown to be largely ineffective and unsatisfactory at reducing pain-related behaviours in goat kids during and after disbudding. For an effective anaesthesia, both cornual branches of the lacrimal and infratrochlear nerves must be blocked (Galatos, 2011). Physiological and behavioural responses have been studied in cautery disbudded goat kids with and without cornual nerve block with 1 mL of 2% lidocaine per site (Alvarez et al., 2015). This injection did not have a significant effect either on the rising cortisol levels, as they were significantly increased immediately after disbudding and remained high for the following 20 to 30 minutes, or on the behavioural response which did not decrease. Similar findings had been previously achieved (Chandrasah et al., 2013) with the local administration of lidocaine not preventing pain related behaviours after the procedure. In a different study, the administration of lidocaine around each horn bud did not reduce cortisol secretion in response to disbudding. In addition, the raise of cortisol secretion was accelerated due to the puncture required to infiltrate the anaesthetic (Alvarez et al., 2009). The use of a higher dosage could potentially be studied although toxicity needs to be considered as approximately 10mg/kg of lidocaine intramuscularly, i.e. 2 mL of 2% lidocaine for a 4 kg goat kid, cause severe toxicity, resulting in lethargy, unwillingness to feed and convulsions (Galatos, 2011).

1.3.3.1.1. Tri-Solfen

Tri-Solfen[®] (TS) is a practical topical anaesthetic and antiseptic formulation used in Australia in painful procedures such as mulesing, castration and tail docking in lambs as well as castration and disbudding in calves. It is only commercially available in Australia. It contains hydrochloride bupivacaine, hydrochloride lidocaine, tartrate adrenaline and cetrimide and comes in the form of a spray-on gel, making it practical and easily applied by farmers immediately after a procedure (Lomax et al., 2008; Windsor et al., 2016).

A rapid onset anaesthesia and prolonged effect is provided with the association of bupivacaine and lidocaine (Lomax et al., 2008) with wounds being desensitised within one minute of the application (Lomax et al., 2013). The presence of a vasoconstrictor (adrenaline) in the preparation also reduces blood loss as haemostasis is rapidly achieved and, through the

reduction of systemic absorption, the local effect of the anaesthetics is prolonged (Lomax et al., 2008; Lomax et al., 2010). The fact that it is a gel can itself be advantageous as it can provide a barrier against environmental agents and touch stimulation, offering protection to nerve endings at the surface of the wound. This, along with the antiseptic agent, results in improvement of wound healing (Emflorgo, 1999, cited by Lomax et al., 2008).

A decrease in cortisol secretion and pain-related behaviours in lambs immediately and for up to 24 hours after mulesing supports TS' effectiveness in pain alleviation and suggests that it inhibits wound nociception for a prolonged time that goes beyond its duration of action (Lomax et al., 2013; Paull et al., 2009). Its use in surgical castration of lambs has also been found to improve wound healing and decrease pain related behaviours with abolishment of primary hyperalgesia. Secondary hyperalgesia has been prevented in animals that were treated with TS post hot-iron tail docking (Lomax et al., 2010).

The management of pain through the administration of this topical anaesthetic immediately after scoop dehorning has also been studied in two-month-old dairy calves with a reduction of pain sensitivity for up to 5 hours following the procedure (Espinoza et al., 2016). However, because the procedure can be associated with considerable haemorrhage, the adherence of TS' substances to the wound might be inadequate (Espinoza et al., 2013 cited by Windsor et al., 2016).

1.3.3.2. General anaesthesia

There is little research on the efficacy of general anaesthesia on pain management in disbudding of goat kids. The use of isoflurane alone and in combination with meloxicam has been studied (Hempstead et al., 2018a) and a reduction in cortisol secretion in kids after disbudding for up to two hours was achieved but only when used in combination. Behaviours indicative of pain were reduced although the effect of isoflurane alone on behaviour was not assessed and further research should be considered. While it showed to be a good method of anaesthesia and provide good pain relief, it may not be practical in terms of equipment requirements and necessity for trained personnel. The development of an on-farm use portable isoflurane delivery service has been suggested.

1.3.3.2.1. Ketamine

N-methyl-D-aspartate (NMDA) is a receptor activated by glutamate that excites post synaptic neurons of the dorsal horn when inflammation or tissue lesion are present and is believed to be a main component in the central sensitization, pain amplification and secondary hyperalgesia's development (Anderson & Muir, 2005; Stilwell, 2017). Ketamine is a dissociative rapid acting

general anaesthetic and NMDA receptor antagonist, responsible for its analgesic properties. It has no depressant effects on the respiratory system, provides stimulation of the sympathetic system, potentially improving cardiovascular function, the peripheral reflexes are maintained and muscle tone is increased (Valverde & Doherty, 2008; Fallis, 2013; Plummer & Schleining, 2013). Regurgitation has occurred in goats following induction with ketamine, so endotracheal intubation should be considered (Prassinis et al., 2005). A good evaluation of the level of anaesthesia should be held as there are reports of animals that do not respond to ketamine (Stilwell, 2017). It should be associated with sedative tranquilizers such as xylazine or diazepam. Nevertheless, in order to avoid prolonged recoveries and an increased risk of regurgitation, sedation is not always performed in ruminants before anaesthesia induction (Prassinis et al., 2005). A combination of ketamine and xylazine given to kids before disbudding has reportedly reduced pain (Ingvast-Larsson et al., 2011) although acute response was not assessed with enough detail.

1.3.3.3. Sedatives

The intramuscular administration of dexmedetomidine hydrochloride in goat kids prior to disbudding (Nfor et al., 2016) both as a single or a combined dose, was found to be successful in reducing the possible long lasting pain and discomfort, as there were no significant increases in baseline cortisol levels and pain-related behaviours had a relatively low incidence. However, because α_2 adrenergic agonists provide an intense sedation and muscular relaxation, absence of movement can be misinterpreted as absence of pain (Stilwell, 2017). Further studies are also required to assess the appropriate dosage for kids.

1.3.3.4. Nonsteroidal anti-inflammatory drugs

1.3.3.4.1. Meloxicam

Meloxicam belongs to the class of nonsteroidal anti-inflammatory drugs (NSAID) and its mechanism of action consists in the inhibition of cyclooxygenase, especially COX-2, and prostaglandin synthesis, having anti-inflammatory, antipyretic and analgesic properties (Fallis, 2013). Its elimination rate is faster in goats than in sheep and cattle (Coetzee et al., 2009, cited by Karademir et al., 2016).

The administrations of meloxicam subcutaneously, intramuscularly and orally in healthy goats have been compared in order to better understand each route's pharmacokinetic parameters. It was found that with subcutaneous administration, plasma concentrations reached a peak faster and a long-term presence of the drug in the goats' plasma was achieved, making it a preferable route of administration (Karademir et al., 2016).

Because of meloxicam's analgesic qualities, its use has been assessed in disbudded kids. In one recent study (Hempstead et al., 2018a), orally given meloxicam was compared with meloxicam administered subcutaneously to kids prior to disbudding. Contrary to what was expected, oral meloxicam had a better effect on behaviour response. Regardless of the administration route, meloxicam did not seem to have a significant effect on the stress response as the kids had similar cortisol concentrations to those experienced by kids disbudded without any pain relief. These findings are corroborated by former literature, in which no significant differences were observed in cortisol levels of kids treated with and without NSAID (Ingvast-Larsson et al., 2011; Nfor et al., 2016). However, prevalence of pain signs was found to be smaller in kids treated with meloxicam on the first 24 hours after disbudding, even though not subsequently and kids given meloxicam in combination with isoflurane had an inferior physiological and behavioural response to the procedure (Hempstead et al., 2018a) which is consistent with earlier suggestions that either in combination or as a single drug, meloxicam may lessen pain related behaviours, therefore improving animal welfare (Chandrasah et al., 2013).

1.4. WOUND HEALING

A disruption or damage to the normal anatomical structure results in a wound (Velnar et al., 2009). As the disbudding wound generally repairs itself within 30 days, it can be classified as an acute wound (Broughton et al., 2006).

The process of wound healing is normally divided into four phases: coagulation and haemostasis, inflammation, proliferation and remodelling with the formation of scar tissue (Velnar et al., 2009). The first step after wounding is bleeding control (haemostasis) through vasoconstriction of the injured blood vessels and activation of the coagulation cascade, resulting in a clot formed by platelets, collagen, fibronectin and thrombin, all of which release growth factors and cytokines that induce the inflammatory response. Within 24 to 36 hours, a neutrophil response that aims to prevent infection is generated during clot formation and is drawn into the wounded area as surrounding blood vessels dilate. Once neutrophils succeed to remove all contaminating bacteria, its population is eliminated and phagocytosed by former monocytes that transform into macrophages generally 48 to 96 hours following damage. These produce important tissue growth factors such as TGF- β that activate fibroblasts, keratinocytes and endothelial cells that will be crucial for the healing process. Interleukin-1 (IL-1) and immunoglobulin G (IgG) draw lymphocytes to the injury site 72 hours after injury and promote collagenase regulation. On the third day the proliferative phase begins and lasts for about 2 weeks. It is characterized by a profuse formation of granulation tissue, provided by fibroblast

migration and deposition of newly produced extracellular matrix supporting cell migration and therefore stimulating re-epithelialization. In the last phase (remodelling), the deposition of collagen in an organized network allows for new epithelium to develop and scar tissue to be formed with an increase in the wound's strength (Broughton et al., 2006; Velnar et al., 2009). Vasculature and cells are often destroyed in burned tissues as happens in cautery disbudding which may complicate the regular healing response. Neutrophils and macrophages' access to the injury site is compromised because of insufficient blood flow as well as angiogenesis, essential for re-epithelialization (Rose & Chan, 2016).

Cautery disbudding causes full thickness burns and ceases circulation leading to decreased tissue perfusion and a longer recovery period (Papp, 2012 cited by Hempstead et al., 2018b). Only one study has been conducted on wound healing after thermal disbudding in goat kids, reporting that at 6 weeks post treatment scabs were still displayed (Hempstead et al., 2018b). A study on disbudded dairy calves (Huebner et al., 2017) has been conducted in order to evaluate the efficacy of aluminum-based aerosol bandage spray immediately after the cautery disbudding on wound healing with the suggestion that it is improved at three weeks following disbudding when compared with no treatment at all.

2. EXPERIMENTAL STUDY

2.1. OBJECTIVES

With this work we aimed to evaluate the behavioural response of goat kids to disbudding under anaesthesia so as to suggest to practitioners and farmers a valid and feasible protocol for pain reduction, which is inexistent at the time. Ketamine will be administered intramuscularly to goat kids with dosage, safety and duration of effect being assessed. After the procedure, behaviour will be observed in order to evaluate the efficacy of this drug in decreasing pain related behaviours either when used alone or in combination with an NSAID.

The effect of Tri-Solfen[®] on preventing wound infection and inflammation after the procedure based on a healing scale will also be assessed.

The conclusions of this study will allow to make progress in pain management and prevention, either by helping to find the best solution or by discarding non-valid ones, therefore contributing to animal welfare.

2.2. MATERIAL AND METHODS

2.2.1. Ethics Statement

All procedures were approved under the ethics committee of University of Lisbon – *Faculdade de Medicina Veterinária*, Ref. 005/2018, with all recommendations being considered.

2.2.2. Description of the farm

The present study was held between October 2017 and February 2018 at *Livretribo leiteira Lda*, an intensive dairy goat farm located in the Ribatejo region with 900 lactating Saneen goats with 2% of cross breeds. Kidding seasons are in January, April, June, September and November. Immediately after birth, kids are separated from the does and kept in loose-housing pens with straw bedding. They are fed with milk *ad libitum* in group buckets until weaning and concentrate is introduced at three weeks of age. Cautery disbudding is routinely done between four and eight days of age.

2.2.3. Safety study

Fourteen healthy goat kids aged between five and seven days were used in this trial, with body weight ranging between 3 and 5.5 kilograms. In order to assess the safety of ketamine, the behavioural response to three different dosages was studied.

The kids were randomly divided into three groups, two with five animals and one with four, and were given 10, 12.5 and 15 mg/kg of ketamine (100 mg/mL) intramuscularly (Table 2). As

to propose a feasible protocol and simulate how a farmer would work in real life, the mean weight was calculated, 4 kg, so that kids were given 0.4 mL of ketamine in Group 1, 0.5 mL in Group 2 and 0.6 mL in Group 3. The kids were individually marked on the back with a coloured spray corresponding to their group to facilitate observation. Behaviours such as vocalization, head scratching, head shaking, tail flicking, allogrooming and self-grooming were visually monitored and registered for one hour every 30 seconds for the first 5 minutes, followed by every 1 minute the next 5 minutes and then every 5 minutes by three observers blind to the dose given (Appendix IV), based on Hempstead et al. (2017) modified ethogram on the behaviour patterns of dairy goat kids (Appendix V). This allowed to determine which may be related to anaesthesia and not to pain, in addition to evaluating the drug's safety and duration of effect. The latter was considered from the moment the animal was recumbent to it being awake and standing unaided.

All behaviours were recorded using a video camera from the beginning of anaesthesia to one hour after.

Table 2 – Treatment description of the experimental groups.

Group	n	Description of the treatment
Group 1	5	Ketamine at 10 mg/kg administered intramuscularly
Group 2	5	Ketamine at 12.5 mg/kg administered intramuscularly
Group 3	4	Ketamine at 15 mg/kg administered intramuscularly

2.2.4. Disbudding study

The same direct and video behaviour observations were used in this trial.

Nine healthy goat kids with body weight ranging between 5 and 6.5 kg, aged between 5 and 12 days were randomly allocated to one of three treatment groups (Table 3): (a) given ketamine (K, n = 3), (b) given ketamine and meloxicam (K+M, n = 3) or (c) simulation of disbudding with a cold iron after being given ketamine (S, n = 3). Two to five minutes prior to disbudding, ketamine was administered intramuscularly to all kids at 10 mg/kg. Group K+M additionally received 0.5 mg/kg of meloxicam subcutaneously five minutes before the procedure. For a safer anaesthesia all kids were weighted and each kid was given the exact amount of ketamine for their bodyweight.

The kids were manually restrained, the head being immobilized and the legs free to move, and the procedure was performed by an experienced farmer with a hot-iron while a trained observer evaluated pain based on an ethogram previously developed, registering the number of

vocalizations, tail flicking and leg movements per animal (Appendix VI). A Kalfarm HORN'UP C iron was applied one to three times per horn, up to 6 seconds at a time. After disbudding, an antibacterial spray was applied to the cauterized horn bud area. Sham disbudded kids were equally handled but a cold disbudding iron was used with no antibacterial spray being needed. All kids were marked on the back with a coloured spray correspondent to their group to facilitate observation.

Table 3 – Intervention and treatment description of the experimental groups.

Group	n	Description of the treatment
Ketamine (K)	3	Ketamine at 10 mg/kg administered intramuscularly 2 to 5 minutes before hot-iron disbudding.
Ketamine plus Meloxicam (K + M)	3	0.5 mg/kg of meloxicam injected subcutaneously 5 minutes before the procedure, in addition to the administration of ketamine as mentioned above.
Sham disbudded (S)	3	A cold disbudding iron was held against the kids horn buds so as to simulate the procedure 2 to 5 minutes after being given ketamine at 10 mg/kg.

After disbudding, all kids were placed in the same pen (Figure 7) with milk buckets available for feeding. Frequency of head shaking, head scratching, feeding, allogrooming, running, head friction, body shaking, jumping, self-grooming and vocalization was registered based on a modified ethogram for three hours at pre-determined intervals (Appendix VII) by three observers who were unaware of the treatment given to each group apart from the S kids as they were identifiable. Behaviour was continuously recorded using a video camera.

Figure 7 – Kids placed in the same pen after disbudding for behaviour observation.



2.2.5. Healing study

Sixteen healthy goat kids aged from 3 to 7 days old with weights ranging from 4 to 6 kg were randomly allocated to one of two groups (Table 4): (i) administration of an antibacterial spray on each cauterized horn bud site immediately after disbudding as is routinely done (n = 8), or (ii) administration of Tri-Solfen[®], an anaesthetic and antiseptic gel (n = 8) with lidocaine, bupivacaine, adrenaline and cetrimide. Two trials were held on separate days with 4 kids in each group to facilitate observation.

For the procedure, kids were handled as in the previous study and behaviours were registered by an experienced observer based on the ethogram previously used. After disbudding and application of the respective treatment kids were placed in the same pen and observed for three hours at pre-determined intervals by two trained observers. Behaviours were registered based on Hempstead et al. (2017) ethogram and were also supported by video recordings. At 15 minutes, 1 hour and 3 hours after the procedure, pain response to pressure was determined using a Wagner FORCE TEN[™] FPX algometer, in Kgf, applied perpendicularly against the center of the cauterized horn bud area. Pressure was increased until each kid showed withdrawal reflex from the stimulus with the respective value being registered. This process was repeated twice for each horn bud. During these instants, no behaviours were registered.

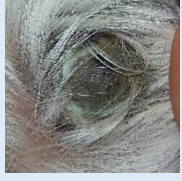


Table 4 – Treatment description of the experimental groups.

Experimental group	n	Description of the treatment
Control (D)	8	Kids were disbudded with a cautery iron and an antibacterial spray was applied on each horn bud site immediately after the procedure.
Tri-Solfen (TS)	8	Kids were handled as above but instead an anaesthetic and antiseptic gel ^a was used.

^aTri-Solfen[®] Bayer Animal Health, Australia.

Three days after the trial and procedure, all kids' wounds were evaluated by three independent observers blind to treatment using a 3-point healing scale (Table Z): (1) normal healing with no scab formation or exudate, (2) a scab is present, and (3) exudate is present. Animals that scored as 3 were treated with antibacterial spray.

Table 5 – Wound evaluation scale description (Photographs taken by the author).

Healing scale	Description	Example photograph
1	Normal healing in which no scab is present nor exudate.	
2	A scab is present.	
3	Exudate is present and visible.	

2.3. STATISTIC ANALYSIS

The R software was used to analyse the data. Using the analysis of variance (ANOVA), the mean difference of the pain-related behaviours between groups of the disbudding study in addition to the positive control of the healing study was assessed in the first half-hour, second half hour, second hour and third hour post disbudding.

Since the variables of the healing study were determined as non-normal by the Shapiro-Wilk normality test, differences between groups were calculated using the non-parametric two-sample Wilcoxon Rank Sum test.

For all the analysis, a value of $p < 0.05$ represented significance.

3. RESULTS

3.1.1. Safety study

In the group given 10 mg/kg of ketamine, the mean duration of effect was of 36 minutes and while under anaesthesia kids performed behaviours such as cycling, vocalization and tail flicking.

Kids given 12.5 mg/kg of ketamine showed almost no behaviours during the first 5 minutes, followed by tail flicking and vocalization throughout the 1-hour observation period. The mean duration of effect was of 39 minutes with all kids being up, bright and walking 52 minutes after the beginning of the anaesthesia.

No behaviours were registered in Group 3 (15 mg/kg) and 3 of the 4 kids were still under anaesthesia at the end of the observation period.

3.1.2. Disbudding study

Two trials were held on distinct days but the first one was not considered due to the young age of the animals with a dosage of ketamine of 15 mg/kg and 0.5mg of meloxicam resulting in kids anaesthetized for more than 3 hours and lethargic afterwards. No similar side effects had been shown in the safety study. Ketamine at 10 mg/kg was then considered as the safest dose and used in the subsequent trial.

3.1.2.1. During the procedure

During disbudding, a variation in leg movements frequency between groups was found, with no movements in the S group and the highest frequency being in D kids, differing significantly when compared with K ($p<0.01$), K+M ($p<0.01$) and S groups ($p<0.001$) (Graph 1). No significant differences were found between groups on tail flicking, with all kids performing this behaviour. All kids vocalized as well, with D kids having the highest frequency and differing significantly from K+M kids, which presented the lowest frequency ($p<0.05$) (Graph 2). Mean frequencies of all behaviours in the different groups are presented in Table 6.

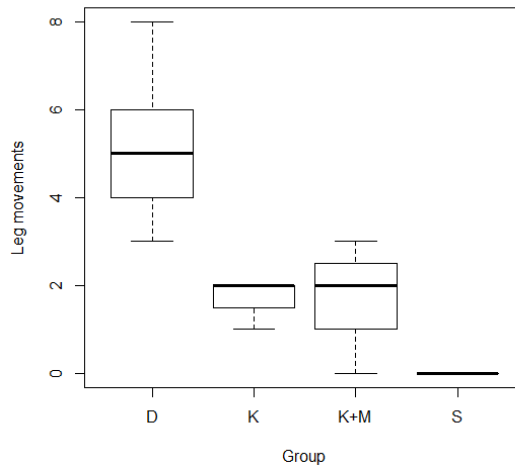
Table 6 – Mean frequency of tail flicking, leg movements and vocalization performed by each group during disbudding. D (disbudded without anaesthesia), K (administration of ketamine prior to disbudding), K+M (disbudded after being administered ketamine and meloxicam), S (sham disbudded after being administered ketamine).

	Tail flicking	Leg movements	Vocalization
D	5.63 ± 1.6	5.13 ± 1.55 ^a	7.13 ± 1.64 ^{a*}
K	4.67 ± 2.31	1.67 ± 0.58 ^{b**}	4.67 ± 0.58
K+M	4.33 ± 3.79	1.67 ± 1.53 ^{b**}	4.33 ± 1.15 ^{b*}
S	4.33 ± 2.08	0 ^{b***}	4.67 ± 0.58

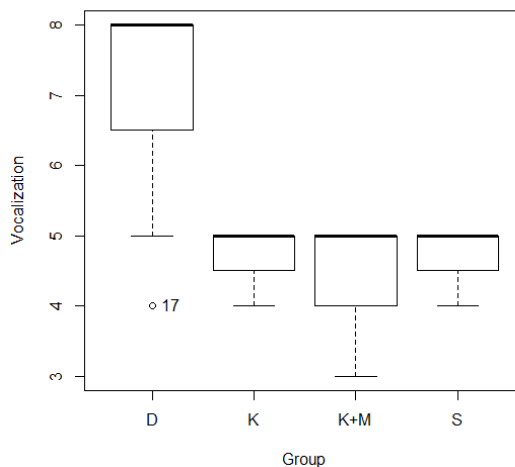
Different superscripts for the same behaviour indicate significant differences between groups ($p<0.05$).

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

Graph 1 – Frequency of “leg movements” within and between groups during disbudding. D (disbudded without anaesthesia) differed significantly from K (disbudded after being administered ketamine), K+M (administration of ketamine and meloxicam prior to disbudding) and S (sham disbudded after being administered ketamine).



Graph 2 – Difference in frequency of “vocalization” between groups during disbudding. D (disbudded without anaesthesia) differed significantly from K+M (disbudded after being administered ketamine and meloxicam). K (administration of ketamine prior to disbudding), S (sham disbudded after being administered ketamine).



3.1.2.2. Post disbudding

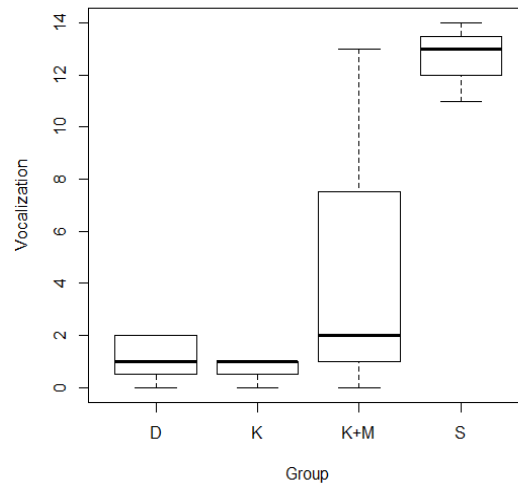
Frequencies (mean \pm S.D) of behaviours throughout the three-hour period are presented in Table 7.

Feeding, jumping, running and head friction behaviours were not considered as relevant due to intragroup variance.

3.1.2.2.1. First half-hour

In the first half-hour following disbudding, all groups performed behaviours such as head shaking and allogrooming and both K and D kids scratched their heads, although no significant differences were found ($p>0.05$). Sham disbudded kids vocalized the most differing significantly from all other groups, especially K kids ($p<0.001$) (Graph 3).

Graph 3 – Difference in frequency of “vocalization” between groups in the first half-hour post disbudding. S (sham disbudded after being administered ketamine) kids differed significantly from K (administration of ketamine prior to disbudding), K+M (disbudded after being administered ketamine and meloxicam) and D (disbudded without anaesthesia).



3.1.2.2.2. Second half-hour

In the second half-hour following the procedure, behaviours such as allogrooming and body shaking were observed in the four groups with no intergroup significant differences ($p < 0.05$). Behaviours focussed in the head were exhibited in all groups, with K kids performing more than 10 times as many head shakes as D ($p < 0.01$) and K+M ($p < 0.05$) kids (Graph 4) and a higher frequency of head scratching in K kids than K+M, S and D kids (Graph 5). Self-grooming was more frequent in S kids than in D and K+M ones ($p < 0.05$) (Graph 6).

Graph 4 – Difference in frequency of “head shaking” between groups in the second half-hour of the observation period, with a significant difference between group K (given ketamine prior to disbudding) and groups D (disbudded without any anaesthesia) and K+M (given ketamine and meloxicam prior to disbudding). S (sham disbudded after administration of ketamine)

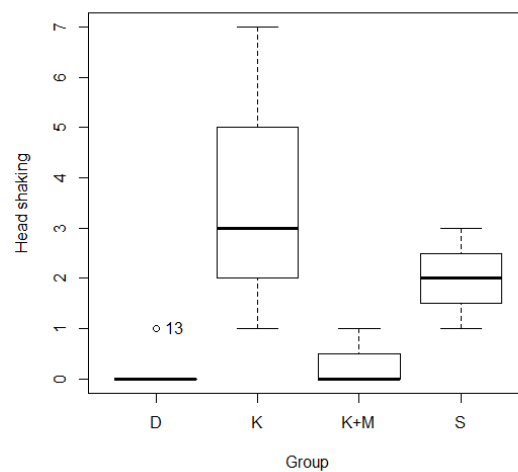


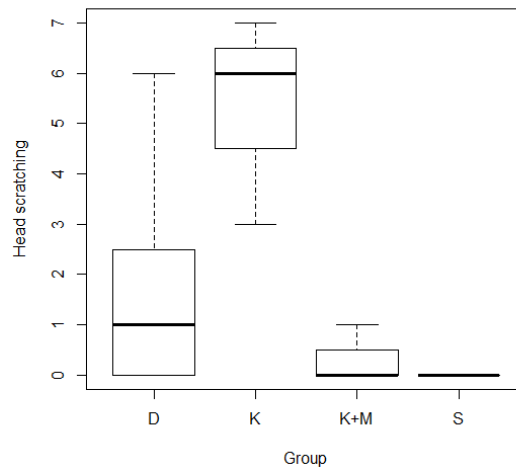
Table 7 – Incidence (mean \pm S.D) of behaviours in four different groups (D – disbudded without anaesthesia; K – disbudded after being given ketamine; K+M – disbudded after being given ketamine and meloxicam; S – sham disbudded after administration of ketamine) during different observational periods of the disbudding study (first half-hour, second half-hour, second hour and third hour).

		Head shaking	Allogrooming	Head scratching	Body shaking	Self-grooming	Vocalization
1 st half-hour	D	3.13 \pm 3.75	0.37 \pm 0.52	3.86 \pm 3.48	0.50 \pm 0.76	1.63 \pm 2.07	1.13 \pm 0.83 ^{a***}
	K	5.00 \pm 3.61	1.67 \pm 1.15	0.33 \pm 0.58	0.67 \pm 1.15	0.00	0.67 \pm 0.58 ^{a***}
	K+M	0.67 \pm 1.15	3.67 \pm 3.21	0.00	0.00	0.00	5.00 \pm 7.00 ^{a*}
	S	2.00 \pm 2.00	2.67 \pm 2.89	0.00	0.00	0.00	12.67 \pm 1.53 ^b
2 nd half-hour	D	0.13 \pm 0.35 ^{a**}	0.25 \pm 0.46	1.63 \pm 2.13 ^{a*}	0.13 \pm 0.35	1.25 \pm 1.75 ^a	1 \pm 1.41
	K	3.67 \pm 3.06 ^b	1.00 \pm 0	5.33 \pm 2.08 ^b	0.33 \pm 0.58	2.00 \pm 2.00	0.00
	K+M	0.33 \pm 0.57 ^{a*}	1.00 \pm 1.73	0.33 \pm 0.58 ^{a*}	0.67 \pm 0.58	0.00 ^{a*}	0.00
	S	2.00 \pm 1.00	2.67 \pm 3.79	0.00 ^{a*}	0.67 \pm 0.58	7.67 \pm 6.66 ^b	0.00
2 nd hour	D	0.00	0.13 \pm 0.35	0.63 \pm 0.92	NA	0.25 \pm 0.46	1 \pm 1.41
	K	0.67 \pm 1.15	0.33 \pm 0.58	1.33 \pm 1.53	NA	0.00	0.00
	K+M	0.33 \pm 0.58	0.00	0.00	NA	0.33 \pm 0.58	0.00
	S	0.00	1.00 \pm 1.73	0.00	NA	1.00 \pm 1.00	0.00
3 rd hour	D	0.38 \pm 0.74	0.75 \pm 0.89	0.63 \pm 0.92	0.00 ^{a***}	0.38 \pm 0.74	0.00 ^{a*}
	K	2.00 \pm 1.73	1.33 \pm 1.53	1.33 \pm 1.53	1.33 \pm 0.58 ^b	1.00 \pm 1.00	0.67 \pm 0.58 ^b
	K+M	0.33 \pm 0.58	2.33 \pm 2.08	0.00	0.00 ^{a***}	1.00 \pm 1.73	0.33 \pm 0.58
	S	0.00	0.00	0.00	0.00 ^{a***}	0.67 \pm 1.15	0.00

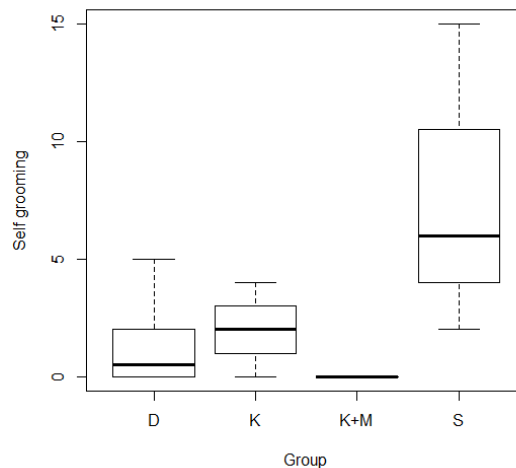
Different superscripts in the same behaviour at a certain observation period indicate significant differences between groups ($p < 0.05$).

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Graph 5 – Difference in frequency of “head scratching” between groups in the second half-hour of the observation period, with a significant difference between group K (given ketamine prior to disbudding) and groups D (disbudded without any anaesthesia), K+M (given ketamine and meloxicam prior to disbudding) and S (sham disbudded after administration of ketamine).



Graph 6 – Difference in frequency of “self-grooming” between groups in the second half-hour of the observation period with a significant difference between S (sham disbudded after administration of ketamine) kids and K+M (ketamine and meloxicam administered prior to disbudding). No significant differences were found between those and D (disbudding without anaesthesia) and K (ketamine given prior to disbudding).



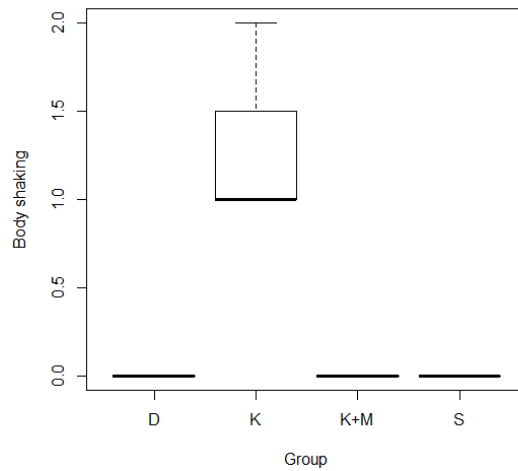
3.1.2.2.3. Second hour

No significant differences were found between groups in the second hour post-disbudding. Only K and K+M kids shook their heads whereas allogrooming and head scratching were behaviours performed exclusively by D and K kids.

3.1.2.2.4. Third hour

In the third-hour of the observation period, head shaking was performed in all groups apart from S and significant differences were found in body shaking, with K being the only group exhibiting this behaviour ($p < 0.001$) (Graph 7).

Graph 7 – Difference in frequency of “body shaking” between groups in the third hour observation period with significant difference between K (ketamine given prior to disbudding) and S (sham disbudded after administration of ketamine), K+M (ketamine and meloxicam administered prior to disbudding) and D (disbudded without anaesthesia).



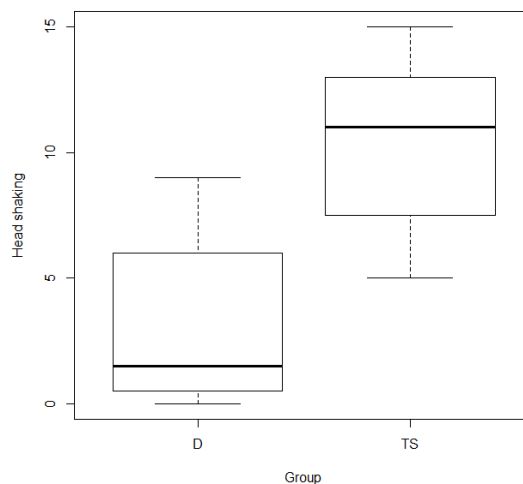
3.1.3. Healing study

In the course of observations, Tri-Solfen[®] gel was noted to be dripping down from the horn bud area to the face of some TS kids and one had to be removed from the trial due to eye irritation following head scratching.

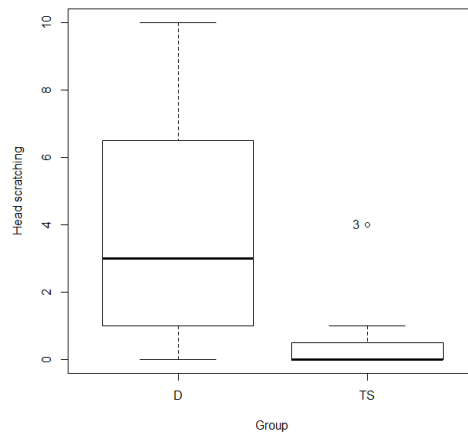
Absolute frequencies (mean ± SD) of behaviours throughout the three-hour period are presented in Table 9. Significant differences were only found in the first half-hour after the procedure. Head shaking was performed by the kids that had received the TS three times more than by kids given the antibacterial spray ($p=0.01$) as is shown in Graph 8, whereas head scratching had higher frequencies in the D group ($p<0.05$) (Graph 9).

The algometry test was performed at 15 minutes, 1 hour and 3 hours after the procedure but the results were not considered due to inconsistent readings.

Graph 8 – Difference in frequency of “head shaking” between groups in the first half-hour of the observation period with significant differences between group D (antibacterial spray after disbudding) and TS (given and an anaesthetic and antiseptic gel after disbudding – Tri-Solfen[®]).



Graph 9 – Difference in frequency of “head scratching” between groups in the first half-hour of the observation period with significant difference between D (antibacterial spray after disbudding) and TS (given an anaesthetic and antiseptic gel after disbudding – Tri-Solfen®).



Three days after disbudding, normal healing, scabs and presence of exudate were found in both groups, with no significant differences (Table 8).

Table 8 – Incidence (mean \pm SD) of lesions presented 3 days after disbudding in two different groups, D (application of an antibacterial spray at the horn bud site after the procedure) and TS (application of Tri-Solfen, an anaesthetic and antiseptic gel after disbudding).

	Absolute frequencies	p-value
D	2.00 \pm 0.93	0.6
TS	1.75 \pm 1.04	

Table 9 – Incidence (mean \pm S.D) of behaviours in two different groups (D – application of an antibacterial spray after disbudding; TS – application of anaesthetic and antiseptic gel after disbudding) during different observational periods (first half-hour, second half-hour, second hour and third hour post-disbudding).

		Head shaking	Allogrooming	Head scratching	Body shaking	Self-grooming	Vocalization
1 st half-hour	D	3.13 \pm 3.76 ^{a**}	0.38 \pm 0.52	3.88 \pm 3.48 ^{a*}	0.5 \pm 0.76	1.63 \pm 2.07	1.13 \pm 0.83
	TS	10.29 \pm 3.82 ^b	0.86 \pm 1.46	0.71 \pm 1.50 ^b	NA	0.71 \pm 1.0	0.43 \pm 0.53
2 nd half-hour	D	0.13 \pm 0.35	.25 \pm 0.46	1.62 \pm 2.13	0.13 \pm 0.35	1.25 \pm 1.75	0.25 \pm 0.71
	TS	0.57 \pm 0.53	0.71 \pm 1.25	0.57 \pm 1.0	0.29 \pm 0.76	1.0 \pm 1.15	NA
2 nd hour	D	0.12 \pm 0.35	0.25 \pm 0.46	1.63 \pm 2.13	0.13 \pm 0.35	1.25 \pm 1.75	0.25 \pm 0.71
	TS	0.57 \pm 0.53	0.71 \pm 1.25	0.57 \pm 0.98	0.29 \pm 0.76	1.00 \pm 1.15	0
3 rd hour	D	0.38 \pm 0.74	0.75 \pm 0.89	0.63 \pm 0.92	NA	0.38 \pm 0.74	NA
	TS	1.14 \pm 1.68	0.57 \pm 0.53	1.29 \pm 1.50	0.28 \pm 0.49	2.14 \pm 2.48	NA

Different superscripts in the same behaviour at a certain observation period indicate significant differences between groups ($p < 0.05$).

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

3.2. DISCUSSION

The small sample was a limitation of the present study as false negatives may occur (type II error), especially when analysing non-parametric data, so it is possible that there existed additional significant differences between the groups.

3.2.1. Safety study

High vocalizations and tail shaking have been previously described as behaviours of pain in goat kids (Alvarez et al., 2009; Chandrahas et al., 2013; Alvarez et al., 2015). In the safety study, those same behaviours were manifested by kids that received 10 mg/kg and 12.5 mg/kg of ketamine although no underlying source of pain was evident, suggesting that either those stand as regular kid behaviour even when sedated or as a result of the anaesthesia and therefore their simple occurrence may not be an indication of pain. Similar findings have been encountered previously with kids exhibiting the same behaviours before and after disbudding (Hempstead et al., 2017). Vocalizations differ among species and may be related to specific behaviour contexts, physiological parameters and environments (Manteuffel et al., 2004). Amplitudes higher than 90 dB have been considered as high intensity and indicative of pain in goat kid disbudding (Alvarez et al., 2009; Alvarez & Gutiérrez, 2010; Alvarez et al., 2015) but further studies should be conducted to better correlate sound intensity and pain in this species. A dose of 15 mg/kg provided a good level of anaesthesia and was considered safe with no side effects such as regurgitation or lethargy being observed. When used in the first trial of the disbudding study, it resulted in a prolonged anaesthesia period and lethargy in kids, especially when used in combination with meloxicam. A recent study showed that younger individuals take longer to recover from anaesthesia than older ones (Wagman et al., 2018). We are unaware of what may have caused this response in our study since K+M kids took the longest to stand. Further research should be held in order to determine if the combination of meloxicam with ketamine along with the young age of kids could possibly lead to an impairment of the renal perfusion resulting in a slower excretion of ketamine metabolites. Because of kids' thin cranium, the possibility of thermal injury resulting in meningoencephalitis and cerebral infarction following hot-iron disbudding (Thompson et al., 2005) cannot be ruled out as a cause for lethargy.

In a farm setting, disbudding is a routine practice that should be cost-effective and not too time-consuming. Since the behaviours shown in the groups given 10 mg/kg and 12.5 mg/kg of ketamine were similar and the duration of effect was slightly inferior in the lowest dosage, this was chosen as the most appropriate and the one we recommend as the safest.

3.2.2. Disbudding study

3.2.2.1. During the procedure

It is not surprising that kids disbudded without any treatment had a higher frequency of leg movements during the procedure, potentially as an attempt to escape, as has been reported before (Alvarez & Gutiérrez, 2010; Chandrahas et al., 2013; Nfor et al., 2016). This behaviour was also expressed by kids in both K and K+M groups but at a lower frequency with no significant difference from sham disbudded kids, suggesting that the treatments may have had some effect in reducing pain related to the procedure.

The same behaviours (tail flicking and vocalization) observed in the safety study were exhibited during disbudding with D kids vocalizing the most during the procedure supporting that it is painful practice. The addition of non-steroidal anti-inflammatory agents to local anaesthesia has been stated to reduce stress related to goat kid disbudding and improve animal welfare (Chandrahas et al., 2013). Similar results were achieved in this study, with the combination of ketamine and meloxicam significantly decreasing vocalizations. Sham disbudded kids also vocalized, possibly as a result of being handled and restrained and not of pain or, as described above, vocalizations can be a result of the anaesthesia itself, which makes it difficult to determine whether the vocalizations performed by the treated groups were associated with pain. All kids flicked their tails, similarly to what happened in the safety study, corroborating that its manifestation may not be used as an indication of pain.

3.2.2.2. Post disbudding

3.2.2.2.1. First half-hour

Hempstead et al (2018a) reported that at 5 minutes post disbudding, the frequency of head shakes in disbudded kids is double the one exhibited by kids in which the procedure was simulated. In the present study, although they did not differ significantly, S and K+M kids did not scratch their head contrary to what happened in D and K groups. Head scratch may be performed as a response to damage and inflammation of the horn buds (Hempstead et al., 2017), therefore an absence of this behaviour in K+M group may be indicative of an inflammation reduction due to the anti-inflammatory properties of meloxicam, consequently improving animal welfare. However, further studies would need to be conducted with a larger sample in order to achieve more accurate results.

Vocalizations were displayed in all four groups, with S kids having the highest frequency. As described before, it could be either a result of the handling or a side effect of the anaesthesia with ketamine. Since K+M and K groups also received ketamine, it is not clear why a lower

frequency of this behaviour occurred in these. As could have happen in the D group, we suggest that it may be a result of an endogenous opioid release after a period of intense pain (Forrest, 2005). This possibility could be explored in the future by measuring beta-endorphins after disbudding.

3.2.2.2.2. Second half-hour

In the second half-hour after the procedure, head source behaviours were performed in higher frequencies in kids disbudded after being administered ketamine alone. This shows that the anaesthesia did not effectively control pain or had a very short action. A study looking at behaviour and cortisol levels after disbudding in calves (Stilwell, 2009) showed an increase in pain-related behaviours and cortisol levels 3 hours after the procedure in animals in which a nerve block was performed. It was suggested that because treated animals had a delayed cortisol release after disbudding, a more intense inflammatory reaction occurred leading to hyperalgesia. This could explain why D kids had a lower incidence of head shaking and scratching in this period than in the first half-hour, and also why few behaviours were performed in the group that received meloxicam in addition to ketamine due to its anti-inflammatory properties.

Significant differences on self-grooming were found between S and K+M groups, with the first having a higher frequency. These findings are not consistent with an earlier study (Hempstead et al., 2017) that suggested that self-grooming may be associated with pain following cauterly disbudding. Further research showed no differences between sham handled animals and others disbudded with no pain relief (Hempstead et al., 2018a). It is unclear why S kids self-groomed more than K+M ones but based on our results and the ones from the studies mentioned above, we do not consider self-grooming to be a reliable measure of pain associated with disbudding.

3.2.2.3. Second hour

No significant differences were found between groups in the second hour following the procedure with all groups seeming to be calmer, showing less behaviours. We could possibly associate this with the animals' need to rest after a period of intense pain (Stilwell, 2009), but that would not explain why S kids also performed fewer behaviours. However, since there was no statistical comparison between observation periods in this study it is not possible to determine whether less behaviours were exhibited or not.

From the second hour on, although no significant differences were found, no head scratching was performed either by S or K+M kids, suggesting less inflammation when meloxicam is used in combination as has been previously described (Hempstead et al., 2018a).

3.2.2.4. Third hour

Body shaking has been shown to be performed at a lower frequency by kids disbudded without anaesthesia or analgesia (Hempstead et al., 2017). Our findings are not consistent with those reports as only K kids performed this behaviour in the third hour after the procedure. Upon standing, after lying down on straw bedding, kids tend to body shake (Hempstead et al., 2017). It is possible that K kids became more active in this period, thus exhibiting body shakes more than other groups but more information on lying behaviour would be needed for more accurate conclusions.

Only groups in which the procedure was performed shook their heads and solely D and K kids scratched their heads which is suggestive of prolonged pain and shows that ketamine is not effective in reducing long term pain related to disbudding when used alone. Nevertheless, larger samples would be needed for more precise results.

3.2.3. Healing study

Tri-Solfen[®] has been shown to reduce pain related to painful procedures such as mulesing, castration and tail docking in lambs (Windsor et al., 2016). On a previous study, the behavioural response to the application of Tri-Solfen[®] immediately after cautery disbudding resulted in inconsistent findings not allowing for conclusions to be drawn on its efficacy in pain reduction following this procedure (Boto, 2015). In our study, significant differences between the group to which Tri-Solfen[®] was applied and the control group were found in the first half-hour. TS kids had a higher frequency of head shakes which could be a consequence of the gel dripping down from the disbudding site to the face as a result from its low adherence to the wound as has been previously described when applied following scoop dehorning (Espinoza et al., 2016). A more viscous form of the gel should be considered. On the other side, head scratching was more frequent in D kids which could stand as an attempt to alleviate pressure and irritation from the inflammation following cauterization and be indicative of pain (Hempstead et al., 2018), suggesting that TS may have some effect in reducing pain.

At 72 h after an injury the proliferative phase characterized by formation of granulation tissue, is expected to have begun (Broughton et al., 2006). However, subcutaneous damage occurs upon cautery disbudding increasing the risk of infection and prolonging the healing process (inflammation, proliferation and remodelling) (Zielins et al., cited by Hempstead et al., 2018b). At the time the present study was conducted there were no reports on wound healing in goat kids following disbudding. Based on Hempstead et al (2018b) recent study, one day after the procedure open and deep wounds with visible bone were observed, erythema of the surrounding tissues by the second day and at 6 weeks post disbudding scabs were still visible. We evaluated

the wound three days after disbudding and individual differences were found. Normal healing, scabs and exudate were present in both groups, suggesting that the cetrimide present in the TS preparation may not have an effect on preventing infection in the first 3 days or is not more effective than an antibacterial spray. However, in a previous study (Huebner et al., 2017) on the effect of aluminium-based aerosol bandage following disbudding in dairy calves improvements on wound healing were only reported at 3 weeks after the procedure so it is possible that TS decreases the normal healing period for disbudding wounds but it could not be assessed after only 3 days. Further studies should be conducted up to 6 weeks post disbudding and with bigger samples.

3.3. CONCLUSIONS

With this study, we aimed to find a protocol that would reduce the pain experienced by goat kids during and following disbudding. Ketamine showed some effect in reducing pain related behaviours during the procedure but was ineffective in reducing post-operative pain. Its use was found to be unsafe with the risk of adverse reactions or even death, not being cost-effective, as well as time-consuming with monitoring being needed, making it unpractical and of low feasibility in a farming setting. There is still no resolution for the pain associated with disbudding and more studies should be conducted regarding this matter for animal welfare improvement but we recommend that an NSAID be always given to reduce post-operative pain related with inflammation. The ideal long-term solution is the development of painless alternatives to such procedures.

Even though the use of Tri-Solfen[®] did not prevent immediate wound infection, it had a similar effect to the antibacterial spray. Since no antibiotics are present in its preparation, its application after disbudding may be a good alternative to the spray with less risk of potentiating antibiotic resistance, a public health concern.

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APPENDICES

APPENDIX I – Poster “Caso atípico de besnoitiose (*Besnoitia besnoiti*) num touro limousine”



XIX JORNADAS DA ASSOCIAÇÃO PORTUGUESA DE BUIATRIA
3|4|5 de Novembro, Ponta Delgada

CASO CLÍNICO ATÍPICO DE BESNOITIOSE (*Besnoitia besnoiti*) NUM TOURO LIMOUSINE

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A besnoitiose bovina é uma doença parasitária provocada pelo protozoário *Besnoitia besnoiti*. A sua prevalência parece estar a aumentar em vacadas de carne sobretudo a Sul do Tejo, embora também tenham sido detetados alguns casos noutras regiões. O parasita tem tropismo para a pele e tecido conjuntivo. Os sinais típicos desta doença surgem em duas fases distintas:

- Fase **aguda** - febre, hiperémia do focinho, pele periorbital e escroto; seguem-se edemas com ligeira tumefação do focinho, espessamento das pregas de pele do pescoço e tórax e anasarca; pele quente, espessa e dolorosa à palpação.
- Fase **crónica** - espessamento e pregueamento progressivo da pele, alopecia progressiva, dermatite com hiperqueratose e formação de crostas; partes da epiderme desprendem-se da pele, deixando áreas acinzentadas, alopécicas e seborreicas; linfadenopatia superficial e esclerodermia. Nos casos graves, a esclerodermia e alopecia persistem

Infeções inaparentes com ligeira hipertermia e diminuição do apetite, representam a maioria dos casos no campo.

Trata-se de uma doença com elevadas mortalidade e morbidade. Em machos, a besnoitiose conduz a uma esterilidade total. Pode causar perdas na produção de carne e de peles, um aumento da taxa de refugo e rejeição no matadouro. Não existe tratamento ou vacina eficazes; isto, adicionalmente à falta de conhecimento dos produtores sobre esta patologia, dificulta a implementação de estratégias de controlo e de prevenção. Os animais afetados normalmente são eutanasiados por razões humanitárias. O aumento da besnoitiose na Europa levou a que a EFSA a caracterizasse como doença emergente

HISTÓRIA CLÍNICA

Um touro de raça Limousine, de 6 anos de idade, quando retirado da vacada, apresentava inapetência, hipertermia e depressão. Foi colocado um iman intra-ruminal e administrados um anti-inflamatório não esteróide e um antibiótico. A resposta à terapêutica foi quase nula.



Figura 1 - áreas extensas de hiperqueratose e pele gretada, sobretudo na zona ventral (parede ventral do abdómen e tórax, virilhas e axilas).



Figura 2 - Área de dermatite bacteriana secundária



Figura 3 - Escroto firme e separado (círculo) do restante testículo



Figura 4 - Testículo atrofiado e com parênquima alaranjado.

QUADRO CLÍNICO

Duas semanas mais tarde, o animal apresentava-se letárgico, prostrado, com baixa condição corporal, áreas extensas de pele espessada, gretada e com hiperqueratose. As zonas afectadas eram sobretudo ventrais (parede ventral do abdómen e tórax, virilhas e axilas), com uma aparente simetria nos dois lados do corpo (Fig. 1). O movimento e a defecação revelava-se doloroso e difícil, bem como. As fezes eram duras e secas.

DECISÃO CLÍNICA

Devido ao elevado sofrimento, decidiu-se não levar o touro à manga, optando-se pela eutanásia imediata.

NECRÓPSIA

Grandes extensões da pele hiperqueratinizadas e espessadas, zonas de dermatite bacteriana secundária (Fig. 2), rins edemaciados e testículos atrofiados (Fig. 3) e com parênquima alaranjado (Fig. 4).

HISTOPATOLOGIA

Testículo com fibrose intersticial, aplasia do epitélio germinativo (orquite crónica fibrosa com infertilidade total), Calcificação no lúmen de alguns tubos. Hiperplasia irregular da epiderme com hiperqueratose ortoqueratótica. Visíveis quistos dispersos, tanto na derme superficial como na média e profunda.

Conclusões: Estaria em curso besnoitiose de forma arrastada com dermatite crónica fibrosa complicada por hiperqueratose severa. As restantes lesões associadas ao processo são a orquite crónica fibrosa.

DISCUSSÃO

Trata-se de um caso de besnoitiose de evolução lenta e com lesões bastante atípicas já que a cabeça e zonas dorsais não foram afectadas. Os quistos normalmente evidentes na esclera também não eram evidentes. Especulamos se as lesões essencialmente ventrais resultam de um tropismo do parasita para locais menos expostos à luz e ao calor. Esta hipótese não é referida na literatura publicada sobre besnoitiose. A descrição destes casos atípicos poderá ser útil para clínicos de campo ao abrir novas hipóteses no diagnóstico diferencial e auxiliar na decisão de envio para abate em tempo útil.

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APPENDIX II – Poster “Aplicação tópica de anestésicos locais para controlo a dor durante a aparagem curativa de lesões podais de vacas leiteiras – dados preliminares”



8º Encontro de Formação da Ordem dos Médicos Veterinários
14 e 15 de Abril de 2018, Lisboa

APLICAÇÃO TÓPICA DE ANESTÉSICOS LOCAIS PARA CONTROLO DA DOR DURANTE A APARAGEM CURATIVA DE LESÕES PODOIS DE VACAS LEITEIRAS – DADOS PRELIMINARES

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Introdução

As patologias podais em vacas leiteiras em regime intensivo têm um enorme impacto, quer sobre a produção quer sobre o bem-estar animal, sendo responsáveis por grande sofrimento e desconforto. Durante o tratamento de lesões, como úlceras da sola ou doença da linha branca, são necessários procedimentos curativos. Tais procedimentos podem levar a grande dor, não só dificultando o maneio para o operador, como sendo uma agressão ao bem-estar imediato do animal por provocar intensificação e agudização da dor. Por norma, estas intervenções são realizadas por técnicos não veterinários sem formação no controlo da dor. O maneio eficaz da dor não só é uma obrigação ética, como também permite uma mais fácil manipulação e um tratamento mais minucioso. Um medicamento composto por anestésicos locais de aplicação tópica, sob a forma de spray gel, demonstrou ter efeito no alívio imediato e prolongado da dor noutros procedimentos, reduzindo os comportamentos relacionados com a dor, através da combinação de lidocaína, como anestésico de rápida ação, e bupivacaína, como anestésico de longa ação, adrenalina, para concentração dos anestésicos locais e redução da hemorragia, e cetrimida, como antisséptico.

Objetivo

Testar a eficácia da formulação de anestésicos locais tópicos, aplicados durante o procedimento, como forma de maneio e controlo da dor durante e após a aparagem curativa de lesões sangrantes dos cascos (Figura 1).

Materiais e Métodos

Vacas no período de secagem

1. Avaliação do grau de claudicação antes da contenção → escala de 1 (sem claudicação) a 5 (claudicação severa);
2. Avaliação da reação do animal a diferentes graus de pressão → Teste de algometria antes da intervenção;
3. Distribuição aleatória a um de dois grupos – C ou T;

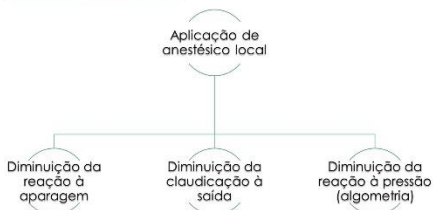
Grupo	Descrição
Controlo (C)	Aparagem sem aplicação de anestésico.
Anestésico local (T)	Aplicação do anestésico local sobre a lesão do córion, seguida de aparagem.

4. Teste de algometria após a intervenção;
5. Avaliação cega dos comportamentos de dor e reação à aparagem curativa → 1- ausente, 2- moderada, 3- severa;
6. Avaliação do grau de claudicação no fim da intervenção.



Figura 1 – Exemplos de patologias podais com possível aplicação do anestésico local.

Resultados preliminares



NOTA: Devido à presença de lidocaína e bupivacaína, está em curso um estudo paralelo para medição da presença de metabólitos excretados no leite.

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Discussão e Conclusão

O estudo demonstra que a utilização de um anestésico local tópico com lidocaína e bupivacaína reduz a dor provocada durante a aparagem curativa de lesões do casco extensas e severas, melhorando o bem-estar animal e aumentando a segurança para o operador, por redução dos comportamentos associados à dor. Considerando a apresentação prática em forma de gel, sem necessidade de procedimentos mais morosos ou mão-de-obra mais especializada, a utilização de um medicamento que favorece conforto e elimina parte do sofrimento induzido durante o tratamento poderá ser uma excelente alternativa para as práticas realizadas até então, tendo o médico veterinário a função de alertar e consciencializar o produtor para boas práticas que visam o bem-estar animal.

APPENDIX III – Abstract “Use of topical anaesthesia to control pain during and after

trimming hoof lesions in dairy cows”

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Treatment of hoof lesions such as sole ulcers in cattle frequently involve trimming of healthy and inflamed tissue (corium). Trimming may cause acute and severe pain resulting in violent reaction with risk for humans' safety as well as potentially affecting the animal's immediate welfare. Specific behaviours are easily identified when pain is caused. Due to technical and practical difficulties and because the procedure is generally carried out by a non-veterinarian, pain management is seldom used during hoof trimming in dairy farms worldwide.

Tri-Solfen® is a “spray and stay” topical anaesthetic formulation containing lidocaine for rapid onset wound anaesthesia, bupivacaine as a long-acting local anaesthetic to provide prolonged duration of effect, adrenalin to concentrate the anaesthetic effect at the wound site and reduce haemorrhage, and cetrimide to provide wound antiseptis, formulated in a viscous gel base. It can be easily applied by non-specialized personnel as a once-only application. Efficacy and welfare studies in mulesing, castration and tail docking in lambs and castration in calves, have shown that Tri-Solfen assists in the immediate alleviation of pain with prolonged effect, reducing both pain related behaviour, and wound pain responses from within 1 minute up to and including 24 hours post procedures.

Our study was designed to determine the effect of Tri-Solfen, applied during the procedure, in attenuating pain during and after trimming bleeding hoof lesions in dairy cows. In treated cows, the gel was applied after removing superficial horn and necrotic tissue and when corium was exposed. Cows selected for hoof trimming at drying off were graded for lameness (1-non lame to 5-severely lame). The hoofs of those with lameness score above or equal to 3 were carefully examined for lesions by an experienced veterinarian. Lesion site, type and size were registered. Animals with horn lesions requiring trimming were randomly distributed to two groups: C – usual trimming with no pain control; T – trimming with Tri-Solfen being applied once live corium was exposed and on completion of trimming. Tri-Solfen application was repeated if deemed necessary (e.g. extensive or deep lesions) with the total quantity being registered. Trimming was resumed one minute after each application. Lameness was scored on the way to the chute, when leaving the chute and three days after trimming. Reaction to trimming was scored (1 to 3) based on the display of the following pain related behaviours: kicking, efforts to release limb and vocalization. A digital algometer was also applied on the lesion area before, during and after trimming. Behaviour observation was done by two persons blinded to treatment.

Across several months this study was repeated in three large dairy farms. In total 28 lame cows were trimmed with Tri-Solfen and 22 were used as control. Two types of statistical analysis tests were used: non-parametric tests and analysis of variance. Treatment significantly influenced reaction to trimming and lameness score after trimming ($p < 0.01$), with better results for those animals treated with Tri-Solfen. Algometer values tended to be higher after application of Tri-Solfen. No differences were shown for lameness after three days. Differences between groups were not influenced by the limb (front or hind) or hoof (outer or inner) affected. Algometry also showed increased pressure threshold in two interdigital skin lesions after application of Tri-Solfen.

In conclusion, this study shows that a topical gel with lidocaine and bupivacaine can reduce pain when trimming severe and extensive hoof lesions, improving dairy cow welfare and eventually trimmer safety.

With thank Animal Ethics, Australia for providing the product for the trials.

APPENDIX IV – Observer’s form (safety study)

Data: __/__/____ Dose: _____ Nº cabritos: _____ Cor do spray: _____ Pesos: _____

Hora da indução: _____ Média do efeito da anestesia: _____ Hora da recuperação: _____

Tempo (minutos)	Agitar cabeça	Agitar cauda	Agitar corpo	Agitar orelhas	Alimentar	Calir desamparado	Cycling	Correr	Levantar	Levantar e deitar	Levantar memb. posteriores	Saltar	Self-grooming	Star-gazing	Vocalizar
00:30															
1:00															
1:30															
2:00															
2:30															
3:00															
3:30															
4:00															
4:30															
5:00															
5:30															
6:00															
7:00															
10:00															
15:00															
20:00															
35:00															
50:00															
60:00															

OBSERVAÇÕES:

APPENDIX V – Ethogram for evaluation of ketamine anaesthesia

ETOGRAMA PARA AVALIAÇÃO DA ANESTESIA COM QUETAMINA	
Comportamento	Descrição
Agitar cabeça	Uma inclinação rápida e contínua da cabeça de um lado para o outro, a acabar num retorno a uma posição neutra.
Agitar corpo	Os pelos do dorso estão levantados o corpo é sacudido de um lado para o outro, a concluir num retorno à posição neutra em pé.
Agitar orelhas	Agitar as orelhas.
Alimentar-se	A boca cobre pelo menos metade da tetina do balde de leite durante, pelo menos, 3 segundos, geralmente seguido de movimentos de sucção.
Cair desamparado	Cair desamparado.
Correr	Fuga repentina para a frente ou para o lado mais rápida que um passo normal, com duração superior a 2 segundos.
Levantar e deitar imediatamente	Levantar e deitar imediatamente.
Levantar membros posteriores em direcção à cabeça	Membro posterior contacta ou quase contacta com alguma parte da cabeça (incluindo a boca, mas excluindo o pescoço)
Saltar	Orelhas para trás com a cabeça elevada seguido de elevação de forma a que os membros anteriores estejam levantados do chão e os joelhos se inclinem em direcção ao corpo. O evento termina com o retorno dos membros anteriores ao chão.
Self-grooming	O focinho do cabrito contacta com qualquer parte do corpo ou membros, com um movimento rítmico (para trás e para a frente).
Star-gazing	Pescoço esticado, olhar para cima.
Vocalizar	Emissão de balidos de boca aberta ou fechada.

APPENDIX VI – Ethogram for observation during disbudding and observer's form

ETOGRAMA PARA AVALIAR DOR DURANTE A DESCORNA	
Comportamento	Descrição
Agitar a cauda	Movimento rápido da cauda de um lado para o outro.
Movimento dos membros	Levantar um membro e voltar a baixar firmemente.
Vocalização	Emissão de balidos de boca aberta ou fechada

AVALIAÇÃO DA DOR DURANTE A DESCORNA

Data: _/ _/ _ Ensaio nº: _____

Cor do spray:

Comportamentos durante a descorna	
Agitar cauda	
Movimento dos membros	
Vocalização	

Cor do spray:

Comportamentos durante a descorna	
Agitar cauda	
Movimento dos membros	
Vocalização	

Cor do spray:

Comportamentos durante a descorna	
Agitar cauda	
Movimento dos membros	
Vocalização	

Comportamentos durante a descorna	
Agitar cauda	
Movimento dos membros	
Vocalização	

Comportamentos durante a descorna	
Agitar cauda	
Movimento dos membros	
Vocalização	

Comportamentos durante a descorna	
Agitar cauda	
Movimento dos membros	
Vocalização	

Comportamentos durante a descorna	
Agitar cauda	
Movimento dos membros	
Vocalização	

Comportamentos durante a descorna	
Agitar cauda	
Movimento dos membros	
Vocalização	

Comportamentos durante a descorna	
Agitar cauda	
Movimento dos membros	
Vocalização	

APPENDIX VII – Observer’s form (disbudding study)

Page 1

Data: / /

Nº cabritos: _____

Cor do spray: _____

Pesos: _____

Idade: _____

AVALIAÇÃO DE COMPORTAMENTOS APÓS A DESCORNA

Tempo (minutos)	Agitar a cabeça	Alimentar-se	Allogrooming	Coçar a cabeça	Correr	Fricção da cabeça	Morder as orelhas	Sacudir o corpo	Saltar	Self-grooming	Vocalização
00:30											
1:00											
1:30											
2:00											
2:30											
3:00											
4:00											
4:30											
5:00											
5:30											
6:00											
7:00											
8:00											

OBSERVAÇÕES:

Page 2

Tempo (minutos)	Agitar a cabeça	Alimentar-se	Allogrooming	Coçar a cabeça	Correr	Fricção da cabeça	Morder as orelhas	Sacudir o corpo	Saltar	Self-grooming	Vocalização
9:00											
10:00											
15:00											
20:00											
25:00											
30:00											
35:00											
40:00											
50:00											
60:00											
75:00											
90:00											
105:00											

OBSERVAÇÕES:

Tempo (minutos)	Agitar a cabeça	Alimentar-se	Allogrooming	Coçar a cabeça	Correr	Fricção da cabeça	Morder as orelhas	Sacudir o corpo	Salar	Self-grooming	Vocalização
120:00											
135:00											
150:00											
165:00											
180:00											
120:00											
135:00											
150:00											
165:00											
105:00											

OBSERVAÇÕES: