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**Disbudding of dairy goat kids:  
Refining protocols to reduce or eliminate pain**

A thesis  
submitted in fulfilment  
of the requirements for the degree  
of

**Doctor of Philosophy in Biological Sciences**

at

**The University of Waikato**

by

**Melissa Nicole Hempstead**

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# Abstract

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The aim of this thesis was to investigate potential improvements for disbudding dairy goat kids to eliminate or reduce pain. Behavioural and physiological measures were used to evaluate: (1) pain associated with four disbudding methods, and (2) the effect of pain mitigation on cauterly disbudded goat kids. Caustic paste and cryosurgical disbudding and clove oil injection initially showed promise as less painful alternatives to cauterly disbudding. However, caustic paste and cryosurgical disbudding were found to be more painful, as cortisol concentrations and the frequencies of head-directed behaviours were higher than for cauterly disbudded kids. Clove oil injection appeared to cause no more pain than cauterly disbudding based on similar cortisol concentrations and head-directed behaviour, but caused less tissue damage. Alternatives to current practices must be efficacious in preventing horn or scur growth, therefore the effect of clove oil injection and two cauterly disbudding techniques (horn bud removed vs. left intact) on horn growth were evaluated. Clove oil was ineffective at preventing horns or scurs compared with cauterly disbudding. In addition, cauterly disbudding by removing the horn bud was more successful at preventing growth than leaving the horn bud intact. As using a cauterly iron to remove the horn bud appeared to be the most efficacious method evaluated in this thesis, the effect of isoflurane and meloxicam on pain associated with cauterly disbudding was investigated. Goat kids disbudded under isoflurane, with or without meloxicam, had lower cortisol concentrations and displayed fewer head-directed behaviours than goat kids that were cauterly disbudded without pain relief, suggesting that isoflurane can reduce pain associated with cauterly disbudding. Overall, the results of this thesis suggest that cauterly disbudding is an effective practice but should ideally be carried out using isoflurane to reduce pain; also, complete horn bud removal lowers the risk of horn or scur development. These results support the continued use of cauterly disbudding by the dairy goat industry but suggest pain relief could significantly improve the welfare of dairy goats on farm.

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# Chapter 1

## General introduction

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My PhD research involved using behavioural, physiological and production measures to evaluate pain associated with disbudding methods, and the effect of pain mitigation on cauterly disbudded dairy goat kids. Chapter 1 will provide the context for my thesis. I will overview the dairy goat industry in New Zealand and highlight the increasing consumer interest in the welfare of production animals. As the central theme of this thesis is the evaluation of pain, I will define pain associated with husbandry procedures and how pain can impact on welfare. In addition, I will describe the cauterly disbudding procedure and why it is performed, before discussing issues associated with the practice. Finally, I will outline the structure of my thesis by detailing the main objectives of each chapter.

## 1.1 Goat farming in New Zealand

Goats are predominantly farmed for milk, meat and fibre production; they also provide weed control of pastures (Batten, 2014). This thesis is focussed on dairy animals, so only they will be considered in detail. Dairy goat farming in New Zealand, is an emerging industry comprising approximately 92 farms running nearly 66,100 dairy goats (Scholtens et al., 2017). The predominant breed is purebred Saanen (or Saanen by Toggenburg crosses), which account for 97.5% of milking does (Solis-Ramirez et al., 2011). The number of farmed dairy goats in New Zealand is small compared with other production animals such as dairy cattle, sheep and beef cattle (totals in 2017: 6,474,500, 27,369,100 and 3,607,600, respectively; Stats NZ, 2017); there are benefits to raising goats for milk (considered below), which may see dairy goat numbers increase. The majority (approximately 80%) of goat milk suppliers belong to the Dairy Goat Co-operative (NZ) Ltd. (DGC), which is based in Hamilton, New Zealand. The key focus of the DGC is to produce nutritional powders for infants and young children (Solis-Ramirez et al., 2011; Stafford and Prosser, 2016). It is common in New Zealand and internationally, to house dairy goats indoors (to reduce parasite load; Stafford and Prosser, 2016) in open-sided barns; fresh cut pasture is brought to the barns and fed out daily (Solis-Ramirez et al., 2011). Dairy goats begin lactating after their first kidding, which is at approximately 1 year of age, a year earlier than dairy cattle. Dairy goats are typically milked twice daily. Furthermore, goats can lactate for multiple years without additional pregnancies (Stafford and Prosser, 2016), which can reduce the amount of stress on the animal associated with yearly mating and pregnancy. In comparison, dairy cows are mated and become pregnant on a yearly basis on New Zealand farms (Back, 2016). A healthy lactating doe will produce on average 2.7 L/d of milk (Anon., 2011), compared with that of dairy cows, which produce 15 L/d (on average) of milk depending on the stage of lactation (Back, 2016). For dairy goats, this equates to 25 – 126 kg of milk solids (kg MS) per lactation, in comparison with dairy cattle, which produce 317 – 416 kg MS yearly (Back, 2016).

### **1.1.1 Benefits of goat milk**

Increasingly, infants and young children are developing intolerances to replacements for human breast milk (e.g., cow milk; Arne, 1994; Heine et al., 2002). Consequently, there is increasing demand for alternatives such as goat milk, that are better for those with allergies or intolerances to cow milk (Bevilacqua et al., 2001; Lara-Villoslada et al., 2004); goat milk is lower in lactose, protein, and casein than cow milk (Jandal, 1996). In addition, goat milk has smaller fat globules (with a greater surface area) than cow milk, meaning goat milk fat is more readily digestible by lipases in the gut (Jandal, 1996). Furthermore, goat milk has other beneficial properties: it is alkaline (whereas cow milk is acidic), it is higher in vitamins A, B<sub>1</sub>, B<sub>12</sub> and C, and with faster protein digestion than cow milk, it is suitable for people suffering from eczema, asthma and stomach ulcers (Jandal, 1996; Haenlein, 2004). Overall, evidence suggests that goat milk may be a useful alternative to cow milk (Jandal, 1996; Haenlein, 2004; Lara-Villoslada et al., 2004; Park, 2007), which could mean that numbers of farmed dairy goats may increase, justifying the need for welfare research.

## **1.2 Animal welfare and pain**

Over recent decades, consumers have become increasingly aware of how production animals are farmed and as a consequence, there is increased demand for high quality products, which encompasses good health, food safety and respect for animal welfare (Verbeke and Viaene, 2000). Farming practices that negatively impact on animal welfare can not only affect farmers' social licence to operate (Barkema et al., 2015), but consumer choice and their willingness to purchase animal products (Verbeke and Viaene, 2000; McEachern et al., 2007; Toma et al., 2011; Heid and Hamm, 2012). Livestock farming in New Zealand is a major industry producing food for both national and international markets. As with many production systems, livestock farming can have negative implications for the environment, food safety and animal welfare (Stafford, 2016).

Defining animal welfare can be difficult as there are multiple interpretations (Stafford, 2013). There are many different philosophies of animal welfare that have been developed to establish how we should treat animals (Fraser, 2008). The Five Freedoms were formulated by the Farm Animal Welfare Council



and include freedom from (i) hunger, thirst and malnutrition, (ii) discomfort, (iii) pain, injury and disease, and (iv) fear and distress, and the freedom to (v) express normal behaviour patterns (FAWC, 1992). Animal welfare can be concerned with subjective animal feelings, particularly unpleasant feelings of suffering and pain (Dawkins, 1980). Suffering can occur when these subjective feelings are acute or last for an extended period (Dawkins, 1990). Generally, there are three overlapping areas that depict the quality of life or welfare of animals: (i) 'natural living', where the animal is provided with an environment to live as it would do naturally, (ii) 'feelings-based', which concerns the affective state or feelings and emotions of animals, including pain, fear, hunger and other negative states but also positive states, and (iii) 'functioning-based', which relates to health and functioning of an animal's biological systems (Fraser et al., 1997). Therefore, husbandry procedures that cause acute or prolonged pain can impact on animal welfare (Bath, 1998); it is important that efforts are made to reduce pain associated with painful procedures commonly undertaken on farm.

Pain in animals can be defined as an aversive sensory and emotional experience where an animal demonstrates awareness of potential or actual damage to tissues (Molony and Kent, 1997). There are difficulties in understanding animal pain due to the inability for animals to communicate in a way that can be easily interpreted by humans (Morton and Griffiths, 1985). Moreover, animals lack the ability to communicate the degree and location of pain (Anil et al., 2005). It is not unreasonable to think that if a negative stimulus would cause pain in humans, then it may cause a similar painful experience in other mammals (Anil et al., 2005). A further complication to understanding animal pain, is whether they outwardly display indicators of pain (Underwood, 2002; Anil et al., 2005). For example, prey species that are injured may conceal their pain to prevent detection by predators (Underwood, 2002). On the other hand, some animal species are quite expressive regarding their state; for example, goats have been observed to vocalise more often than other farmed species when separated from conspecifics (Lyons et al., 1993), and therefore their behaviour may be incorrectly interpreted as indicating a painful or stressful situation. Pain caused by husbandry procedures such as tail docking, castration, disbudding and dehorning has been evaluated for lambs (Mellor and Murray, 1989; Mellor et al., 1991), calves (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Faulkner and Weary, 2000; Stafford et al.,

2000; Sutherland et al., 2002a; Stewart et al., 2008; Coetzee et al., 2012), piglets (McGlone et al., 1993; Prunier et al., 2005; Carroll et al., 2006), adult goats (Ajadi et al., 2012; Hartnack et al., 2018) and goat kids (Alvarez and Gutiérrez, 2010; Ingvast-Larsson et al., 2011; Alvarez et al., 2015; Nfor et al., 2016; Hempstead et al., 2017, 2018).

Pain is a natural part of life and functions so that animals can learn and become motivated to avoid certain negative stimuli that may cause damage to their tissues (McMillan, 2003). Furthermore, pain can cause an animal to rest, allowing for recovery or repair of tissues (Bateson, 1991). In the case of disbudding, pain cannot be easily avoided but disbudding usually only occurs once. The long-term implications of pain associated with disbudding of goat kids are not well understood. In this thesis, I will examine potential indicators of pain that can be used to evaluate less painful (but equally efficacious) alternatives for disbudding goat kids, and pain mitigation strategies for cautery disbudding to improve the welfare of farmed dairy goats.

### **1.2.1 Identifying and interpreting animal pain**

There are different types of pain. Acute and chronic pain occur over different time courses; acute pain is usually immediate, whereas chronic pain can last for a longer period of time (Molony and Kent, 1997). Somatic pain is generally localised and associated with tissue damage that activates peripheral sensory neurons, whereas visceral pain is usually internal and leads to stimulation of the central nervous system (Molony and Kent, 1997; Vinuela-Fernandez et al., 2007). There is a difference between pain and nociception; nociceptors, which relay electrical impulses to the central nervous system, can detect stimuli and generate a response (or reflex) independent of the conscious experience of pain; pain can exist without nociception (Garry et al., 2004; Vinuela-Fernandez et al., 2007).

The first step to reducing pain is to correctly identify it. Identifying and assessing pain in animals can be difficult as responses to pain can vary not only between but also within a species (e.g., differences in sex, age, prior experiences, rearing environments), making consistent assessments difficult (Anil et al., 2002). As an example, the behaviour of goat kids in response to cautery disbudding has

been reported to be highly variable (Ingvast-Larsson et al., 2011; Hempstead et al., 2017), which may be associated with individuals experiencing different stages of ontogeny (Bekoff, 1972). A further difficulty in assessing pain, is that some measures may reflect other emotional states such as fear, stress or excitement (Colborn et al., 1991; Fazio et al., 2006); therefore, it is important to be conservative and cautious when interpreting indicators of pain. There is no single measure of pain, but many different measures that can be used in combination to give a better understanding of the internal experience of pain (Molony and Kent, 1997). Pain has been previously assessed using behavioural (Stafford et al., 2000; Sylvester et al., 2004), physiological (Sutherland et al., 2002b; Prunier et al., 2005) and production parameters (Carroll et al., 2006; Bates et al., 2016). Specific measures of pain associated with disbudding of calves and goat kids are reviewed in Chapter 2.

### **1.3 Cautery disbudding of dairy goat kids**

Usually within the first week of life, dairy goat kids will be cauterly disbudded to prevent horn growth (Smith and Sherman, 2009). Horns can be impractical in the farming context due to an increased risk of injury to other goats (Waiblinger et al., 2011) as well as human handlers, and increased space requirements in feeding and lying areas (Loretz et al., 2004). Cautery disbudding is most commonly carried out on doe kids that are used as replacement stock for milking (Smith and Sherman, 2009). Less buck kids are raised to replace the small number of adult bucks (kept for breeding purposes) and as a result, doe kids are more commonly disbudded. Therefore, I will refer to disbudding as being carried out on doe kids, unless otherwise stated. The most common method of disbudding involves using a hot cauterly iron to thermally destroy the horn buds; the practice is generally considered to cause acute and post-operative pain (Alvarez and Gutiérrez, 2010; Ingvast-Larsson et al., 2011; Alvarez et al., 2015; Hempstead et al., 2017, 2018). In addition to causing pain, cauterly disbudding can have other negative consequences for goat kids such as burns to the skin surrounding the horn buds, thermal injury to the skull and brain, necrosis of the skull and brain and meningoencephalitis (Wright et al., 1983; Sanford, 1989; Thompson et al., 2005), all of which impact on welfare. Therefore, as part of responsible animal

care and husbandry, ways to improve the practice that can reduce the negative effects on dairy goat kid welfare must be explored.

## **1.4 Aims of the thesis**

In this thesis, I use an experimental approach to investigate pain and pain management, with the ultimate goal of improving the welfare of goat kids undergoing disbudding. Welfare can be reduced when an animal experiences intense or prolonged pain (Bath, 1998). If painful husbandry procedures are necessary to improve management, then pain should be minimised.

In practical terms, I will identify potential improvements for disbudding of dairy goat kids to reduce or eliminate pain associated with the practice.

## **1.5 Thesis structure**

This thesis has investigated (i) alternatives to cautery disbudding that may cause less pain (and their efficacy), and (ii) pain mitigation strategies to reduce or eliminate pain associated with cautery disbudding.

Chapter 2 presents a review of the literature on cautery disbudding of goat kids. I will discuss the negative consequences of the procedure, variation in cautery techniques, pain assessment, whether less painful and efficacious alternatives exist and pain mitigation strategies to reduce or eliminate pain.

Chapters 3 and 4 are companion studies that evaluated alternatives to cautery disbudding that may cause less pain. The alternative methods that were evaluated were modelled on procedures developed for calves (i.e. cryosurgical and caustic paste disbudding, and clove oil injection). In Chapter 3, I investigated physiological measures of immediate and longer-term pain including (i) serum cortisol concentrations, (ii) serum haptoglobin concentrations, (iii) temperature of the skin surrounding the horn buds using infrared thermography and (iv) body weight. In Chapter 4, behavioural measures of post-treatment pain were investigated. The behaviours assessed were those that changed most in response to cautery disbudding (based on the work of Hempstead et al. [2017]): head shaking and scratching, self-grooming, body shaking and feeding behaviour.

The aim of the study presented in Chapter 5 was to evaluate the effect of alternative methods of disbudding on pain sensitivity around the horn buds, along

with skin, skull and brain injury. Chapter 5 represents the first study to use pressure algometry to measure pain sensitivity in goat kids, and to quantify skull or brain injury associated with disbudding methods (note that clove oil injection was not evaluated in this study as goat kids used for human meat consumption were used).

The study described in Chapter 6 evaluated the efficacy of cautery disbudding methods and clove oil injection on preventing horn or scur development in goat kids. A recent study reported that clove oil was successful in preventing horn growth of goat kids (Molaei et al., 2015); however, these reports required validation using a larger sample size and more systematic protocols.

Chapter 7 describes a study that evaluated the effect of isoflurane, with or without meloxicam, on the behaviour and physiology of goat kids following cautery disbudding. The overall objective was to identify pain mitigation strategies that may reduce or eliminate pain associated with cautery disbudding of goat kids. Studies have shown that nerve or ring blocks using local anaesthesia are ineffective at reducing pain associated with cautery disbudding of goat kids (Alvarez et al., 2009, 2015; Nfor et al., 2016). Therefore, other pain relief options including general anaesthesia and non-steroidal anti-inflammatory drugs were used. A further aim of Chapter 7 was to evaluate the effect of oral or injected meloxicam on pain associated with cautery disbudding, as it was anticipated that meloxicam would be more practical for use on farm.

Chapter 8 provides a summary of the research of this thesis and discusses the animal welfare implications and overall conclusions. Furthermore, I will consider limitations and areas of future research that have become apparent while completing this PhD.

Other than Chapters 1 and 8, all remaining chapters, of which I am first author, have co-authors; for each chapter, the nature of the contribution of each co-author has been described. There will be some repetition of topic introductions, methodologies and themes or concepts across chapters, as they are presented as separate research articles; however, every effort has been made to reduce repetition.

## 1.6 Ethical statement

Prior to each experiment, ethics approval from the Ruakura Animal Ethics Committee was granted. Power analyses were performed (as a requirement of applying for animal ethics) to estimate the minimum number of animals required to detect biologically important differences in the response variable. Every effort was made to reduce pain experienced by animals where appropriate by providing pain relief, except when the responses to pain were required using control animals. There are no alternatives to using animals, as the only models of active horn growth are on living animals. However, deceased animals were used initially to develop disbudding protocols before live animals were used.

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## Chapter 2

# Cautery disbudding of goat kids: Less painful alternatives and pain mitigation can improve goat welfare

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## 2.1 Introduction

Disbudding is one of many common painful husbandry procedures (e.g., castration, ear tagging) performed on goat kids usually with a hot cauterizing iron and within the first week of life. Cautery disbudding involves thermal destruction of the horn buds to prevent horn growth. The horn bud is a homogeneous, cartilage-like mass situated under an outer epidermal layer, extending to the periosteum of the frontal bone (Dove, 1935). With increasing age, the horn bud fuses with the frontal bone and the horn (which is made of keratin) becomes a bony extension of the skull. The horn eventually becomes hollow and forms a large cavity that leads to the frontal sinus. Goats have large sinuses that provide cushioning when their head collides with other goats during aggressive interactions (Farke, 2008). Adults with horns pose a risk to other goats (and their human handlers) during agonistic encounters (Tolu and Savas, 2007), leading to injuries (Waiblinger et al., 2011, 2012; Hartnack et al., 2018); horned goats can also damage farm facilities. Furthermore, horns increase the amount of space required at the feed rails and in lying areas (Loretz et al., 2004). It is for these reasons that goat kids are routinely disbudded.

Cautery disbudding of goat kids is widely considered to cause acute and post-operative pain (Alvarez et al., 2009; Ingvast-Larsson et al., 2011; Hempstead et al., 2017, 2018a). Pain experienced by production animals is a significant welfare concern of consumers, and as a consequence, interest in 'pain research' is increasing (Anil et al., 2005). Pain in humans is generally defined as an unpleasant sensory or emotional experience associated with actual or potential tissue damage (IASP, 1994). However, pain is a subjective experience that is difficult to measure reliably in animals, which are incapable of communicating in a way that humans can easily interpret (Morton and Griffiths, 1985). Therefore, the definition provided by Molony and Kent (1997) will be used for this review: "animal pain is an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal's physiology and behaviour to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery" (p. 266). The most effective way to evaluate animal pain is to use a multi-faceted approach, using a combination of indicators (Anil et al., 2002). Common measures of animal pain

include behaviour (e.g., changes in posture and temperament, stereotypical movements), physiology (e.g., plasma cortisol and lactate concentrations) and production (e.g., weight gain, reproductive success) (Faulkner and Weary, 2000; Anil et al., 2002; Landa, 2012).

In addition to causing pain, cautery disbudding can have other negative consequences for goat kids such as second- or third-degree burns (with associated ulcerations), thermal injury to and necrosis of the skull and brain (Allen et al., 2013a; Denler et al., 2014), and either thermal or bacterial meningoencephalitis (Wright et al., 1983; Sanford, 1989; Thompson et al., 2005). At worst, cautery disbudding can lead to kid mortality if performed incorrectly, in terms of excessive use (e.g., duration of application and force/pressure) of the cautery iron. Goat kids may have an increased risk of mortality compared to calves, as cautery disbudding was originally designed for use in calves, which are larger than goat kids. The skulls of kids are also thinner and more fragile than those of calves (Bowen, 1977; Mobini, 1991; Hartnack et al., 2018). Furthermore, the sinuses of goats are generally more developed than those of calves (Mobini, 1991). Therefore, penetration into the sinus by a cautery iron can occur more easily, which can lead to open cavities that increase the risk of infection (Hartnack et al., 2018).

Cautery disbudding is commonly carried out on goat kids less than a week of age as the horn buds are not yet attached to the underlying skull. Historically, it was believed that neonates did not feel pain or at least as much pain as adults due to an underdeveloped nervous system (Anand and Hickey, 1987). The conscious experience of pain involves a complex interaction between many neural regions and not merely a single pain-experiencing 'centre' (Derbyshire, 1999), which may explain the belief that neonates do not experience as much pain. However, neonates may experience even more pain than adults based on intense and prolonged responses to challenges (Mellor and Murray, 1989; Fitzgerald, 1991). In humans, neonates have dense nerve endings in the skin similar to or greater than that of adult skin, which suggests an enhanced capacity to sense noxious stimuli (Fitzgerald, 1991). There are many indicators of pain such as behavioural, hormonal and cardiovascular changes in neonates experiencing a noxious stimulus (Anand and Hickey, 1987). Note however, that castrated piglets of differing ages show little difference in their pain response (Taylor et al., 2001). Overall, it



appears that neonates may experience pain to a similar or even higher degree than adults; therefore, it is necessary to explore pain mitigation strategies for painful husbandry procedures performed on neonates, such as cautery disbudding.

Our aim is to critically review approaches that may reduce or eliminate pain associated with cautery disbudding of goat kids. Where goat kid literature is sparse, we will include reference to calf disbudding literature; however, while the calf literature is extensive, caution is taken in its application due to physical and behavioural differences between goat kids and calves. We will start by outlining (i) cautery disbudding techniques, (ii) how existing methods can vary, (iii) differences in disbudding success across methods and (iv) recommended best practice. Then, we will evaluate (v) how pain associated with cautery disbudding of goat kids can be measured and (vi) alternatives to cautery disbudding of goat kids that may be less painful but equally, or more, efficacious; these alternatives include the development of novel disbudding methods (e.g., clove oil injection), pain mitigation strategies for cautery disbudding, management of goats with horns, breeding polled goats and dehorning later in life.

## **2.2 Cautery disbudding techniques**

Disbudding using a cautery iron is the most common method employed worldwide for calves (Misch et al., 2007; Gottardo et al., 2011; Cozzi et al., 2015; Winder et al., 2016; Staněk et al., 2018) and goat kids (Alvarez and Gutiérrez, 2010). Cautery irons are usually heated to extreme temperatures (i.e. reaching 600°C) and are designed to quickly sear the skin and destroy the horn buds. There are two techniques of cautery disbudding.

### **2.2.1 Removing the horn bud**

The horn bud is usually cut by pressing the hot, sharp edge of the tip of the iron through the skin for a total of approximately 6 s (our unpublished data); the iron is usually twisted to facilitate a rapid, clean cut. The ring of tissue containing the horn bud is then removed by being forcibly flicked off the head (Stewart et al., 2009; Mintline et al., 2013; Alvarez et al., 2015; Huebner et al., 2017). By removing the horn bud, it is easier to verify complete destruction of the horn bud; horn bud removal can also reduce the occurrence of scurs (Smith and Sherman,

2009a). Scurs are defined as secondary regrowth of the horn bud cells that were not completely destroyed during disbudding (Hartnack et al., 2018).

### **2.2.2 Leaving the horn bud intact**

Another cautery technique commonly used for calves (Vickers et al., 2005; Stock et al., 2015) and goat kids (Alvarez et al., 2009; Alvarez and Gutiérrez, 2010) involves leaving the horn bud intact after cauterisation; this technique may cause less pain than burning, cutting and removing the skin containing the horn bud (but note that due to the destruction of the nociceptors innervating the horn bud, this point is arguable). A cautery iron, usually with a bunt tip, is pressed down to cauterise the horn bud (with or without cutting the skin). Disbudding in this manner is typically considered successful when a copper-coloured ring around the horn bud is observed (Smith and Sherman, 2009a). However, with this method, it can be more difficult to determine whether sufficient cauterisation of the horn bud has been carried out to prevent scurs.

### **2.2.3 Which is more efficacious: Horn bud removal or buds left intact?**

Recently, we investigated whether disbudding using an iron to cauterise and remove the horn bud was more effective in preventing scurs than leaving them intact (our unpublished data). Removing the horn bud had a higher probability of success (with exact 95% CI) in preventing horns or scurs (0.77 [0.63 – 0.87]) than leaving the bud intact (0.20 [0.11 – 0.34];  $P \leq 0.05$ ). Unusual growths termed ‘scorns’ (neither horn nor scur) that resembled a lump of fibrous tissue, often grew in place of a horn when horn buds were left intact; in this case, the probability of scorns was 0.41 (0.25 – 0.60). Overall, removing the horn bud appears more efficacious for preventing horns and scurs but more research is required to evaluate pain associated with each cautery disbudding technique.

### **2.2.4 Regulations and recommendations for cautery disbudding**

Countries belonging to the European Union generally have high animal welfare standards yet there is no legislation pertaining to the use of pain relief for disbudding of goat kids. In the United Kingdom, disbudding is considered a

veterinary surgery meaning that only veterinarians are permitted to disbud goat kids (Veterinary Surgeons Act, 1966) and usually with pain relief. Although there are no legislations for disbudding goat kids in New Zealand yet (current policies are under review), it is recommended that pain relief is used (National Animal Welfare Advisory Committee, 2005). Australian welfare standards include the use of pain relief for disbudding calves that are more than 6 months of age (Animal Health Australia, 2014) but there is no standard for goats. In the United States, there are few laws or regulations for production animals other than for during transport and at slaughter (Matheny and Leahy, 2007).

Standard cautery disbudding practice differs worldwide depending on many factors such as legislation, public perception, type of iron used, age of kids, veterinary input and training received. The steps and characteristics of cautery disbudding in goat kid and calf studies are presented in Tables 2.1 and 2.2; these tables show the level of variation in technique across studies, which may affect the amount of pain or injury caused. For example, the amount of time the iron is applied to each horn bud can vary between 4 and 30 s for goat kids and 3 and 60 s for calves. Furthermore, the information in Tables 2.1 and 2.2 highlight the information not provided; for example, almost half of the publications referenced do not state whether the horn bud was or was not removed).

Evidence suggests that disbudding of calves is largely performed by farm personnel (Misch et al., 2007; Cozzi et al., 2015; Winder et al., 2016). In addition, one study reported that 70% of farm personnel did not receive any specific training on how to perform cautery disbudding (Gottardo et al., 2011). The benefit of improved technical skill has been demonstrated by those that received hands-on versus on-line training to administer local anaesthetic and disbud a calf (Winder et al., 2017a, 2018). To the authors' knowledge, there are no published scientific papers on the level of training received by farm staff or contractors for disbudding goat kids. It is vital that operators receive training from a veterinarian or other qualified person on how to safely and effectively disbud goat kids to reduce pain and injury (Gottardo et al., 2011; Staněk et al., 2018).

Restraint can cause stress that may add to the pain associated with cautery disbudding (Goonewardene et al., 1999; Graf and Senn, 1999; Stafford and Mellor 2011). There seems to be little variation in the type of restraint across studies; goat kids are generally held with their heads immobilised (Table 2.1), whereas

calves, on account of their size, are typically restrained using devices such as a head bale or calf crush (Table 2.2). Therefore, careful handling and proper restraint by using either a restraint device or another handler is best.

The iron should reach maximal temperature (usually around 600°C), which has been used in multiple calf (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Faulkner and Weary, 2000) and kid studies (Alvarez and Gutiérrez, 2010; Ingvast-Larsson et al. 2011; Hempstead et al., 2018a) as this ensures proper cauterisation of the horn bud and surrounding skin and reduces the risk of bleeding (Petrie et al., 1996; Gottardo et al., 2011). There is little variation in the temperature of the iron used to disbud calves (Table 2.2) or goat kids (Table 2.1). Although, more recently goat kids were disbudded using an iron that reached a maximum temperature of 326°C (Nfor et al., 2016). Future research should evaluate the effect of different temperatures on pain, horn bud tissue damage and the efficacy in preventing horns or scurs for goat kids. It is vital that excessive pressure or application times of the iron is not used as this can damage the skull and transmit heat to the brain (Wright et al., 1983; Sanford, 1989; Thompson et al., 2005).

Operators generally apply the iron once to each horn bud of calves (Table 2.2), but up to 3 – 4 times have been reported for goat kids (Table 2.1). To reduce the risk of heat transference to the brain, it is recommended that time is allowed for the head to cool between applications of the iron (Alvarez and Gutiérrez, 2010). Ideally, pain relief should be administered. For disbudded calves, it has been shown that giving a local anaesthetic under sedation (making administration of a local anaesthetic easier) and a systemic analgesic (i.e. non-steroidal anti-inflammatory drug; NSAID) can markedly reduce pain (Faulkner and Weary, 2000; Stafford and Mellor, 2005). General anaesthetic (isoflurane) with or without an NSAID (meloxicam) has been shown to reduce pain associated with cautery disbudding of goat kids (Hempstead et al., 2018a).

Application of a topical antibiotic to the horn buds once the procedure is complete, may reduce the risk of infection (Nfor et al., 2016; Hempstead et al., 2018a, b). It seems to be much more common in goat kids than calves to apply topical antibiotic to the cautery wounds (Tables 2.1 and 2.2). Occasionally, producers will administer a prophylactic antibiotic to further reduce the risk of infection (Smith and Sherman, 2009a). Careful monitoring of the goat kids for up

to 3 wk after disbudding can help to identify infection or other negative consequences (Wright et al., 1983; Sanford, 1989; Thompson et al., 2005). From the reviewed literature for cautery disbudding of calves and goat kids, it appears that cautery disbudding methodology can be hugely variable (Tables 2.1 and 2.2). Key factors that may affect the amount of pain and injury associated with the cautery iron include operator training, restraint, maximum temperature, duration of application and pressure of the iron, use of pain relief, and post-operative care.

### **2.3 Measuring pain associated with disbudding**

Pain associated with cautery disbudding can be measured by assessing behaviour, physiology or production. Behavioural measures of pain can be useful as they are non-invasive (i.e. can be monitored without manipulating the animal), and human influences on behaviour can be minimised by concealing observers or using cameras (Graf and Senn, 1999; Chandrahas et al., 2013; Hempstead et al., 2017). Furthermore, behaviour can be specific to a noxious stimulus and directed at the site of the stimulus or wound (Rutherford, 2002); for example, head shaking increases after cautery disbudding of goat kids (Hempstead et al., 2018a) and leg extensions increase following ring castration in lambs (Mellor and Murray, 1989). However, identifying and quantifying behaviour can be subjective as it is dependent on the interpretation of the observer; this makes tests of intra- and inter-observer reliability crucial. Many papers discussed in this review did not provide a measure of reliability (e.g., Chandrahas et al., 2013; Nfor et al., 2016); analysis of reliability should be applied more widely in future studies to ensure accuracy of the data. While behaviour can be a useful measure of pain in animals, it is best used in conjunction with other measures to provide a more complete understanding of animal pain.

**Table 2.1.** Cautery disbudding methodology reported in the goat kid publications noted. Categories include age (d) and sex of animal, type of restraint, hair removal (Y/N), type of iron, iron temperature (°C), total application time per horn bud (s), number of applications per horn bud, horn bud removal (Y/N) and anti-bacterial use (Y/N).

Age of animal (d)	Sex (F/M or both)	Restraint type	Hair clipped (Y/N)	Type of iron	Iron temperature (°C)	Application time per horn bud (s)	Number of applications	Horn bud removed (Y/N)	Anti-bacterial use (Y/N)	Reference
7 - 28	Both	NA	NA	NA	NA	15 - 30	NA	NA	NA	Greenwood and Shutt (1990)
11 - 20	NA	Head immobilised by handler with legs free to move	Y	Electric - 220 V	600	9 - 16	3 - 4	N	Y	Alvarez et al. (2009)
< 20	NA	Held by a handler, head immobilised	Y	Electric - 220 volts	600	9 - 16	3 - 4	N	Y	Alvarez and Gutiérrez (2010)
17 - 19	Both	NA	NA	Electric	600	7 - 15	1	NA	NA	Ingvast-Larsson et al. (2011)

14 - 15	Both	NA	NA	NA	NA	NA	NA	NA	NA	NA	Chandrabhas et al. (2013)
10 - 20	Both	Held by the handler	NA	Electric - Goat dehorner, Lenk®	NA	6 - 16	3 - 4	Y	Y	Alvarez et al. (2015)	
4 - 9	Both	Head raised and legs loosely held	Y	Electric	295 - 326	4 - 7	1	NA	Y	Nfor et al. (2016)	
2 - 6	F	Restraint device and head immobilised	Y	Electric - 'Quality' debudder, 230 volts	600	≤ 8	1	Y	Y	Hempstead et al. (2017)	
2 - 7	F	Restraint device and head immobilised	Y	Electric - 'Quality' debudder, 230 volts	600	≤ 6	1	Y	Y	Hempstead et al. (2018a)	
9 - 14	F	Restraint device and head immobilised	Y	Electric - 'Quality' debudder, 230 volts	600	5 - 7	1	Y	Y	Hempstead et al. (2018b)	

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NA: not available as the information was not provided.

**Table 2.2.** Cautery disbudding methodology reported in the calf publications noted. Steps include age (wk) and sex of animal, type of restraint, hair removal (Y/N), type of iron, iron temperature (°C), total application time per horn bud (s), number of applications per horn bud, horn bud removal (Y/N) and anti-bacterial use (Y/N).

Age of animal (wk)	Sex (F/M or both)	Restraint type	Hair removal (Y/N)	Type of iron	Iron temperature (°C)	Application time per horn bud (s)	Number of applications	Horn bud removed (Y/N)	Antibacterial use	Reference
8	M	NA	NA	Electric	600	60	NA	NA	NA	Morisse et al. (1995)
6 - 8	M	Calf crush	NA	Gas-powered	NA	3 - 5	1	NA	NA	Petrie et al. (1996)
4 - 6	Both	Handler held calf against pen wall	NA	Electric - 'Safety-First' A-Super-Vario	600	10 - 20	1	Y	NA	Graf and Senn (1999)
4 - 6	Both	NA	NA	Electric - Kruuse 190 W	600	15	1	NA	NA	Grondahl-Nielsen et al. (1999)
4 - 8	Both	None - sedated	NA	Electric - Rhinehart X30	600	35	NA	NA	NA	Faulkner and Weary (2000)
1 - 5	F	NA	NA	Electric - Rhinehart X30	600	15	NA	N	NA	Vickers et al. (2005)



5 - 7	F	Head bail	NA	Gas-powered - ABER LPG debudder	NA	NA	NA	Y	NA	Stewart et al. (2008)
4 - 6	Both	Head bail	NA	Gas-powered - ABER LPG debudder	NA	NA	NA	Y	NA	Stewart et al. (2009)
4 - 6	F	NA	NA	Butane- powered	NA	20 - 30	NA	NA	NA	Stilwell et al. (2010)
6 - 12	F	NA	NA	Electric - Rhinehart X30	600	NA	NA	NA	NA	Heinrich et al. (2010)
8 - 10	M	NA	NA	Electric - Rhinehart X30	600	NA	NA	NA	NA	Allen et al. (2013b)
4 - 5.5	Both	NA	NA	Gas-powered - ABER LPG debudder	NA	NA	NA	Y	NA	Mintline et al. (2013)
4 - 6	Both	NA	NA	Butane- powered - Express dehorner	600	10	1	N	NA	Stock et al. (2015)
3 - 6	F	Calf crush	NA	Cordless, gas-filled	NA	12 - 15	1	Y	Y	Bates et al. (2016)

1 - 3	F	Handler stood over calf	Y	Butane- powered - Portasol Calf Dehorner III	NA	5	NA	Y	Y	Huebner et al. (2017)
1 - 4	M	NA	NA	Electric - LötKolben 230 V	600	NA	NA	NA	NA	Mirra et al. (2018)

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NA: not available as the information was not provided.

Physiological measures of pain are objectively quantifiable and are usually analysed in commercial laboratories (that can be made blind to treatments) using standardised assays. Cortisol is a useful indicator of pain in goat kids; however, cortisol is an indirect measure of pain (Molony and Kent, 1997; Anil et al., 2002), and should be used together with other physiological and behavioural measures. However, physiological changes usually occur in response to a suite of factors, not just pain (e.g., fear, stress or excitement) (Colborn et al., 1991; Fazio et al., 2006); physiological assessments can also be costly to obtain, as they usually require either multiple samples for each animal or specialised personnel, equipment and software.

Production measures such as reductions in body weight or slow growth rates can result as a consequence of pain and can be objectively measured (Morton and Griffiths, 1985); body weight changes are usually associated with reduced feed intake (Landa, 2012), which may reflect pain. However, these changes in body weight can be due to factors in addition to food and fluid consumption, including changes in metabolic rate and decreased gut absorption (Morton and Griffiths, 1985).

## **2.3.1 Behaviour**

### **2.3.1.1 During disbudding**

Goat kids that were disbudded struggled more frequently and vocalised more intensely than handled controls (Alvarez et al., 2009, 2015; Nfor et al., 2016). Furthermore, cautery disbudded kids kicked more often than those that were only handled (Alvarez and Gutiérrez, 2010; Chandrahas et al., 2013). In contrast, calves have been reported to attempt to escape by moving backwards, rearing up and ventral falling (Morisse et al., 1995; Graf and Senn, 1999). Escape behaviour may not be observed in goat kids due to comparative ease of restraining small goat kids. Calves have also been reported to exhibit frequent ear flicks, head jerks, vocalisations and tail shaking or flapping as well as leg stretching during the procedure (Morisse et al., 1995; Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Stilwell et al., 2010).

### 2.3.1.2 Post-disbudding

Head shaking, head scratching durations, body shaking and head rubbing were more frequent in cauterly disbudded kids than in sham-handled controls (Hempstead et al., 2017, 2018a). Head-directed behaviours such as head rubbing or scratching may be an attempt to reduce pain associated with a cauterly burn, by activating low-threshold mechanoreceptors. In human examples, a sharp pain can be reduced by vigorously rubbing the site of injury (Purves et al., 2001). Greenwood and Shutt (1990) reported that head shaking and isolation from other kids was observed post-disbudding, but lasted only a short period (i.e. 5 min). More recently, kids have been shown to vigorously shake and scratch their heads for up to 1 h post-treatment (Hempstead et al., 2018a). Similarly, calves performed higher rates of head shaking, head scratching and ear flicking and were generally more active, in comparison to handled controls or calves provided pain relief (Morisse et al., 1995; Grondahl-Nielsen et al., 1999; Heinrich et al., 2010; Stilwell et al., 2010). Calves disbudded without pain relief have been reported to perform more head shakes and ear flicks than calves disbudded with pain relief for up to 44 h post-treatment (Faulkner and Weary, 2000; Heinrich et al., 2010). Note that ear flicking, a useful indicator of pain in calves (Faulkner and Weary, 2000), has not been found to be a useful indicator of pain in goat kids; this may be associated with the relatively rapid ear movement of kids, making reliable recording of this behaviour difficult (Hempstead et al., 2017).

Other post-disbudding behavioural changes observed in disbudded goat kids included greater standing bouts with higher instances of exploring and biting structures within their pen, compared with kids disbudded and provided with meloxicam (Chandrasah et al., 2013); Chandrasah et al (2013) suggested these behavioural responses were due to increased restlessness or reduced comfort. Calves that were disbudded with either a cauterly iron or caustic paste performed frequent periods of standing up and lying down, which may also reflect restlessness (Morisse et al., 1995). Interpretations of lying behaviour can be difficult as changes can reflect pain in some species but not others; for example, cryosurgically disbudded calves lay more than cauterly disbudded calves suggesting less discomfort (Stewart et al., 2014), whereas castrated piglets spent more time lying than uncastrated piglets suggesting greater pain was experienced (McGlone et al., 1993).

Ingvast-Larsson et al. (2011) used a visual analogue scale (VAS) to rank animals on a scale of 1 (no signs of pain) to 10 (severe signs of pain); scores were based on a suite of behaviours (e.g., vocalisations, head shakes, scratches) and were used to measure pain in kids disbudded with and without pain relief (i.e. meloxicam). The VAS scores were higher for kids that were disbudded without meloxicam, suggesting more pain was experienced (Ingvast-Larsson et al., 2011). However, Ingvast-Larsson et al. (2011) reported that individual differences in behaviour were large, perhaps as the kids were sedated for up to an hour after disbudding; sedation may have prevented the expression of behaviour within this time. Variation in behaviour within treatment groups has also been reported by others (Greenwood and Shutt, 1990; Hempstead et al., 2017), which indicates the variable nature of the behaviour of young goats; a multifaceted approach to evaluating pain may be required in such cases. Play behaviour (e.g., running, bucking, head-to-head contact) in disbudded calves was less frequent than in handled controls over a 3 h period (Mintline et al., 2013), but similar studies have not been performed for goat kids. In summary, changes in frequency or duration of select behaviours appear to indicate pain associated with cautery disbudding across species and studies.

Pressure algometry has been used in cows, humans, and horses to objectively measure pain associated with inflammation or soft tissue injury (Varcoe-Cocks et al., 2006; Fitzpatrick et al., 2013; Pelfort et al., 2015). Pressure algometry measures the amount of pressure tolerated by an animal before it withdraws from the algometer; this is called the mechanical nociceptive threshold (MNT). Disbudded calves had lower MNT values and therefore were more sensitive to pressure after cautery disbudding than handled controls (Heinrich et al., 2010; Allen et al., 2013b; Stock et al., 2015). Similarly, goat kids showed increased sensitivity (i.e. lower MNT) of the tissues surrounding the horn buds after cautery disbudding (our unpublished data). Von Frey monofilaments can also be used to measure wound sensitivity associated with disbudding; this method was developed using the same principles as pressure algometry and involves assessing the animal's response (i.e. move away) to a painful stimulus (Mintline et al., 2013). The monofilaments use a predetermined and repeatable force; this procedure has been used to quantify pain sensitivity in disbudded calves (Mintline et al., 2013; Mirra et al., 2018), tail docked pigs (Sandercock et

al., 2011) and mulesing in lambs (Lomax et al., 2009). Pressure sensitivity tests show promise for evaluating pain in goat kids.

### **2.3.2 Physiology**

Physiological changes, such as increases in cortisol concentrations have been frequently measured to assess pain in cauterly disbudded goat kids (Greenwood and Shutt, 1990; Alvarez et al., 2009; Ingvast-Larsson et al., 2011; Nfor et al., 2016; Hempstead et al., 2018a, b) and calves (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Faulkner and Weary, 2000; Allen et al., 2013b). The activity of the hypothalamic pituitary adrenal axis can be activated by stressful experiences such as pain. The main glucocorticoid hormone released is cortisol, which can be measured, for example, in saliva, plasma or serum (Landa, 2012). Plasma cortisol concentrations of disbudded goat kids were elevated above the levels of handled controls (by approximately 40 nmol/L) for up to 2 h after disbudding, reaching a maximum of 190 nmol/L (Greenwood and Shutt, 1990; Alvarez et al., 2009; Alvarez and Gutiérrez, 2010; Nfor et al., 2016). Our recent work showed that plasma cortisol concentrations of disbudded kids were numerically higher, on average, than sham-handled kids 15 min post-treatment, and peaked at nearly 300 nmol/L (Hempstead et al., 2018a). Differences in methodology may account for differences in cortisol concentrations across studies, such as the age of kids used (10 – 20 d old kids by Alvarez et al. [2015] vs. 3 d old kids by Hempstead et al. [2018a]). In some cases, no differences in plasma cortisol concentrations occur between cauterly disbudded and sham-handled kids for up to 3 d post-treatment (Ingvast-Larsson et al., 2011; Hempstead et al., 2018b). Cortisol can be highly variable depending on the stage of development of goat kids (Chen et al., 1999). In order to take invasive samples (e.g., blood), animal restraint is often required, which has also been shown to influence cortisol concentrations (Goonewardene et al., 1999; Graf and Senn, 1999). Measuring cortisol in saliva may reduce stress associated with handling and blood sampling; however, collecting the required volume over consecutive samplings is difficult in goat kids (Greenwood and Shutt, 1990).

Immunoreactive beta-endorphin concentrations of cauterly disbudded kids peaked 5 min post-disbudding; however, no comparisons with handled controls were made (Greenwood and Shutt, 1990). Beta-endorphins increased in response

to mulesing of lambs (Paull et al., 2008), but the same trend was not observed in amputation dehorned cattle (Cooper et al., 1995). Higher beta-endorphin concentrations were found in calves disbudded at 1 wk of age than 4-wk old calves (Mirra et al., 2018). Beta-endorphins act to suppress the flow of noxious stimuli and can dampen the experience of pain (Derbyshire, 1999); therefore beta-endorphins should be further explored in disbudded goat kids as increases may indicate pain.

Plasma lactate and glucose concentrations have not been shown to increase in response to cautery disbudding of goat kids (Ingvast-Larsson et al., 2011; Hempstead et al., 2018a). Glucose is synthesised by gluconeogenesis, which is stimulated by cortisol and other glucocorticoids (Khani and Tayek, 2001). Lactate is utilised during gluconeogenesis in the liver, and once converted to glucose, is transported to the muscles, where glucose is reduced to lactate (Exton and Park, 1967). Lactate has been shown to increase in response to cortisol secretion in pigs, as stress causes immobilisation of glycogen stores (Brown et al., 1998; Hambrecht et al., 2004). Prunier et al. (2005) reported an increase in lactate in response to castration of piglets, but this same response was not observed in disbudded goat kids (Hempstead et al., 2018a). Lactate may not be a sensitive enough measure of stress or pain in goat kids. Glucose did not increase in response to castration of piglets (Prunier et al., 2005) or cautery disbudding of goat kids (Ingvast-Larsson et al., 2011; Hempstead et al., 2018a). These results may be due to insufficient glycogen stores in young animals (Heymann and Modic, 1939), or the maintenance of constant blood glucose by insulin (Steffens, 1970).

Haptoglobin is an acute phase protein that acts as an inflammatory biomarker as it increases in response to inflammation either from disease or non-infectious causes such as tissue injury (Heller and Johns, 2015). No increases in haptoglobin concentrations associated with cautery disbudding have been observed in goat kids (Hempstead et al., 2018b) or calves (Allen et al., 2013b; Ballou et al., 2013); however, surgically castrated calves showed an increase in haptoglobin 24 h after the procedure (Ballou et al., 2013). The increase in both cortisol and haptoglobin of surgically castrated calves (Ballou et al., 2013) may be associated with a greater intensity of acute pain compared with cautery disbudding. Cortisol release is known to affect inflammatory processes (e.g., suppression of proinflammatory cytokine production) associated with wound healing (Derbyshire, 1999; Christian et al., 2006), which may explain the lack of

differences observed in haptoglobin concentrations of cautery disbudded goat kids and calves. In addition, elevation in skin temperature measured by infrared thermography can indicate inflammation of the procedural site in cautery disbudded goat kids (Hempstead et al., 2018b) and hot-iron branded calves (Schwartzkopf-Genswein and Stookey, 1997). Cautery disbudded goat kids had higher skin temperatures around the horn bud than 'handled only' kids 72 h post-treatment (Hempstead et al., 2018b). Cattle that were hot-iron branded showed higher skin temperatures around the site of branding than calves that were freeze-branded (Schwartzkopf-Genswein and Stookey, 1997). Therefore, infrared thermography appears to detect inflammatory pain across species and may be a more sensitive measure compared to elevations of acute phase proteins.

Heart and respiration rates are regulated by the sympathetic nervous system and can increase in response to stressful or painful stimuli (Porges, 1995); these increases have not been observed in response to cautery disbudding of goat kids (Alvarez et al., 2009; Alvarez and Gutiérrez, 2010; Nfor et al., 2016). Although, Nfor et al. (2016) reported that heart rate was lower in kids disbudded under sedation with dexmedetomidine hydrochloride, which is likely an effect of sedation on the cardiovascular system (i.e. bradycardia; Dugdale, 2011). Heart rate increased in calves after cautery disbudding (Grondahl-Nielsen et al., 1999; Stewart et al., 2009) as measured using continuous heart rate monitors and electrocardiogram recordings; in comparison, the goat kid studies used a stethoscope, which requires handling that can affect heart rate (Alvarez et al., 2009; Alvarez and Gutiérrez, 2010; Nfor et al., 2016). In future goat kid studies, automated heart/respiration rate monitors attached to the kid may more accurately detect differences in heart or respiration rate and may serve as a useful non-invasive measure of pain in goat kids.

Physiological estimators of pain that have been evaluated in calves, but not yet in kids, include cytokines (Mirra et al., 2018), substance P (Coetzee et al., 2012), prostaglandins (Stock et al., 2015), adrenocorticotrophic hormone (ACTH) and vasopressin concentrations (Graf and Senn, 1999) and ocular temperature (Stewart et al., 2008, 2009). There was no difference in cytokines (tumour necrosis factor alpha, interleukin-1 $\beta$ , and interleukin-6) or substance P across cautery disbudded and control calves (Stock et al., 2015; Mirra et al., 2018). Inflammatory pain resulting from tissue injury generally causes a release of chemicals including substance P, serotonin and histamine (Dahl and Kehlet, 1991),



but inflammation associated with cautery disbudding may not be severe enough to elevate these chemicals. Prostaglandin E<sub>2</sub> was reported to be lower in calves disbudded with pain relief than disbudded controls (Stock et al., 2015). Both ACTH and vasopressin were elevated in calves disbudded without pain relief in comparison to those provided pain relief (Graf and Senn, 1999). Furthermore, eye temperature rapidly decreased immediately following disbudding of calves compared with calves provided a local anaesthetic (Stewart et al., 2009). It is uncertain whether these measures of pain would be practical for use in goat kids as blood constituents can be variable in young kids (Chen et al., 1999); however, infrared thermography of the eye may be useful for goat kids as it can detect elevations in skin temperature (Hempstead et al., 2018b).

### **2.3.3 Production**

To the authors' knowledge, production measures (e.g., weight gain, reproductive success) have yet to be compared between goat kids that have been disbudded and those that have not, or between goat kids disbudded with pain relief and those without. Therefore, it is necessary to turn to the calf literature to gain insight into how disbudding methodologies might impact on goat production measures. Bates et al. (2016) reported that calves disbudded without pain relief showed lower weight gains than calves disbudded with pain relief. However, others have reported no differences in growth rate or feed intake between disbudded and control calves (Grondahl-Nielsen et al., 1999; Stock et al., 2015). Generally, changes in body weight are associated with changes in feeding motivation and feed intake (Morton and Griffiths, 1985). There is evidence to suggest that pain associated with castration of piglets can cause an increase in feeding behaviour as the process of suckling can have analgesic effects that may impact weight gain (Noonan et al., 1994). In addition to feed intake and impaired growth associated with acute pain, chronic pain can have negative effects on other productivity measures such as immune functions, milk yield and fertility (Anil et al., 2005). Clearly, research effort needs to be directed at examining correlations between pain associated with practices such as disbudding and both goat production and overall well-being.

### **2.3.4 Current and future indicators of pain associated with disbudding of goat kids**

Behavioural measures such as head shaking and head scratching, wound sensitivity tests (e.g., pressure algometry) and cortisol concentrations have shown to differ between cauterly disbudded and handled kids consistently across studies (Alvarez et al., 2009; Alvarez and Gutiérrez, 2010; Alvarez et al., 2015; Hempstead et al., 2018a, b), therefore they appear to be useful indicators of pain. In addition, cytokines, substance P, prostaglandins, ACTH, vasopressin and ocular temperature appear to detect pain in calves (Graf and Senn, 1999; Stewart et al., 2009; Coetzee et al., 2012; Stock et al., 2015; Mirra et al., 2018) and may have application for goat kids in future research.

## **2.4 Alternatives to cauterly disbudding**

### **2.4.1 Raising horned goats**

As previously discussed, adult goats with horns can be problematic for farmers (e.g., injuries to handlers and other goats, increased space requirements), explaining why goat kids are commonly disbudded. Nevertheless, one alternative to cauterly disbudding that can eliminate pain associated with the practice, is to raise goats with horns. Not only would this save producers time and money (e.g., reducing time requirements and the need to employ contractors) at a very stressful time (i.e. kidding), it would eliminate pain and injury associated with disbudding. However, this would require farmers to adapt facilities and management systems for horned animals. Careful facility design can reduce the risk of aggressive encounters that may cause injury (Waiblinger et al., 2011). For example, in commercial dairy goat systems, greater space allowances in lying areas (or a lower stocking density) within barns or the provision of outdoor space would allow goats to move away from instigators of conflict. Furthermore, having wider horizontal feed rails (or palisade feed barriers) to accommodate for horned goats, may also reduce the risk of injury. Although horned goats showed a higher incidence of udder injuries, farms that had lower numbers of milking does with less mixing of mobs during lactation, showed lower rates of injury (Waiblinger et al., 2011). Other research showed that horned goats displayed more avoidance behaviour and threats than hornless animals (Aschwanden et al., 2008; Hillmann

et al., 2014); furthermore, hornless animals attacked other goats more frequently (Aschwanden et al., 2008; Hillmann et al., 2014). Cattle with horns can also be managed to reduce the risk of agonistic behaviour and injury (Menke et al., 1999).

#### 2.4.2 Polled goats

Another alternative to cauterly disbudding is to breed polled animals that do not grow horns. Polled goats tend to have bony ‘knobs’ in place of horn buds (Figure 2.1), which can occasionally be mistaken as horns and disbudded unnecessarily (Dove, 1935). A polled line of dairy cattle is available to farmers (LIC, 2018) but some evidence suggests that farmers have concerns with sire quality and therefore this line is rarely used (Winder et al., 2016). A polled line of goats is not yet available due to developmental abnormalities; polled intersex syndrome (PIS) occurs frequently in goats (Szatkowska et al., 2014) and the PIS mutation links polledness and intersexuality or hermaphroditism (Pailhoux et al., 2001), which affects fertility of both the does and bucks. New genetic techniques or selective breeding to establish a polled line of goats that is not associated with PIS, may prevent the need for disbudding.



**Figure 2.1.** A naturally polled goat with bony ‘knobs’ in place of horns.

### **2.4.3 Dehorning**

Adult goats that were not disbudded as kids can be dehorned by removing horns with either a saw or obstetrical wire (Smith and Sherman, 2009a). Dehorning causes significantly more pain than disbudding (Hague and Hooper, 1997; Stafford and Mellor, 2005). Furthermore, dehorning can prolong healing, and lead to complications such as discharge or infection, inflammation, horn regrowth, dehiscence or even death (Hague and Hooper, 1997; Hartnack et al., 2018). For these reasons, disbudding is the preferred method over dehorning.

### **2.4.4 Alternative disbudding methods**

Alternatives to cautery disbudding need to be equally effective at preventing horns or scurs while causing less pain; alternative methods should also promote rapid healing. Caustic paste disbudding usually involves rubbing a sodium or calcium hydroxide-based paste (Stafford and Mellor, 2011) into the horn buds. The corrosive action of caustic paste will generally last for as long as it is in contact with the skin (Palao et al., 2010). Caustic paste appears to cause pain in calves for up to 4 h as evidenced by higher cortisol concentrations, head rubs and shakes, heart rate and sensitivity around the horn buds in comparison to sham-disbudded controls (Vickers et al., 2005; Stilwell et al., 2009; Winder et al., 2017b). Although, in comparison to cautery disbudding, caustic paste has been suggested to cause less pain in calves due to less head shaking post-treatment (Vickers et al., 2005). Conversely, Morisse et al. (1995) suggested that cautery disbudding resulted in less pain than caustic paste, evidenced by lower cortisol levels in cautery disbudded calves; however, by the authors' own admission, comparisons were questionable due to differences in age across treatments. Our recent studies, evaluating pain associated with different disbudding methods for goat kids, showed that caustic paste promoted large increases in cortisol concentrations, skin temperatures surrounding the horn buds and frequencies of head shaking and scratching; these increases were greater than that of cautery disbudded kids, indicating more pain associated with caustic paste disbudding (Hempstead et al., 2018b, c). Caustic paste can have other negative consequences: Winder et al. (2017b) observed the paste running into the eyes of the calves or being transferred by the animal's own leg to other areas of the body or rubbed onto other animals or pen structures. Calves may be housed individually to

prevent transference of caustic paste to other animals (Vickers et al., 2005), but this is not practical for group housing systems, for example in New Zealand, due to the seasonal nature of farming and the high number of young born over a short period. Altogether, it appears as though caustic paste disbudding of goat kids may not be a suitable alternative to cautery disbudding.

Cryosurgical disbudding involves directing a pressurised spray of liquid nitrogen onto the horn buds. The low temperatures ( $\leq -20^{\circ}\text{C}$ ) freeze the horn bud tissue and with a slow thaw (up to  $10^{\circ}\text{C}/\text{min}$ ), kill the horn bud cells (Krunic and Marini, 2015). For calves, cryosurgical disbudding may be less stressful than cautery disbudding (Bengtsson et al., 1996; Stewart et al., 2014); however, our recent research on goat kids showed higher cortisol concentrations, skin temperatures surrounding the horn buds and frequencies of head scratching of cryosurgically versus cautery disbudded kids (Hempstead et al., 2018b, c). Initially, there were no breaks in the skin associated with cryosurgical disbudding (Hempstead et al., 2018b), which is commonly seen in kids experiencing cautery or caustic paste disbudding. However, 24 h after cryosurgical disbudding, ulcerations or vesicles were observed, resulting in open wounds and delayed healing in calves (Bengtsson et al., 1996) and kids (Hempstead et al., 2018b). In addition, there are impracticalities associated with using liquid nitrogen on farm, such as the need to store liquid nitrogen, the expense (and maintenance) of the spray applicator(s) and the requirements of additional training and safety equipment. Branding is commonly used on cattle for permanent marking; the two most common methods are either freeze or cautery branding. Cautery branding is suggested to be more painful than freeze branding due to the severity of tissue damage (Schwartzkopf-Genswein and Stookey, 1997; Underwood, 2002). Although initial reports on calves suggested cryosurgical disbudding may be a promising alternative to cautery disbudding (Bengtsson et al., 1996; Stewart et al., 2014), further research is required.

A novel method of disbudding involves injecting clove oil into the horn bud, which was reported to prevent horn growth in goat kids (Molaei et al., 2015) and calves (Molaei et al., 2014). More recently, it has been suggested that clove oil is initially less painful than cautery disbudding of calves (Sutherland et al., 2018). Clove oil is derived from clove spice and has complex properties; clove oil is a well-established fish anaesthetic (Sladky et al., 2001; Javahery et al., 2012), has anaesthetic activity in humans (Markowitz et al., 1992), and antibacterial

(Briozzo et al., 1989), cytotoxic (Babich et al., 1993; Prashar et al., 2006) and anti-carcinogenic properties (Zheng et al., 1992). The main component of clove oil is eugenol, which at high concentrations, causes cellular necrosis of the oral mucosa of rats (Kozam and Mantell, 1978) and isolated human skin cells (Prashar et al., 2006). Goat kids that were injected with clove oil showed a similar cortisol response, had a similar change in skin temperature and frequency of head shaking and scratching to sham-handled controls and to those of cautery disbudded kids, indicating a similar experience of pain (Hempstead et al., 2018b, c). Furthermore, clove oil injection may result in faster healing rates than cautery disbudding due to less tissue damage (Hempstead et al., 2018b). The exact mechanisms of action of clove oil on horn bud tissue is not well understood; however, the histological results of one goat kid study (Molaei et al., 2015), suggested the cytotoxicity of clove oil may be associated with membrane damage and subsequent cell lysis or cell apoptosis (Prashar et al., 2006). Clove oil can also inhibit cellular enzymes involved in cell transport processes, which may cause cell death (Kreydiyyeh et al., 2000). Clove oil shows promise as a method of disbudding if it proves to cause less pain than cautery disbudding, and is efficacious, with few or no deleterious side effects.

Further investigations into alternatives to cautery disbudding that are less painful and equally or more effective in preventing horns and scurs are needed. Initial reports suggest that clove oil injection may cause a similar amount of pain as cautery disbudding and lead to faster healing (Hempstead et al., 2018b, c); however, additional studies are needed to validate these findings and to assess whether injecting clove oil into the horn bud can successfully prevent horns or scurs.

#### **2.4.4.1 Efficacy of disbudding methods in preventing horns or scurs**

There is little research evaluating the effectiveness of different disbudding methods on preventing horns or scurs in goat kids or calves; furthermore, horn and scur incidence within farms is not well documented. Scurs are soft, partial regrowths of horn bud tissue that can be easily broken, whereas horns have grown normally with a hard, keratinised outer shell that has fused with the underlying skull (Dove, 1935). To the authors' knowledge, there is no published literature on

cautery disbudding efficacy of calves, although cautery disbudding of goat kids can be effective at preventing scurs (Hempstead et al., 2018b).

A study evaluating the effect of local anaesthetic on caustic paste disbudding of calves reported that 30% of treated calves (6/32 calves) grew scurs (Winder et al., 2017b). Cryosurgical disbudding in calves has been reported to be effective in preventing horn growth in 50% (9/16 horns) of cases (Bengtsson et al., 1996). One group reported that horn growth in calves (Molaei et al., 2014) and kids (Molaei et al., 2015) was prevented after injection of clove oil in 100% of treated animals (sample size: 12 calves and 16 goat kids). Our recent work showed that 3 wk after treatment, scurs grew on 6/10 caustic paste disbudded kids, 8/10 cryosurgically disbudded kids and 9/10 clove oil injected kids (no scurs were observed in cautery disbudded kids; Hempstead et al., 2018b); as the study focused on the evaluation of pain, and not the likelihood of horns or scurs, 10 d old kids were used. Horn growth of goat kids at 10 d of age is advanced, and the buds would have begun to fuse with the skull (Dove, 1935), potentially explaining scurs in all but the cautery disbudded group (where the horn bud was removed). More recently, we found clove oil injection to be ineffective as a disbudding agent: 87% of treated horn buds (136/156 horn buds) led to the growth of horns or scurs (our unpublished data). Clearly, more research is required on the efficacy of different disbudding methods at preventing horns or scurs.

## **2.5 Pain mitigation for cautery disbudding of goat kids**

### **2.5.1 Local anaesthesia**

A common pain mitigation strategy used for cautery disbudding of calves is the administration of local anaesthesia (e.g., lidocaine), which causes a lack of sensation to a localised area (Dugdale, 2011). Lidocaine can reduce pain associated with cautery disbudding of calves as evidenced by virtually abolishing the behavioural responses during disbudding; treated calves perform less head shaking and have lower cortisol concentrations for up to 2 h post-treatment compared with calves disbudded without pain relief (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Stafford and Mellor, 2005). The advantages of using a local anaesthetic is that it (i) is not cost prohibitive, (ii) requires less equipment than general anaesthetic and (iii) can be performed (with training) by farm personnel (Dugdale, 2011).

Interestingly, when administered to goat kids prior to cautery disbudding (using either a ring or nerve block), lidocaine does not appear to eliminate or reduce acute pain; kids disbudded with lidocaine had similar frequencies of vocalisations and leg shakes, and similar cortisol concentrations as kids disbudded without lidocaine (Alvarez et al., 2009, 2015; Nfor et al., 2016). The apparent difference in efficacy between calves and kids is likely due to a difference in the number of nerves supplying the horn buds. Goat kids have two nerves innervating each horn bud (the lacrimal and infratrochlear nerves), meaning that multiple injections are required per horn bud to achieve a successful block. Calves on the other hand, have only one nerve (lacrimal) supplying each horn bud (Vitums, 1954; Dugdale, 2011). Due to differences in ages/weights of the calves and kids at the point of disbudding, dosage may affect the success of the nerve block; kids were administered 1 – 2 mL of lidocaine (Alvarez et al., 2009, 2015; Nfor et al., 2016) whereas, calves typically receive 5 mL of lidocaine (Graf and Senn, 1999). In addition, goat kids have an increased risk of toxicity compared to other ruminants due to the young age at which they are disbudded (Smith and Sherman, 2009b; Dugdale, 2011). Therefore, the dose required to reduce sensitivity of the horn bud area may be above levels that goat kids can tolerate. The toxic dose of lidocaine for young kids and lambs is approximately 4 – 10 mg/kg, with a suggested dose of 4 mg/mL (Dugdale, 2011). Furthermore, the injection of a local anaesthetic likely causes pain itself and should only be performed if it clearly reduces pain associated with cautery disbudding. Alternatives to injected lidocaine such as topical formulations, or local anaesthesia with reduced toxicity for goat kids should be evaluated in future research.

### **2.5.2 General anaesthesia**

General anaesthesia produces a state of unconsciousness that is a “controlled and reversible intoxication of the central nervous system, whereby the patient neither perceives nor recalls noxious (or other) stimuli” (Dugdale, 2011, p. 1). Goat kids that were provided isoflurane during disbudding performed less frequent head shakes and displayed lower cortisol concentrations up to 2 h post-treatment than kids disbudded without pain relief (Hempstead et al., 2018a). Practically, isoflurane can be useful on farm as portable units can be used, the anaesthetic depth can be adjusted quickly and recovery is faster than other



inhalation anaesthetics (e.g., halothane and sevoflurane). Furthermore, restraint is made easier for the handlers due to the lack of struggling and disbudding can be carried out more efficiently (Hempstead et al., 2018a). However, limitations include the requirements of veterinary administration of general anaesthesia and supervision of the animal while unconscious (increasing costs associated with the practice), and complications associated with the use of such drugs that can cause regurgitation, aspiration pneumonia, hypoventilation and hypotension (Dugdale, 2011). Initial reports indicate that general anaesthesia can reduce pain associated with cautery disbudding of goat kids; however, more practical pain mitigation strategies that can be easily administered by farm personnel at a minimal cost to the farmer should be investigated.

### **2.5.3 Sedatives**

Adrenergic alpha-2 agonists or sedatives (e.g., xylazine), when used with lidocaine, can effectively reduce pain in disbudded calves as evidenced by lower frequencies of head jerks, leg movements and struggles and lower cortisol concentrations than disbudded controls (Grondahl-Nielsen et al., 1999; Stilwell et al., 2010). Xylazine has a short withholding time and produces dose-dependent sedation but may cause cardiovascular depression (Khan et al., 1999). Xylazine and ketamine have been used together to sedate goat kids prior to disbudding (Ingvast-Larsson et al., 2011); however, the effect of these drugs on pain was not assessed. Goat kids that were disbudded under sedation using dexmedetomidine hydrochloride had lower cortisol concentrations than those disbudded without sedation for up to 30 min post-treatment (Nfor et al., 2016). Sedatives function to bind to alpha-2 adrenalin receptors in the central and peripheral nervous systems, inhibiting noradrenalin release and impeding transmission of further nerve impulses; this can provide the dual effect of sedation and analgesia (Dugdale, 2011). Sedatives such as xylazine and dexmedetomidine hydrochloride may show promise as pain mitigation strategies for disbudding of goat kids. However, sedatives can have negative cardiovascular (e.g., bradycardia and central suppression of thermoregulation in goat kids; Dugdale, 2011; Nfor et al., 2016) and respiratory effects (e.g., pulmonary oedema, hypoxaemia; Dugdale, 2011) on small ruminants. Therefore, the appropriate dosage to induce sedation, but prevent deleterious effects, should be investigated.

#### **2.5.4 Non-steroidal anti-inflammatory drugs**

Non-steroidal anti-inflammatory drugs block cyclooxygenase activity to inhibit synthesis of prostaglandins that mediate inflammation and associated pain (Dahl and Kehlet, 1991; Del Tacca et al., 2002). Meloxicam has been suggested to reduce inflammatory pain associated with disbudding of goat kids as the VAS was lower in kids disbudded with meloxicam 24 h post-treatment (Ingvast-Larsson et al., 2011). Furthermore, kids administered meloxicam immediately before cautery disbudding spent a similar amount of time head scratching as sham-handled kids for 2 h post-treatment, indicating meloxicam reduced pain associated with disbudding (Hempstead et al., 2018a).

An oral formulation has been suggested as a practical alternative on farm as meloxicam can be added to milk prior to disbudding (in calves; Faulkner and Weary, 2000); however, the dose per animal would be difficult to control in group-housed animals. In summary, accumulated evidence suggests that NSAIDs may reduce post-operative pain associated with cautery disbudding of goat kids and be practical for use on farm. Future research should investigate the optimal timing for administration of NSAIDs prior to disbudding for effective pain mitigation.

## **2.6 Conclusions and implications**

Cautery disbudding of goat kids not only causes immense acute and post-operative pain, but the practice can cause thermal injury and necrosis of the brain and meningoencephalitis, which can negatively impact on welfare. Cautery disbudding techniques vary considerably between operators, which may affect pain and injury associated with the practice. Further research should more clearly describe disbudding methodologies to improve comparisons of results across studies. In addition, future research should evaluate the level of training received by goat kid disbudding operators and the effect of temperature, application duration or pressure of the iron on pain, injury and efficacy.

Pain and injury associated with disbudding could be eliminated by either changing herd management strategies or facility design to allow for horned goats, or by breeding polled animals. Based on the literature reviewed here, it appears that other disbudding methods including caustic paste and cryosurgical disbudding are more painful than cautery disbudding and may not be useful

alternatives. Although clove oil injection appears to cause a similar experience of pain as cautery disbudding, the method is ineffective at preventing horns and scurs and may not be a viable alternative to cautery disbudding.

Lidocaine does not appear to reduce pain associated with disbudding in goat kids; therefore, the effect of dosage, formulation or topical application should be investigated. General anaesthesia or sedation and NSAIDs can reduce acute and post-operative pain in cautery disbudded goat kids. Pain relief that is affordable, practical, easy to administer and safe for both humans and the treated goats is most likely to be adopted by farmers.

Bath (1998) suggested that the minimisation of pain caused by a husbandry procedure requires that it is done for the right reasons, by the best method, using correct equipment, at the right time, with correct follow up and with proper training. Until a less painful and efficacious alternative is realised, it appears that cautery disbudding with pain relief is the best option available.

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# Chapter 3

## Evaluation of alternatives to cautery disbudding of dairy goat kids using physiological measures of immediate and longer-term pain

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## **Abstract**

We evaluated alternatives to cauterization disbudding of goat kids using physiological measures of immediate and longer-term pain. Fifty Saanen doe kids were randomly assigned to one of five treatments (n = 10/treatment): (1) cauterization disbudding (CAUT), (2) caustic paste disbudding (CASP), (3) liquid nitrogen disbudding (CRYO), (4) clove oil injected into the horn bud (CLOV), or (5) sham disbudding (SHAM). Serum cortisol and haptoglobin concentrations were measured from blood samples collected immediately before treatment (baseline) and at 15, 30, 60 and 120 min and then again at 6 and 24 h post-treatment. An infrared thermography camera was used to take images of the horn buds 24 h pre- and 24, 48 and 72 h post-treatment to measure skin temperature. Body weight was measured daily for 1 wk to assess weight change post-treatment. Images of the horn buds were taken at d 1, 2 and 7, and at 6 wk post-treatment to assess tissue damage and wound healing. Mean cortisol concentrations were elevated in CASP kids 1 h post-treatment relative to CAUT kids. Cortisol concentrations of CRYO kids were higher than those of CAUT kids 30 min post-treatment; concentrations for CLOV kids were similar to CAUT kids post-treatment. Mean haptoglobin concentrations were similar across treatments over time; however, CLOV kids had higher concentrations at 24 h post-treatment than all other treatments. Skin temperatures of CASP kids were elevated relative to SHAM kids at all time points post-treatment, and all disbudded kids (except for CLOV kids) had skin temperatures above those of SHAM kids at 72 h post-treatment. Treatment did not influence weight gain. The CAUT kids had large, open wounds exposing bone; small scabs were still evident 6 wk post-treatment. The CASP kids had red and open, raw wounds that generated large eschars, apparent for up to 6 wk. The CRYO kids had closed, dry wounds initially, but over time ulcerations appeared that caused open wounds; small scabs were present 6 wk post-treatment. The CLOV kids had closed, dry wounds with blackened skin; healed skin and minimal scabs were present 6 wk post-treatment. Caustic paste and cryosurgical disbudding appeared to cause more pain compared with cauterization disbudding; thus, these methods may not provide good alternatives to cauterization disbudding. Clove oil appeared to cause a similar pain response as cauterization disbudding and smaller wounds with earlier tissue repair; this method shows promise as an alternative to cauterization disbudding.

### 3.1 Introduction

Disbudding of goat kids and calves is performed to prevent horn growth, as horns can cause injuries to other farmed animals and human handlers. Cautery disbudding of kids is a common practice but is painful and can cause health issues (Thompson et al., 2005; Alvarez et al., 2009; Hempstead et al., 2017). Pain in disbudded kids can be assessed using behavioural and physiological responses such as frequent intense vocalisations (Alvarez and Gutiérrez, 2010), increased frequencies of head and body shaking, longer bouts of head scratching (Greenwood and Shutt, 1990; Hempstead et al., 2017, 2018), and elevated plasma beta-endorphin (Greenwood and Shutt, 1990) and cortisol concentrations (Alvarez et al., 2009, 2015; Hempstead et al., 2018). Calves show defensive behaviour during disbudding, such as rearing or dropping to the ground, and responses post-procedure, such as head shaking and ear flicking (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Heinrich et al., 2010). Moreover, disbudded calves display elevated cortisol concentrations and heart and respiratory rates (Grondahl-Nielsen et al., 1999; Heinrich et al., 2009), a more variable heart rate, a rapid decrease in eye temperature (Stewart et al., 2008), reduced weight gain when pain relief is unavailable (Bates et al., 2016), and heightened sensitivity to pressure applied to the disbudding wounds (Heinrich et al., 2010).

Haptoglobin is an acute phase protein that is produced in response to inflammation and has previously been measured in disbudded calves provided non-steroidal anti-inflammatory drugs (NSAID; Allen et al., 2013). Infrared thermography has also been used in cattle to measure inflammation; higher skin temperatures in tissues of either hot-iron or freeze-branded cattle were found relative to unbranded controls measured at the same position (Schwartzkopf-Genswein and Stookey, 1997). Currently, research assessing inflammatory responses to disbudding in kids is limited. Hence, there is a need to evaluate pain mitigation strategies and alternative methods to cautery disbudding that can cause less pain and tissue damage and improve wound healing.

Alternative methods to cautery disbudding have been evaluated in calves but have not been assessed in kids. Methods include the application of caustic paste (Morisse et al., 1995; Vickers et al., 2005; Stilwell et al., 2009), liquid nitrogen (i.e., cryosurgical disbudding; Bengtsson et al., 1996; Stewart et al., 2014), and clove oil (Molaei et al., 2014) to horn buds. Caustic paste techniques involve the application of a sodium, calcium, or potassium hydroxide paste that

chemically burns the horn bud area; application of these pastes has been reported to be less painful than cauterisation disbudding in calves based on lower frequencies of head shakes (Vickers et al., 2005). Furthermore, the frequencies of pain-related behaviours (e.g., head rubs, head shakes, tail flicks) were much lower in calves disbudded with caustic paste and a local anaesthetic corneal block than in calves treated with caustic paste alone (Winder et al., 2017). Cryosurgical disbudding involves spraying liquid nitrogen on the horn buds to destroy cells and may be less painful than cauterisation disbudding as it causes a reduced inflammatory response (Bengtsson et al., 1996; Stewart et al., 2014). Clove oil has traditionally been used in dentistry as a mild topical anaesthetic (Markowitz et al., 1992) and has antibacterial and anticarcinogenic properties (Chaieb et al., 2007). Furthermore, clove oil is a well-established fish anaesthetic (Sladky et al., 2001). Recent studies on calves (Molaei et al., 2014) and goat kids (Molaei et al., 2015) have used clove oil as a novel method of disbudding and have shown arrested growth in horn buds injected with clove oil compared with saline injection. Clove oil, which contains eugenol, has properties (e.g., cytotoxic, necrotising agent, anaesthetic; Markowitz et al., 1992; Prashar et al., 2006) that may have application for disbudding. These methods have the potential to improve goat kid welfare compared with cauterisation disbudding.

We evaluated alternatives to cauterisation disbudding (i.e., the application of caustic paste, liquid nitrogen, and clove oil) for goat kids using physiological measures of immediate and longer-term pain. We predicted that the application of caustic paste would cause the greatest pain response and that cryosurgical disbudding and clove oil injection would have the lowest pain response relative to cauterisation disbudding. We also expected caustic paste to cause the greatest tissue damage and that kids injected with clove oil would benefit from the anaesthetic properties of eugenol, a main component of clove oil (Markowitz et al., 1992).

## **3.2 Materials and methods**

### **3.2.1 Animals and housing**

Our study was conducted on 50 female Saanen and Saanen cross dairy goat kids (mean  $\pm$  SD;  $5.2 \pm 0.66$  kg) aged 9 to 14 d ( $10.6 \pm 0.91$  d) at the Ruakura Research Farm, Waikato region (latitude  $37^{\circ}47'S$ , longitude  $175^{\circ}19'E$ ), New Zealand, during July and August 2016. The study was approved by the

Ruakura Animal Ethics Committee (Protocol No. 13899). All kids received goat colostrum at birth and were separated from their dam after 24 h. Kids were transported to the Research Farm when approximately 2 d old. Once kids arrived, they were weighed and given an identification collar. Kids were also vaccinated subcutaneously (s.c.) (Covexin, Schering-Plough Animal Health Ltd., Wellington, New Zealand) per routine farm practice and prophylactically administered an antibiotic s.c. (Norocillin, 30% wt/vol, Norbrook Laboratories Ltd., Northamptonshire, UK).

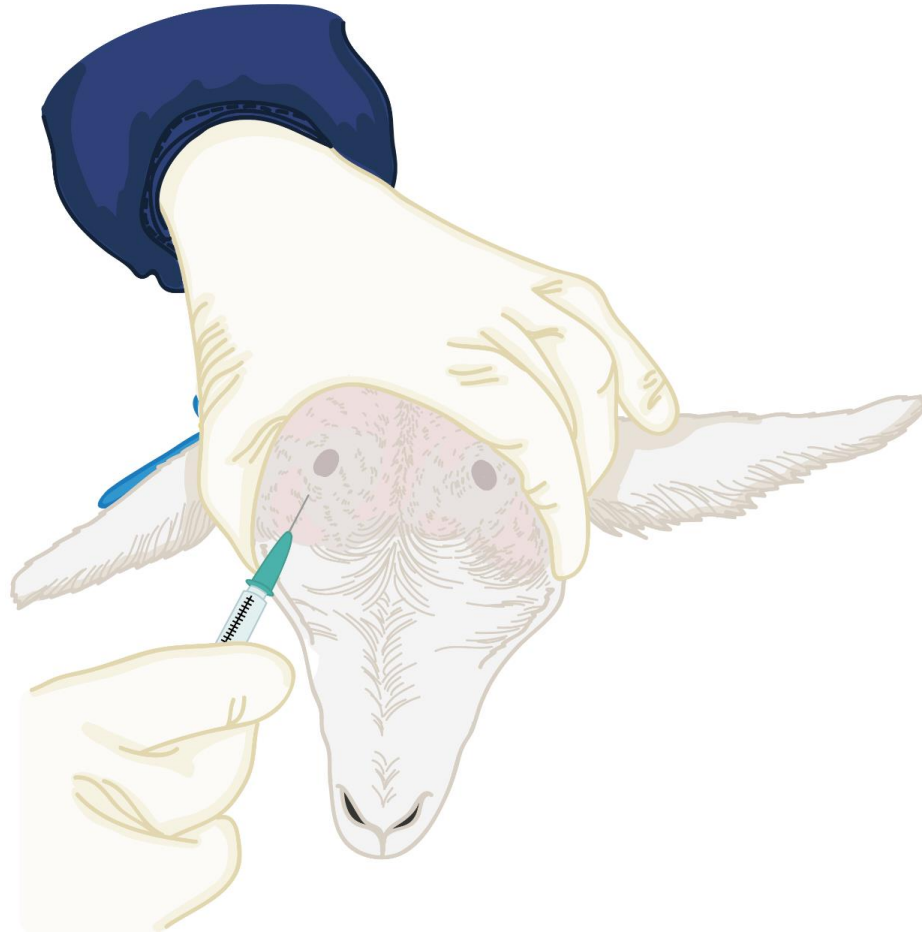
The animals were housed in groups of five in pre-treatment pens (2.4 x 1.6 m). The pen floors were covered with clean, dry bedding (wood shavings, PGG Wrightsons, Hamilton, New Zealand) 10 cm deep. The kids remained with the same pen-mates for the entire trial. Kids were habituated to the pens for 1 d before baseline (pre-treatment) data collection. Kids had access to at least 600 mL of milk replacer/d per kid, which was increased over the study period to 1 L/d per kid (Anlamb, Fonterra Ltd., Auckland, New Zealand), via a feeder. Fresh water was supplied in a bucket attached to the pen wall. Overnight, feeders were removed to reduce gut fill affecting body weight (BW) measurements the following morning. The feeders were then replaced at approximately 0700 h. Once the BW measurements concluded at 2 wk post-treatment, feeders were left in the pens overnight. Daily temperature and relative humidity within the goat barn ranged between 6.0 and 24.5°C ( $12.9 \pm 0.03^\circ\text{C}$ ) and 37 and 93% ( $69.5 \pm 0.18\%$ ), respectively.

### **3.2.2 Experimental design**

We used a randomised complete block design blocked by treatment day and pen within treatment day. Kids were randomly allocated to one of five treatment groups balanced for age ( $n = 10/\text{treatment}$ ). Only one kid per treatment was represented in each pen. The experimental component of the study was conducted over four treatment days within a 2-wk period. Each kid was fed approximately 1 h before treatment and then collected from their pre-treatment pen and placed into a restraint device, which held the animal 90 cm off the ground for operator ease. The device consisted of a rigid plastic pipe (0.35 m x 0.85 m) sectioned lengthways with holes for the kids' legs and 2 straps (Velcro, London, UK) used to secure the kid across the shoulder and back. Treatments were carried

out in the same room containing the pre- and post-treatment pens. The order of treatment was randomly assigned. Hair covering the horn buds was removed with an electric clipper (Laube, 505 cordless kit, Shoof International, Cambridge, New Zealand) to expose the horn buds. Treatments were performed by the same veterinarian between 0900 and 1030 h each day and included the following:

1. Sham treatment (SHAM): Kids were sham-handled and a finger was used to massage each horn bud in a circular motion for 10 s (i.e., not disbudded).
2. Cautery disbudding (CAUT): Kids were cautery disbudded using an iron (Quality electric debudder, 18-mm tip, 230 V, 190 W; Lister GmbH, Ludenscheid, Germany), which was heated to approximately 600°C for 20 min before being held to each horn bud for 5 to 7 s. Horn buds were then removed by pressing the iron down and then rotating it so the skin was cut and the buds forcibly flicked out. The cautery disbudding treatment was modified from the procedure described by Hempstead et al. (2017).
3. Caustic paste (CASP): Kids were disbudded using a sodium hydroxide-based caustic paste (Hornex, Shoof International Ltd.) that was rubbed onto each horn bud (0.16 mL/bud) using a fingertip on a gloved hand. A ring of petroleum jelly was spread around each horn bud before application to stop the paste from running into the kids' eyes. The caustic paste treatment was modified from the protocol described by Vickers et al. (2005) for calves.
4. Liquid nitrogen (CRYO): Kids were cryosurgically disbudded using a commercial applicator (CryAc B-700, 500-mL capacity, Brymill Cryogenic Systems, Ellington, CT), spraying liquid nitrogen onto each horn bud for 10 s. A device that consisted of a rubber cone (1-cm-diameter touching the head and 2-cm-diameter at the other end) connected to a metal handle was pressed against the head of the kid to localise the spread of the spray and to protect the kids' eyes.
5. Clove oil (CLOV): Kids were injected with 0.2 mL (as used by Molaei et al., 2015) of clove oil (C8392, 100 mL, 83 – 85% eugenol; Sigma-Aldrich, Saint Louis, MO) laterally into the centre of each horn bud at a 45° angle between the ear and muzzle (Figure 3.1). A well inside the horn bud was made by inserting and rotating the needle and then pulling backward slightly to create space for the clove oil to be injected.



**Figure 3.1.** Needle position used to inject clove oil into kids' horn buds.

No pain mitigation was provided as part of any treatment because the aim of the study was to evaluate the pain response relative to the different procedures. After treatment, the CAUT kids' wounds were sprayed with antibacterial spray (AluSpray, Neogen Corp., Lexington, KY) to prevent infection. The horn buds of CASP, CRYO, and CLOV kids were not sprayed to avoid interference with the treatments. The animals were then placed into their post-treatment pens (adjacent to their pre-treatment pens). The kids remained at the Research Farm in holding pens until they were returned to the farm of origin 6 wk after treatment, once health monitoring had concluded.

### **3.2.3 Blood sampling**

Serum cortisol and haptoglobin concentrations were measured from 4-mL blood samples collected by venepuncture from both jugular veins immediately before treatment, and at 15, 30, 60 and 120 min. Additional samples were taken at

6 and 24 h following treatment. Each kid was firmly restrained by a handler while samples were collected using 22-G, 2.54-cm needles (PrecisionGlide, Becton Dickinson, Franklin Lakes, NJ) and kept in serum tubes (Becton Dickinson). Blood samples were centrifuged at approximately 1,500 x g for 10 min at 20°C after leaving the samples to sit for approximately 1.5 h. The serum was separated and stored at -20°C until analysed.

Samples were analysed by a commercial laboratory using standard quality control methodologies; technicians were blind to the treatment each kid received. Cortisol concentrations were determined by electrochemiluminescence immunoassay using a commercial kit (Roche Diagnostics GmbH, Mannheim, Germany). Sensitivity of the assay was 1.5 nmol/L. Haptoglobin concentrations were determined by colorimetric assay using a Tridelta phase haptoglobin kit (Tridelta Development Ltd., Maynooth, Ireland). Sensitivity of the assay was 0.005 mg/mL.

### **3.2.4 Skin temperature**

Images of the horn buds were taken using a handheld infrared thermography camera (FLIR T650sc; FLIR Systems Inc., Wilsonville, OR). Two images were taken immediately before treatment (0900 h) and again at 24, 36 and 72 h post-treatment (~1500 h), and the clearest image of the pair at each sampling point was selected for analysis. Images were not taken within 24 h of treatment to avoid handling and associated stress affecting other measures. Kids were restrained so that the head remained horizontal to the floor, and images of the horn bud area were taken (~3-cm diameter around each horn bud). Handlers took care not to touch the horn bud area during restraint. All images were taken at 1 m directly in front of the animal. Ambient temperature (°C) and relative humidity (%) within the facility were recorded continuously using a data logger (EL-USB-2-LCD+, Lascar Electronics, Salisbury, UK), and temperature and humidity data were entered into the ThermoCam Researcher Pro 2.10 software (FLIR Systems AB, Taby, Sweden) used for thermal image analysis. The maximum skin temperature (°C) was calculated from an approximately 2-cm-diameter area surrounding each horn bud.

### **3.2.5 Body weight measurements**

Kids were weighed daily before feeding; this was done for 3 d before treatment, on the day of treatment, and daily for 1 wk after treatment. A final BW measurement was taken 2 wk after treatment. Kids were placed in a large fabric bag and weighed using free-standing digital hanging scales (model WS603, Wedderburn, Auckland, New Zealand).

### **3.2.6 Healing images**

Images of the horn bud area of each goat kid were taken using a Nikon camera (Coolpix L840; Nikon Corp., Tokyo, Japan) at d 1, 2 and 7, and at 6 wk after treatment to provide visual aids for descriptions of wound healing over time. The following criteria were used to assess tissue damage and wound healing: (1) whether the wound was open (broken skin with dermal layers visible) or closed (skin intact), (2) the presence of fluid (wet or dry), (3) whether an eschar (brown or black deep tissue damage that is flush with skin) or a scab (brown, thin layer of dried blood cells that sits on top of the skin surface) was present, and (4) the colour of tissues (i.e., redness/erythema or blackened).

### **3.2.7 Statistical analysis**

Data were analysed using Genstat software (version 17, VSN International, Hemel Hempstead, UK). Residual plots were assessed to detect departures from the assumptions of normality and constant variance. No transformations were required. One animal from the CAUT group was removed from the trial due to a leg injury not related to treatment. Serum cortisol and haptoglobin concentrations and BW were analysed using a repeated measures model fitted by restricted maximum likelihood. The model included fixed effects for treatment, time (day for BW), and their interaction and random effects for treatment day, pen, kid and age. The correlation in measurements taken on the same kid over time was modelled with a power model of order 1 for serum cortisol and haptoglobin concentrations and for BW. The model for skin surface temperature included the same fixed and random effects as above with the addition of random effects for horn bud (left or right) within kid. The correlation in measurements taken on the same horn bud over time was modelled with an



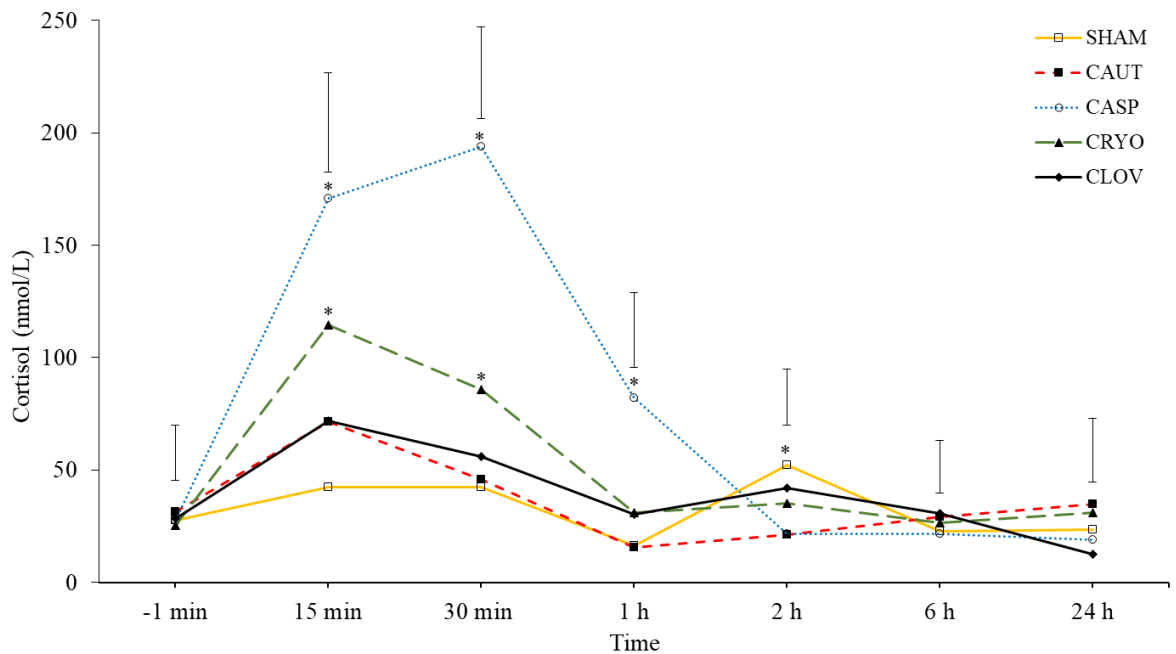
autoregressive model of order 1. Differences between and within treatments were detected using Fisher's least significant differences test. Mean values were provided with standard errors of the difference (SED). The level of significance was set at  $P \leq 0.05$ .

### 3.3 Results

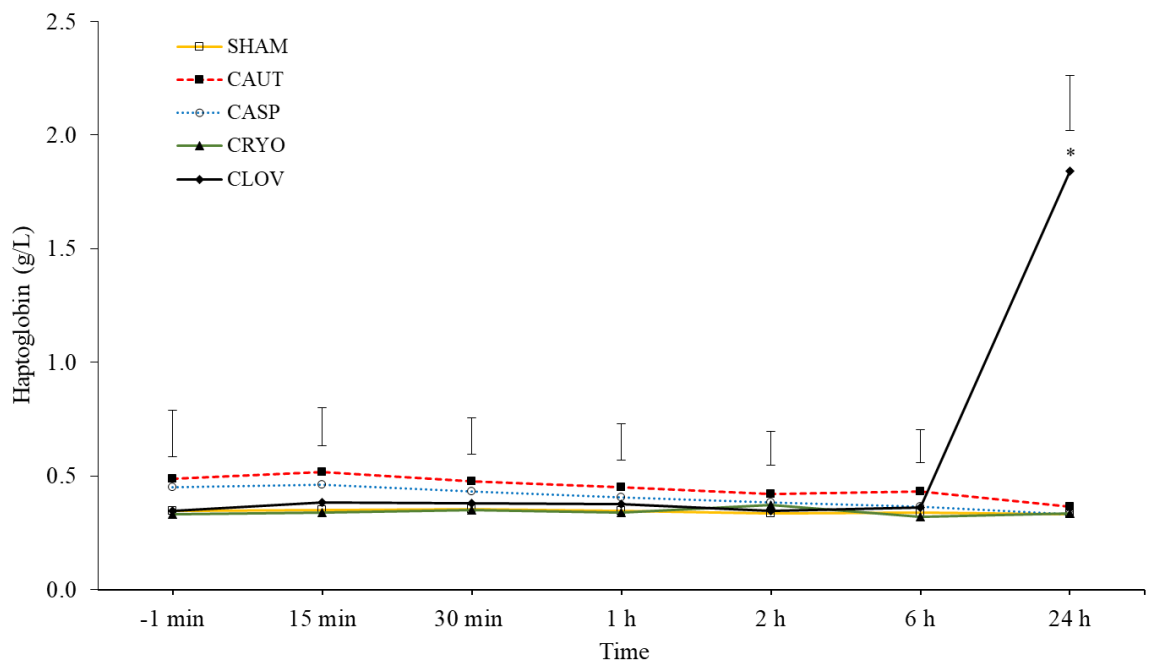
#### 3.3.1 Blood sampling

There was a treatment by time interaction for cortisol concentrations ( $F_{24, 163} = 6.6$ ,  $P < 0.001$ ; Figure 3.2). Mean baseline cortisol concentrations were not different across treatments (27.8, 31.4, 27.7, 25.4, and  $28.3 \pm 10.89$  nmol/L [pooled SED] for SHAM, CAUT, CASP, CRYO, and CLOV kids, respectively;  $P > 0.50$ ). Mean cortisol concentrations did not differ across time for SHAM kids ( $P > 0.10$ ) except at 2 h, where cortisol concentrations were elevated above baseline levels ( $P \leq 0.05$ ). Mean cortisol concentrations of CAUT kids increased from baseline ( $P \leq 0.01$ ) and were not different from SHAM kids 15 min post-treatment ( $P > 0.10$ ). Mean cortisol concentrations of CLOV kids increased from baseline 15 min post-treatment ( $P \leq 0.01$ ) and did not differ from those of SHAM kids for up to 1 h post-treatment ( $P > 0.10$ ). Furthermore, CLOV kid cortisol concentrations were not different from those of CAUT kids over 24 h post-treatment ( $P > 0.10$ ). Mean cortisol concentrations of CRYO kids were elevated above baseline ( $P \leq 0.01$ ) and CAUT kid levels 30 min post-treatment ( $P \leq 0.05$ ). Mean cortisol concentrations of CASP kids were elevated above baseline and CAUT kid levels for up to 1 h post-treatment ( $P \leq 0.01$ ). By 6 h post-treatment, there were no differences between groups for mean cortisol concentrations, which had returned to baseline levels ( $P > 0.10$ ).

A treatment by time interaction was observed for haptoglobin concentrations ( $F_{24, 181} = 10.9$ ,  $P < 0.001$ ; Figure 3.3). Mean baseline haptoglobin concentrations were similar across treatments (0.43, 0.53, 0.50, 0.37, and  $0.37 \pm 0.095$  g/L [pooled SED] for SHAM, CAUT, CASP, CRYO, and CLOV kids, respectively;  $P > 0.05$ ). For SHAM kids, haptoglobin concentrations did not differ over time ( $P > 0.50$ ). Post-treatment haptoglobin concentrations for CAUT kids were not different from baseline concentrations ( $P > 0.10$ ). There was an increase in mean haptoglobin concentrations from baseline for CLOV kids at 15 min post-treatment (0.40 g/L;  $P \leq 0.05$ ) and then again at 24 h (1.86 g/L;  $P \leq 0.01$ ).



**Figure 3.2.** Mean (error bars represent maximum SED for each time point) serum cortisol concentrations (nmol/L) over 24 h in goat kids (n=10/treatment) that were disbudded using caustic paste (CASP), liquid nitrogen (cryosurgical disbudding; CRYO), clove oil (CLOV) or a cautery iron (CAUT). For the control (SHAM), the horn buds were massaged but not disbudded. Asterisk indicates means that differ from CAUT kid means at  $P \leq 0.05$ .



**Figure 3.3.** Mean (error bars represent maximum SED for each time point) serum haptoglobin concentrations (g/L) over 24 h in goat kids (n = 10/treatment) that were disbudded using caustic paste (CASP), liquid nitrogen (CRYO), clove oil (CLOV), or a cautery iron (CAUT). For the control (SHAM), the horn buds were massaged but not disbudded. Asterisk indicates means that differ from CAUT kid means at  $P \leq 0.05$ .

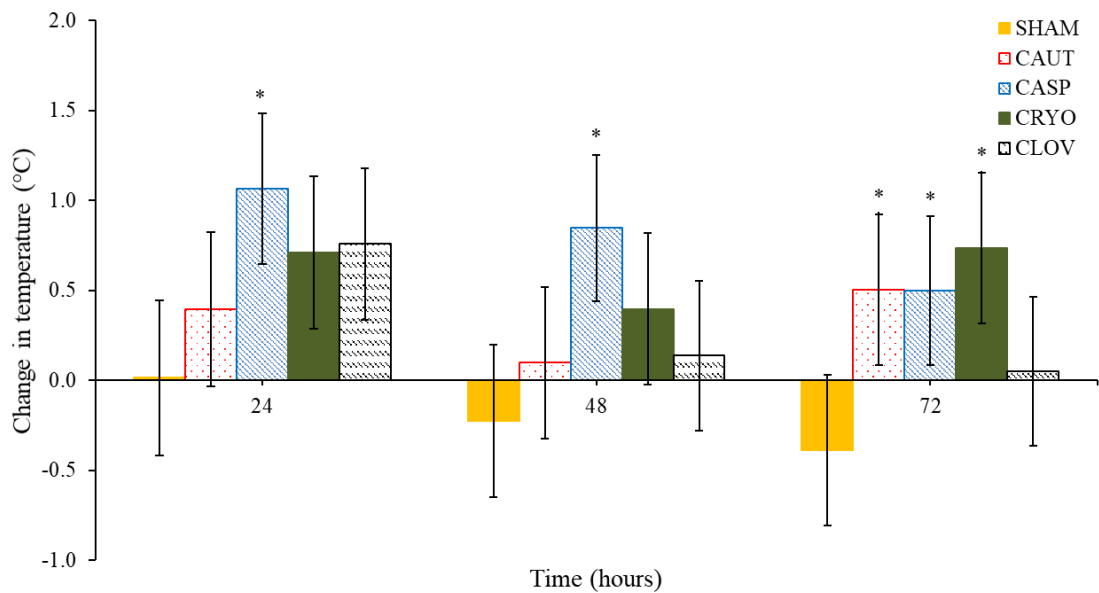
Furthermore, CLOV kids had higher haptoglobin concentrations than kids experiencing the other treatments at 24 h post-treatment ( $P \leq 0.01$ ). Kids experiencing CRYO and CASP treatments had no difference in mean haptoglobin concentrations across time ( $P > 0.10$ ).

### 3.3.2 Skin temperature

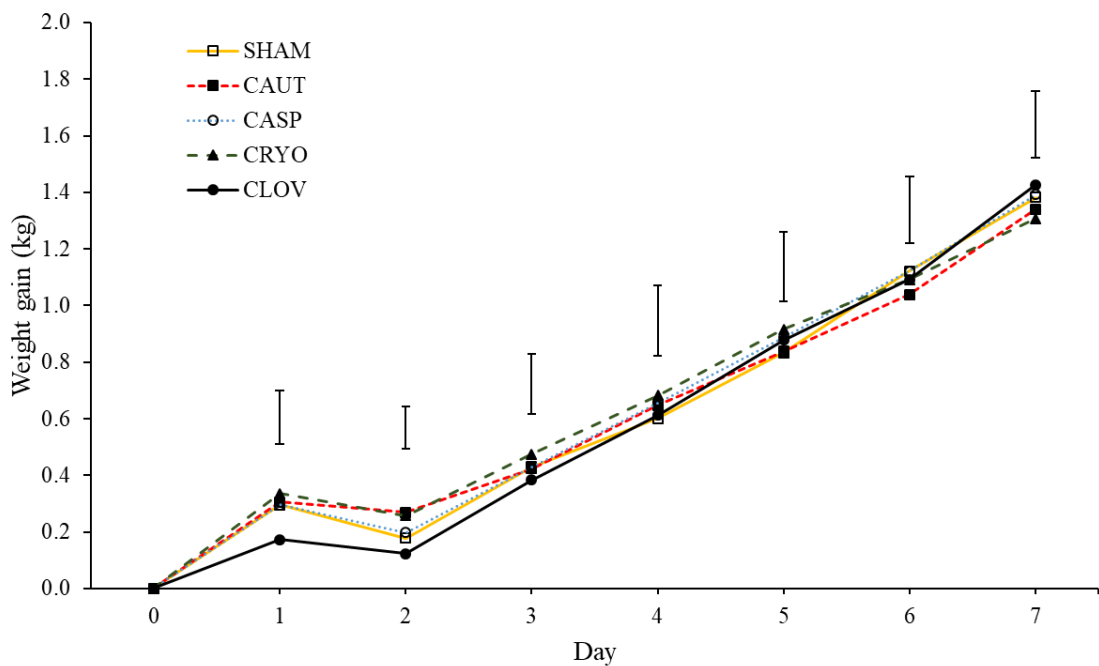
Due to missing baseline data, the skin temperatures from 21 kids were excluded from analysis. There was a treatment by time interaction for change in maximum skin temperature ( $F_{8,75} = 2.8$ ;  $P = 0.009$ ; Figure 3.4). Skin temperature of CASP kids was warmer than SHAM kids for 72 h post-treatment ( $P \leq 0.05$ ). The change in skin temperature did not differ between CAUT and SHAM kids up to 48 h post-treatment ( $P > 0.50$ ); however, at 72 h post-treatment, the increase in skin temperature was higher in CAUT than SHAM kids ( $P \leq 0.05$ ). The change in skin temperature did not differ between CRYO and SHAM kids up to 48 h post-treatment ( $P > 0.10$ ); however, at 72 h post-treatment, the increase in skin temperature was higher in CRYO kids than in SHAM kids ( $P \leq 0.05$ ). Skin temperatures of CLOV kids were not different from SHAM kids for 72 h post-treatment ( $P > 0.10$ ).

### 3.3.3 Body weight

There was no effect of treatment on mean BW over the 3 pre-treatment days ( $F_{8,43} = 1.5$ ,  $P = 0.20$ ), and mean BW was not different across groups 1 d pre-treatment (5.1, 5.1, 5.3, 5.2, and  $5.6 \pm 0.32$  kg [pooled SED] for SHAM, CAUT, CASP, CRYO, and CLOV kids, respectively;  $P > 0.10$ ). There was a day effect on mean weight gain over 7 d post-treatment ( $F_{6,128} = 163.8$ ,  $P < 0.001$ ). At 2 d post-treatment, all kids had lower weight gains than 1 d post-treatment (0.28 and  $0.21 \pm 0.029$  kg, respectively;  $P \leq 0.05$ ). There was no evidence that mean weight gain over 7 d post-treatment differed between groups ( $F_{24,124} = 0.8$ ,  $P = 0.68$ ; Figure 3.5). At 2 wk post-treatment, there were no statistically significant differences in mean BW across treatments (10.6, 10.3, 11.3, 11.2, and  $11.3 \pm 0.82$  kg [pooled SED] for SHAM, CAUT, CASP, CRYO, and CLOV kids, respectively;  $F_{4,44} = 0.7$ ,  $P = 0.58$ ).



**Figure 3.4.** Maximum ( $\pm$  SED) change in skin temperature ( $^{\circ}$ C) from baseline (immediately before treatment) of goat kids over 24 h pre- and 72 h post-treatment that have been disbudded using caustic paste (CASP), liquid nitrogen (CRYO), clove oil (CLOV), or a cautery iron (CAUT). For the control (SHAM), the horn buds were massaged but not disbudded. Asterisk indicates treatments that differ from SHAM kid means at  $P \leq 0.05$ .



**Figure 3.5.** Mean (error bars represent maximum SED for each time point) weight gain (from baseline; kg) over 7 d post-treatment of goat kids ( $n = 10$ /treatment) that have been disbudded using caustic paste (CASP), liquid nitrogen (CRYO), clove oil (CLOV), or a cautery iron (CAUT). For the control (SHAM), horn buds were massaged but not disbudded.

### 3.3.4 Healing descriptions









Figure 3.6 displays examples of wound healing for the disbudding treatments over time. On d 1 post-treatment, CAUT wounds were open and deep with bone clearly visible. Erythema of the tissue surrounding the wounds was observed by d 2. After 7 d, an eschar below the outer layer of skin formed. At 6 wk post-treatment, scabs were apparent.

On d 1, CASP wounds were open and wet; the tissue surrounding the wound was deep red. From 2 d onward eschars formed, with large scabs remaining at 6 wk.

On d 1, CRYO wounds were closed and dry. However, on d 2 lesions developed and the wounds were red, wet, and open. Scabs formed by 7 d and were still visible 6 wk post-treatment.

On d 1, CLOV wounds were closed and dry. Three of the 10 CLOV kids had inflammation around the upper eye area (not apparent from images), which was visible for up to 2 d post-treatment. Erythema of the tissue surrounding the site of injection was observed, with some blackened areas at 2 d post-treatment.

At 6 wk, small scabs were present on the healed skin. Scurs (partial regrowth of horns) were not observed in CAUT kids; however, from approximately 3 wk after treatment, scurs grew on 6/10 CASP kids, 8/10 CRYO kids, and 9/10 CLOV kids. Our study was not designed to be an efficacy study.

Treatment	Day 1	Day 2	Day 7	Week 6
CAUT				
	Open, wet wound with redness and inflammation of surrounding skin	Similar to d 1. Note: silver colour produced by antibacterial spray	Closed, dry wound with eschars formed	Healing wound with small scabs present
CASP				
	Open, wet wound with redness and inflammation of skin	Closed, dry wound with eschars formed	Similar to d 2	Large scabs present

CRYO



Closed, dry wound with redness of skin



Open, wet wound with redness of skin



Closed, dry wound with eschars formed



Small scabs present

CLOV



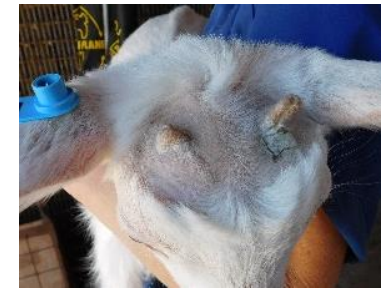
Closed, dry wound with redness and inflammation of skin



Closed, dry wound with areas of redness and blackened skin



Similar as d 2 with eschars formed



Small scab present. Note scur formation

**Figure 3.6.** Images of wound healing over 6 wk in goat kids (n = 10/treatment) that were disbudded using caustic paste (CASP), liquid nitrogen (CRYO), clove oil (CLOV), and a cautery iron (CAUT). Sham-handled kids have not been included.

### 3.4 Discussion

We evaluated three alternatives to cauterisation (the application of caustic paste, liquid nitrogen and clove oil) of goat kids using physiological measures of immediate and longer-term pain. The elevated cortisol levels of kids disbudded with caustic paste suggest that this treatment caused more pain than the other disbudding methods for up to 1 h post-treatment. Similarly, researchers studying calves have reported that animals disbudded with caustic paste had higher cortisol concentrations 1 h post-treatment than those that were cauterised (Morisse et al., 1995; Stilwell et al., 2009). In contrast, Vickers et al. (2005) suggested that reductions in behavioural responsiveness of calves disbudded with caustic paste relative to those that were cauterised indicated that caustic paste was less painful; however, these calves were sedated with xylazine, which may have prevented the initial expression of pain-related behaviours. Moreover, cortisol concentrations peaked at 425 nmol/L after an adrenocorticotrophic hormone challenge was performed on 1-wk-old goat kids to stimulate a maximal cortisol response (our unpublished data). This response was approximately 55% greater than the response elicited in CASP kids, which peaked at 194 nmol/L, suggesting that although the cortisol response to caustic paste was large, it was not maximal. However, the cortisol response to caustic paste was markedly larger than the response elicited by cauterisation.

Cortisol concentrations were higher in CRYO kids than in CAUT kids, which indicates that the application of liquid nitrogen caused more acute pain than cauterisation. Calves experiencing cryosurgical disbudding elicit defensive behaviours during the procedure such as struggling, with movements of the head and legs as well as vocalisations (Bengtsson et al., 1996), suggesting that the procedure caused pain. However, calves that underwent cryosurgical disbudding also spent more time lying than cauterised calves, which may reflect less discomfort and pain (Stewart et al., 2014). Interpretation of lying behaviour is difficult, as increased lying times (compared with controls) may reflect pain in some species but not others; for example, castration of young pigs caused increased lying time compared with intact controls, which suggests that greater pain was experienced (McGlone et al., 1993). Further research is required to evaluate the pain responses of goat kids to cryosurgical disbudding.



The cortisol response of CLOV kids was not different from that of CAUT kids over the 24 h post-treatment period, which indicates that clove oil caused a similar amount of pain as the application of the cautery iron. It was unclear whether elevated cortisol concentrations were in response to damage caused by the needle, the displacement of tissues by the clove oil, or the action of clove oil on the cells. Currently, little research exists on the use of clove oil for disbudding. To our knowledge only two efficacy studies exist: one in calves (Molaei et al., 2014) and one in kids (Molaei et al., 2015). These studies reported 100% success in preventing horn growth but did not assess the pain response associated with injecting clove oil. Moreover, the time course of pain associated with the injection of clove oil is unknown; animals treated with clove oil may experience pain for longer than those that are cautery disbudded. The cautery iron instantly destroys nociceptors, whereas eugenol (the main component of clove oil) can cause cellular necrosis up to 1 h after treatment (Kozam and Mantell, 1978). However, clove oil may cause less pain than cautery disbudding as eugenol has similar properties to local anaesthesia (i.e., a loss of feeling or sensation within a given area); for example, eugenol can block conduction of action potentials of nerves, reduce synaptic transmission at the neuromuscular joint, and inhibit nerve activity (Markowitz et al., 1992). However, the appropriate dose to induce anaesthetic effects is unknown. In the future, pain responsiveness to the injection of clove oil over a 24-h post-treatment period should be measured more intensively. In our study, more blood samples were not taken to avoid affecting kid behaviour (measured as part of Chapter 4).

The increase in cortisol in CAUT kids was larger than that in SHAM kids, which indicates that cautery disbudding causes acute pain. An increase in cortisol post-disbudding compared with sham-handled controls has previously been reported for kids (Alvarez and Gutiérrez, 2010; Alvarez et al., 2015; Hempstead et al., 2018) and calves (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Heinrich et al., 2009). In the present study, the geometric mean serum cortisol concentrations of CAUT kids were not significantly higher than those of SHAM kids 15 min post-treatment. Large individual variation may explain the lack of significant differences between CAUT and SHAM kids; considerable individual variation was previously reported in a similar kid study (Ingvast-Larsson et al., 2011). These earlier results, together with the present study, suggest that cautery disbudded kids experienced more pain than those exposed to handling alone. The

acute pain response for all disbudding treatments appeared to be relatively short lived, as cortisol concentrations typically returned to baseline levels by 1 h post-treatment; in calves, cortisol can remain elevated for up to 4 h post-treatment (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Heinrich et al., 2010). Interestingly, the cortisol concentrations of SHAM kids were higher than baseline concentrations at 2 h post-treatment; this was unlikely to be associated with handling, as cortisol concentrations were not elevated within the hour following treatment, when most of the handling occurred. Elevated cortisol concentrations may have been associated with a higher motivation to feed compared with the disbudded groups, as dairy cattle that anticipated feed showed increases in plasma cortisol concentrations (Willett and Erb, 1972).

Both haptoglobin and skin temperature (measured using infrared thermography) can detect inflammatory responses, which are usually associated with pain (Schwartzkopf-Genswein and Stookey, 1997; Ballou et al., 2013). In the present study, a small increase in haptoglobin concentrations of CLOV kids immediately post-treatment and the large increase in haptoglobin at 24 h post-treatment suggest that clove oil can cause inflammation over this time. Interestingly, CLOV kids showed no increase in skin temperature post-treatment compared with SHAM kids, and heat is usually associated with inflammation (Palmer, 1981; Schwartzkopf-Genswein and Stookey, 1997). Changes in temperature may not have been detected because the skin of CLOV kids was intact, whereas the other treatments resulted in broken skin, which may affect heat loss (Stewart et al., 2005). It is important to note the limitations of each measure of inflammation; infrared thermography should be used on skin that is free from dirt, moisture, or foreign material because these can alter emissivity and conductivity and increase local heat loss to the environment (Palmer, 1981). Haptoglobin can be produced in response to systemic disease states (Gonzalez et al., 2008; Olumee-Shabon et al., 2013); however, changes in haptoglobin concentrations may not be sensitive enough to measure injury to localised soft tissue.

Normal haptoglobin concentrations for adult goats range between 0.39 and 1.26 g/L (Heller and Johns, 2015; Saidu et al., 2016), which can increase to 1.70 g/L with mastitis (Simplicio et al., 2017), which causes inflammation of the mammary tissues and is considered to be painful (Leslie and Petersson-Wolfe, 2012; Fitzpatrick et al., 2013). The values we observed following clove oil

injections were substantial, highlighting potentially undesirable side effects. However, pain associated with these inflammatory responses may be alleviated by analgesics such as NSAID (e.g., meloxicam). One CAUT kid excluded from the trial 2 h post-treatment due to a leg joint injury had haptoglobin concentrations of 9.72 g/L; this value was more than five-times higher than the values observed for CLOV kids. Haptoglobin concentrations have been shown to increase in response to surgical castration in 3-month-old bull calves but not in dehorned calves (Ballou et al., 2013) or dairy cows with mastitis (Gronlund et al., 2005); this highlights that more sensitive measures of inflammation are required to fully evaluate the inflammatory response associated with different methods of disbudding.

Up to 72 h post-treatment, CASP kids had warmer skin surrounding the horn buds than SHAM kids, indicating that tissue damage and associated inflammation occurred over this time. The skin was warmer on CAUT and CRYO kids than on SHAM kids 72 h post-treatment, suggesting that inflammation and associated pain may worsen over time for these disbudding methods. It appears that CASP, CRYO, and CAUT kids had burns of similar severity at 72 h post-treatment. Skin temperatures were higher in cattle that were hot-iron branded rather than freeze-branded for up to 144 h following branding, which may reflect burn severity (Schwartzkopf-Genswein and Stookey, 1997). From our results, it is apparent that some inflammation is caused by all disbudding methods. Segregation between the acute stress response and the inflammatory response to dehorning in cattle was clearly shown by McMeekan et al. (1998). Inflammatory pain can be reduced by NSAID in cautery disbudded calves (Faulkner and Weary, 2000; Milligan et al., 2004) and goat kids (Ingvast-Larsson et al., 2011; Hempstead et al., 2018). Accumulated research clearly emphasises the importance of providing animals with NSAID to reduce post-operative pain associated with inflammation.

Local anaesthesia can reduce the acute response of calves to cautery disbudding (Graf and Semm, 1999; Grondahl-Nielsen et al., 1999); however, local anaesthesia does not appear to be effective for kids. The cortisol response of kids administered local anaesthesia before disbudding was similar to that of kids disbudded without anaesthesia (Alvarez et al., 2009, 2015). The horn buds of calves are innervated by the lacrimal nerve, whereas in goats the cornual branches of two nerves (lacrimal and infratrochlear) innervate the horn buds (Dugdale,

2011); therefore, it may be more difficult to achieve an effective block in goat kids. Because local anaesthesia, as administered, appears to be ineffective at reducing pain in goat kids, there is a need to evaluate alternatives that are less painful than cauterisation.

Comparing the degree of pain associated with the different disbudding methods evaluated in this study can be difficult because the mechanism of tissue destruction differed. The severe heat and physical removal of tissue associated with cauterisation instantly destroyed nociceptors usually associated with intense acute pain; for this reason, there may be less post-operative pain compared with other methods due to reduced sensation. Immediate pain associated with caustic paste may be lower than the other methods due to the time required for the paste to penetrate the dermis; however, longer-term pain may be more intense than the other methods due to the corrosive action of the paste lasting for as long as it is in contact with the skin (Palao et al., 2010). To effectively destroy tissue, cryosurgical disbudding must rapidly generate low temperatures (approximately  $-20^{\circ}\text{C}$ ) and thaw slowly (up to  $10^{\circ}\text{C}/\text{min}$ ). The application of liquid nitrogen caused an immediate pain response associated with cell damage and excitation of nociceptors (Vinuela-Fernandez et al., 2007). We hypothesised that kids would have a lower pain response to cryosurgical than cauterisation due to the response observed in calves disbudded with liquid nitrogen, which appeared to experience less pain compared with a cauterisation iron (Bengtsson et al., 1996). A difference in the time required to complete the technique (i.e., 10 s for CRYO and 5 to 7 s for CAUT) may have affected the level of pain experienced. Based on histological results of an earlier pilot study (our unpublished data), 10 s of liquid nitrogen spray was sufficient to destroy horn bud cells. Clove oil can be cytotoxic to human skin cells, causing membrane lysis and consequent necrosis or cell apoptosis (Prashar et al., 2006). Clove oil can also inhibit certain cellular enzymes involved in cell transport processes (Kreydiyyeh et al., 2000), which may cause cell death. However, clove oil has beneficial properties such as anaesthetic and anti-inflammatory actions (Markowitz et al., 1992). Further research is necessary to better understand the effect of clove oil on horn bud tissue and associated pain.

All disbudding methods except for clove oil caused burns to the horn buds and surrounding tissue; however, resultant wounds and characteristics of wound healing were quite different among methods. Cauterisation and caustic paste disbudding appeared to generate more tissue damage than the other two

disbudding methods. Cautery disbudding caused deep dermal or full thickness burns, which extended through all layers of the skin (Benson et al., 2006; Papp, 2012). Thermal injury denatures surrounding extracellular proteins and causes instant cell death. Circulation is ceased immediately, which leads to decreased tissue perfusion, both of which prolong recovery (Papp, 2012). Furthermore, the horn buds were removed, resulting in subcutaneous damage (an 18-mm open wound; Wright et al., 1983), which increases not only time required for the stages of healing (inflammation, proliferation, and remodelling), but the risk of infection (Zielins et al., 2015). Bacteria and bacterial by-products can delay wound healing by disrupting healing processes (Robson, 1997). At 6 wk post-treatment, CAUT kids still displayed scabs, indicating prolonged healing.

The corrosive action of caustic paste causes cellular dehydration and consequent liquefaction necrosis of tissue that usually produces a soft eschar (Palao et al., 2010). The resultant chemical burns are typically superficial to mid-dermal burns characterised by blistering, which can erupt, revealing a red, shiny, moist, and painful wound bed (Papp, 2012). Kids treated with caustic paste had a larger area of tissue damage than CAUT kids, with associated eschar formations that were apparent for up to 6 wk post-treatment. It is difficult to control the spread of caustic paste as it can smear easily; animals may rub against others or scratch the horn bud area with the hind foot (Smith and Sherman, 2009).

Cryosurgical disbudding causes freezing of the tissues and produces intracellular ice and cell dehydration (Gage et al., 1982; Krunic and Marini, 2015). Similar to caustic burns, cryosurgical disbudding causes superficial dermal burns, resulting in erythema and oedema. Although cryosurgical disbudding does not initially cause broken skin, consequent blistering and ulcerations were apparent for at least four CRYO kids. Broken skin increases the risk of infection, which can delay wound healing (Robson, 1997). Wounds appeared similar to those of calves experiencing cryosurgical disbudding, which displayed vesicle formations (Bengtsson et al., 1996). Cryosurgical disbudding resulted in smaller scabs than caustic paste 6 wk post-treatment.

Clove oil can be cytotoxic in isolated cells (Prashar et al., 2006); however, the mechanisms of wound healing after injection of clove oil into tissue are not well understood. Clove oil caused patches of blackened skin around the injection site (which was likely necrotic tissue) but no open wounds over the 6-wk observation period; this suggests that disbudding with clove oil may allow more

efficient healing, potentially reducing the occurrence of infection and damage to the skull and brain caused by the cautery iron (Wright et al., 1983; Thompson et al., 2005). Clove oil injection generated smaller scabs than the other disbudding methods, suggesting that less tissue damage occurred around the horn buds. Further research is required to better understand wound repair after injection of clove oil into the horn buds of goat kids.

Three of the 10 CLOV kids had inflammation of the eye lid (and surrounding area) and haptoglobin concentrations twice their baseline levels, which lasted approximately 24 h post-treatment; incorrect placement of the needle and consequent movement of clove oil closer to the upper eyelid area may explain this result.

From our monitoring of wound characteristics, caustic paste and cautery disbudding appear to generate the most tissue damage (and a prolonged healing phase), followed by cryosurgical disbudding. Disbudding with clove oil resulted in less tissue damage and appeared to lead to earlier tissue repair than the other treatments; therefore, we suggest that clove oil shows the most promise as an alternative to cautery disbudding.

Bates et al. (2016) reported that calves disbudded without pain relief had slower growth rates than calves disbudded with pain relief. In the present study, there were no differences in growth rates across treatments over 7 d post-treatment. However, there was lower weight gain 2 d after treatment for all kids, suggesting that handling stress (e.g., blood sampling, restraint) can also affect weight gain. A potential explanation may be a lack of motivation to feed or that feeding was disrupted by blood sampling and associated handling. We ensured that milk feeders were removed from the pens at least 12 h before BW measurements were taken to reduce the effect of gut fill. From our results, it appears that none of the disbudding methods evaluated in this study had a negative effect on the growth rates of goat kids.

Several CLOV, CASP, and CRYO kids developed scurs in our study (no CAUT kids developed scurs as the technique destroys and removes the horn buds); scurs in kids exposed to the alternative disbudding methods were likely due to the use of older animals with mature horn bud cells. Studies using younger animals (as young as 5 d old) to assess the effectiveness of cryosurgical disbudding and clove oil injection reported greater success in preventing scurs (Bengtsson et al., 1996; Molaei et al., 2015). As our study was intended to

measure the acute pain response, older animals were required due to high variability of cortisol of young goat kids (Chen et al., 1999). Future research should assess how clove oil volume, eugenol concentration, injection technique, and kid age affect scur growth.

### 3.5 Conclusions

Based on our results, disbudding goat kids with caustic paste is more painful and causes greater tissue damage than the other disbudding methods we assessed. Cryosurgical disbudding appears to cause intermediate pain (i.e., between caustic paste and clove oil) and moderate wounds; however, this method may not be practical due to difficulties associated with managing liquid nitrogen on farm. Clove oil may serve as a valuable alternative to cautery disbudding because it appears to generate a similar acute pain response as cautery disbudding but results in less tissue damage. Future research is required to evaluate the behavioural responses of goat kids experiencing different methods of disbudding; in addition, research is required to validate the efficacy of clove oil disbudding in preventing horn regrowth in dairy goat kids.

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# Chapter 4

## Evaluation of alternatives to cautery disbudding of dairy goat kids using behavioural measures of post-treatment pain

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Author contributions

Funding acquisition: MAS, MS. Conceptualisation: MNH, MAS, MS, JRW.

Experimental design: MNH, MAS, MS, JRW, VMC. Performed the experiments: MNH. Analysed the data: VMC, MNH. Interpretation of findings: MNH, JRW, MAS, MS. Wrote the paper: MNH, JRW, MAS, MS, VMC.

## Abstract

Alternatives to cauterly disbudding (caustic paste and cryosurgical disbudding, and clove oil injection) were evaluated using behavioural measures of post-treatment pain in dairy goat kids. Fifty Saanen doe kids were randomly assigned to one of five treatments ( $n=10/\text{treatment}$ ): (i) cauterly (CAUT), (ii) caustic paste (CASP), (iii) cryosurgical (liquid nitrogen; CRYO), (iv) clove oil (CLOV) or (v) sham disbudding (SHAM). Head and body shaking, head scratching, self-grooming and feeding were video-recorded for 24 h pre- and post-treatment. Frequencies of each behaviour were measured over 1 h pre- and post-treatment, as were the durations of head scratching, self-grooming and feeding. Accelerometers measured lying bouts and lying time for 24 h pre- and post-treatment. CASP kids displayed more head shakes ( $73.7$  vs.  $38.5 \pm 11.06$  No./h) and head scratches ( $35.1$  vs.  $13.1 \pm 6.62$  No./h) but less self-grooming ( $1.3$  vs.  $10.8 \pm 2.00$  No./h) and body shakes ( $1.6$  vs.  $4.3 \pm 0.88$  No./h), and shorter feeding durations ( $1.0$  vs.  $2.4 \pm 0.61$  min/h), than CAUT kids ( $P \leq 0.05$ ). CRYO kids performed more head scratches ( $28.8$  vs.  $13.1 \pm 6.62$  No./h) but less body shakes ( $2.1$  vs.  $4.3 \pm 0.88$  No./h), and spent less time lying ( $15.8$  vs.  $17.0 \pm 0.32$  h/24h) but with more bouts ( $32.8$  vs.  $26.3 \pm 2.25$  No./24h) than CAUT kids ( $P \leq 0.05$ ). Head shaking, scratching and self-grooming frequencies in CLOV kids ( $34.0 \pm 11.06$ ,  $16.7 \pm 6.62$  and  $12.6 \pm 2.00$  No./h, respectively) were no different to those for CAUT kids ( $P > 0.10$ ). CLOV kids spent less time lying ( $16.1$  vs.  $17.0 \pm 0.32$  h/24h) (but with more bouts [ $33.4$  vs.  $26.3 \pm 2.25$  No./24h]) than CAUT kids ( $P \leq 0.05$ ), which suggests less pain, as SHAM kids spent less time lying than CAUT kids ( $16.2$  vs.  $17.0 \pm 0.32$  h/24h;  $P \leq 0.05$ ). Our results suggest that caustic paste and cryosurgical disbudding were more painful than cauterly disbudding and may not be suitable alternatives for goat kids. During the first hour after treatment, clove oil injection appeared to cause less pain than caustic paste or cryosurgical disbudding, but a similar behavioural response as cauterly disbudding. Clove oil injection may show promise as an alternative to cauterly disbudding. However, future research should first evaluate the efficacy of clove oil in preventing horn growth; if effective, further research on the long-term effects of clove oil on goat welfare should be conducted.

## 4.1 Introduction

Cautery disbudding is a common husbandry practice performed on calves (Morisse et al., 1995; Graf and Senn, 1999; Grondahl-Nielsen et al., 1999) and goat kids (Alvarez et al., 2009; Ingvast-Larsson et al., 2011) to prevent horn growth, but causes pain and distress. Kids perform higher frequencies of leg shaking during disbudding as well as intense and frequent vocalisations compared with handled controls; these responses appear to be indicative of pain (Alvarez and Gutiérrez, 2010; Alvarez et al., 2015; Nfor et al., 2016). Immediately following disbudding, kids also perform frequent head and body shakes, and longer head scratching episodes than handled controls, which may also reflect pain (Greenwood and Shutt, 1990; Hempstead et al., 2017, 2018a). In addition, lying behaviour may be a useful measure of pain for kids; calves disbudded without pain relief spent less time lying than calves disbudded with pain relief (Heinrich et al., 2010). Therefore, behavioural changes, such as head-related and lying behaviours can be used to estimate pain in relation to disbudding.

If cautery irons are used excessively during disbudding, in terms of pressure and application duration (associated with poor training), thermal injury to the skull and brain may result, which can lead to bacterial infection and consequent meningoencephalitis and mortality in goat kids (Wright et al., 1983; Sanford, 1989; Thompson et al., 2005). Therefore, it is necessary to investigate potential alternatives to cautery disbudding for goat kids, which cause less post-treatment pain and reduce the risk of injury to the skull and brain. Alternatives to cautery disbudding have been established for calves, but not for goat kids. These methods include caustic paste (Morisse et al., 1995; Vickers et al., 2005; Stilwell et al., 2009; Winder et al., 2017) or cryosurgical disbudding (Bengtsson et al., 1996; Stewart et al., 2014) and clove oil injection (Molaei et al., 2014; Sutherland et al., 2018). Caustic paste has been suggested to be less painful than cautery disbudding in calves based on the evidence of lower rates of head shaking (Vickers et al., 2005). The corrosive action of caustic paste (usually a sodium or calcium hydroxide formulation; Stafford and Mellor 2011) causes chemical burns and destroys horn bud cells and surrounding tissue (Palao et al., 2010; Papp, 2012). Cryosurgical disbudding, which involves spraying liquid nitrogen (under pressure) onto the horn buds, may also be less painful than cautery disbudding as the skin is not broken and resultant wounds are less severe (Bengtsson et al., 1996; Stewart et al., 2014). Clove oil injection into the horn bud is a novel disbudding



technique, which was reported to prevent horn growth in calves (Molaei et al., 2014) and kids (Molaei et al., 2015); Molaei et al. (2015) suggested that the technique was less stressful than cauterly disbudding, although no specific measures of pain were presented. Moreover, Sutherland et al. (2018) suggested that clove oil injected under the horn bud of calves was initially less painful and did not cause any more pain than cauterly disbudding 48 h post-treatment. Clove oil has been used in dentistry as a mild anaesthetic (Markowitz et al., 1992) and is a well-established fish anaesthetic (Soto and Burhanuddin, 1995; Munday and Wilson, 1997; Sladky et al., 2001). The anaesthetic, anti-inflammatory and cytotoxic properties of clove oil, which contains eugenol as its major component (Markowitz et al., 1992; Prashar et al., 2006), may have application for disbudding.

The objective of this study was to evaluate alternatives to cauterly disbudding (caustic paste and cryosurgical disbudding, and clove oil injection) for goat kids using behavioural measures of post-treatment pain. We hypothesised that the application of caustic paste would cause the greatest amount of post-treatment pain due to the extended time course of a caustic burn compared with the other methods (Hettiaratchy and Dziewulski, 2004). We also expected cryosurgical disbudding and clove oil injection to result in less pain than cauterly disbudding due to a reduction in tissue damage caused by liquid nitrogen compared with the cauterly iron, and the anaesthetic properties of clove oil (Markowitz et al., 1992).

## **4.2 Materials and methods**

This study was part of a larger study that also evaluated the physiological responses (e.g., serum cortisol and haptoglobin concentrations, skin temperature, and weight gain) to the four disbudding methods considered here (Hempstead et al., 2018b). The same animals were used for both studies.

### **4.2.1 Animals and housing**

This study used 50 female Saanen or Saanen cross dairy goat kids (mean  $\pm$  SD, 5.2  $\pm$  0.66 kg) aged 10.6  $\pm$  0.91 d old (mean  $\pm$  SD) at treatment, and was conducted at the Ruakura Research Farm, Waikato (latitude 37°47'S, longitude 175°19'E), New Zealand during July and August, 2016. The Ruakura Animal

Ethics Committee approved the use of animals prior to the commencement of the study (Protocol No. 13899). Goat colostrum was fed to all kids at birth; they were separated from their dam after 24 h. Kids were collected from a commercial farm at approximately 2 d of age and transported to the Ruakura Research Farm. Upon arrival, kids were weighed and given an identification collar, which corresponded with the treatment they received (described below). The kids were vaccinated subcutaneously (s.c.) (Covexin, Schering-Plough Animal Health Limited, Wellington, New Zealand) and, as part of routine farm practice, were administered a prophylactic antibiotic s.c. (Norocillin, 30% w/v, Norbrook Laboratories Ltd., Northamptonshire, England). The kids were marked with paint to identify individuals in video-recordings (i.e. a line across the shoulders, or along the spine, a double line across the rump, a cross on the rump or left unmarked).

Kids were housed in groups of five in pre-treatment pens (2.4 x 1.6 m) with concrete floors covered with a 10 cm deep layer of clean, dry bedding (wood shavings, PGG Wrightson, Hamilton, New Zealand). Kids were kept with the same pen-mates for the duration of the trial. Each kid had access to at least 600 mL/d of milk replacer, which was increased gradually to 1 L/d (Anlamb, Fonterra Ltd., Auckland, New Zealand) via a 10-space kid feeder (Milk Bar, Waipu, New Zealand). Milk replacer was provided at approximately 0700 and 1600 h with *ad libitum* access within this period of time. Following the afternoon feeding time, feeders were removed to reduce gut fill impacting body weight measurements the following morning (for the physiological study; Hempstead et al., 2018b). The feeders were then replaced in time for morning feeding. Water was provided in a bucket attached to the pen wall. The daily temperature and relative humidity inside the facility ranged between 6.0 and 24.5°C (mean  $\pm$  SEM: 12.9  $\pm$  0.03°C) and 37 and 93% (69.5  $\pm$  0.18%), respectively.

#### **4.2.2 Experimental design**

Our experiment used a randomised complete block design, blocked by treatment day and pen within treatment day. The kids were randomly allocated to one of five treatments balanced for age (n = 10/treatment). Treatment order was randomly generated by the project manager using Genstat software (Version 17, VSN International Ltd., Hemel Hempstead, UK). Only one kid per treatment was

represented per pen and all kids from the same pen were treated on the same day. Treatments were conducted over four treatment days within a 2-wk period. Kids were fed approximately an hour before treatment, and then collected from their pre-treatment pens and restrained in a device described by Hempstead et al. (2018a). For all treatments, hair was removed with an electric clipper (Laube, 505 cordless kit, Shoof, Cambridge, New Zealand) prior to treatment to clearly identify the horn buds.

Treatments were carried out in the same room containing the pre- and post-treatment pens by the same veterinarian between 0900 and 1030 h each day. Treatments were the same as those described in Hempstead et al. (2018b):

1. SHAM: Kids were sham-handled and a finger was used to massage each horn bud in a circular motion for 10 s.
2. CAUT: Kids were cautery disbudded using an iron (“Quality” electric debudder, 18 mm tip, 230 V, 190 W; Lister GmbH, Lüdenscheid, Germany), which was heated (to approximately 600°C) for 20 min prior to being held to each horn bud for 5 – 7 s using methodology described by Hempstead et al. (2017).
3. CASP: Kids were caustic paste disbudded using a sodium hydroxide-based paste (Hornex, Shoof International Ltd., Cambridge, New Zealand) that was rubbed onto each horn bud (0.16 mL/bud) using a fingertip (of a gloved hand); a ring of petroleum jelly was spread around each horn bud prior to application to stop the paste from running into the kids’ eyes. The caustic paste treatment was based on the protocol described by Vickers et al. (2005) for calves.
4. CRYO: Kids were cryosurgically disbudded using a commercial applicator (CryAc® B-700, 500 mL capacity, Brymill Cryogenic Systems, Ellington, CT), which sprayed liquid nitrogen onto each horn bud for 10 s. A device, which consisted of a rubber cone (1 cm diameter touching the head and 2 cm diameter at the other end) connected to a metal handle, was pressed against the head of the kid to localise the spread of the spray and also to protect the kids’ eyes.
5. CLOV: Kids were injected with 0.2 mL (Molaei et al., 2015) of clove oil (C8392, 100mL, 83-85 % eugenol, Sigma-Aldrich, Saint Louis, MO) laterally into the centre of each horn bud at a 45° angle between the ear and muzzle; a well inside the horn bud was made by inserting and rotating the

needle and then pulling backwards slightly to create space for the clove oil to be injected.

A pilot experiment was carried out prior to commencing the present study to determine the amount of time liquid nitrogen should be sprayed on the bud and to validate the volume of clove oil Molaei et al. (2015) applied to destroy horn bud cells (using histology; our unpublished results). As the objective of this study was to evaluate pain in response to the different methods of disbudding, pain mitigation was not used for any treatment. Following treatment, the horn buds of CAUT kids were sprayed with antibacterial spray (AluSpray, Neogen Corporation, KY) to reduce the risk of infection. To reduce observer bias, SHAM kids were also sprayed. However, CASP, CRYO and CLOV kids were not sprayed to prevent interference with the treatments. After treatment, kids were placed into their post-treatment pens (adjacent to the pre-treatment pens). Kids remained at the Research Farm for 6 wk until they were returned to the farm of origin after health monitoring had concluded.

#### **4.2.3 Behavioural measurements**

Prior to pre-treatment (baseline) data collection, there was a 1 d habituation period to reduce the effects of handling and transport on behaviour. The behaviour of each animal was monitored continuously using video recorders (HC-V270, Panasonic Corp., Osaka, Japan) during 24 h pre- and post-treatment. Behaviour was then measured for 1 h pre-treatment (i.e. the same time as the post-treatment hour except the previous day) and the hour immediately post-treatment; these two hour periods were then compared. The first hour post-treatment was monitored because goat kids were found to be most active within this time period (Hempstead et al., 2018a). In addition, the behavioural observations for each kid were grouped into twelve 5-min periods within the first hour post-treatment, to better understand the acute behavioural response following treatment. The cameras were set 1.85 m above the pens at an angle of 30° to the vertical attachment pole. The cameras were fitted with fisheye lenses (Raynox, Insta-Wide lenses, QC-303, Yoshida Industry Co. Ltd. Tokyo, Japan) to enable a full view of each pen. Red floodlights (80 W, 30° beam, Philips Ltd., Auckland, New Zealand) were placed 2 m above each pen to assist with night observations.

Behaviours recorded were based on the ethogram described by Hempstead et al. (2017) but modified to improve inter-observer reliability (for comparison of ethograms, see Hempstead et al., 2018a). Behaviours measured were head and body shaking, head scratching, self-grooming and feeding (Table 4.1). Feeding was included in the present study as changes in feeding behaviour have been reported in similar calf studies (Graf and Senn, 1999). Behaviours directed at other kids (e.g., allogrooming) were not included as no differences were observed between treatments by Hempstead et al. (2017). Total frequencies of each of the behaviours during the pre- and post-treatment periods were recorded in addition to the total time spent head scratching, self-grooming and feeding during the same two periods. Behaviours during disbudding (e.g., leg shaking, vocalising; Alvarez and Gutiérrez, 2010) were not included as the method of restraint prevented a clear view of the legs and also mouth movements.

**Table 4.1.** Ethogram of behaviour patterns of dairy goat kids (modified from Hempstead et al. [2018a]) observed during video analysis, recorded during the 1 h pre- and post-treatment observation periods.

<b>Behaviour</b>	<b>Description</b>
Head Shaking	Rapid continuous tilting of the head from side to side concluding with a return to neutral position. Head shakes separated by > 1 s were considered separate events.
Body Shaking	Hackles on the back were raised and the body shook from side to side. Body shakes separated by >1 s were considered separate events.
Head Scratching	The rear foot touched any part of the head or neck (including collar). Scratches separated by > 1 s were considered separate events.
Self-Grooming	The kid's muzzle contacted any part of the body or legs (excluding hoof) with a rhythmic back and forth motion. A separate grooming event was considered to have occurred after a pause of > 1 s.
Feeding	The mouth covered at least half of the nipple of the feeder for > 3 s, usually followed by suckling motions. A separate feeding event occurred after the mouth was not in contact with the nipple for > 3 s.

Behaviours were not recorded when handlers were collecting or returning kids to their pens (usually < 15 s per pen visit). One trained observer analysed the video-recordings using Adobe Premiere Pro software (CS6, Version 6.0.0, Adobe Systems Incorporated, San Jose, CA). The observer remained blind to the treatment each kid received except that SHAM and CAUT kids could be identified as they were sprayed with silver antibiotic spray. Frequency and duration data were recorded against kid identification. Intra-observer reliability

was assessed on three kids selected at random; their behaviour was recorded for 1 h and then re-examined ( $\kappa = 0.73$  for head shaking;  $\kappa = 0.82$  for head scratching;  $\kappa = 0.96$  for self-grooming;  $\kappa = 0.80$  for feeding and  $\kappa = 1.00$  for body shaking).

Lying behaviour was measured using accelerometers (HOBO Pendant G data logger, 64k, Onset Computer Corporation, Bourne, MA) set to record x and z axes at 30 s intervals. The accelerometer was placed into a durable fabric pouch and strapped, with Velcro®, to the lateral side of the left hind leg above the metatarsophalangeal joint. At the conclusion of the trial, the data were downloaded using HOBOWare Pro software (Version 3.4.1, Onset Computer Corporation, Bourne, MA) and converted to hourly summaries of lying behaviour in SAS (Version 9.3, SAS Institute Inc., Cary, NC) using code designed for this purpose (AWP UBC, 2016) and validated for use in goats (Zobel et al., 2015). Lying bouts, bout duration and total lying time over 24 h pre- and post-treatment were presented. Due to the extended period of monitoring, compared with the video analysis (i.e. 24 vs. 1 h), time when the kids were being handled was not taken into consideration.

#### **4.2.4 Statistical analysis**

Genstat software (Version 17, VSN International Ltd., Hemel Hempstead, UK) was used to analyse our data. Residual plots were examined to determine whether there were any departures from the assumptions of normality and constant variance. Logarithmic transformations were required for 5-min period data within the first hour (except for feeding frequency). A kid from the CAUT group was removed from the trial due to a leg injury unrelated to treatment.

Behaviour frequency and duration data were analysed using a one-way analysis of covariance (blocked by treatment date and pen) adjusted for the pre-treatment values. Further analyses were carried out on behaviour data within the first hour post-treatment (twelve 5-min periods) using a repeated measures model fitted by restricted maximum likelihood. The model included the fixed effects for treatment, time (5-min intervals) and their interaction and the random effects for kid, age, weight, farm, treatment date and pen. The correlation between measurements taken on the same kid over time was modelled with a power model of order 1. The average of the twelve 5-min periods 1 h pre-treatment was used as

the covariate. Only treatment by time interactions were presented as the effects of treatment were observed for the total hour analysis.

Lying bouts, bout duration and total lying time over 24 h post-treatment were analysed using a one-way analysis of covariance blocked by treatment date and pen with pre-treatment lying behaviour used as the covariate.

Fisher's least significant differences test was used to detect differences between and within treatments. Mean values (back-transformed if required, with exact 95% CI) were provided with standard errors of the difference (SED) and the level of significance was set at  $P \leq 0.05$ .

### 4.3 Results

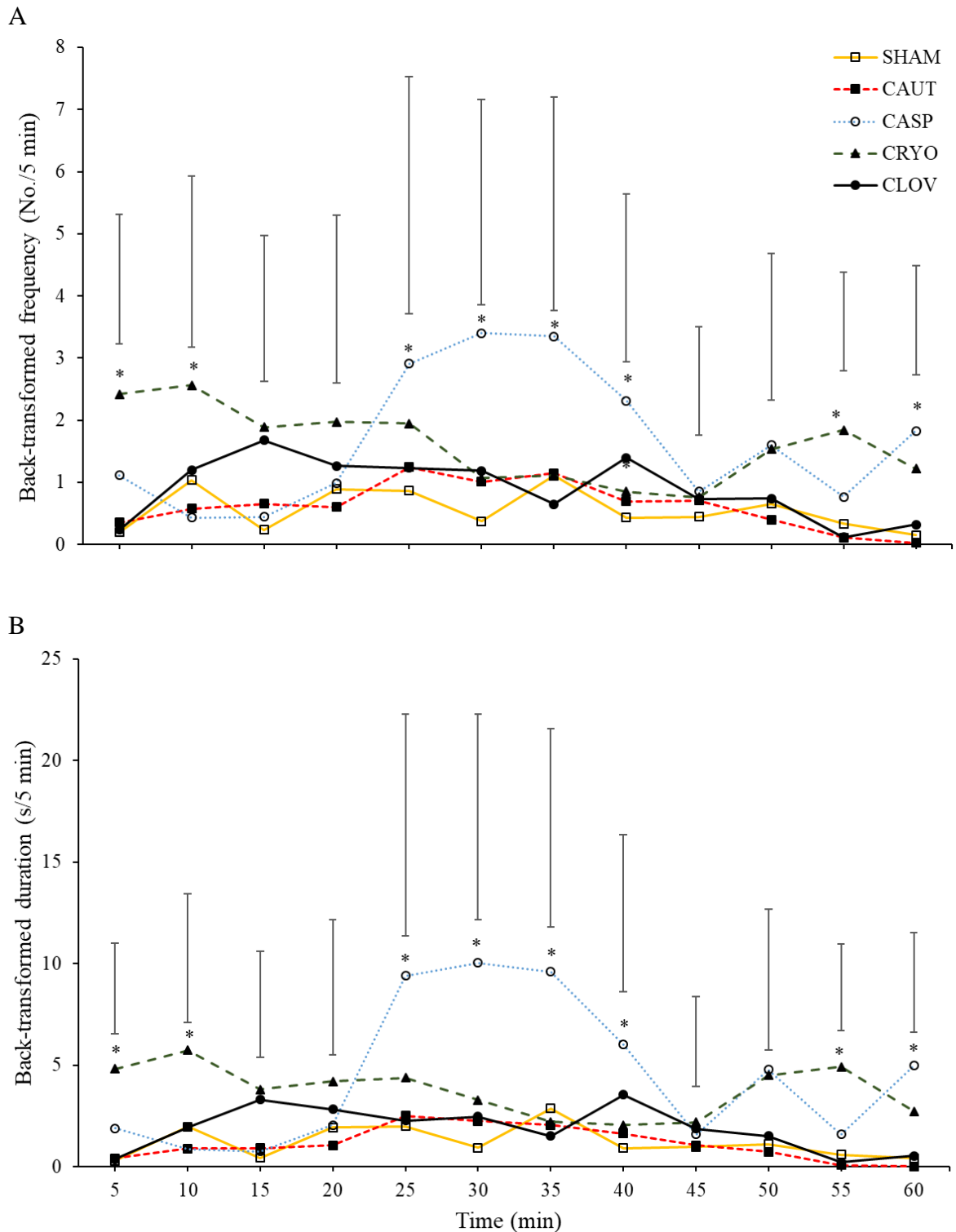
There was a strong treatment effect for head shaking frequency 1 h post-treatment ( $F_{4,34} = 4.5$ ,  $P = 0.005$ ; Table 4.2). The frequency of head shakes of CASP were higher than those of CAUT kids ( $P \leq 0.05$ ), but there was no difference in head shakes between SHAM, CAUT, CRYO and CLOV kids ( $P > 0.50$ ). For the 5-min periods within the 1 h post-treatment period, there was no treatment by time interaction ( $F_{44,443} = 1.2$ ,  $P = 0.20$ ).

There was a strong treatment effect for head scratching frequency ( $F_{4,34} = 5.2$ ,  $P = 0.002$ ) and duration ( $F_{4,34} = 5.0$ ,  $P = 0.003$ ) 1 h post-treatment (Table 4.2). CRYO kids performed more head scratching than CAUT kids ( $P \leq 0.05$ ). In addition, head scratching was more frequent and longer in duration for CASP than CAUT kids ( $P \leq 0.01$ ). There was no difference in head scratching frequency or duration for SHAM, CAUT and CLOV kids ( $P > 0.10$ ). There was a treatment by time interaction for head scratching frequency ( $F_{44,443} = 1.6$ ,  $P = 0.01$ ; Figure 4.1A) and duration ( $F_{44,453} = 1.4$ ,  $P = 0.05$ ; Figure 4.1B) across the 5-min periods 1 h post-treatment. Head scratching frequency and duration of CRYO and CASP kids were above CAUT kid levels frequently post-treatment ( $P \leq 0.05$ ; Figure 4.1A and 4.1B); frequency and duration did not differ between SHAM and CAUT kids post-treatment ( $P > 0.10$ ). Furthermore, the frequency and duration of head scratches for CLOV kids was similar to that of CAUT kids post-treatment ( $P > 0.10$ ).

**Table 4.2.** Mean ( $\pm$  SED) frequency (No./h) of head shaking, body shaking, head scratching, self-grooming and feeding, and duration (min/h) of head scratching, self-grooming and feeding over 1 h post-treatment; mean ( $\pm$  SED) number of lying bouts (No./24 h), bout duration (min) and total lying time (h/24 h) over 24 h post-treatment (adjusted for pre-treatment behaviour). Goat kids (n = 10/treatment) were disbudded using caustic paste (CASP), liquid nitrogen (cryosurgical disbudding; CRYO), clove oil (CLOV) and a cautery iron (CAUT) or the horn buds were massaged but not disbudded (SHAM). Means with differing superscripts were significantly different at  $P \leq 0.05$ .

Behaviours	CASP	CRYO	CLOV	CAUT	SHAM
<b>Head shaking</b>					
Frequency (No./h)	73.3 $\pm$ 11.06 <sup>b</sup>	55.9 $\pm$ 11.06 <sup>ab</sup>	34.0 $\pm$ 11.06 <sup>a</sup>	38.5 $\pm$ 11.06 <sup>a</sup>	38.1 $\pm$ 11.06 <sup>a</sup>
<b>Body shaking</b>					
Frequency (No./h)	1.6 $\pm$ 0.88 <sup>a</sup>	2.1 $\pm$ 0.88 <sup>a</sup>	1.7 $\pm$ 0.88 <sup>a</sup>	4.3 $\pm$ 0.88 <sup>b</sup>	2.6 $\pm$ 0.88 <sup>ab</sup>
<b>Head scratching</b>					
Frequency (No./h)	35.1 $\pm$ 6.62 <sup>b</sup>	28.8 $\pm$ 6.62 <sup>b</sup>	16.7 $\pm$ 6.62 <sup>a</sup>	13.1 $\pm$ 6.62 <sup>a</sup>	10.5 $\pm$ 6.62 <sup>a</sup>
Duration (min/h)	2.4 $\pm$ 0.47 <sup>b</sup>	1.5 $\pm$ 0.47 <sup>ab</sup>	0.9 $\pm$ 0.47 <sup>a</sup>	0.7 $\pm$ 0.47 <sup>a</sup>	0.5 $\pm$ 0.47 <sup>a</sup>
<b>Self-grooming</b>					
Frequency (No./h)	1.3 $\pm$ 2.00 <sup>a</sup>	7.9 $\pm$ 2.00 <sup>b</sup>	12.6 $\pm$ 2.00 <sup>c</sup>	10.8 $\pm$ 2.00 <sup>bc</sup>	8.7 $\pm$ 2.00 <sup>bc</sup>
Duration (min/h)	0.08 $\pm$ 0.21 <sup>b</sup>	0.6 $\pm$ 0.21 <sup>a</sup>	0.9 $\pm$ 0.21 <sup>a</sup>	0.9 $\pm$ 0.21 <sup>a</sup>	0.7 $\pm$ 0.21 <sup>a</sup>
<b>Feeding</b>					
Frequency (No./h)	3.3 $\pm$ 2.59 <sup>a</sup>	7.3 $\pm$ 2.59 <sup>a</sup>	6.0 $\pm$ 2.59 <sup>a</sup>	7.9 $\pm$ 2.59 <sup>a</sup>	9.2 $\pm$ 2.59 <sup>a</sup>
Duration (min/h)	1.0 $\pm$ 0.61 <sup>b</sup>	1.7 $\pm$ 0.61 <sup>ab</sup>	1.2 $\pm$ 0.61 <sup>ab</sup>	2.4 $\pm$ 0.61 <sup>a</sup>	2.6 $\pm$ 0.61 <sup>a</sup>
<b>Lying</b>					
Bouts (No./24h)	29.2 $\pm$ 2.25 <sup>ab</sup>	32.8 $\pm$ 2.25 <sup>a</sup>	33.4 $\pm$ 2.25 <sup>a</sup>	26.3 $\pm$ 2.25 <sup>b</sup>	29.9 $\pm$ 2.25 <sup>ab</sup>
Bout duration (min)	36.3 $\pm$ 5.96 <sup>a</sup>	28.5 $\pm$ 5.96 <sup>a</sup>	30.4 $\pm$ 5.96 <sup>a</sup>	44.3 $\pm$ 5.96 <sup>a</sup>	34.1 $\pm$ 5.96 <sup>a</sup>
Time (h/24h)	16.5 $\pm$ 0.32 <sup>b</sup>	15.8 $\pm$ 0.32 <sup>a</sup>	16.1 $\pm$ 0.32 <sup>a</sup>	17.0 $\pm$ 0.32 <sup>b</sup>	16.2 $\pm$ 0.32 <sup>a</sup>





**Figure 4.1.** Back-transformed mean (error bars represent exact 95% CI for each time point) head scratching (A) frequencies (No./5 min) and (B) durations (s/5 min) over 1 h post-treatment in goat kids ( $n = 10/\text{treatment}$ ) that were disbudded using caustic paste (CASP), liquid nitrogen (cryosurgical disbudding; CRYO), clove oil (CLOV) or a cautery iron (CAUT). For the control, the horn buds were massaged but not disbudded (SHAM). Asterisk indicates means that differ from CAUT kid means at  $P \leq 0.05$ .

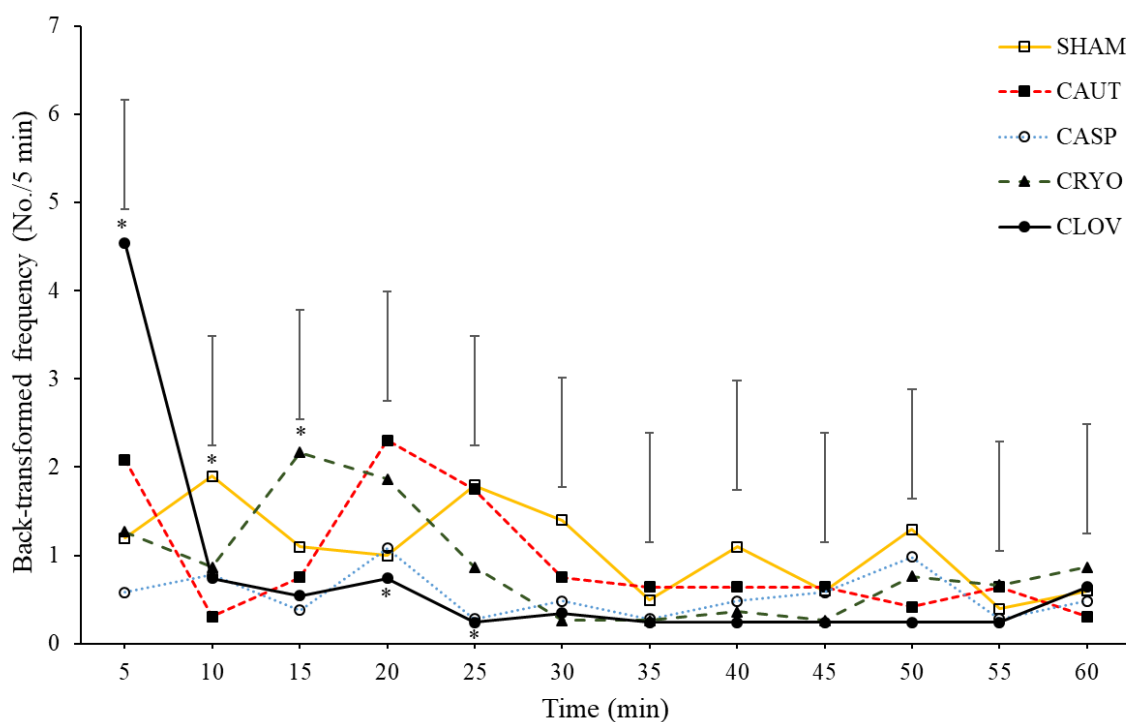
There was a strong treatment effect for self-grooming frequency ( $F_{4,34} = 9.4, P < 0.001$ ) and duration ( $F_{4,34} = 4.9, P = 0.003$ ) 1 h post-treatment (Table 4.2).

Self-grooming frequency ( $P \leq 0.01$ ) and duration ( $P \leq 0.05$ ) of CASP kids was lower than that of all other treatments. There was no difference in self-grooming frequency or duration across CAUT and SHAM kids ( $P > 0.10$ ). Self-grooming frequency and duration of CLOV ( $P > 0.10$ ) and CRYO ( $P > 0.50$ ) kids were no different to that of CAUT kids. For the 5-min periods 1 h post-treatment, there was no treatment by time interaction for self-grooming frequency ( $F_{44, 486} = 1.1, P = 0.38$ ) or duration ( $F_{44, 455} = 1.3, P = 0.13$ ).

There was a treatment effect for body shaking frequency ( $F_{4, 34} = 3.1, P = 0.03$ ; Table 4.2) 1 h post-treatment. Kids that experienced CAUT performed more body shakes than SHAM kids ( $P \leq 0.06$ ). CAUT kids performed more body shakes than CASP, CRYO and CLOV kids ( $P \leq 0.05$ ). Due to the low numbers of body shakes over the 1 h post-treatment period, the 5-min period data was not presented.

There was no effect of treatment for feeding frequency ( $F_{4, 34} = 1.5, P = 0.22$ ) but there was an effect of treatment on feeding duration 1 h post-treatment ( $F_{4, 34} = 2.6, P = 0.05$ ; Table 4.2). CASP kids spent less time feeding than CAUT kids ( $P \leq 0.05$ ). There was no difference in feeding duration for SHAM and CAUT kids ( $P > 0.05$ ). Furthermore, there was no difference in feeding duration for CAUT, CLOV and CRYO kids ( $P > 0.10$ ). For the 5-min periods within 1 h post-treatment, there was a treatment by time interaction ( $F_{44, 465} = 2.2, P < 0.001$ ), but there was no consistent pattern of feeding among groups (Figure 4.2) as there were periodic fluctuations across time. There was no treatment by time interaction ( $F_{44, 465} = 1.3, P = 0.07$ ) on feeding duration.

There was an effect of treatment for mean lying time over 24 h post-treatment ( $F_4 = 3.4, P = 0.02$ ; Table 4.2). Kids that experienced CAUT and CASP spent more time lying than SHAM, CLOV and CRYO kids ( $P \leq 0.05$ ). There was an effect of treatment for the number of lying bouts over 24 h post-treatment ( $F_4 = 3.3, P = 0.02$ ; Table 4.2). Kids that experienced CAUT displayed a lower number of lying bouts than CLOV and CRYO kids ( $P \leq 0.01$ ). There was no difference in the number of lying bouts for SHAM kids compared with any other treatments ( $P > 0.50$ ). There was no difference in lying bout frequency between CLOV and CASP kids ( $P > 0.10$ ). Bout duration was not different across treatments over 24 h post-treatment ( $F_4 = 2.2, P = 0.09$ ; Table 4.2).



**Figure. 4.2.** Back-transformed mean (error bars represent exact 95% CI for each time point) feeding frequencies (No./5 min) over 1 h post-treatment in goat kids (n = 10/treatment) that were disbudded using caustic paste (CASP), liquid nitrogen (cryosurgical disbudding; CRYO), clove oil (CLOV) or a cautery iron (CAUT). For the control, the horn buds were massaged but not disbudded (SHAM). Asterisk indicates means that differ from CAUT kid means at  $P \leq 0.05$ .

## 4.4 Discussion

Alternatives to cautery disbudding (caustic paste and cryosurgical disbudding, and clove oil injection) were evaluated using behavioural measures of immediate post-treatment pain in goat kids. Until recently, there was limited understanding of behavioural indicators of pain in goat kids but there is now evidence to suggest that changes in head shaking, head scratching, self-grooming, body shaking and lying behaviour (Greenwood and Shutt, 1990; Heinrich et al., 2010; Hempstead et al., 2017, 2018a) may indicate pain associated with disbudding.

Kids that experienced the CASP treatment shook and scratched their heads more often and performed lower rates of self-grooming and feeding durations compared with CAUT kids. Moreover, CASP kids had elevated plasma cortisol concentrations compared to CAUT kids 1 h post-treatment (Hempstead et al., 2018b), which supports the interpretation that these behavioural responses are indicative of pain. We hypothesised that CASP kids would experience more post-treatment pain than CAUT kids, as the corrosive action of the caustic paste

usually lasts for a longer period of time (Hettiaratchy and Dziewulski, 2004; Smith and Sherman, 2009; Palao et al., 2010) than cautery disbudding, which is usually completed within a minute (Ingvast-Larsson et al., 2011; Hempstead et al., 2017, 2018a), although inflammation and associated pain can last for longer. It appeared that CASP kids had a stronger behavioural response for up to 40 min post-treatment, as evidenced by longer periods of, and more frequent head scratching than CAUT kids, possibly reflecting more pain over this time. Furthermore, caustic paste can be rubbed off by the animal's foot, and may prevent sufficient destruction of the nociceptors, but leaving the area highly sensitised. Following disbudding with caustic paste, calves performed more head shaking and rubbing and plasma cortisol concentrations were elevated compared to cautery disbudded calves, which suggests that this procedure caused more pain in calves (Morisse et al., 1995; Stilwell et al., 2009). However, others have found that calves disbudded with a cautery iron performed more head shakes than those disbudded with caustic paste (Vickers et al., 2005). This may be explained by the use of local anaesthesia and sedation for up to 4 h after disbudding (Vickers et al., 2005), which likely affected calf behaviour over this time. Furthermore, we observed a number of CASP kids frequently rubbing their heads along the walls of the pen or the pen floors, which suggests the kids were trying to remove the irritant. Accumulated evidence suggests that caustic paste causes more post-treatment pain than cautery disbudding and therefore may not provide a useful alternative for disbudding goat kids.

Over the hour after disbudding, CRYO kids performed a greater number of head-directed behaviours and lying bouts, and performed less self-grooming and feeding, suggesting they may have experienced more pain than CAUT kids. At closer inspection, the behavioural response of CRYO kids was higher than CAUT kids for 10 min post-treatment, then decreased to the same levels as CAUT kids for 40 min, and increased again at 55 min post-treatment. This fluctuation in the behavioural response to disbudding may indicate that cryosurgical disbudding initially causes acute pain associated with nociceptor activation followed by longer-term pain caused by inflammatory mediators. However, evaluation of inflammatory markers in goat kids post-disbudding is required to confirm whether this behavioural response is related to inflammatory pain or some other mechanism. In a previous study, kids that experienced cryosurgical disbudding had higher plasma cortisol concentrations than CAUT kids 15 min post-treatment

(Hempstead et al., 2018b), which further supports the suggestion that cryosurgical disbudding causes more pain than cautery disbudding in goat kids. However, the CRYO treatment may cause less tissue damage and longer-term pain than cautery disbudding in calves (Bengtsson et al., 1996). Our hypothesis that the CRYO treatment would cause less pain due to less resultant tissue damage than CAUT was not supported. The different behavioural responses seen across CAUT and CRYO treatments may be associated with the different mechanisms of tissue destruction. Cautery disbudding results in the complete destruction of the nociceptors innervating the horn buds and the tissue containing the horn buds is typically removed. The freezing process involves (i) direct tissue/cell injury by a build-up of extra- and intra-cellular ice, which damages cell components and causes cell death, (ii) vessel injury by thrombosis of all vessels, and (iii) inflammation as lymphocytes migrate to the area (Krunic and Marini, 2015). Furthermore, the depth of frozen tissue may vary with horn bud and skin thickness across kids. In addition, cryosurgical disbudding may be impractical for use on farm due to difficulties with storing liquid nitrogen, the expense of the spray device(s), and repeated use causing device malfunction (e.g., the spray nozzle can freeze, causing a blockage). Based on the results of our study, it appears that cryosurgical disbudding causes more post-treatment pain than cautery disbudding in kids and may be impractical for use on farm.

Kids disbudded with clove oil performed a similar number of head-related behaviours and feeding and self-grooming events, and spent less time lying, but with more bouts, than cautery disbudded kids, which indicates that clove oil may cause no more acute pain than cautery; we hypothesised that clove oil injection would cause less pain than cautery disbudding due to the anaesthetic effects of eugenol in clove oil (Markowitz et al., 1992). We found that CAUT kids spent more time lying than SHAM kids, which suggests that cautery disbudding may cause more discomfort than handling alone. As CLOV kids spent a similar amount of time lying as SHAM kids, CLOV kids may have been more comfortable than CAUT kids, although the higher number of bouts for CLOV kids may indicate restlessness; however, the effect of pain on lying behaviour in goat kids is not well understood, and caution should be taken when interpreting changes in lying behaviour.

Clove oil has cytotoxic properties, which can cause membrane lysis or inhibit cellular enzymes involved in cell transport processes (Kreydiyyeh et al.,

2000) and consequent cellular necrosis or apoptosis (Prashar et al., 2006). It is not clear whether the behavioural response of CLOV kids was associated with the needle injection, the displacement of tissue caused by the clove oil or the action of the clove oil on the cells; however, in future studies, additional controls using a carrier oil (e.g., olive oil) or saline could help to clarify which aspects of this procedure causes pain. It appears that clove oil injection may show some promise as an alternative to cauterisation; however, the evaluation of longer-term behavioural responses are required to identify any effects of clove oil beyond 24 h post-treatment. The methodology used in this study to inject clove oil has its limitations; some kids struggled during treatment, which made consistent needle insertion into the same location across kids difficult. Furthermore, at times, struggling affected the volume of clove oil injected under the skin, as the needle could become dislodged. Further refinement of this technique is required. Ideally, an applicator could be developed, which quickly administers the clove oil to improve consistency of needle placement and volume across kids. Future research on clove oil injection should investigate the exact mechanisms of cell destruction in goat horn bud tissue and whether the method of disbudding is efficacious (prevents horn regrowth).

An interesting finding of this study is that the apparent onset of pain may differ across the disbudding methods. As mentioned earlier, it appears that CRYO kids experienced more initial pain or irritation after treatment, and then again at 55 min post-treatment, compared to CAUT kids, which may indicate that the wounds associated with cryosurgical disbudding caused more pain at these times. Pain or irritation associated with caustic paste appears to reach peak levels 25 min after application (well above levels of CAUT kids) based on frequent head scratches at that time. Moreover, caustic paste may cause more prolonged pain than cauterisation evidenced by more head scratching over the entire 1 h post-treatment period. Although not significant, CLOV kids had numerically greater head scratching frequencies 15 min after treatment than CAUT kids, but was no different thereafter. Therefore, it appears that, like cauterisation, the behavioural response to injecting clove oil is short-lived and that post-treatment pain may be less than for the other methods assessed. To gain a better understanding of post-treatment inflammatory pain over time, future research should measure the behavioural and physiological responses of goat kids experiencing these disbudding methods for longer durations as has been done for

disbudded calves (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Doherty et al., 2007). Furthermore, a better understanding of the mechanisms of tissue damage and inflammatory pain following disbudding in goat kids can help farm managers make informed decisions about the use of post-treatment pain mitigation such as non-steroidal anti-inflammatory drugs.

We expected all disbudded groups to spend less time lying than handled controls, but the reverse was apparent for CAUT and CASP kids; these kids may spend more time lying, perhaps so as not to disturb their wounds and cause further pain. Increased time spent lying can be associated with increased comfort in calves (Heinrich et al., 2010; Sutherland et al., 2014). However, for castration of piglets and lambs, which is also known to be painful, lying times were higher than for handled controls (McGlone et al., 1993; Molony and Kent, 1997). McMeekan et al. (1999) also reported that calves that underwent amputation dehorning spent more time lying than handled controls for up to 4 h post-treatment. Others have found that disbudded calves displayed no differences in the ratio of standing to lying behaviour between the pre- and post-treatment periods (Morisse et al., 1995; Doherty et al., 2007). Therefore, further research is required to understand the relationship between lying behaviour and pain in dairy goat kids and other animals.

Interestingly, SHAM kids performed a similar number of head shakes and scratches to CAUT kids. It is generally considered that cautery disbudding causes intense, acute pain during the procedure as demonstrated by frequent struggles and high intensity vocalisations in kids (Alvarez et al., 2009) and escape or avoidance behaviour (e.g., head jerks, rearing) in calves (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999). In another study conducted by our group, the frequency of head shaking and scratching behaviour increased after disbudding compared with sham-handled kids (Hempstead et al., 2018a). The difference in results between studies may be due to the differing lengths of observation periods (e.g., 24 vs. 1 h), or the mean age of kids (4 vs. 10 d old); furthermore, these differences highlight the difficulties in evaluating goat kid behaviour, and that other measures in addition to behaviour should be used to assess pain associated with disbudding.

It is important to acknowledge the limitations of measuring behaviour and physiology within the same study. The effects of the alternative methods of disbudding on physiological measures of pain (e.g., cortisol and haptoglobin

concentrations) were presented in a companion study (Hempstead et al., 2018b). The handling associated with blood sampling is likely to have influenced kid behaviour; however, the effects of handling are largely consistent across treatments as kids were handled for a similar amount of time. Furthermore, there were no significant increases in frequency or duration of the behaviours monitored at the 5-min level post-treatment. In order to reduce the effect of handling on behaviour, it would have been ideal to use different animals for each study.

## 4.5 Conclusions

Kids that were disbudded using caustic paste performed more head shaking and scratching, and lower self-grooming and feeding durations, than those experiencing cautery disbudding, which suggests they experienced more post-treatment pain. Cryosurgical disbudding caused higher head scratching frequencies and less lying time (but with more lying bouts) than cautery disbudding indicating that cryosurgery caused more pain. Therefore, caustic paste and cryosurgical disbudding may not be suitable alternatives to cautery disbudding in goat kids. Clove oil appeared to cause a similar amount of pain as cautery disbudding based on similar head shaking and scratching frequencies and lower lying time (but with more lying bouts), therefore this method may show promise as an alternative to cautery disbudding. The findings of this study used in conjunction with physiological measures of pain (as evaluated in our companion study), may provide a better understanding of pain associated with the different disbudding methods. Future research should evaluate clove oil efficacy in preventing horn growth before assessing the long-term effects of clove oil on goat welfare.

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## Chapter 5

# Pain sensitivity and injury associated with three methods of disbudding goat kids: Cautery, cryosurgical and caustic paste

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## **Abstract**

We evaluated pain sensitivity and skull or brain injury associated with cautery, cryosurgical and caustic paste disbudding of goat kids. We randomly assigned 280 kids (reared for meat) to one of four treatments (n=70/treatment): (1) sham-handling (SHAM) or (2) cautery (CAUT), (3) cryosurgical (CRYO) or (4) caustic paste (CASP) disbudding. A pain sensitivity test using a pressure algometer was carried out 15 min pre- and 1 h post-treatment. Skull and brain injury were assessed by post-mortem examination. Kids with evidence of injury to the skull or brain (discolouration or indentation), as well as a random sample of kids (n = 15/treatment) without evidence of skull or brain injury, were selected for histological examination of brain tissue. Average daily gains (ADG) were calculated from body weight measurements taken 10 min pre-treatment and then at 2, 7 and 14 d post-treatment as a measure of the potential effects of pain or injury on growth. CASP and CRYO kids displayed higher pain sensitivity post-treatment than CAUT or SHAM kids ( $P < 0.001$ ), suggesting they experienced more acute pain 1 h post-treatment. One of 70 CAUT kids had a perforated skull but there was no histological evidence of brain injury; a further 9 CAUT kids displayed hyperaemia of the skull. None of the other treatments resulted in injury to the skull or brain. There was no evidence of a difference in ADG across treatments ( $P = 0.73$ ). Caustic paste and cryosurgical disbudding resulted in greater acute pain sensitivity than cautery disbudding; however, cautery disbudding has the potential to cause skull injury if performed incorrectly.

## 5.1 Introduction

Disbudding of goat kids is typically carried out within the first week of life with a hot cautery iron to prevent horn growth. Cautery disbudding of goat kids causes physiological (Alvarez et al., 2009, 2015; Hempstead et al., 2018a) and behavioural (Greenwood and Shutt, 1990, Hempstead et al., 2017, 2018a) changes indicative of pain. Pressure algometry has been used to measure pain sensitivity in calves that were cautery disbudded (Heinrich et al., 2010; Allen et al., 2013); the studies illustrated that disbudded calves had heightened sensitivity of tissues surrounding the procedural sites than sham-handled controls.

Pain can lead to changes in body weight through reduced feeding motivation (Borderas et al. 2009; National Research Council Committee [US], 2010). Calves that were cautery disbudded without pain relief displayed lower weight gains for up to 2 wk compared to calves disbudded with pain relief (Faulkner and Weary, 2000; Bates et al., 2015). Pain sensitivity and average daily gains (ADG) associated with disbudding have not been evaluated in goat kids.

Cautery disbudding of goats, if performed incorrectly, can cause damage to the skull and thermal injury to the brain and consequent necrosis (Wright et al., 1983; Sanford, 1989; Thompson et al., 2005). Bacterial infection of the brain or meninges can result from the weakened or damaged skull, which in turn, can lead to meningoencephalitis and mortality (Thompson et al., 2005). Therefore, it is necessary to investigate alternatives to cautery disbudding of goat kids that are less likely to cause injury to the skull and brain.

Alternatives to cautery disbudding have been evaluated in calves; caustic paste (Vickers et al., 2005; Stilwell et al., 2009) or cryosurgical disbudding (Bengtsson et al., 1996; Stewart et al., 2014) may cause less pain and skull or brain injury than cautery disbudding as suggested by lower rates of head shaking in caustic paste disbudded calves and reduced lying times in cryosurgically disbudded calves (Vickers et al., 2005; Stewart et al., 2014); however, cautery disbudding remains the most commonly used method for disbudding calves between 4 – 6 wk old (Stafford and Mellor, 2011). Effective alternatives should reduce pain sensitivity, have no adverse effect on ADG and generate no skull or brain injury. The aim of our study was to evaluate pain sensitivity, ADG and skull and/or brain injury associated with cautery, cryosurgical and caustic paste disbudding. We predicted that (i) pain sensitivity would be highest and (ii) ADG



would be lowest for caustic paste disbudded kids based on higher skin temperatures (indicating inflammation and associated pain) than the other disbudding methods 24 h post-treatment (Hempstead et al., 2018b), and (iii) skull and/or brain injury would be observed in cautery disbudded kids, which has been reported previously (Thompson et al., 2005).

## **5.2 Materials and methods**

### **5.2.1 Animals and housing**

Our study was conducted on a dairy goat farm in the Waikato region, New Zealand. The study was approved by the Ruakura Animal Ethics Committee (Protocol No. 13907). A total of 280 Saanen doe ( $n = 58$ ) and buck ( $n = 222$ ) kids aged  $4 \pm 1.0$  d (mean  $\pm$  SD), with an average body weight (BW) of  $4.6 \pm 0.86$  kg, were enrolled. The kids were reared for meat and slaughtered after  $19.0 \pm 5.41$  d, once they reached an average BW of  $7.1 \pm 0.83$  kg. Kids were housed in pens (9.2 x 3.0 m), which contained approximately 50 kids each. Kids were selected for inclusion if they were between 2 and 7 d of age and had horn buds (i.e. they were not polled). Clinical examinations were only performed by a veterinarian if an animal showed clinical signs of illness.

The kids were reared as per routine farm practice. The ground within the pens was covered with pine shavings (approximately 15 cm deep). The kids had *ad libitum* access to goat milk colostrum for at least 1 wk and were then provided with Sprayfo milk powder (AgriVantage, Hamilton, New Zealand) mixed according to package instructions. Each pen was provided with approximately 100 L of milk in a plastic drum feeder with six teats per drum. Fresh water was always available in a trough.

### **5.2.2 Experimental design and treatments**

Due to logistical reasons, we used a randomised unbalanced incomplete block design, with treatment day as the blocking variable. Kids were randomly assigned to one of four treatments ( $n = 70$ /treatment; described below). Treatments were balanced for sex and age and on each treatment day, treatment order was randomly generated. On average,  $35 \pm 4.6$  ( $\pm$  SEM) kids were disbudded per treatment day.

To determine sample size, a power analysis with 5% significance level and 80% power was carried out. The primary outcome was disbudding-related deaths (15%) from a study by our group (our unpublished data), assumed to be binomially distributed.

On treatment days, kids (mean  $\pm$  SD;  $6.0 \pm 2.21$  d old) were moved one at a time from the home pen to the treatment area and placed in a restraint device (see description in Hempstead et al., 2018a). For all treatments, hair was removed using clippers (Laube, 505 cordless kit, Shoof International Ltd., Cambridge, New Zealand) to clearly locate the horn buds. All treatments were performed between 1000 and 1400 h by the same operator (trained by a veterinarian), with the exception of cautery disbudding, which was performed by a professional contractor. Treatments included:

1. SHAM: Kids were sham-handled and a finger was used to massage each horn bud in a circular motion for 10 s.
2. CAUT: Kids were cautery disbudded using a liquefied petroleum gas powered iron ('Heavy Duty' brand, 18 mm tip; Shoof International Ltd.). The iron (approximately 600°C) was applied for approximately 6 s. The horn bud and surrounding tissue was removed by using downwards pressure and circular motions to forcibly lift off the horn bud tissue. Iodine spray (Vetadine PVP, Bomac Laboratories Ltd., Manakau, New Zealand) was then applied to the wounds.
3. CRYO: Kids were cryosurgically disbudded using a commercial applicator (Cry-Ac® B-700, Bry-Mill Cryogenic Systems, Ellington, CT) that sprayed liquid nitrogen onto each horn bud for 10 s. To localise the contact area and protect the kid's eyes, a device consisting of a rubber cone (1 cm diameter touching the head and 2 cm diameter at the Cry-Ac end) connected to a metal handle, was pressed against the head.
4. CASP: Kids were caustic paste disbudded using a sodium hydroxide-based paste (Hornex, Shoof International Ltd.) that was rubbed into each horn bud (0.16 mL/bud) for approximately 30 s using the fingertip of a gloved hand. Prior to the application of the paste, petroleum jelly was spread as a ring around each horn bud to prevent caustic paste from running into the kids' eyes.

Following treatment, the kids were placed into a small holding pen (2.5 x 1.5 m) within their home pen until measurements were taken. CASP kids were

housed within the same pen as the other kids but any transference of paste between kids was quickly removed. The kids were monitored for up to 2 h immediately after treatment and regularly (every 3 d) for up to 14 d post-treatment to ensure there were no negative effects (e.g., infection) of the disbudding treatments.

### **5.2.3 Pain sensitivity**

Pain sensitivity was measured using a digital pressure algometer (Force one FDIX 50, Wagner Instruments, Greenwich, CT) equipped with a 1 cm diameter rubber tip, applied to four locations around each horn bud (Figure. 5.1) 15 min pre- (baseline) and 1 h post-treatment as previously described by Heinrich et al. (2010). The horn bud (left or right) tested first was randomly determined but the locations around each horn bud were measured in order from 1 to 4 for each horn bud (Figure 5.1). The amount of pressure a kid tolerated before it withdrew its head was measured in kilograms of force (kgf) and was referred to as the mechanical nociceptive threshold (MNT). Downwards pressure was applied at a steady rate of approximately 1 kgf/s. The algometer automatically read the highest level of pressure applied, and was then manually reset before the next measurement was taken. To maintain consistency, one trained operator performed all of the algometry measurements.

### **5.2.4 Skull and brain assessment**

Prior to slaughter at the abattoir, kids were ear tagged (sheep tags, Allflex, Palmerston North, New Zealand) so that the head of each kid could be identified and linked with its treatment. Gross and histological examination was performed by a veterinary pathologist. The horn bud sites were grossly examined to assess exterior tissue damage to the skin and skull (i.e. ulcerations, necrosis and haemorrhage); the heads were then cut transversely, just caudal to the horn buds, using a commercial meat band saw. The brain was removed from the front of the skull and the inner surface of the frontal bone was examined for evidence of injury (i.e. discoloration, indentation) or inflammation beneath the horn bud sites. The dorsal surfaces of the cerebral hemispheres beneath these sites were examined for ulcerations or discoloration.



**Figure 5.1.** Locations around the goat kid horn buds where the pressure algometer was applied. The horn bud (left or right) tested first was randomly determined but the order of locations tested remained the same (from 1 through to 4).

Fifteen heads were randomly selected for histologic examination from the SHAM, CASP and CRYO treatments. For the CAUT group, 10 heads that displayed evidence of skull and brain injury were selected, together with 5 heads randomly selected. The anterior part of the brain was collected and fixed in 10% buffered formalin. Sections of both the right and left dorsal cerebral hemispheres from beneath the horn bud sites were embedded in paraffin wax for histological examination. Histologic sections were cut at 5 $\mu$ , stained with haematoxylin and eosin, and examined for pathologic changes using light microscopy.

### **5.2.5 Body weight measurements**

Kids were weighed immediately pre-treatment (baseline) then at 2, 7 and 14 d post-treatment. Kids were weighed using a hanging digital scale (Kamer, Shoof International Ltd.) attached to a weigh cradle. Body weights for each kid were converted to ADG (g/d).

## **5.3 Statistical analysis**

Data were analysed using Genstat statistical software (Version 17, VSN International, Hemel Hempstead, UK). No transformations of the data were required. Change in MNT from baseline was analysed using a mixed model fitted by restricted maximum likelihood (REML). The model included fixed effects for

treatment, location and their interaction and the random effects for kid, sex, treatment day, horn bud (left or right) within kid, horn bud order (first or second) and location within kid.

The body weights were excluded from analysis for 89 kids that either died, were missed at the time of measurement or sent to the abattoir before 2 wk of age. Average daily gains were analysed using a repeated measures model fitted by REML. The model included the fixed effects for treatment, time and their interaction and the random effects for sex, treatment day and kid within time. The correlation in measurements taken on the same kid over time was modelled with a power model of order 1.

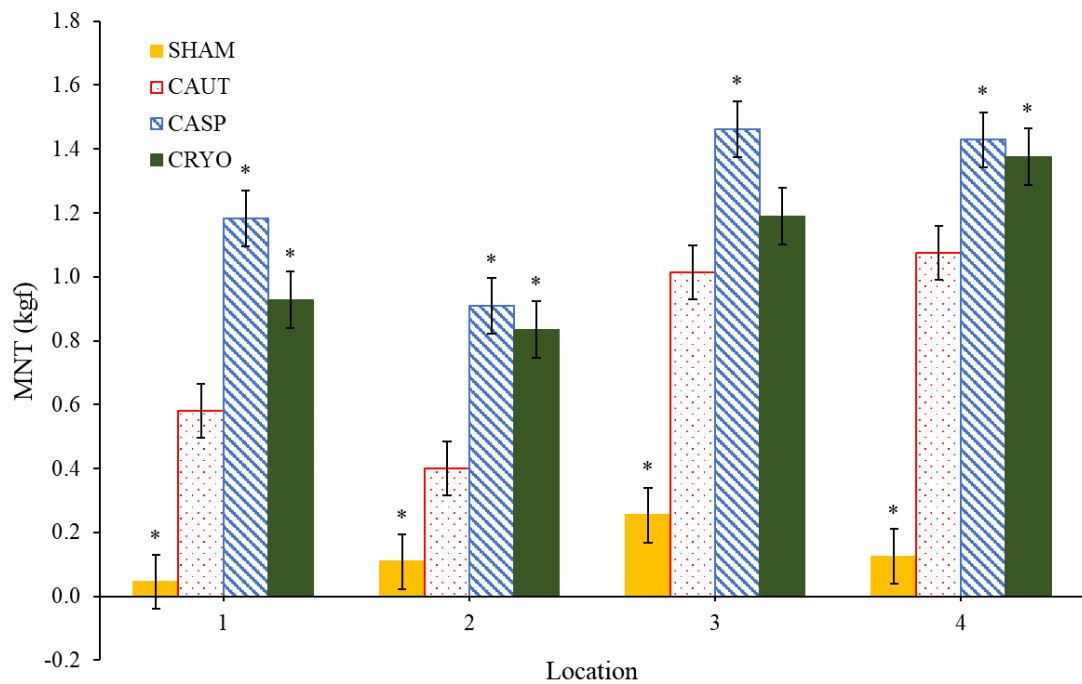
Differences between treatments were detected using Fisher's least significant differences test. Mean values are provided with standard error of the difference (SED). The level of significance was set at  $P \leq 0.05$ .

## 5.4 Results

### 5.4.1 Pain sensitivity

There was a treatment effect on the mean change in MNT ( $F_{3,258} = 132.5$ ,  $P < 0.001$ ). Kids treated with CASP were more sensitive than SHAM and CAUT kids ( $P \leq 0.001$ ), and SHAM kids were less sensitive than CAUT and CRYO kids 1 h post-treatment ( $0.13, 0.77, 1.25$  and  $1.08 \pm 0.069$  kgf for SHAM, CAUT, CASP and CRYO respectively;  $P \leq 0.001$ ).

There was a treatment by location interaction for mean change in MNT ( $F_{9,777} = 8.8$ ,  $P < 0.001$ ; Figure 5.2). Kids treated with CASP were more sensitive than CAUT for all locations ( $P \leq 0.001$ ). Furthermore, CASP kids displayed more sensitivity than CRYO kids for locations 1 and 3 ( $P \leq 0.01$ ) but there was no difference in sensitivity for locations 2 and 4 ( $P > 0.50$ ). Kids treated with CRYO were more sensitive than CAUT kids for all locations ( $P \leq 0.05$ ). Kids experiencing the SHAM treatment displayed less sensitivity than the other treatments at all locations ( $P \leq 0.001$ ).



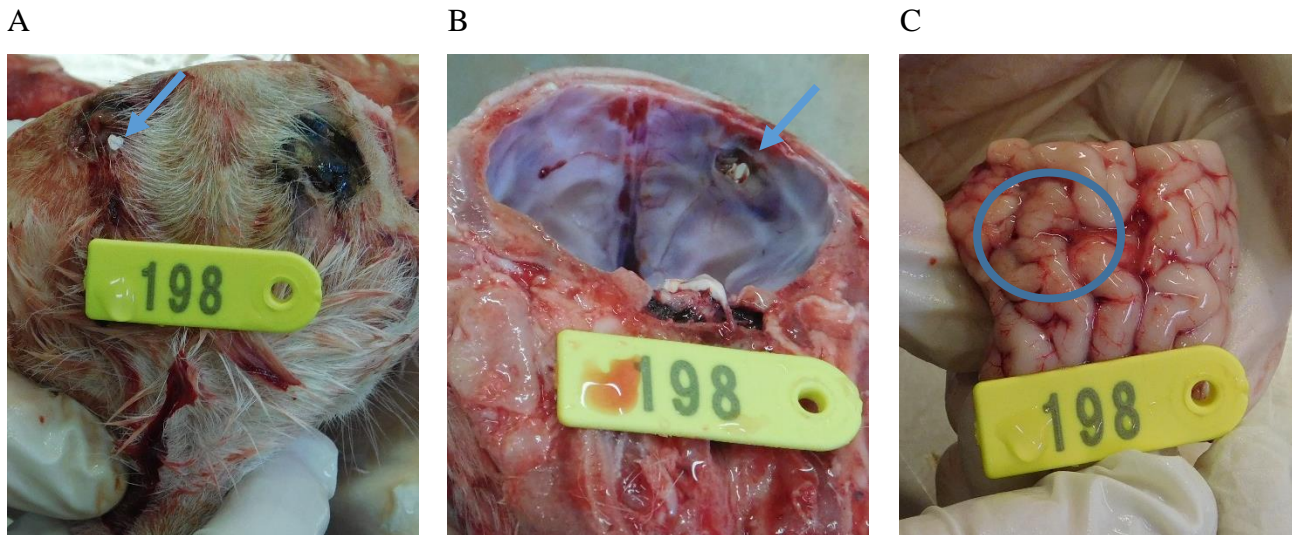
**Figure 5.2.** The change in mechanical nociceptive threshold (MNT; kg/f; mean  $\pm$  SED) measured using pressure algometry on goat kids (n = 70/treatment) that were either handled only (SHAM) or disbudded using a cautery iron (CAUT), liquid nitrogen (CRYO) or caustic paste (CASP). Asterisk indicate means that differ from CAUT kid means at  $P \leq 0.05$ .

#### 5.4.2 Skull and brain assessment

There was no gross or histological evidence of skull or brain injury associated with SHAM kids. Kids in the CAUT group had a ring of necrotic tissue surrounding the holes (Figure 5.3) exposing bone. One doe kid in the CAUT group (1/70) had a perforated skull (approximately 5 mm diameter) corresponding with the placement of the cautery iron (Figure 5.4). A further 9 CAUT kids (2 doe and 7 buck kids) displayed hyperaemia of the inside of the skull under the disbudding sites (Figure 5.5). The 5 randomly selected heads showed no evidence of injury. There was no histological evidence of brain injury for CAUT kids. All CRYO kids had circular scabs covering the horn buds (Figure 5.6) with little apparent damage to underlying tissue, and no gross or histological evidence of injury to the skull or brain. The CASP kids had superficial burns to the epidermis (Figure 5.7) that resulted in scabs forming a ring-pattern around the horn buds; there was no gross or histological evidence of skull or brain injury.



**Figure 5.3.** The skin has been lifted from the skull to show tissue damage associated with the cautery iron: holes where the iron burnt through the skin tissue with a surrounding ring of necrotic tissue (discoloration). Dark scabs remain on the skull at 2 wk post-treatment.



**Figure 5.4.** Skull of a cautery disbudded kid with a hole through the outside (A) and interior (B) of the skull and brain (C) corresponding with the cautery iron wound. Blue arrows indicate a hole in the skull and the blue circle highlights the area of the brain corresponding with the hole.



**Figure 5.5.** Hyperaemia in a circular pattern on the inside of the skull of a goat kid corresponding with placement of the cautery iron. The blue arrow points to hyperaemia.



**Figure 5.6.** Scabs in a ring pattern around each horn bud of a goat kid approximately 2 wk after it was cryosurgically disbudded.



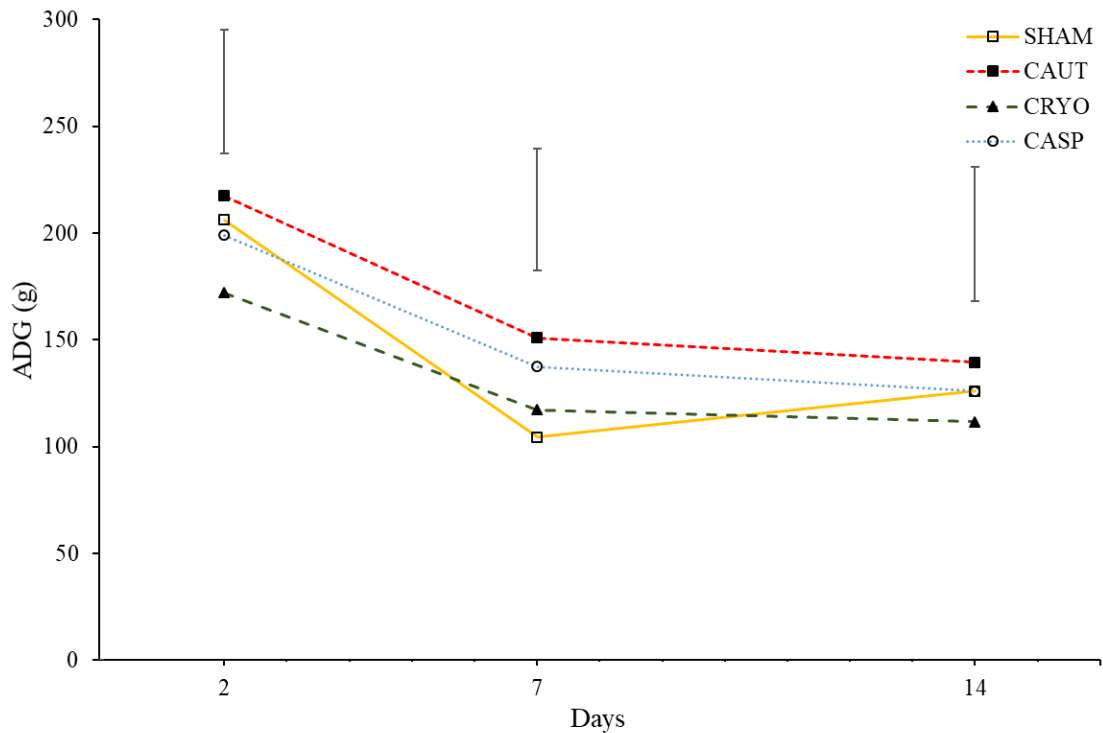


**Figure 5.7.** A goat kid with tissue damage associated with caustic paste disbudding, approximately 1 h post-treatment.

### 5.4.3 Body weight

There was an effect of day on ADG for all kids ( $F_{2,254}=9.8$ ,  $P < 0.001$ ). There was an overall ADG of 199 g/d at 2 d, 128 g/d at 7 d and 126 g/d at 14 d ( $\pm 20$  g/d;  $P \leq 0.001$ ).

There was no evidence of an overall effect of treatment on ADG ( $F_{3,246}=0.9$ ,  $P = 0.44$ ; SHAM = 146 g/d, CAUT = 169 g/d, CRYO = 134 g/d and CASP = 154 g/d [ $\pm 21$  g/d]) or a treatment by day interaction ( $F_{6,333}=0.6$ ,  $P = 0.73$ ; Figure 5.8).



**Figure 5.8.** Average daily gains (g/d; error bars represent maximum SED for each time point) of goat kids over 14 d post-treatment that were either handled only (SHAM) or disbudded using a cautery iron (CAUT), liquid nitrogen (CRYO) or caustic paste (CASP).

## 5.5 Discussion

We evaluated pain sensitivity, ADG and skull or brain injury associated with cautery, cryosurgical and caustic paste disbudding of goat kids. All disbudding methods caused increased pain sensitivity compared to sham-handling, suggesting that all methods of disbudding cause acute post-operative pain. Behavioural and physiological changes following cautery disbudding of goat kids (Alvarez et al., 2015; Hempstead et al., 2017, 2018a) support the interpretation that the disbudding methods tested are associated with increased pain sensitivity. The horn bud areas of CASP and CRYO kids were more sensitive post-treatment compared with CAUT kids. Therefore, reduced tissue sensitivity displayed by CAUT kids, compared to CASP or CRYO kids suggests that cautery disbudding may cause less acute post-operative pain; however, this response is likely a function of the differing mechanisms of cell destruction and resultant wounds among disbudding methods. Future work should examine the effects of disbudding methods on pain sensitivity beyond 1 h post-treatment.

Local anaesthesia, when used for calves can reduce pain associated with cautery disbudding (Morisse et al., 1995; Graf and Senn, 1999); however, it is

largely ineffective at reducing pain in kids (Alvarez et al., 2015; Nfor et al., 2016). Also, for young goat kids, there are issues of toxicity associated with high doses of lidocaine (Smith and Sherman, 2009a). Our intention was to measure pain sensitivity, which may have been affected had pain relief been used; therefore, pain relief was not used in the current study.

In some studies that have compared caustic paste to cautery disbudding, it has been suggested that caustic paste disbudding is less painful (Vickers et al., 2005; Stillwell et al., 2009). Differences in methodology between studies may explain these results. Calves treated with caustic paste were sedated and provided local anaesthesia, which may have reduced the natural expression of pain. Furthermore, the type of caustic paste used (e.g., calcium vs. sodium hydroxide) may affect the amount of pain experienced as the penetration rate is faster for sodium than calcium hydroxide (Kuckelkorn et al., 2002).

Ten cautery disbudded kids out of 70 had injury to the skull (including skull perforation and hyperaemia), which suggests the procedure weakened or damaged the skull. Interestingly, three out of 15 doe kids (20%) that were cautery disbudded showed evidence of skull injury (including the kid with skull perforation) compared with seven buck kids out of 55 with skull injury (13%). Sexual dimorphism is prominent in the frontal bones of goats, as males usually have stronger and larger horns with a wider horn attachment to the skull than females (Shawulu et al., 2011); therefore, it is possible that doe kids have weaker skulls than those of buck kids, which may explain the higher incidence of injury in doe kids.

During post-mortem examination, no evidence of infection of the meninges or brain was observed; however, the dura layer of the meninges remained intact, which may have prevented pathogens from entering the brain. A case study that examined five cautery disbudded kids post-mortem, reported necrosis of the frontal bones that extended to the internal surface of the skull (Wright et al., 1983). A similar study that examined two cautery disbudded kids post-mortem 10 d after disbudding, had a 2 cm diameter of skin and skull cap that could be lifted off a disbudding site (Sanford, 1989). Furthermore, yellow circular patches were prominent on the brain underneath the disbudding sites (Sanford, 1989). Other studies have reported brain damage or meningoencephalitis in 12/150 (8%) kids that died as a result of cautery disbudding (Thompson et al., 2005). This sort of damage may be caused by incorrect technique (e.g., excessive

pressure or extended application of the cautery iron) or possibly associated with an inexperienced operator.

Together with the existing literature on disbudding, our results highlight the severity of injuries that can occur if care is not taken (e.g., appropriate pressure and application duration) when cautery disbudding goat kids. Cautery disbudding techniques were originally designed for calves, which have much thicker skulls and more developed sinuses than goats (Wright et al., 1983; Sanford, 1989); calves are also typically disbudded at a much older age (up to 12 wk; Graf and Senn 1999; Grondahl-Nielsen et al., 1999; Stafford and Mellor, 2011) than kids (1 wk; Smith and Sherman, 2009b). These factors may explain why there is little documented evidence of skull and brain damage associated with cautery disbudding in calves. Based on the work of Thompson et al. (2005), we had anticipated more complications associated with cautery disbudding in the present study; the lack of issues may be a function of the contractor being well-trained and experienced. It is important to note that even when using appropriate pressure and timing of the cautery iron, a small number of kids did experience complications, which highlights the potentially dramatic consequences if the procedure is done by an inexperienced individual.

There was no evidence that treatment affected ADG over the study period. Disbudding causes intense acute pain in kids but may not impact on motivation to feed and consequent growth rates. A study from our group evaluating pain associated with alternative methods of disbudding found no difference in growth rates between sham-handled kids and kids disbudded with either a cautery iron, clove oil, caustic paste or cryosurgery (range:  $187 \pm 16$  to  $204 \pm 17$  g/d; Hempstead et al., 2018b). Disbudding of kids, regardless of method, may not cause enough of a disturbance to affect weight gain.

## **5.6 Conclusions**

Goat kids that were cryosurgically or caustic paste disbudded displayed higher pain sensitivity than cautery disbudded kids, indicating these methods may be associated with more acute post-operative pain. Cautery disbudding resulted in 10/70 of kids with skull injuries, highlighting the potential for injury even with a trained practitioner. It appeared ADG was not affected by disbudding method. Cautery disbudding may cause less pain than caustic paste and cryosurgical

disbudding, however the potential for complication exists with the former method. Therefore, it is imperative operators receive proper training to ensure cautery disbudding is performed properly to limit skull and brain injury. Furthermore, there is need to develop alternative methods to cautery disbudding that cause less pain and are associated with a lower risk of causing skull or brain injury.

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## Chapter 6

# The effectiveness of clove oil and cautery disbudding techniques on horn growth in dairy goat kids

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Funding acquisition: MAS MS. Conceptualisation: MAS MNH MS JRW.

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## Abstract

The effectiveness of clove oil and cautery disbudding on horn growth was evaluated in goat kids. The study used 243 Saanen doe kids ( $4 \pm 1.0$  d old; mean  $\pm$  SD) on two commercial dairy goat farms, and were disbudded with either (i) clove oil injection (CLOVE), (ii) a cautery iron and horn bud removed (BUDOFF), or (iii) a cautery iron with horn bud left intact (BUDON). Each kid received a different treatment per horn bud, which were balanced between buds (left and right) and randomly allocated. A trained observer monitored horn bud growth following treatment for 3 months recording either: N: no growth, H: normal horn, S: abnormal horn (scur), or SC: soft, fibrous lump (scorn). After the final observation, horn buds were assessed for the probability of detecting (i) success (no growth), (ii) scurs, (iii) horns or (iv) scorns [with exact 95% CI]. The probability of success for BUDOFF (0.77 [0.63 – 0.87]) was higher than for BUDON (0.20 [0.11 – 0.34]) and CLOVE (0.09 [0.04 – 0.18];  $P \leq 0.05$ ). Furthermore, the probability of success for BUDON was higher than for CLOVE ( $P \leq 0.05$ ). The probability of scurs was higher for CLOVE (0.72 [0.63 – 0.80]) than BUDOFF (0.25 [0.17 – 0.34]) and BUDON (0.30 [0.21 – 0.39];  $P \leq 0.05$ ). There was no difference in the probability of scurs for BUDOFF and BUDON ( $P > 0.05$ ). The probability of horns was higher for CLOVE (0.21 [0.15 – 0.29]) than BUDON (0.02 [0.01 – 0.06];  $P \leq 0.05$ ); horns were not observed for BUDOFF. The probability of scorns for BUDON (the only treatment that led to scorns) was 0.41 (0.25 – 0.60). These results suggest that BUDOFF was more effective at preventing growth than CLOVE and BUDON and appears the most effective method, of the methods tested, for disbudding kids. Future research should explore different clove oil administration methods or other alternatives to cautery disbudding that may be both efficacious and cause less pain.

## 6.1 Introduction

Dairy goat kids are routinely disbudded, usually within the first week of life (Smith and Sherman, 2009); the practice involves the destruction of the horn buds to prevent horn growth. The predominant method involves using a hot cautery iron to cauterise and remove horn buds. Disbudding is carried out to reduce the risk of injury to other goats (Waiblinger et al., 2011) and their human handlers, and allows for more space at the feed rail or in lying areas (Loretz et al., 2004). Current disbudding methods for goat kids have been adapted from those used for calves, such as cautery disbudding, caustic agents and surgical methods (Graf and Senn, 1999; Sylvester et al., 2004; Stafford and Mellor, 2005).

It is generally accepted that cautery disbudding causes pain and distress in goat kids as evidenced by intense and frequent vocalisations, leg shakes during the procedure (Alvarez et al., 2015), elevated cortisol concentrations and increased frequencies of head shaking, rubbing and scratching post-disbudding (Hempstead et al., 2017, 2018a, b). Furthermore, potential complications associated with cautery disbudding include second- or third-degree burns, inflammation, thermal injury to the skull and brain (causing necrosis), infection and an increased risk of mortality (Wright et al., 1983; Sanford, 1989; Thompson et al., 2005).

If disbudding of goat kids is not performed, then adult goats can be dehorned; this involves surgical removal of the horns, which creates an opening into the frontal sinus and causes more pain than disbudding (Stafford and Mellor, 2005). It can also lead to complications such as prolonged healing, discharge/infection, inflammation, regrowth of horns, dehiscence or even death (Hague and Hooper, 1997; Hartnack et al., 2018). Therefore, disbudding is the preferred method for preventing horn growth.

There is limited research on the efficacy of different disbudding methods (including cautery disbudding) on horn or scur growth in goat kids or calves. Disbudding is considered successful if no horns or scurs grow (i.e. all horn bud tissue is destroyed). Scurs can be defined as distorted horn regrowth following disbudding (Hartnack et al., 2018). Problems associated with scurs include being aesthetically unpleasing, breaking off easily causing open wounds and increasing the risk of infection and potential abnormal growth back towards the animal's head, requiring surgical removal. Cautery disbudding can be performed by either

(i) totally removing the ring of tissue containing the horn bud cells or (ii) cutting/burning a circular ring around the horn bud but leaving it intact. It is unclear which method of cautery disbudding is most effective in preventing scurs or horns.

A recent study used the physiological and behavioural responses of dairy goat kids to evaluate alternatives to cautery disbudding (i.e. caustic paste and cryosurgical disbudding, and clove oil injection; Hempstead et al., 2018a). Clove oil injection elicited a similar cortisol response and number of head shakes and scratches as cautery disbudding, indicating a similar experience of pain (Hempstead et al., 2018a). Even though the pain response to clove oil injection appeared to be similar to that generated by the cautery iron, the clove oil method caused less tissue damage (Hempstead et al., 2018a). Consequently, clove oil injection may result in faster healing times and lower rates of infection or skull or brain injury. Caustic paste and cryosurgical disbudding appeared to cause more pain than cautery disbudding (Hempstead et al., 2018a), and therefore were not included as treatments in the present study.

Clove oil, which was traditionally used in dentistry as a topical analgesic and antiseptic (Markowitz et al., 1992), contains a high concentration of eugenol (i.e. 80 – 85%), which has been shown to cause cellular necrosis (and inflammation) of the oral mucosa of rats (Kozam and Mantell, 1978); it can also be highly cytotoxic for human skin cells (Prashar et al., 2006). Recently, clove oil has been shown to cause local cellular necrosis of horn bud tissue resulting in arrested horn growth in calves (Molaei et al., 2014) and goat kids (Molaei et al., 2015). Further research is required to evaluate the effect of clove oil on horns and scurs in goat kids.

The objective of this study was to evaluate the effectiveness of clove oil injection and two cautery disbudding techniques (i.e. horn buds removed vs. left intact) on horn bud growth in dairy goat kids. We predicted that clove oil injection would result in similar levels of horns and scurs as cautery disbudding, based on the success rates (100%) reported by Molaei et al. (2015). We also predicted that cautery disbudding with horn buds removed, would result in less horn or scur growth than leaving the horn buds intact due to increased potential for complete cell destruction.

## 6.2 Materials and methods

The Ruakura Animal Ethics Committee approved the use of animals prior to the commencement of the study (Protocol No. 14213).

### 6.2.1 Pilot study

A pilot experiment was carried out to ensure that administration of clove oil into the horn bud had no detrimental effect on the skull and brain of goat kids before further studies were conducted. On a commercial dairy goat farm in the Waikato region of New Zealand, 10 Saanen doe kids were selected from unwanted stock based on age (i.e. 2-3 d old) in June 2017. The animals were injected with 0.2 mL of clove oil into each horn bud using the procedure described in Hempstead et al. (2018a). Kids were reared in a small barn separate from the farmer's replacement stock and fed using a 10 L bucket feeder with six teats. Two weeks after clove oil injection, five kids were euthanized by a veterinarian. The remaining five kids were euthanized 1 wk later (i.e. 3 wk after clove oil injection) to assess the effects of clove oil over time. After euthanasia, the bodies were transported to a post-mortem facility at the Ruakura Research Centre, in Hamilton, New Zealand, and gross examination was then performed by a veterinary pathologist.

Firstly, the skin over the head was visually assessed and then the skin was removed so that the outside of the skull could be examined. Next, the head was cut transversely, just caudal to the horn buds using a commercial meat band saw. The brain was removed from the front part of the skull and the inner surface was examined for evidence of damage (e.g., perforation, hyperaemia) or inflammation beneath the horn bud sites. The dorsal surfaces of cerebral hemispheres beneath these sites were examined for ulcerations.

Large black scabs covered the horn buds 2 wk post-injection of clove oil. There were localised dark patches on the skull below the horn buds for the five kids euthanized 2 wk after treatment, with no evidence of damage on the inside of the skull or the brain. At 3 wk post-injection, patches of newly healed skin were observed as well as scurs in three out of the five kids. Discolouration of the skull beneath the horn buds was apparent in only one kid. There was no evidence of inflammation, perforation or infection in the skull, meninges or brain associated with the clove oil injection for any of the kids.

### 6.2.2 Animals and housing

This study was conducted on two private commercial dairy goat farms within the Waikato region in New Zealand between June and December 2017. A total of 243 Saanen doe kids ( $4 \pm 1.0$  d old; mean  $\pm$  SD) were used (Farm A: 189 kids; Farm B: 54 kids) and were selected for inclusion based on age (2 – 6 d old) and size of horn buds ( $< 16$  mm). Kids had an average body weight of  $4.0 \pm 0.55$  kg (range: 2.7 – 6.1 kg). Kids were housed in pens (3.5 x 2.0 m) with approximately 15 kids/pen until they were 2 wk old or were feeding independently, at which point they were moved to larger pens (9.0 x 5.0 m) with approximately 45 kids/pen.

The kids were reared as per routine practice on each farm (Dairy Goat Co-operative, 2012). The ground within the pens was covered with untreated pine shavings (approximately 10 cm deep). Each pen had *ad libitum* access to milk replacer (SprayFo, AgriVantage, Hamilton, New Zealand; mixed according to packet instructions) in feeders (Milk Bar, Waipu, New Zealand) with 10 teats/feeder (36 L capacity) and fresh water in a trough.

### 6.2.3 Experimental design

A power analysis for a binary outcome bio-equivalence trial was conducted based on 80% power, a 5% significance level and an equivalence range of  $\pm 0.15$ . We used a randomised split-plot design with a different treatment per horn bud, with treatment day, kid and farm as blocking variables. A replicate consisted of all pairs of treatments and whole replicates were completed per treatment day. Horn buds were randomly allocated treatments ( $n = 162$  horn buds/treatment) balanced for kid age. Kids were given a coloured collar for treatment identification within the pens as they were housed with others not experiencing any treatment. The same veterinarian performed all treatments. The experiment was conducted on eight treatment days over a 2.5-wk period. Kids were fed approximately 1 h before treatment and then taken (one at a time) from their home pen and placed in a restraint device (described in Hempstead et al. [2018b]). Treatments were performed in an alleyway alongside the home pens and treatment order was randomly assigned. Hair covering the horn buds was removed with an electric clipper (Laube, 505 cordless kit, Shoof, Cambridge, New Zealand) to expose the horn buds. Prior to treatment, kids received an oral non-steroidal

anti-inflammatory drug (Loxicom 0.5 mg/mL oral suspension for dogs, Norbrook Laboratories Ltd, Newry, UK; 0.2 mg/kg BW) and a corneal nerve block using lidocaine (Lopaine 2%, 20 mg/mL, Ethical Agents, Auckland, New Zealand; 0.1 mL/horn bud) to reduce pain associated with treatment.

Treatments included:

1. BUDOFF: Disbudding using a cautery iron (“Quality” electric debudder, 230 V, 190 W; Lister GmbH, Lüdenscheid, Germany), which was heated for 20 min (to reach approximately 600°C) prior to being pressed to each horn bud for a total of  $5.9 \pm 1.09$  s (mean  $\pm$  SD). Horn buds were then removed by pressing the iron down and rotating so the skin was cut and the buds forcibly flicked out, as described in Hempstead et al. (2017).
2. BUDON: The same procedure as for BUDOFF, except that the horn buds were cut but not removed. The iron was held to each horn bud for a total of  $4.8 \pm 1.08$  s on average.
3. CLOVE: Clove oil (C8392, 100mL, 83-85% eugenol, Sigma-Aldrich, Saint Louis, MO) was injected (0.2 mL; Molaei et al., 2015) laterally into the centre of each horn bud at a 45° angle between the ear and muzzle ( $20.9 \pm 8.39$  s; mean completion time  $\pm$  SD); details of the procedure are described in Hempstead et al. (2018a).

After treatment, BUDON and BUDOFF wounds were sprayed with antibacterial spray (Tetravet, Bayer New Zealand Ltd., Auckland, New Zealand) to prevent infection. Horn buds treated with CLOVE did not receive spray as there were no open wounds. Kids were then returned to their home pen and were monitored for 2 h post-treatment to ensure no complications associated with treatments occurred. The health status and horn bud growth of the goat kids was assessed for 5 months post-treatment. Any kids that died over the course of the experiment were examined post-mortem by a veterinarian to determine cause of death.

#### **6.2.4 Horn bud growth categories**

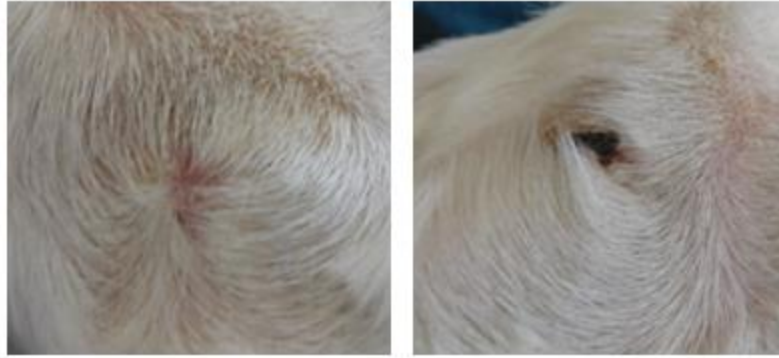
Horn bud growth categories were defined before the start of the experiment. Each horn bud was categorised based on whether it displayed normal horn growth (H), abnormal growth or scurs (S) or no evidence of growth (N; Figure 6.1). An extra category was added after the first farm visit 2 wk after

treatment as there were growths that could not be categorised as either H or S – a scorn (SC; Figure 6.1). A horn was defined as having normal growth without abnormalities. A scur was defined as any abnormal growth with a hardened (keratinised) surface that could be felt by hand in the horn bud area. A scorn was defined as a soft and fibrous (observed when cut) growth with a wide base and usually a rounded tip. N was recorded when the skin was smooth and there was no horn growth; this was considered a success. Horn buds were categorised into the four groups fortnightly for 2 months and then monthly for a further 3 months by a trained observer. The observer remained blind to the treatments each kid received. Once growth was observed, that treatment was considered unsuccessful and if both horn buds had evidence of growth, the animal was no longer monitored in subsequent checks. The probability of success, scurs, horns or scorns for each treatment at the final observation are presented.

### **6.2.5 Statistical analysis**

Genstat statistical software (version 18, VSN International, Hemel Hempstead, UK) was used to analyse the data. The binary response variables used for analyses included success (no growth = N), scurs (growth = S), horns (growth = H) and scorns (growth = SC); each response variable was assumed to be binomially distributed. Analysis of the differences between treatments were performed independently for all response variables. In addition, a bio-equivalence analysis was performed for the probability of success, with 80% power and an equivalence range of  $\pm 0.15$ . Bio-equivalence was assessed for each treatment with BUDOFF (considered the reference treatment) and also for CLOVE and BUDON (with BUDON as the reference treatment). We used a generalised linear mixed model for the analyses with a logit link. The fixed effects were for treatment and the treatment on the other horn bud (i.e. of the same kid). The random effects were for kid, farm, horn bud (left or right) and treatment date within farm. Differences between treatment means were compared using Fisher's protected least significant difference test at the 5% significance level.

N



S



H



SC



**Figure 6.1.** Categories of horn growth in dairy goat kids. No growth = N, scur = S, horn = H and scorn = SC. Goat kids were disbudded using either a clove oil injection (CLOVE), a cauterizing iron with the horn buds removed (BUDOFF) or a cauterizing iron with the horn buds left intact (BUDON).



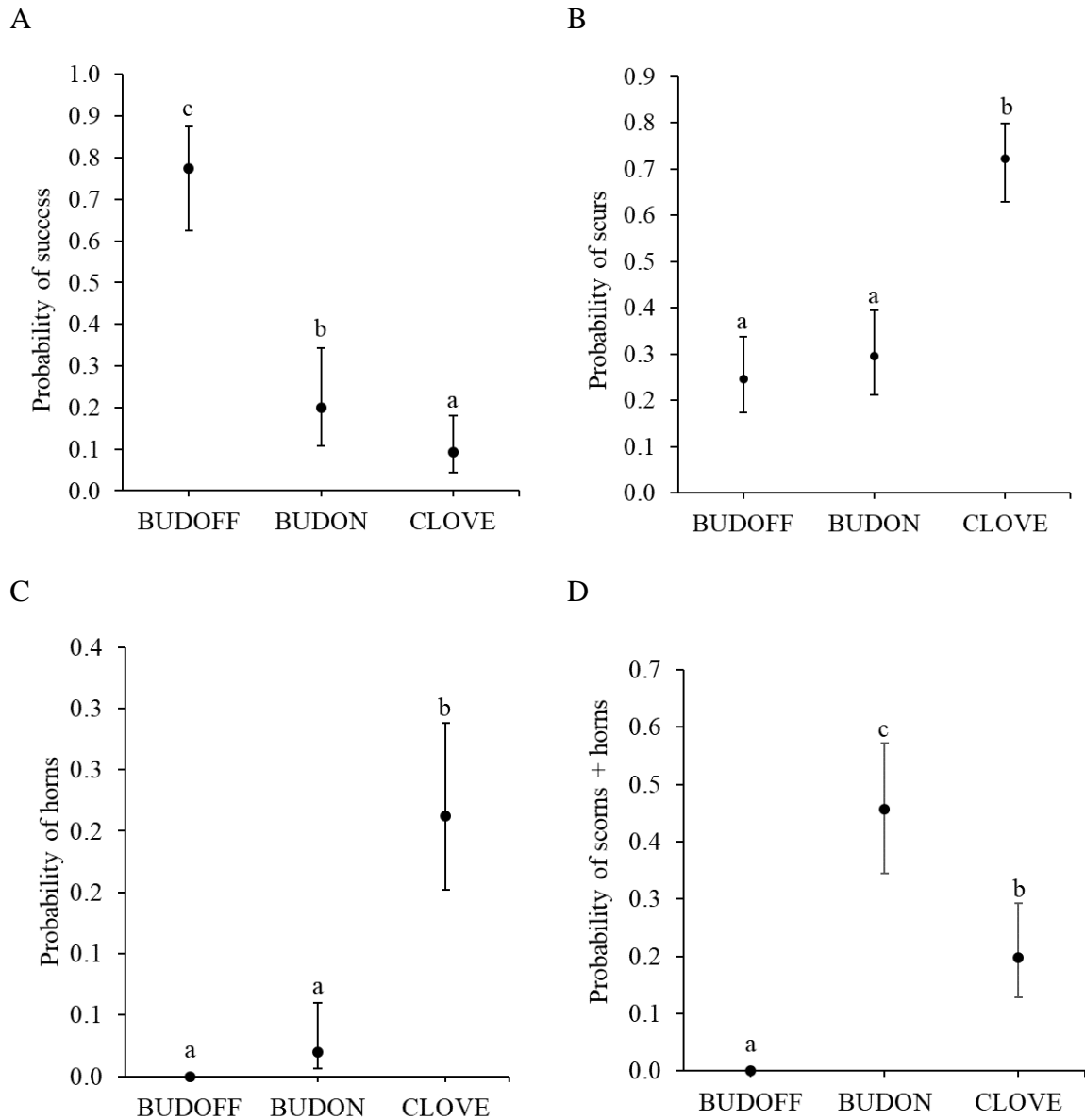
### 6.3 Results

Of the 243 kids were enrolled in the study, 12 died before their 24 horn buds could be assessed 2 wk following treatment; one animal (BUDON/BUDOFF) died 2 wk post-treatment as a result of meningitis below the horn bud but the others died from complications not related to treatment (i.e. pneumonia, digestion issues). Data was missing for 9 BUDON and BUDOFF horn buds and 6 CLOVE horn buds. The differences in the probabilities of success, scurs, horns or scorns are presented in Figure 6.2. There was an effect of treatment on the probability of success ( $F_{2,443} = 43.3, P < 0.001$ ). The probability of success for BUDOFF horn buds was higher than for BUDON and CLOVE horn buds ( $P \leq 0.05$ ). Furthermore, the probability of success for BUDON horn buds was higher than that of CLOVE horn buds ( $P \leq 0.05$ ). There was no evidence of bio-equivalence for the probability of success between BUDOFF and CLOVE, nor between BUDOFF and BUDON; however, the probabilities of success for BUDON and CLOVE horn buds were bio-equivalent.

There was a treatment effect on the probability of developing scurs ( $F_{2,452} = 28.3, P < 0.001$ ). The probability of scurs was higher on CLOVE than BUDOFF and BUDON horn buds ( $P \leq 0.05$ ). There was no difference in the probability of scurs for BUDOFF and BUDON horn buds ( $P > 0.05$ ).

There was a treatment effect for the probability of horns ( $F_2 = 8.9$  [chi-square test used as denominator degrees of freedom (ddf) were not estimable],  $P < 0.001$ ). The probability of horns was higher for CLOVE than BUDON horn buds ( $P \leq 0.05$ ); horns were not observed for BUDOFF horn buds.

Scorns were only observed for BUDON horn buds (0.41 [0.25 – 0.60]). There was an effect of treatment on the probability of horns and scorns combined ( $F_2 = 11.2$  [chi-square test used as ddf not estimable],  $P < 0.001$ ). The probability of scorns and horns was higher for BUDON than CLOVE and BUDOFF horn buds ( $P \leq 0.05$ ).



**Figure 6.2.** Probability of the four categories of horn growth with exact 95% CI for goat kids disbudded using three different techniques. (A) Success (no growth = N), (B) scurs (growth = S), (C) horns (growth = H) and (D) scorns and horn combined (growth = SC or H). Goat kids were disbudded using either clove oil injection (CLOVE;  $n = 156$  horn buds), a cautery iron with the horn buds removed (BUDOFF;  $n = 153$  horn buds) or a cautery iron with the horn buds left intact (BUDON;  $n = 153$  horn buds). Means with differing subscripts are significantly different at  $P \leq 0.05$ .

## 6.4 Discussion

The effectiveness of clove oil injection and two cautery disbudding techniques (i.e. horn buds removed vs. left intact) in preventing horn growth were evaluated in dairy goat kids. Clove oil injection has been previously reported to be 100% successful in preventing horn growth in kids (Molaei et al., 2015) as well as

calves (Molaei et al., 2014). In the present study, the CLOVE treatment appeared to be less effective at preventing horn growth than either of the cautery disbudding techniques based on the high incidence of horns and scurs. Clove oil injection is a novel method of disbudding for goat kids compared with cautery disbudding (adapted from use in calves), which is the most commonly used method for disbudding goat kids worldwide (Smith and Sherman, 2009). Higher proportions of scurs on horn buds treated with clove oil compared with cautery disbudding may be associated with difficulties in restricting movement of the head during treatment. Clove oil was applied using a needle injected laterally into the buds whereas cautery disbudding involved pressing an ergonomic cautery iron down on the head. Consistent administration of the full volume of clove oil to the correct location (centre of the horn bud) was not always possible. The injection is likely to cause discomfort or pain (Hempstead et al., 2018a) and kids generally struggled (rapid jerks of the head) during the procedure, resulting in the needle becoming dislodged. The creation of an applicator that can quickly deliver a consistent volume of clove oil to the right location may improve efficacy.

Potential explanations for the differences in efficacy of clove oil between Molaei et al. (2015) and the present study include differences in methodologies. Molaei et al. (2015) used a small sample size (16 vs. 243 kids in the present study), and their own clove oil distilled from the spice (clove oil used in the present study was sourced from a commercial manufacturer). The distilled clove oil appeared to be more effective as it totally prevented horn growth in both doe and buck kids, which is notable as horn growth is more precocious in bucks (Dawson et al., 2007). The exact method of clove oil administration was not completely described in Molaei et al. (2015) (e.g., it was not clear how the clove oil was injected or whether the hair was clipped so that any growth could be clearly observed). Perhaps more importantly, Molaei et al. (2015) measured horn growth, whereas we evaluated any growth including horns, scurs (of any size) and scorns. It appears as though our methodology for injecting clove oil does not consistently prevent horns or scurs in goat kids and may not be a useful alternative to cautery disbudding with horn buds removed.

Interestingly, there was a similar level of success in preventing scurs or horns between CLOVE and BUDON horn buds, indicating that leaving the horn bud intact may also not prevent horn regrowth. The method used in the present study to evaluate horn bud growth may be more comprehensive than methods

used by farmers or farm staff and previous studies (Molaei et al., 2014, 2015). Future research should examine the exact mechanisms of clove oil action on the horn bud tissue of goat kids.

We observed some animals with inflammation of the upper eye lid area below the horn bud associated with the CLOVE treatment. This is interesting, as eugenol, the main component of clove oil, has anti-inflammatory properties (Markowitz et al., 1992; Daniel et al., 2009). Perhaps blood flow to the horn bud is reduced due to localised cellular necrosis and the observed swelling was pooling of blood below the horn bud. Measures of tissue sensitivity such as pressure algometry or Von Frey monofilaments, could be used to evaluate whether this apparent inflammatory response is painful.

We also had one kid (treated with BUDOFF and BUDON) die as a result of meningitis, which is likely associated with cautery iron use. It is well-established that cautery iron use can cause damage and thermal injury to the skull and brain, resulting in meningitis (Wright et al., 1983; Sanford, 1989; Thompson et al., 2005). Although only one out of 243 kids died as a result of cautery disbudding, this demonstrates the capacity for cautery disbudding to not only cause pain, but mortalities.

To the authors' knowledge, this is the first study to quantify the efficacy of cautery disbudding methods for goat kids. The BUDOFF treatment appeared to be most effective in preventing horns, scurs or scorns compared with the other two methods; this may be due to more complete horn bud destruction as the buds were cauterised and removed. By removing the horn bud, it is easier to ensure that all of the horn bud tissue is destroyed. It is generally considered more efficacious to remove the horn buds than leaving them intact (Smith and Sherman, 2009) and this method has been favoured in other studies (Mintline et al., 2013; Alvarez et al., 2015; Huebner et al., 2017).

The BUDON method resulted in abnormal growths that were soft, fibrous and fitted into neither the horn nor scur categories. The cautery iron used in the present study had a hollow centre with hot edges that cut into the skin, which may have allowed for the inner cells of the horn bud to continue to grow. This result has implications for farmers and contractors as it suggests that removing the horn buds may be more effective for preventing scurs than burning alone.

There is limited information available on horn growth in domesticated farmed goats, therefore comparisons with the literature are difficult. There is

however, information on the horn growth of wild adult populations of goats (Côté et al. 1998), ibex (Büntgen et al., 2014) and chamois (Rughetti et al., 2011), usually with respect to environmental effects. Further research on scur or horn growth rates in goat kids after disbudding could help quantify the efficacy of cautery disbudding methods.

In order to understand regrowth associated with disbudding, it is important to understand how horns grow. The process involves keratinisation of the horn bud epidermis and ossification of the underlying dermis and hypodermis (Dove, 1935). Goat horn anatomy is similar to that of cattle horns; the horn comprises tightly packed tubules produced by corium and germinal epithelium, which is attached to the frontal bone. The cornual diverticulum of the frontal sinus forms a cavity within the horn (Smith and Sherman, 2009). If the horn bud epidermis is not completely destroyed then keratinisation of some epidermal cells can occur resulting in scurs. Scurs usually result from inadequate burning of the horn bud site in an effort to reduce the risk of thermal injury to the skull and brain (Dawson et al., 2007) or insufficient removal of the germinal tissue from the base of the horn bud (Hartnack et al., 2018). If the base of the horn bud, which can be hard to see, is wider than the diameter of the cautery iron and not all tissue is destroyed, then scurs may also develop. Interestingly, in many cattle breeds, there is a gene for scurs (Mariasegaram et al., 2010; Capitan et al., 2011; Ward, 2015), as well as polled and horned genes (Prayaga, 2007), meaning that scurs can grow naturally without disbudding. It is unknown whether a gene for scurs exists in goats. If so, herds with high rates of scurs may be associated with the genetic potential to grow scurs.

The best alternative to reduce pain and complications associated with cautery disbudding, would be to breed polled goats to eliminate the need for this procedure. There are hurdles to achieving this however, as the gene for polledness is linked with the intersex gene in goats (Pailhoux et al., 2001). This means that there is a high probability that naturally polled goats will be infertile. Until a polled line can be established, mitigating pain associated with cautery disbudding or further alternatives that cause less pain or injury than cautery disbudding should be considered.

## 6.5 Conclusions

Clove oil injection did not appear to be as successful at preventing scurs or horns as cautery disbudding and therefore the methodology used in this study may not be useful for disbudding goat kids (if having no scurs is a concern). Interestingly cautery disbudding by removing the horn bud germinal tissue was more efficacious than leaving the horn buds intact, suggesting that the former method may be more effective for preventing horns, scurs or scorns. Future research should explore different clove oil formulations and administration methods or other alternatives to cautery disbudding that may be efficacious and cause less pain.

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# Chapter 7

## Effect of isoflurane alone or in combination with meloxicam on the behaviour and physiology of goat kids following cautery disbudding

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## Abstract

Cautery disbudding of goat kids is painful, but may be alleviated with pain mitigation. We therefore evaluated the effect of administering general anaesthesia (isoflurane) or a non-steroidal anti-inflammatory drug (meloxicam) on goat kid behaviour and physiology following cautery disbudding. Trial 1 (n = 12/treatment) evaluated behavioural responses in 72 female Saanen dairy goat kids (mean  $\pm$  standard error of the mean;  $3.9 \pm 0.15$  d old) and trial 2 (n = 10/treatment) evaluated physiological responses in 60 female Saanen dairy goat kids ( $4.3 \pm 0.14$  d old). Goat kids were randomly assigned to one of six treatment groups that were either (1) sham-handled only (SHAM) or disbudded with (2) no pain relief (CAUT), (3) isoflurane gas (ISO), (4) isoflurane and s.c. meloxicam combined (ISO+MEL), (5) meloxicam s.c. (0.5 mg/kg of body weight; I-MEL), or (6) oral meloxicam (0.2 mg/kg of body weight; O-MEL). Head shaking, head scratching, self-grooming, feeding, and body shaking were continuously video recorded for 24 h pre- and post-treatment. Lying behaviour was recorded continuously for 24 h pre- and post-treatment using accelerometers. Plasma cortisol, glucose, and lactate concentrations were measured from blood samples collected immediately before treatment (baseline) and at 15, 60 and 120 min post-treatment. Body temperature was measured immediately after blood sampling at all blood sampling time points. Head shaking and body shaking frequencies were 50% higher in CAUT than SHAM kids 5 min post-treatment; ISO+MEL and ISO kids performed 25% less body shakes than CAUT kids. Head scratching durations 1 h post-treatment were higher in CAUT than SHAM kids, whereas O-MEL were similar to SHAM kids from 2 h post-treatment. Self-grooming, feeding, and lying did not differ between groups. Cortisol concentrations were higher in CAUT than SHAM kids ( $156.4 \pm 26.41$  and  $104.1 \pm 26.41$  nmol/L, respectively), whereas ISO+MEL and ISO kids ( $88.3 \pm 26.41$  and  $113.2 \pm 26.41$  nmol/L, respectively) had lower cortisol concentrations than CAUT kids over the 2-h sampling period. Moreover, O-MEL and I-MEL kids ( $163.0 \pm 26.41$  and  $130.9 \pm 26.41$  nmol/L, respectively) had similar cortisol concentrations to CAUT kids. We found no evidence that plasma glucose and lactate concentrations or body temperature were affected by treatment. The administration of isoflurane, with or without meloxicam, appeared to reduce pain associated with cautery disbudding.

## 7.1 Introduction

In commercial housing systems, horned goats risk injuring themselves and conspecifics during agonistic interactions (Patt et al., 2012) and stockmen during handling (Smith and Sherman, 2009c). Furthermore, horned goats require more space in lying areas and at feed barriers (Loretz et al., 2004). Therefore, goat kids are routinely cauterily disbudded within the first week of life (Smith and Sherman, 2009b). Disbudding causes burning and necrosis of tissue (increasing the risk of bacterial infection; Thompson et al., 2005), inflammation, and subcutaneous damage (Wright et al., 1983), all of which can cause pain. However, the use of pain relief is uncommon, and few studies (Alvarez et al., 2009; Ingvast-Larsson et al., 2011) have investigated its use during disbudding of kids.

Disbudding is associated with changes in behaviour and physiology indicative of pain and distress. Behavioural responses during disbudding include frequent high-intensity vocalisations and struggling (Alvarez et al., 2009). Post-disbudding behavioural changes include increased head shaking, head scratching, head rubbing, self-grooming, and body shaking (Hempstead et al., 2017) as well as isolation from pen-mates and increases in time spent motionless (Greenwood and Shutt, 1990; Ingvast-Larsson et al., 2011); therefore, the pain response may be characterised by vigorous head-oriented movements punctuated by motionless periods. Respiration rate, heart rate, and plasma cortisol, glucose, and beta-endorphin concentrations have also been investigated in response to disbudding (Greenwood and Shutt, 1990; Ingvast-Larsson et al., 2011; Alvarez et al., 2015). Cortisol and beta-endorphin concentrations were elevated in disbudded kids compared with controls (Greenwood and Shutt, 1990; Alvarez et al., 2015). With such evidence, it is necessary to evaluate pain relief strategies to reduce or eliminate pain associated with the practice.

Local anaesthesia, such as lidocaine, can markedly reduce behavioural and physiological responses caused by disbudding in calves (Morisse et al., 1995; Graf and Senn, 1999); however, lidocaine does not consistently reduce pain-related behaviours (Chandrabhas et al., 2013) or plasma cortisol concentrations (Alvarez et al., 2015; Nfor et al., 2016) in disbudded kids. An effective block using lidocaine may be difficult to achieve in kids, as the horns are supplied by the cornual branches of lacrimal and infratrochlear nerves, whereas calves only have the cornual branch of the lacrimal nerve innervating each horn bud (Dugdale, 2011). Additionally, young kids are sensitive to lidocaine and have an increased

risk of toxicity at high doses (Smith and Sherman, 2009a). For these reasons, lidocaine was not investigated in the present study.

General anaesthesia induced before disbudding may prevent acute pain. Isoflurane gas, a general anaesthetic, can be used to safely anaesthetise goats (McEwen et al., 2000; Dzikiti et al., 2011), but its use during disbudding of kids has not been evaluated. Analgesics, such as meloxicam (a nonsteroidal anti-inflammatory drug; NSAID), administered to kids after cautery disbudding resulted in fewer signs of pain (using a visual analogue scale from 1 – 10, where 1 = no signs and 10 = severe signs of pain) than those disbudded without meloxicam (Ingvast-Larsson et al., 2011). These drugs are advantageous for on farm use, as they can be administered orally or injected by farm staff under veterinarian supervision. However, NSAIDs reduce pain associated with inflammation, not acute pain caused by the destruction of nociceptors during disbudding (Ingvast-Larsson et al., 2011). The objective of our study was to evaluate isoflurane, either alone or in combination with meloxicam, and meloxicam administered orally or subcutaneously (s.c.) on goat kid behaviour and physiology following cautery disbudding. We hypothesised that the behavioural and physiological responses to disbudding would be lower in kids administered isoflurane and meloxicam due to induced unconsciousness and mediation of inflammatory pain. We also hypothesised that kids administered s.c. meloxicam would display a reduced behavioural and physiological response to disbudding than kids administered oral meloxicam due to faster absorption rates (Karademir et al., 2016).

## **7.2 Materials and methods**

This study was composed of two trials; trial 1 measured behavioural responses and trial 2 measured physiological responses. The trials were run separately to avoid having the physiological sampling protocols (e.g., blood sampling) affect the behavioural responses of animals.

### **7.2.1 Animals and location**

Trials were conducted in July and August (southern hemisphere winter) of 2014 (behaviour) and 2015 (physiology) at the Ruakura Research Farm, Waikato, New Zealand (latitude 37°47'S, longitude 175°19'E), and approved by the Ruakura Animal Ethics Committee (Protocol No. 13255). At 1 to 2 d of age,

female Saanen cross dairy goat kids were collected from commercial farms, having been separated from their dams within 24 h of birth. Female kids were selected based on the following criteria: horn buds present (i.e., not polled), 2 to 7 d of age, of good health, and weighing > 2.5 kg. On arrival at the Research Farm, kids were weighed and given an identification number. Stock-marking paint (Donaghys Sprayline, Christchurch, New Zealand) was used across the back of some animals (in a line or cross pattern), whereas others were left unmarked to enable individual identification during video analysis. Kids were fed approximately 300 mL of Anlamb milk replacer (Fonterra Ltd., Auckland, New Zealand) via a lamb feeding bucket, twice daily (at 0800 and 1600 h). The feeders remained within the pens after feeding time with *ad libitum* access to further milk replacer.

### 7.2.2 Study facility

The study facility had walls on all four sides and corrugated iron roofing. One large room contained 12 equal sized stalls (3.60 x 3.40 x 1.15 m high). The layout for trial 1 included four stalls, which comprised six smaller pens (1.20 x 1.64 x 0.62 m high) that housed animals pre- and post-treatment. Six additional stalls held the animals after a 2-d observation period ended. Another stall was allocated for kids that suffered from illness, and a final stall acted as the location for treatment administration. The pens that housed the animals for behavioural measurements were reconfigured for trial 2. Five equal-sized stalls (3.60 x 3.40 x 1.15 m high) comprised two smaller pens (1.64 x 2.40 x 0.62 m high): one pen housed animals pre-treatment and one housed the animals post-treatment. The other stalls remained the same as described for trial 1.

The pens were numbered and colour coded to facilitate handling and identification of kids during video analysis. The concrete pen floors were covered with untreated wood shavings (*Pinus radiata*, PGG Wrightsons, Hamilton, New Zealand), 10 cm deep. Pens contained a plastic water trough (15 x 15 x 17 cm deep) fastened to the wall, and a milk feeder was placed on the dividing wall between adjacent pens.

### 7.2.3 Experimental design

Our experiment used a randomised incomplete block design, using pen groups of three kids as the blocking variable. Treatments were balanced for age and the order of treatment was randomly generated by our biometrician using CycDesigN 5 (VSN International, Hemel Hempstead, UK). All kids that fulfilled the criteria described earlier were enrolled by the project manager (a research technician). At the time of enrolment, kids were allocated at random to one of six treatment groups (described below). The project manager was not blind to the treatments the kids received; however, handlers and other farm staff were blind to the treatment each kid received during both trials. Kids were fitted with a coloured collar (blue, green, yellow, red, orange, or pink), associated with the treatment they received (handlers were blind to the treatment-colour combinations), and were placed into their pre-treatment pen-groups (only one kid per treatment per pen as determined by the incomplete block randomisation). The animals remained with the same pen-mates for the entire trial period. Both trials employed the same process to allocate treatments to kids.

A power analysis was carried out to determine the sample size required for both trials. The power analysis was based on a 5% significance level and with 80% power. The primary outcomes used to determine power size were struggling frequency and log<sub>10</sub> blood cortisol, which were assumed to be normally distributed. The standard deviation and minimum response to be detected used were obtained from Alvarez et al. (2009) for struggling frequency (2.32 and 2.8, respectively) and from an ACTH challenge performed by our group (our unpublished data) for cortisol (0.358 and 0.5, respectively).

On treatment days, kids were moved one at a time from the pre-treatment pens into the treatment area and placed in a restraint device. This device was raised off the ground (0.90 m) and consisted of a rigid plastic pipe (0.35 x 0.85 m) sectioned lengthways with holes for the kids' legs and straps to secure the kid across the shoulder and back. Hair covering the horn buds was removed with electric hair clippers (Laube, 505 cordless kit, Shoof, Cambridge, New Zealand) to expose the horn buds and to reduce hair burning. The restraint prevented movement at the time of treatment, so only the behaviours pre- and post-restraint were recorded. Kids were fed before treatment. All treatments were performed by a veterinarian between 0800 and 1600 h for trial 1 and between 0800 and 1000 h for trial 2.

The treatments were as follows.

1. Cautery disbudded (CAUT) using a modified version of the procedure described by Ingvast-Larsson et al. (2011) and without pain relief. The cautery iron (Quality electric debudder, 230 V, 190 W; Lister GmbH, Ludenscheid, Germany) was heated for at least 20 min before use (approximately 600°C) and held on each horn bud for  $\leq 6$  s. The use of downwards pressure (and a circular motion of the cautery iron) on the animal cut the tissue, and with additional downward pressure, and a flick, forcibly lifted off the ring of tissue containing horn bud cells.
2. Isoflurane gas (ISO) administered at a rate of 4% via a face mask (in oxygen); delivery continued until the animal lost consciousness (determined by dilation of pupils and loss of palpebral reflex), at which point the isoflurane mixing rate was reduced to 2%. Kids were then disbudded using the same procedure as for CAUT. Following disbudding, isoflurane was removed from the gas supply, allowing the inhalation of pure oxygen for several seconds. The oxygen was then turned off, the face mask removed, and the animal remained in the restraint under supervision until it regained consciousness ( $\leq 5$  min).
3. Isoflurane gas administered as in treatment 2 and meloxicam s.c. (ISO+MEL) administered using the same procedure described for I-MEL below, followed by cautery disbudding.
4. Meloxicam (Loxicom 20mg/mL solution for injection for cattle, pigs and horses, Norbrook Laboratories Ltd., Newry, UK), injected s.c. (IMEL; 0.5 mg/kg of BW) over the ribs, (dorsal thorax, caudal to the scapula), after which kids were disbudded using the same procedure as for CAUT.
5. Meloxicam (Loxicom 0.5 mg/mL oral suspension for dogs, Norbrook Laboratories Ltd.) administered orally (O-MEL; 0.2 mg/kg of BW), after which kids were disbudded using the same procedure as for CAUT.
6. Sham-handled (SHAM; simulated disbudding) with a cold disbudding iron applied to the horn buds (i.e., not disbudded) for  $\leq 6$  s and no pain relief given.

After horn bud removal, antibacterial spray (Tetravet, Bayer New Zealand Ltd., Auckland, New Zealand) was applied to the open wounds to prevent infection (this was not done on SHAM kids). The kids were then moved into the post-treatment pens.



### 7.2.3.1 Trial 1: Behavioural responses

Seventy-two female Saanen cross dairy goat kids ( $n = 12/\text{treatment}$ ) were used in this trial, consisting of six treatment days over 30 d with at least six kids treated per experimental day. Kids were aged between 2 and 7 d (mean  $\pm$  SEM;  $3.9 \pm 0.15$  d) with BW ranging between 2.6 and 5.1 kg ( $3.8 \pm 0.07$  kg).

Behaviours were continuously recorded using video cameras (HDR-CX220E, Sony Corp., Shanghai, China) for 24 h pre- and post-treatment. Due to the New Zealand industry standard age for disbudding kids, no time was allocated for habituation to the new environment before behavioural measurements commenced. The cameras were positioned at a height of 1.85 m above the pens (so that one camera could monitor three adjacent pens) and set at an angle of  $30^\circ$  to the vertical attachment pole. Each camera was fitted with a fisheye lens (Raynox, Insta-Wide lenses, QC-303, Yoshida Industry Co. Ltd., Tokyo, Japan) to enable the whole width of each pen to be viewed. Red floodlights (80 W,  $30^\circ$  beam angle, Philips Ltd., Auckland, New Zealand) were placed 2 m above each stall to assist with night observations.

Behaviours recorded were based on the ethogram described by Hempstead et al. (2017) but were modified to improve inter-observer reliability (see Table 7.1 for ethogram comparison). Feeding was included in the present study as changes in feeding behaviour have been reported in similar calf studies (Graf and Senn, 1999). Body shaking was consistently reliable between observers and therefore was not modified. The frequency of all behaviours was measured with the addition of duration of head scratching, self-grooming and feeding as these behaviours could be reliably measured.

One trained observer analysed all video-recordings using Adobe Premier Pro software (CS6, Version 6.0.0, Adobe Systems, San Jose, CA). When analysing recordings, the observer remained blind to the treatment each kid received, except that SHAM kids could be identified as they were not disbudded or sprayed with blue antibiotic spray. Frequency and duration data were recorded against kid identification, which corresponded with the markings on the kids' backs (previously described) in the video-recordings. Intra-observer reliability was performed on three kids over the same 1-h period, selected randomly from active periods, for each behaviour ( $\kappa = 0.77$  for head shaking;  $\kappa = 0.88$  for head scratching;  $\kappa = 0.98$  for self-grooming;  $\kappa = 0.95$  for feeding; and  $\kappa = 0.94$  for body shaking). Inter-observer reliability between three observers was performed

throughout observations by selecting a further three animals at random for each behaviour (mean kappa among three observers,  $\kappa = 0.88$  for head shaking;  $\kappa = 1.00$  for head scratching;  $\kappa = 0.79$  for self-grooming;  $\kappa = 0.93$  for feeding; and  $\kappa = 1.00$  for body shaking).

Lying behaviour was measured using accelerometers (64k Pendant G data loggers, Onset Computer Corporation, Bourne, MA) set to record the x and z axes at 60-s intervals. Lying duration was recorded continuously for 24 h pre- and post-treatment. The logger was placed into a durable fabric pouch and strapped (with hook and loop fasteners) to the lateral side of the left hind leg above the metatarsophalangeal joint. After the trial, data were downloaded using Onset HOBOWare Pro software (Onset Computer Corporation, version 3.4.1) and converted to hourly summaries of lying behaviour using SAS software (version 9.4, SAS Institute Inc., Cary, NC) code designed for this purpose; based on the work of Zobel et al. (2015), we corrected for single standing and lying events.

### **7.2.3.2 Trial 2: Physiological responses**

Sixty female Saanen cross dairy goat kids ( $n = 10/\text{treatment}$ ) were used; the trial included four treatment days over an 8-d period with a minimum of 12 kids treated per experimental day. Kids were aged between 3 and 6 d (mean  $\pm$  SEM;  $4.3 \pm 0.14$  d) with BW ranging between 2.3 and 4.4 kg ( $3.3 \pm 0.06$  kg). Kids were given 24 h to habituate to their home pen before the trial commenced; they were therefore older than trial 1 animals.

Plasma cortisol, glucose, and lactate concentrations were measured using blood samples (4 mL) collected by venipuncture from the jugular veins immediately pre-treatment (baseline; before hair clipping) and at 15, 60 and 120 min post-treatment. Two handlers, trained by a veterinarian, collected the blood samples from each kid; the process took  $\leq 2.5$  min/kid. Each kid was firmly restrained by a handler while samples were collected using 21-gauge 1-inch needles (PrecisionGlide, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ); samples were then held in fluoride oxalate tubes (Becton Dickinson Vacutainer Systems) on ice. Blood samples were centrifuged at 3,000 rpm (approximately  $1,500 \times g$ ) for 10 min at  $4^\circ\text{C}$  immediately after sampling and the plasma was separated and stored at  $-20^\circ\text{C}$  until analysed.

**Table 7.1.** Comparison of ethograms from Hempstead et al. (2017) and the current paper.

Behaviour	Description	
	Hempstead et al. (2017)	Current paper
Head Shaking	A rapid tilting of the head from side to side a minimum of three times concluding with a return to neutral position. Head shakes separated by > 1 s are considered separate events.	Rapid continuous tilting of the head from side to side concluding with a return to neutral position. Head shakes separated by > 1 s are considered separate events.
Head Scratching	Tilting of the head so the rear foot scratches any part of the head (excluding neck). The rear fetlock must reach the shoulder and includes attempts (i.e. the foot does not contact the head but is raised off the ground). Head scratches separated by > 1 s are considered separate events.	The rear foot touches any part of the head or neck (including collar). Scratches separated by > 1 s were considered separate events.
Self-Grooming	The kid's mouth contacts any part of the body or legs. A separate self-grooming event is considered to occur after a pause of > 1 s.	The kid's muzzle contacts any part of the body or legs (excluding hoof) with a rhythmic back and forth motion. A separate grooming event was considered to occur after a pause of > 1 s.
Feeding	The mouth covers at least half of the nipple of the feeding bucket for > 3 s, usually followed by suckling motions. Repetitions following separation of the mouth from the nipple of > 3 s are considered separate events.	The mouth covers at least half of the nipple of the feeding bucket for > 3 s, usually followed by suckling motions. Repetitions following separation of the mouth from the nipple of > 3 s were considered separate events.

Plasma cortisol, glucose, and lactate concentrations were analysed in an independent laboratory by technicians blind to the treatments. Plasma concentrations of cortisol were determined by electrochemiluminescence immunoassay using a commercial kit (Roche Diagnostics GmbH, Mannheim, Germany). Sensitivity of the assay was 1.5 nmol/L, and intra- and inter-assay coefficients of variation were 2.1 and 5.2%, respectively. Plasma glucose concentrations were determined by the hexokinase method using a commercial kit (Roche Diagnostics GmbH) and the sensitivity of the assay was 0.1 mmol/L. Plasma lactate concentrations were determined by enzymatic methods using a commercial kit (Roche Diagnostics GmbH) and the sensitivity of the assay was 0.2 mmol/L. Body temperature was measured immediately after blood sampling at all four time points using a rectal Rapid Digital Thermometer (Vet Temp, Advanced Monitors Corp., San Diego, CA).

#### **7.2.4 Statistical analysis**

Data were analysed using Genstat software (Version 17, VSN International, Hemel Hempstead, UK). We inspected the residual diagnostic plots to detect departures from the assumptions of normality and constant variance. Log transformations were required for all behaviour data except for head shaking and lying. Transformations of the physiological data were not required. We recorded no adverse events for trial 1, hence no kids were excluded. One animal was removed from trial 2 due to illness and another was excluded from the cortisol analysis due to a processing error. Total frequencies and durations of behaviours were grouped into 1-h periods across the 48-h observation period (pre- and post-treatment). In addition, frequency and duration data were grouped into twelve 5-min periods 1 h pre- and post-treatment to assess the acute behavioural response following treatment. Acute behavioural responses within 1 h post-treatment (during daylight hours) were chosen, as kids were most active within this period and plasma cortisol concentrations of goat kids typically return to baseline levels 1 h after disbudding (Alvarez et al., 2015).

Head shaking, head scratching, self-grooming, feeding, body shaking, body temperature, and plasma cortisol, glucose, and lactate concentrations from pre- to post-treatment were assessed using a repeated measures model fitted by restricted maximum likelihood (REML). The model included the fixed effects of

treatment, time, and their interaction and the random effects of kid identification, age, weight, farm, treatment date and pen. The correlation between measurements taken on the same kid over time was modelled with a power model of order 1. Further analyses were carried out on behaviour data within the first hour post-treatment (twelve 5-min periods) using a repeated measures model fitted by REML. The model used the same variables as for the hourly analysis, except, in this case, the average of the twelve 5-min periods 1 h pre-treatment was used as a covariate.

Lying behaviour was modelled using a repeated measures model fitted by REML, with the average of the pre-treatment lying duration per hour as a covariate. The fixed and random variables were the same as above. To meet the assumptions of constant variance and normality, the lying data required rank transformation.

Differences between and within treatments were detected using Fisher's least significant differences test. Mean values (back-transformed if required, with exact 95% CI) were provided with standard errors of the difference (SED). The level of significance was set at  $P \leq 0.05$ .

## 7.3 Results

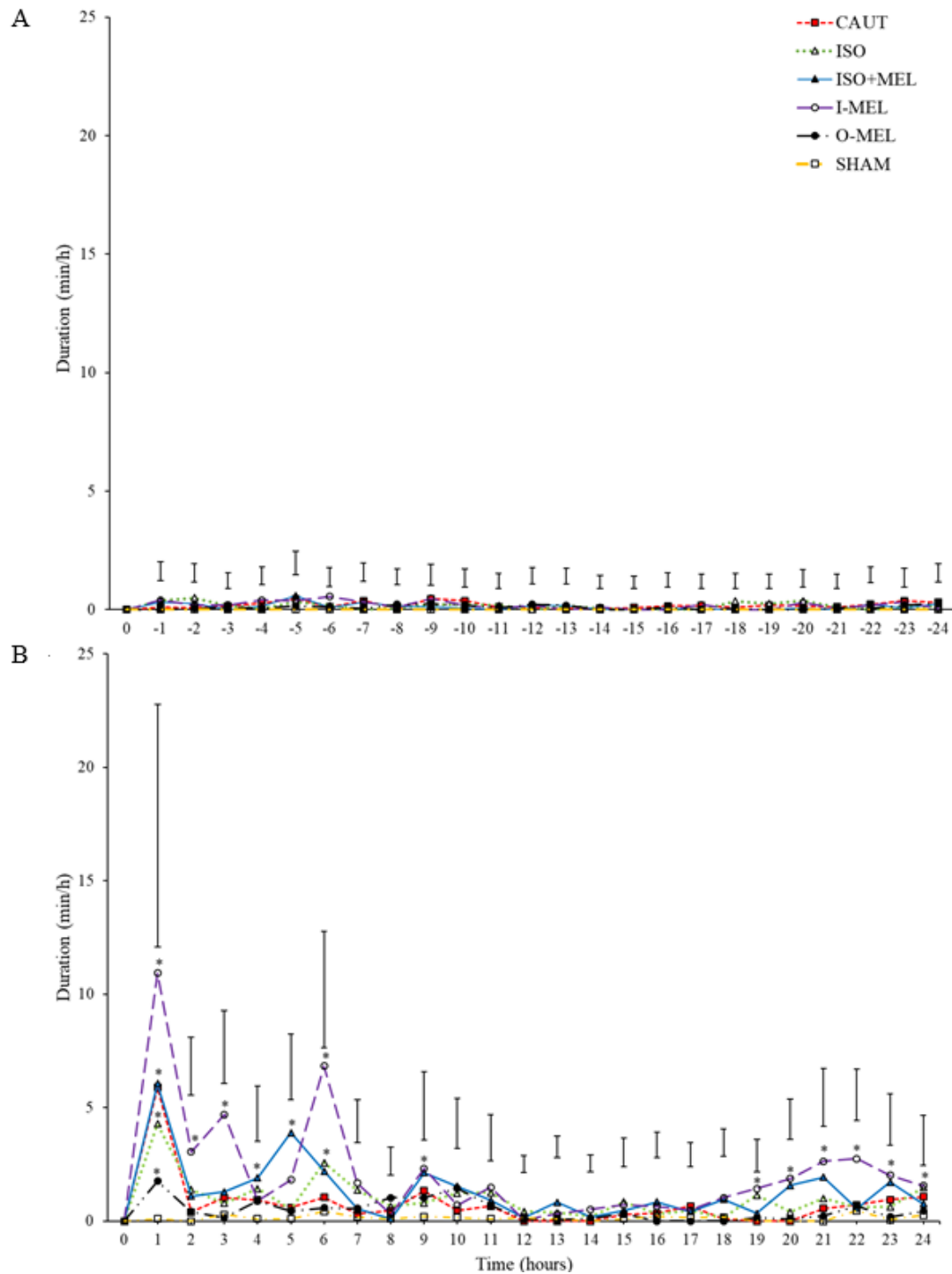
### 7.3.1 Trial 1: Behavioural responses

We found no treatment by time interaction on the mean number of head shakes per hour over the 48-h observation period ( $P = 1.00$ ); however, we noted an overall effect of time ( $F_{48, 3033} = 18.3; P < 0.001$ ). Head shakes were 10-times more frequent 1 h post-treatment compared with pre-treatment and all other post-treatment hours ( $P \leq 0.05$ ). For the 5-min periods within 1 h post-treatment, we observed a treatment by time interaction ( $F_{55, 672} = 1.6; P = 0.005$ ). At 5 min post-treatment, CAUT kids performed over double the number of head shakes as SHAM kids ( $P \leq 0.05$ ) but displayed similar levels as SHAM kids by 10 min post-treatment ( $P = 0.93$ ). In addition, ISO+MEL kids performed half as many head shakes as CAUT kids 5 min post-treatment and ISO kids performed one-third the number of head shakes as CAUT kids for up to 10 min post-treatment ( $P \leq 0.05$ ). Both ISO+MEL and ISO kids performed less head shaking than I-MEL and O-MEL kids at 5 min post-treatment ( $P \leq 0.05$ ). Both I-MEL and O-MEL kids performed similar frequencies of head shaking to CAUT and SHAM kids over the

twelve 5-min periods within 1 h post-treatment ( $P > 0.08$ ) except for I-MEL kids, which performed twice as many head shakes as SHAM kids at 15 min post-treatment ( $P = 0.05$ ).

We found no treatment by time interaction on the mean number of body shakes per hour over 48 h (pre- and post-treatment;  $P = 1.00$ ); however, we did note an effect of time ( $F_{48, 3030} = 16.3$ ;  $P < 0.001$ ). Overall, kids had a 3-fold increase in body shakes 1 h post-treatment compared with pre-treatment hours ( $P \leq 0.05$ ). There was a treatment by time interaction across the 5-min periods 1 h post-treatment for body shaking frequencies ( $F_{55, 672} = 2.1$ ;  $P < 0.001$ ). At 5 min post-treatment, CAUT kids performed, on average, 3-times as many body shakes as SHAM kids ( $P \leq 0.05$ ), but declined to SHAM kid frequencies during the remainder of the hour ( $P > 0.15$ ). Furthermore, ISO+MEL and ISO kids performed less than one-quarter the number of body shakes as CAUT kids 5 min post-treatment ( $P \leq 0.05$ ). At 10 min post-treatment, ISO kids performed one-third the number of body shakes as CAUT kids ( $P \leq 0.05$ ). Additionally, ISO+MEL and ISO kids performed one-fifth the number of body shakes as I-MEL and OMEL kids at 5 min post-treatment ( $P \leq 0.05$ ).

We observed a treatment by time interaction for hourly head scratching durations over 48 h ( $F_{235, 2952} = 1.4$ ;  $P < 0.001$ ; Figure 7.1). At 1 h post-treatment, CAUT kids spent more time head scratching than SHAM kids ( $P \leq 0.05$ ). In addition, I-MEL and ISO+MEL had higher head scratching durations than SHAM kids ( $P \leq 0.05$ ), and O-MEL kids spent more time head scratching than SHAM kids but reduced to SHAM kid levels at 2 h post-treatment ( $P > 0.24$ ; Figure 7.1). Over the post-treatment period, ISO and ISO+MEL kids performed similar head scratching durations as CAUT kids ( $P > 0.18$ ); however, ISO+MEL kid durations were elevated above CAUT kid levels 5 h post-treatment ( $P = 0.001$ ; Figure 7.1). For O-MEL kids, time spent head scratching was less than for I-MEL kids at many time points post-treatment ( $P \leq 0.05$ ; Figure 7.1). There was no treatment by time interaction on mean head scratching frequencies over 48 h ( $P = 0.27$ ), although we did note an overall effect of time ( $F_{48, 3034} = 9.9$ ;  $P < 0.001$ ). All kids performed twice as many head scratches 1 h post-treatment than 1 h pre-treatment ( $P \leq 0.05$ ). We found no difference between treatments within 1 h post-treatment for head scratching frequencies across the twelve 5-min periods ( $P = 0.44$ ).



**Figure 7.1.** Back-transformed mean head scratching durations (min/h; error bars represent exact 95% CI for each time point) over 24 h pre-treatment (A) and 24 h post-treatment (B) of goat kids exposed to experimental treatments (n = 12/treatment). Asterisks indicate means that differ from SHAM kid means at  $P < 0.05$ . CAUT: disbudded with no pain relief; ISO: disbudded with isoflurane gas; ISO+MEL: disbudded with isoflurane and s.c. meloxicam; I-MEL: disbudded with meloxicam s.c. (0.5 mg/kg of BW); O-MEL: disbudded with oral meloxicam (0.2 mg/kg of BW); SHAM: sham-handled only.

We noted no treatment or treatment by time interaction on self-grooming frequencies and durations over 48 h ( $P > 0.92$ ). There was an effect of time over 48 h on self-grooming, with a two-fold increase in the activity over 12 h post-treatment compared with the pre-treatment period ( $F_{48, 3035} = 11.6$ ;  $P < 0.001$ ).

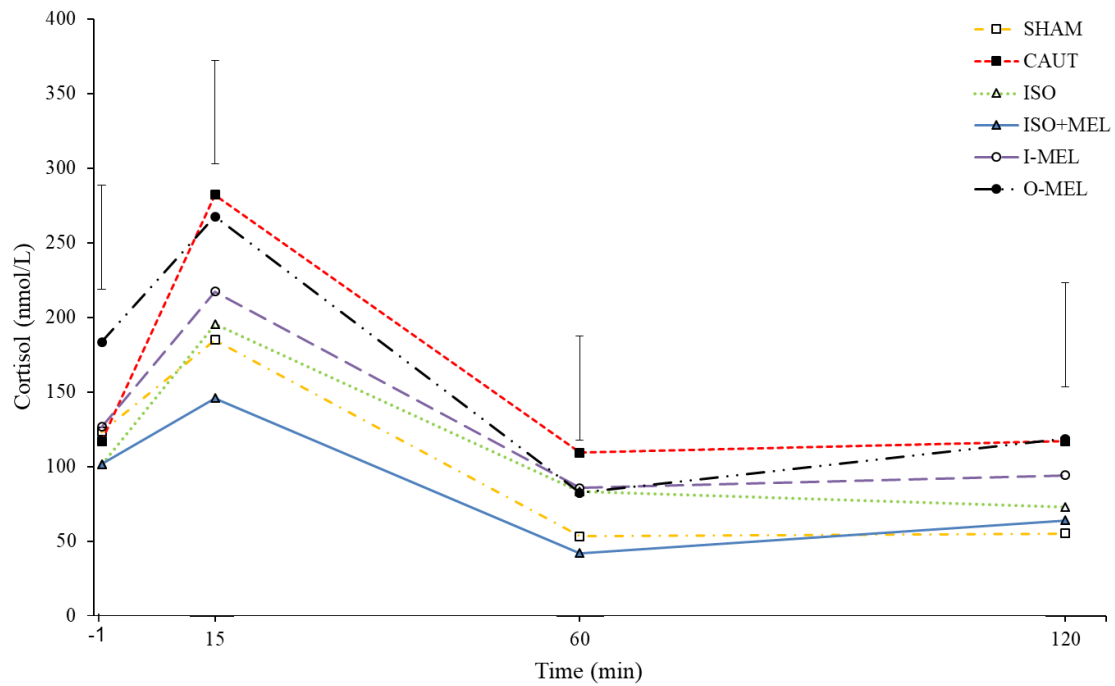
No effect of treatment or treatment by time interaction was observed on feeding frequency and duration over 48 h ( $P = 1.00$ ), but we noted a separate effect of time on feeding ( $F_{48, 3034} = 7.33$ ;  $P < 0.001$ ). Kids fed more often 2 h pre-treatment compared with 1 h post-treatment ( $P \leq 0.05$ ). Furthermore, feeding frequency was higher 4 h post-treatment compared with 1 h post-treatment ( $P \leq 0.05$ ). Kids spent more time feeding during the first half of the post-treatment period compared with the pre-treatment period ( $P \leq 0.05$ ).

Lying duration was similar across treatments over 24 h post-treatment ( $P = 1.00$ ), although we found an effect of time on lying behaviour ( $F_{23, 606} = 14.6$ ;  $P < 0.001$ ). For all treatments, kids spent more time on average lying (3.5-times) 2 h post treatment than 1 h post-treatment ( $P \leq 0.05$ ).

### 7.3.2 Trial 2: Physiological responses

We observed no treatment by time interaction for cortisol concentrations ( $P = 0.24$ ); however, there were separate effects of time ( $F_{3, 118} = 58.8$ ;  $P < 0.001$ ) and treatment ( $F_{5, 43} = 2.5$ ;  $P = 0.04$ ; Figure 7.2). Baseline cortisol concentrations were similar across treatments ( $125.5 \pm 11.81$  nmol/L;  $F_5 = 1.1$ ;  $P = 0.37$ ). Overall, cortisol concentrations peaked at 15 min post-treatment and dropped below baseline at 60 min post-treatment ( $P \leq 0.05$ ). Cortisol concentrations for CAUT kids were higher than SHAM kids over the 2-h post-treatment sampling period ( $P \leq 0.05$ ). Moreover, cortisol concentrations were similar between ISO+MEL and SHAM kids ( $P = 0.47$ ) but lower than CAUT kids ( $P \leq 0.05$ ); O-MEL kids had similar cortisol concentrations to CAUT kids ( $P = 0.86$ ). Furthermore, ISO and I-MEL kids had similar cortisol concentrations to both CAUT and SHAM kids ( $P > 0.24$ ).





**Figure 7.2.** Plasma cortisol concentrations (nmol/L; error bars represent maximum SED for each time point) over 2 h post-treatment of goat kids exposed to experimental treatments (n = 12/treatment). CAUT: disbudded with no pain relief; ISO: disbudded with isoflurane gas; ISO+MEL: disbudded with isoflurane and s.c. meloxicam; I-MEL: disbudded with meloxicam s.c. (0.5 mg/kg of BW); O-MEL: disbudded with oral meloxicam (0.2 mg/kg of BW); SHAM: sham-handled only.

We found no treatment by time interaction for glucose concentrations ( $P = 0.68$ ; Table 7.2) or an effect of treatment ( $P = 0.12$ ); however, a separate effect of time was noted ( $F_{3,162} = 5.3$ ;  $P = 0.002$ ). Glucose concentrations were higher ( $P \leq 0.05$ ) 2 h post-treatment than at baseline. There was no treatment by time interaction for lactate concentrations ( $P = 0.36$ ; Table 7.2) or separate effects of time ( $P = 0.30$ ) and treatment ( $P = 0.45$ ). We observed no treatment by time interaction for body temperature ( $P = 0.28$ ; Table 7.2) or an effect of treatment ( $P = 0.70$ ); however, we did note a separate effect of time ( $F_{3,116} = 3.5$ ;  $P = 0.02$ ). Body temperature was higher for all kids at 15 min post-treatment than baseline ( $P \leq 0.05$ ).

**Table 7.2.** Plasma glucose (mmol/L; mean  $\pm$  SED) and lactate concentrations (mmol/L; mean  $\pm$  SED) and body temperatures ( $^{\circ}$ C; mean  $\pm$  SED) of goat kids over 2 h, from 1 min pre-treatment to 120 min post-treatment (n = 12/treatment).

Item <sup>1</sup>	Time (Min)			
	-1	15	60	120
<sup>a</sup> Glucose (mmol/L)				
CAUT	6.3	6.7	7.1	7.9
ISO	6.1	6.0	6.4	6.8
ISO+MEL	6.6	6.4	6.7	7.4
I-MEL	5.2	5.7	6.1	6.4
O-MEL	5.7	6.1	6.1	5.9
SHAM	5.7	6.2	6.3	6.4
Average SED	0.70	0.62	0.66	0.68
<sup>b</sup> Lactate (mmol/L)				
CAUT	2.4	2.7	2.2	2.3
ISO	2.3	2.3	2.5	2.4
ISO+MEL	2.3	2.8	2.9	2.6
I-MEL	1.9	2.5	2.4	2.4
O-MEL	2.9	2.6	2.5	2.5
SHAM	2.2	2.6	2.4	3.0
Average SED	0.42	0.39	0.41	0.41
<sup>c</sup> Body temperature ( $^{\circ}$ C)				
CAUT	38.6	39.2	39.0	38.9
ISO	38.8	38.9	38.8	38.9
ISO+MEL	39.1	39.1	39.2	39.2
I-MEL	38.8	39.0	38.9	38.8
O-MEL	39.0	39.3	38.9	38.8
SHAM	38.9	39.0	38.9	39.1
Average SED	0.25	0.23	0.23	0.23

<sup>1</sup>Treatment by time interaction for glucose  $P = 0.68$ ; lactate  $P = 0.36$ ; and body temperature  $P = 0.28$ . CAUT: disbudded with no pain relief; ISO: disbudded with isoflurane gas; ISO+MEL: disbudded with isoflurane and s.c. meloxicam; I-MEL: disbudded with meloxicam s.c. (0.5 mg/kg of BW); O-MEL: disbudded with oral meloxicam (0.2 mg/kg of BW); SHAM: sham-handled only.

## 7.4 Discussion

Our study is the first to examine the effects of inducing general anaesthesia using isoflurane, either alone or in combination with an NSAID (meloxicam), on the behavioural and physiological responses of goat kids following cautery disbudding. General anaesthetic (alone or in combination with an oral NSAID) reduced the behavioural response of kids immediately after disbudding, particularly head and body shakes; therefore, our hypothesis, that unconsciousness reduces subsequent responsiveness, was supported. However, it is possible that behaviour was affected by the isoflurane alone. An additional

sham-handled group provided anaesthesia but not disbudded may have displayed a similar response to the ISO group; however, this treatment was not included in the present study due to logistical reasons. Isoflurane may have affected kid behaviour for up to 2 h post-treatment, as has been observed in calves that had undergone umbilical surgery (Offinger et al., 2012). Therefore, future research is required to establish the longer-term effects of isoflurane on kid behaviour.

The behavioural response of disbudded kids appears relatively short-lived in comparison with disbudded calves, which exhibit higher frequencies of head shaking than sham controls for up to 4 h after disbudding (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Heinrich et al., 2010). In the present study, the behavioural response diminished 1 h after treatment. A potential explanation is the difference in age that disbudding is typically performed, as kids are usually disbudded within a week of age (Smith and Sherman, 2009b) and calves usually between 4 to 6 wk; therefore, the horn buds of calves are more developed than those of 1-wk-old kids. Furthermore, the amount of time taken to disbud calves and kids can differ; in calves, disbudding can take up to 20 s per horn bud (Graf and Senn, 1999) compared with approximately 6 s per horn bud for kids in the present study. Thus, cautery disbudding in kids may be less painful than disbudding in calves.

In the present study, isoflurane, whether in combination with meloxicam or not, reduced the cortisol response to disbudding in kids for up to 2 h, which indicates a reduction in acute pain associated with the practice. This is in line with research that found calves disbudded with local anaesthetic with or without sedation showed a reduced cortisol response (Grondahl-Nielsen et al., 1999). Yet others reported that local anaesthetic did not reduce the cortisol response of disbudded calves compared with calves not provided pain relief (Morisse et al., 1995). The difference in results may be associated with difficulty in administering a successful nerve block, which is also apparent in goat kids (Alvarez et al., 2009). Furthermore, in the present study, isoflurane (with or without meloxicam) is more effective than meloxicam alone, regardless of the route of administration. Interestingly, Heinrich et al. (2009) reported calves disbudded with meloxicam had lower serum cortisol than saline controls 6 h after disbudding, indicating that meloxicam reduced the physiological stress response to disbudding. As the response of goat kids to disbudding appears short in duration, perhaps the long-term action of meloxicam is not as apparent as it is for calves. Isoflurane, with or

without meloxicam, shows promise as a method of pain relief for disbudding goat kids; however, the use of isoflurane anaesthesia on farm may not be practical due to the time required for recovery and equipment and personnel requirements (e.g., veterinary administration), but a portable isoflurane delivery device could be developed for on farm use. Meloxicam is better suited to on farm use, as it can be administered by farm staff, but may be less effective.

We hypothesised that kids provided injected meloxicam would have a lower behavioural and physiological response to disbudding than kids provided oral meloxicam due to rapid absorption rates. However, oral meloxicam appeared to generate a lower behavioural response than injected meloxicam. It is unclear why oral meloxicam would be more effective than injected meloxicam at reducing pain, as the subcutaneous route should be absorbed into the blood quicker than oral meloxicam (Ingvast-Larsson et al., 2011; Karademir et al., 2016). Ingvast-Larsson et al. (2011) reported that disbudded goat kids administered injected meloxicam showed less signs of pain (using a visual analogue score) than groups disbudded without meloxicam. Therefore, evidence suggests that oral meloxicam may reduce post-operative pain associated with cautery disbudding in goat kids. Plasma cortisol concentrations of kids receiving the NSAID were similar to cautery disbudded kids without pain relief, which suggests that meloxicam, regardless of the route of administration, may not reduce the acute stress response to disbudding. This is in line with earlier literature reporting that cortisol concentrations were similar between kids disbudded with and without an NSAID (Ingvast-Larsson et al., 2011). In comparison with calf literature, meloxicam or ketoprofen administered 10 min before disbudding resulted in lower cortisol concentrations than calves without NSAID for up to 6 h (Milligan et al., 2004; Heinrich et al., 2009) and reduced head shaking from 3 to 24 h post-disbudding (Faulkner and Weary, 2000). In the present study, on average, kids given meloxicam along with isoflurane had a lower behavioural and physiological response to disbudding; therefore, used in combination with anaesthesia, meloxicam may reduce pain. In the present study, both injected and oral meloxicam were given immediately before disbudding to model on farm application (i.e., to avoid double handling of animals). Future research is required to establish the optimal time of meloxicam administration before disbudding in goat kids.

Our earlier findings suggested that head scratching and self-grooming frequencies may reflect pain associated with cautery disbudding (Hempstead et al., 2017). Yet in the present study, we found no statistically significant differences between treatments either (1) over 48 h pre- and post-treatment or (2) within 1 h post-treatment for head scratching and self-grooming frequencies. A key difference between these studies was the use of different ethograms, which were modified to increase inter-observer reliability. However, the mean values for head scratching frequency in the present study tended to be higher in cautery disbudded than sham-handled controls.

No differences in feeding behaviour were found across treatments, which is consistent with our earlier study (Hempstead et al., 2017); however, in calves, feeding behaviour is affected for up to 4 h after disbudding (Graf and Senn, 1999). It appears that all kids, regardless of treatment, fed more often after handling or treatment than before, which may be associated with the lack of sufficient habituation before behaviour recording commenced or that the kids had learned how to feed independently. In addition, lying behaviour of kids over 24 h post-treatment did not differ significantly between treatments, which contrasts with Chandrahas et al. (2013), who reported that sleeping duration was higher in kids provided meloxicam than kids disbudded only. Differences in results could be due to differences in how lying or sitting behaviour were measured; Chandrahas et al. (2013) recorded behaviour manually by observation with a point sampling method, whereas we measured lying behaviour continuously using accelerometers. It has also been observed in disbudded calves that lying behaviour was similar between groups provided with pain relief or without (Faulkner and Weary, 2000; Duffield et al., 2010; Sutherland et al., 2016).

In the present study, basal cortisol concentrations were higher 1 min pre- than 1 h post-treatment for all kids, which may be related to the timing of feeding and associated handling of the kids approximately half an hour before disbudding. Adult goats have shown increased plasma cortisol within 60 min of feeding (Eriksson and Terävainen, 1989). In dairy cows, cortisol is known to be released in response to feeding (Willett and Erb, 1972). In future studies, a longer settling period after feeding may be required to enable cortisol concentrations to return to basal levels before testing. Periodic fluctuations (e.g., circadian rhythms) in glucocorticoid levels, that have been previously described in other mammalian species such as pigs (Andersson et al., 2000) and horses (Irvine and Alexander,

1994), may explain the difference in cortisol over the blood sampling period; however, goats do not appear to show this daily rhythm in cortisol (Alila-Johansson et al., 2003).

Gluconeogenesis, which is the process of synthesising glucose, is stimulated by cortisol and other glucocorticoids. Prunier et al. (2005) reported elevations in cortisol in castrated pigs but no increase in plasma glucose compared with uncastrated controls, which was most likely due to insufficient glycogen stores in young pigs. We found no statistically significant differences in mean glucose concentrations between treatments, which is consistent with previous goat studies (e.g., Ingvast-Larsson et al., 2011); furthermore, we found no statistically significant differences among treatments for mean lactate, which typically increases with stress or pain in pigs (Prunier et al., 2005) and dairy cattle (El-Ghoul and Hofmann, 2002). The difference in glucose and lactate elevation between disbudding and castration may be associated with castration causing more visceral pain than disbudding, which may cause more somatic pain (Dunckley et al., 2005) or high variability of blood constituents in goat kids due to early life development (Chen et al., 1999). In addition, we found no evidence that mean body temperature was affected by our treatments; however, overall kids had higher body temperature after treatment, indicating that handling and associated stress affected body temperature rather than the treatments themselves. This contrasts with the findings of Nfor et al. (2016), who reported that body temperature decreased post-disbudding compared with baseline values in kids that were disbudded either under sedation (dexmedetomidine hydrochloride) or provided a local ring block (1 mL s.c.). However, body temperature was not reduced in kids disbudded without pain relief (injected saline); therefore, differences were likely caused by the pain relief treatments.

## **7.5 Conclusions**

Cautery disbudding causes elevated cortisol concentrations, increased head shaking and body shaking frequencies, and greater head scratching durations. Isoflurane alone or in combination with meloxicam reduced the behavioural and cortisol responses to cautery disbudding and therefore may significantly reduce pain. Furthermore, meloxicam appears to reduce postoperative pain, but more research on the optimal time to administer meloxicam before disbudding is

needed. Although the use of isoflurane (with or without meloxicam) can reduce pain associated with cautery disbudding, the practicality of using isoflurane on farm needs to be addressed; other pain relief options should also be evaluated to improve the welfare of disbudded goat kids.

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# Chapter 8

## General discussion

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Here I provide a summary of the results of my thesis, their animal welfare implications and my overall conclusions. I will also discuss the limitations of my research and identify areas that require further research. I have investigated potential improvements for disbudding of dairy goat kids by firstly identifying useful behavioural and physiological indicators of pain associated with the practice. I then explored alternatives to cauterisation (i.e. caustic paste, cryosurgical disbudding and clove oil injection) that may cause less pain, and based on these results, I assessed the efficacy of cauterisation techniques (horn bud removed vs. left intact) and clove oil injection. Finally, I assessed pain mitigation strategies for cauterisation.

## **8.1 Useful measures of pain associated with disbudding of goat kids**

Throughout my PhD, I have used numerous techniques to assess pain associated with disbudding of goat kids. Head shaking, head scratching, head rubbing, self-grooming, body shaking frequency and lying time (as well as number of lying bouts) all changed in response to disbudding (Chapters 4 and 7), suggesting the increases or decreases in the performance of these behaviours indicates pain. Similarly, head shaking and rubbing are commonly used measures of pain in disbudded calves (Graf and Semm, 1999; Grondahl-Nielsen et al., 1999; Faulkner and Weary, 2000). Pressure algometry was used to measure the mechanical nociceptive threshold (MNT), which is the amount of pressure an animal can tolerate before moving from the stimulus, and can indicate pain in disbudded calves (Heinrich et al., 2010; Allen et al., 2013). Disbudding of goat kids resulted in a lower MNT around the horn buds compared with sham-handled kids, indicating the reduced tolerance of pressure was indicative of pain (Chapter 5).

Social behaviours directed at other animals (e.g., allogrooming), were not shown to change in response to cautery disbudding of goat kids and therefore may not reflect pain (Hempstead et al., 2017). Feeding behaviour was not shown to change in response to disbudding (Chapters 4 and 7), which suggests pain associated with disbudding does not affect feeding motivation and may not reflect pain. However, other behaviours such as standing or lying in contact with others was not evaluated in Hempstead et al. (2017) or this thesis. Greenwood and Shutt (1990) observed goat kids standing alone shortly after cautery disbudding, although this was not quantified. Castrated piglets were reported to spend more time lying isolated from other piglets or the sow compared with sham-castrated piglets (Sutherland et al., 2012), which may be an attempt to avoid contact with conspecifics that may elicit pain (Mellor et al., 2000). Therefore, assessment of time spent isolated from conspecifics (either standing or lying) could be evaluated in future studies estimating pain associated with different methods of disbudding of goat kids.

Cortisol concentrations, which are commonly used as an indirect measure of pain, have been shown to increase in response to painful husbandry procedures such as castration in lambs (Mellor and Murray, 1989), disbudding in calves

(Morisse et al., 1995; Graf and Senn, 1999; Grondahl-Nielsen et al., 1999) and amputation dehorning in calves (Sylvester et al., 1998). The results of my thesis show that cortisol concentrations were higher in disbudded kids than sham-handled controls (Chapters 3 and 7), indicating that changes in cortisol can reflect pain in goat kids. In addition, haptoglobin concentrations and skin temperature (measured by infrared thermography) were elevated above sham-handled kid levels in clove oil and cauterly disbudded kids indicating pain associated with inflammation (Chapter 3).

There were no differences in glucose concentrations between cauterly disbudded or sham-handled kids (Chapter 7). Another goat kid study also found no difference in glucose concentrations 24 h after cauterly disbudding with or without meloxicam (Ingvast-Larsson et al., 2011). There was also no difference in lactate concentrations between cauterly disbudded and sham-handled kids (Chapter 7). Castrated piglets however, showed an increase in lactate after the procedure, suggesting that lactate may be useful for evaluating pain in piglets (Prunier et al., 2005). Glucose and lactate concentrations may not be useful when assessing pain associated with cauterly disbudding of goat kids, as blood glucose is regulated by insulin (Steffens, 1970) and blood constituents in neonatal kids can be highly variable (Chen et al., 1999). In addition, for the studies presented in this thesis, there was no difference in weight gain over the post-treatment periods, indicating that body weight changes may not be indicative of pain associated with disbudding in kids (Chapters 3 and 5).

Overall, I have presented useful measures of pain associated with disbudding of goat kids (e.g., head shaking, scratching, rubbing, cortisol, haptoglobin, skin temperature) based on differences between disbudded and sham-handled kids. More studies are required to further validate these measures in goat kids. In addition, further research on measures of pain *during* disbudding (evaluated in Alvarez et al. [2009] and Alvarez and Gutiérrez [2010]) are also required (when the response to pain is likely to be the greatest).

## **8.2 Cautery disbudding techniques**

As discussed in Chapter 2, there is significant variation in cauterly disbudding techniques used across operators that can affect the efficacy of preventing horns or scurs. For example, there can be substantial variation in the

amount of time the iron is applied for, the pressure used and the age of the kids that are disbudded. Excessive application durations and pressure of the cautery iron has been suggested to cause thermal injury to, and necrosis of, the brain of goat kids (Wright et al., 1983; Sanford, 1989; Thompson et al., 2005); however, exact lengths of time or the amount of force that is considered excessive and what effect this would have on efficacy is unclear. There was also a wide range in the age of kids disbudded (from 2 to 20 d of age; Ingvast-Larsson et al., 2011; Alvarez et al., 2015; Nfor et al., 2016; Hempstead et al., 2018b). With increasing age, the width of the horn bud increases and begins to fuse with the underlying skull (Dove, 1935), hence it can be more difficult to destroy all horn bud tissue and subsequently prevent horn or scur development.

Further variation in technique includes the use of the cautery iron to cut the skin (with applied pressure) and either (i) remove the horn bud (Alvarez et al., 2015), or (ii) leave the horn bud intact (Alvarez et al., 2009; Alvarez and Gutiérrez, 2010). The amount of skin containing the horn bud that is cut or removed may differ across operators as the diameter of cautery iron tips can range between 13 and 22 mm (Petrie et al., 1996; Graf and Senn, 1999; Hempstead et al., 2017; Huebner et al., 2017; Hempstead et al., 2018a). The results of this thesis show that removing the horn bud completely (18 mm tip) led to higher success in preventing horns or scurs compared with leaving the horn bud intact (Chapter 6); this is likely associated with complete removal of all horn bud cells. Furthermore, leaving the horn bud intact resulted in 'scorns', which were not observed in kids with the horn bud removed. Scorns were different to horns or scurs; scorns were soft, fibrous lumps, whereas horns and scurs were keratinised, normal or abnormal growths. Scorns were present in 41% of horn buds that were left intact (Chapter 6). Other than the results described in Chapter 6, there is little information available on the effect of removing the horn bud (including the amount of skin removed) or leaving it intact on subsequent growth of horns or scurs in calves or goat kids. Future research on cautery techniques should describe and quantify the types of horn growth associated with cautery techniques. In addition, studies should evaluate the effect of temperature, application duration and pressure of the iron and the age of goat kids at disbudding on cautery disbudding efficacy.

Removing the horn bud appears to improve disbudding success in goat kids and may be better than leaving the horn bud intact. Removing the horn bud may be beneficial for both farm personnel and goat kids as a lower chance of

scurs (or horns) means that secondary removal (with additional and unnecessary pain) is not required. However, removing the horn buds causes large, open wounds exposing the bone; these wounds can take up to 6 wk to heal (Chapter 3). Prolonged healing increases the risk of infection (Zielins et al., 2015), which can lead to increased goat kid mortalities (Sanford, 1989; Thompson et al., 2005). Future research should evaluate the effect of horn bud removal on acute and post-operative pain and rates of infection and healing.

This thesis did not assess pain or efficacy associated with cautery disbudding of buck kids, as larger numbers of doe kids are disbudded to become replacement milking does (Stafford and Prosser, 2016). However, a small number of buck kids are disbudded and kept for breeding purposes. Horn growth of bucks is typically more precocious than that of doe kids (Dawson et al., 2007) and consequently it may be more difficult to prevent horns or scurs in bucks, although this was not investigated in my thesis. Future research should evaluate pain associated with methods of disbudding and their efficacy in preventing horns or scurs in buck kids.

### **8.3 Alternative methods to cautery disbudding of goat kids**

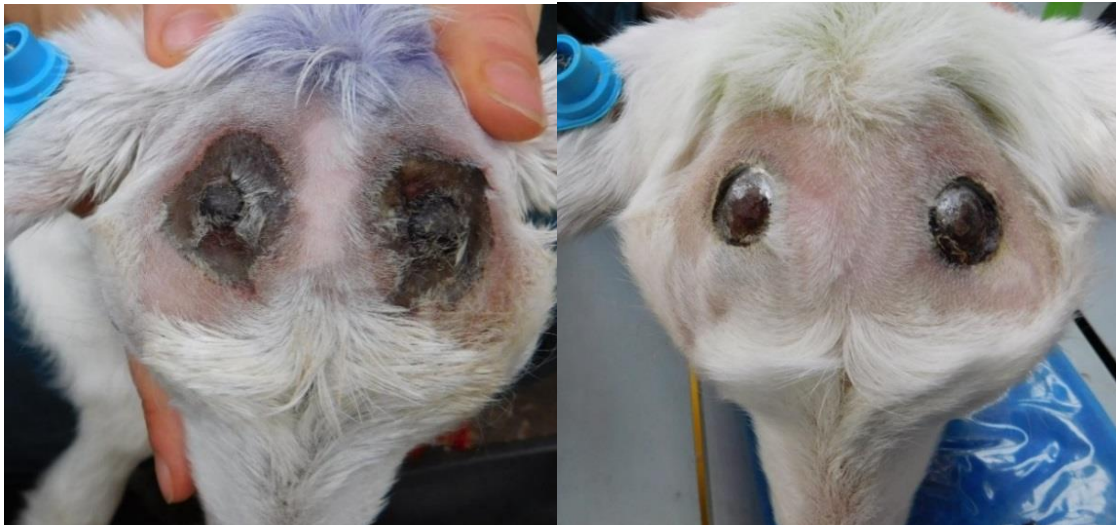
An alternative to cautery disbudding should cause less pain and injury, and be equally or more efficacious. Other methods of disbudding include caustic paste and cryosurgical disbudding and clove oil injection.

#### **8.3.1 Caustic paste disbudding**

Caustic paste disbudding of calves was suggested to cause less pain than cautery disbudding (Vickers et al., 2005); although, the contrary was reported by Morisse et al. (1995). Caustic paste disbudded kids had higher cortisol concentrations, performed more head scratching, and had more sensitive tissues surrounding the horn bud than cautery disbudded kids (Chapters 3, 4 and 5). Together, these results indicate that caustic paste caused significantly more pain than cautery disbudding of goat kids; this may be due to different mechanisms of action. Cauterisation and physical removal of the horn bud rapidly destroys the nociceptors, whereas the slow, corrosive action of caustic paste degrades the skin for as long as it is in contact (Palao et al., 2010). Furthermore, cautery iron wounds are generally localised, whereas caustic paste is spread over a larger area



(see Figure 8.1), potentially stimulating a larger number of nociceptors. Caustic paste can also run into the eyes (Winder et al., 2017) causing further pain.



**Figure 8.1.** Caustic paste (left) and cautery disbudding wounds (right) on the horn buds of goat kids 1 d after the procedures.

Different caustic paste formulations were used across calf studies and those used in my research, which may explain differences in the level of pain reported. Chapters 3 and 4 described a study that used a sodium hydroxide-based paste (up to 60% concentration), while Vickers et al. (2005) used a paste consisting of sodium hydroxide (45%) and calcium hydroxide (45%); Morisse et al. (1995) used a potassium hydroxide-based paste and Stilwell et al. (2009) disbudded calves using a sodium hydroxide-based paste. Calcium hydroxide penetrates the skin the slowest, potassium hydroxide is intermediary and sodium hydroxide has the fastest rate of penetration (Kuckelkorn et al., 2002). Pain responses to these different formulations should be systematically investigated in future research on both calves and goat kids.

A limitation of the studies presented in Chapters 3 and 4 is that both behavioural and physiological measurements were performed on the same animals, meaning that blood sampling and associated handling likely affected behavioural responses. A separate group of animals should be used in future research to reduce the impact of handling on behaviour. Furthermore, pain sensitivity of disbudded calves has been measured up to 4 h post-treatment (Heinrich et al., 2010), whereas I only measured sensitivity of the wounds for 1 h post-treatment; therefore, it would be useful to evaluate pain sensitivity beyond this time to determine how long the wounds remain sensitive.

Pain mitigation strategies such as local anaesthetic, sedation and non-steroidal anti-inflammatory drugs can reduce pain associated with caustic paste disbudding of calves (Vickers et al., 2005; Stilwell et al., 2009; Winder et al., 2017). Therefore, it is worth investigating pain mitigation options for caustic paste disbudding of goat kids in future research. Due to the high levels of pain associated with caustic paste disbudding of kids (Chapters 3, 4 and 5), efficacy was not evaluated (note 60% of kids disbudded with caustic paste grew scurs in Chapter 3, but 10 d old kids with mature horn buds were used). If pain relief can reduce pain to cautery disbudding levels, then the effect of caustic paste disbudding on horn growth should be investigated. Together, the results presented in this thesis suggest that caustic paste, as it is currently used, may not be a suitable alternative to cautery disbudding of goat kids.

### **8.3.2 Cryosurgical disbudding**

Cryosurgical disbudding has been suggested to be less painful than cautery disbudding of calves based on greater lying times and less tissue damage (Bengtsson et al., 1996; Stewart et al., 2014). Furthermore, freeze branding (with liquid nitrogen) was reported to be less painful than hot-iron branding (Schwartzkopf-Genswein and Stookey, 1997). Cryosurgically disbudded kids had higher cortisol concentrations and pain sensitivity around the horn buds and more frequent head scratches than cautery disbudded kids, indicating a greater experience of pain (Chapters 3, 4 and 5). In addition, although the wounds associated with cryosurgical disbudding were initially dry and closed, subsequent ulcerations resulted in open wounds, which may have increased the risk of infection (Chapter 3). Ulcerations have also been reported in cryosurgically disbudded calves (Bengtsson et al., 1996).

Cryosurgical disbudding may be more painful for goat kids than cautery disbudding due to incomplete nociceptor destruction; cryosurgical efficacy can be affected by the depth of the epidermal layers and also underlying structures (Kronic and Marini, 2015) (e.g., horn buds). Perhaps the horn bud thickness prevented adequate liquid nitrogen contact to destroy the nociceptors completely. I conducted a pilot study (in Chapter 3) to determine the optimum length of time to spray liquid nitrogen to cause necrosis of horn bud cells (i.e. 10 s); however, 10 s may not have been long enough to destroy the nociceptors innervating the horn

bud. Altogether, the results presented in this thesis suggest that cryosurgical disbudding of goat kids is more painful than cautery disbudding and may therefore not be a useful alternative.

### **8.3.3 Clove oil injection**

To my knowledge, there are no published reports of pain associated with clove oil injection in goat kids; however, it appears that clove oil causes less initial pain than cautery disbudding of calves (Sutherland et al., 2018). The results of Chapters 3 and 4 showed no differences in head shaking, head scratching, self-grooming or cortisol concentrations for clove oil injected and cautery disbudded kids within an hour after treatment, suggesting that the amount of pain caused by each method was similar. However, kids injected with clove oil showed elevated haptoglobin concentrations in comparison to cautery disbudded kids at both 15 min and 24 h post-treatment, indicating more inflammation and likely pain at these times. Using haptoglobin concentrations to assess inflammation associated with disbudding of goat kids is novel. Haptoglobin is usually produced in response to disease (Gonzalez et al., 2008; Olumee-Shabon et al., 2013); although haptoglobin may change in response to clove oil, haptoglobin may not be sensitive enough to detect localised inflammation resulting from cautery disbudding. Skin temperatures were higher for cautery disbudded than clove oil injected kids for up to 72 h post-treatment (Chapter 3); this suggests that cautery disbudded kids experienced inflammation with associated pain until this time. However, infrared thermography has limitations, as emissivity and conductivity can be affected by dirt, moisture or foreign bodies on the skin surface (Stewart et al., 2005). Cautery disbudded kids had open wounds, whereas clove oil injected kids had intact skin, which may have affected heat loss detected. Future research should assess pain sensitivity surrounding the horn buds of kids injected with clove oil. A limitation of the study described in Chapter 4 is that behaviour was only evaluated for up to 1 h after treatment; it would be useful to evaluate the longer-term effects of clove oil on behaviour to gain a more complete understanding of pain associated with its use in goat kids.

Based on the success rates reported by Molaei et al. (2015), clove oil injection was anticipated to be as effective as cautery disbudding at preventing horns or scurs; however, clove oil injection was not as efficacious as cautery

disbudding (Chapter 6). In fact, clove oil injection resulted in far lower success in preventing horns or scurs than cauterly disbudding (i.e. 9% vs. 77%, respectively). Preliminary results of another study reported horns (9%) and scurs (91%) grew in all buck kids 45 d after clove oil injection (Still-Brooks et al., 2017). In addition, Still-Brooks et al. (2017) reported that 5 out of 11 kids showed necrosis of the skull underneath the horn buds along with meningitis, which resulted in death approximately 6 – 8 wk after clove oil injection; a further two kids also had meningitis and one kid had severe orbital swelling 1 d after clove oil injection. Apart from short-term swelling of the tissue surrounding the eye in 3 out of 10 kids (Chapter 3), these deleterious consequences of clove oil were not observed in my research. A key difference between the studies in this thesis and Still-Brooks et al. (2017) is the angle of the clove oil injection, which may explain the differences in observations; studies presented in this thesis injected clove oil laterally into the horn bud at a 45° angle between the ear and muzzle, whereas Still-Brooks et al. (2017) inserted the needle into the horn bud orthogonally to the plane of the skull. Still-Brooks et al. (2017) may have injected the clove oil directly into the skull or sinus; additionally, they observed 3 out of 11 kids with transient paralysis immediately after clove oil injection. Clove oil injection into the horn bud that is perpendicular to the skull, may not be appropriate for future use.

The differences in success between the studies in presented in Chapters 3 and 4, Still-Brooks et al. (2017) and Molaei et al. (2015) may be due to different methodologies. Molaei et al. (2015) used their own oil distilled from clove spice and reported complete necrosis of the horn bud of all kids 5 d after injection. Still-Brooks et al. (2017) and Chapters 3 and 4 described studies that used clove oil from a commercial manufacturer and incomplete necrosis of the horn buds in the majority of kids was observed; the clove oil used by Molaei et al. (2015) may have been more concentrated than the commercial clove oil used. Furthermore, the clove oil used may have differed in viscosity, which may have affected the rate of spread under the skin and subsequent variation in the level of contact with the horn bud cells. Molaei et al. (2015) measured horn growth, whereas growth including scurs, horns and scorns were measured in this thesis and by Still-Brooks et al. (2017). It is unclear whether the amount of eugenol in clove oil can affect horn bud cell necrosis (range: 83 – 85% in this thesis and in Molaei et al. (2015)); eugenol appears to be the key component of clove oil that causes cytotoxicity

(Babich et al., 1993). Clove oil appears to be non-cell specific as it can degrade rat oral mucosa (Kozam and Mantell, 1978), human skin cells (Prashar et al., 2006), and the horn bud cells (Molaei et al., 2015) and bone (Still-Brooks et al., 2017) of goat kids. Using clove oil as a disbudding method does have benefits, as there is comparatively less tissue damage caused than that of cautery disbudding resulting in faster rates of healing (Chapter 3) and there is no chance of thermal injury to the brain (Thompson et al., 2005). However, caution is required when injecting clove oil to avoid the negative effects of skull damage and mortality.

It is apparent from the results of this thesis that even though the experience of pain may be similar, the clove oil methodology used is ineffective at preventing horns or scurs and therefore requires refinement. There are many factors that may have contributed to the apparent inefficiency of clove oil in preventing horns or scurs including: the concentration of eugenol (within clove oil), the technique used (i.e. best location to administer the clove oil safely) and the age and sex of goat kids. Further research should explore factors that may affect the rate of spread under the skin (e.g., type of clove oil used) and undesirable effects (e.g., mortality). Until the methodology is refined, clove oil does not appear to be a useful alternative to cautery disbudding of goat kids on farm.

#### **8.4 Pain relief for cautery disbudding**

Local anaesthetic such as lidocaine is commonly used in calves to eliminate acute pain associated with cautery disbudding (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999). However, lidocaine appears to be ineffective at reducing pain in goat kids (Alvarez et al., 2009, 2015; Nfor et al., 2016) and for this reason, was not included as a treatment in the study presented in Chapter 7. Young goat kids can be susceptible to high doses of lidocaine (Smith and Sherman, 2009; Dugdale, 2011); therefore, the doses used in previous studies (Alvarez et al., 2015; Nfor et al., 2016) may not have been adequate to induce localised anaesthesia. Furthermore, there are two nerves that innervate each horn bud of goat kids (lacrimal and infratrochlear), whereas calves have only one nerve supplying each horn bud (Dugdale, 2011). Therefore, goat kids would require two injections per horn bud to achieve a successful block. Local anaesthetic methodologies require refinement for use in goat kids. Future investigations into lidocaine efficacy should evaluate dosage, injection location and the number of

injections and the structure of the lacrimal and infratrochlear nerves at different stages of development. In addition, there may be other formulations of injectable local anaesthetics or even topical anaesthetics more suitable for use in goat kids. Furthermore, combinations of pain relief (i.e. xylazine and lidocaine) can reduce pain associated with cautery disbudding of calves (Grondahl-Nielsen et al., 1999, Faulkner and Weary, 2000) and therefore, may be more effective than using lidocaine alone for disbudding goat kids.

General anaesthesia such as isoflurane can be safely used to anaesthetise goats (McEwen et al., 2000; Dziki et al., 2011), but until my research, had not been used to provide anaesthesia during disbudding of goat kids. Kids disbudded under isoflurane (with or without meloxicam) had lower cortisol concentrations over the 2 h post-treatment period and performed lower rates of head and body shaking up to 10 min post-treatment than kids cautery disbudded without pain relief, indicating less pain was experienced (Chapter 7). As the goat kids that were anaesthetised with isoflurane were unconscious during cautery disbudding, the ability to perceive or recall pain associated with the practice was likely minimised (Dugdale, 2011) and could explain the lower physiological and behavioural responses to disbudding than kids disbudded without isoflurane. There are drug combinations, such as xylazine and ketamine that can induce anaesthetic effects and have been used previously in goat kids (Ingvast-Larsson et al., 2011). However, goats can have cardiovascular and respiratory complications associated with sedative use (Dugdale, 2011). Future research should investigate safe, but practical injectable general anaesthetic options or sedatives to reduce pain associated with cautery disbudding of goat kids.

The effect of meloxicam administration (either oral or injected) on pain was also evaluated in Chapter 7. I had anticipated that injected meloxicam would act faster than oral meloxicam due to faster absorption rates (Karademir et al., 2016); however, kids provided oral meloxicam spent less time head scratching (similar to sham-handled kid levels) than kids administered injected meloxicam, indicating less pain was experienced by kids that received oral meloxicam. It is not clear why oral meloxicam appears to be more effective than injected meloxicam in reducing pain associated with cautery disbudding. In the study presented in Chapter 7, both oral and injected meloxicam were administered immediately before cautery disbudding to simulate common practice on farm (i.e. handling the kids only once). The amount of time that meloxicam is administered

to kids prior to disbudding may influence its' analgesic effect; therefore, the effect of time of meloxicam administration before disbudding on pain could be investigated in future studies. Meloxicam is more practical than isoflurane for use on farm, as it can be administered by farm staff without veterinary supervision. However, meloxicam provides relief from post-operative inflammation (Del Tacca et al., 2002) and not the acute pain caused by cauterisation and cutting of the skin, therefore, practical pain relief during disbudding is still required.

A limitation of the study presented in Chapter 7 was that a control for either isoflurane or meloxicam (i.e. without disbudding) was not used. The effect of isoflurane and meloxicam on goat kids is not well understood and therefore should be explored in future work. It appears that isoflurane can eliminate or reduce pain associated with cautery disbudding and may be a 'gold-standard' method of providing pain relief; however, this should be validated by future studies. More practical pain management strategies for eliminating or reducing both acute and post-operative pain should be explored in future research.

## **8.5 Animal welfare implications**

The results presented in this thesis have implications for management of cautery disbudded goat kids on farm. I have presented useful indicators of pain in goat kids that changed in response to cautery disbudding and are be useful for future experiments evaluating pain.

Cautery disbudding under isoflurane (with or without meloxicam), reduces pain compared to cautery disbudding alone, and removing the horn bud is more efficacious at preventing horns or scurs than leaving the horn bud intact; these results mean that the welfare of kids undergoing disbudding can be improved as pain can be reduced and further disbudding events required to remove scurs can be avoided. The results presented in this thesis may promote the use of pain relief and horn bud removal for cautery disbudded kids on farm. In addition, the findings of this thesis can inform policy makers regarding the use of pain relief for cautery disbudding of goat kids.

Caustic paste and cryosurgical disbudding appear to cause significantly more pain than cautery disbudding and may further reduce animal welfare; therefore, caustic paste and cryosurgical disbudding should be avoided until refinements to these techniques can be made. Clove oil may cause a similar

experience of pain but less tissue damage than cauterly disbudding; however, clove oil is ineffective at preventing horns or scurs using the methodology described in this thesis.

Another alternative management practice that could be adopted by farmers, which would eliminate pain, is by preventing the need to perform disbudding altogether. Currently, a polled line of goats is not available due to a high risk of polled intersex syndrome (PIS; Szatkowska et al., 2014); goats with PIS have a higher risk of infertility than those with horns (Pailhoux et al., 2001). Future research into developing polled lines of goats that are not associated with PIS can prevent the need for disbudding. In addition, disbudding is not required if farmers are able to modify the ways in which they manage their goats (e.g., not mixing goat mobs after returning from the milking parlour) and have well-designed facilities (e.g., vertical feed rails instead of horizontal) to allow for adult goats with horns (Waiblinger et al., 2011).

Until a less painful and efficacious alternative method of disbudding is developed, cauterly disbudding is the best available method of preventing horns; however, cauterly disbudding causes acute and post-operative pain and consequently reduces welfare, therefore pain relief such as isoflurane and meloxicam should be provided.

## **8.6 Final conclusions**

This thesis has used a multifaceted approach and provided useful indicators of acute and post-operative pain associated with disbudding of goat kids, which can be included in future research. Caustic paste, cryosurgical disbudding and clove oil injection, although initially showing promise, were not less painful or as efficacious as cauterly disbudding and therefore are not suitable replacements for cauterly disbudding at this time. Overall, the results presented in my thesis suggest that cauterly disbudding by removing the horn bud is an effective practice, but should be undertaken using isoflurane and meloxicam to reduce acute and post-operative pain. Future research should investigate more practical pain mitigation strategies for cauterly disbudding that are cost effective and can be easily used on farm or management practices that eliminate the need for disbudding.



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