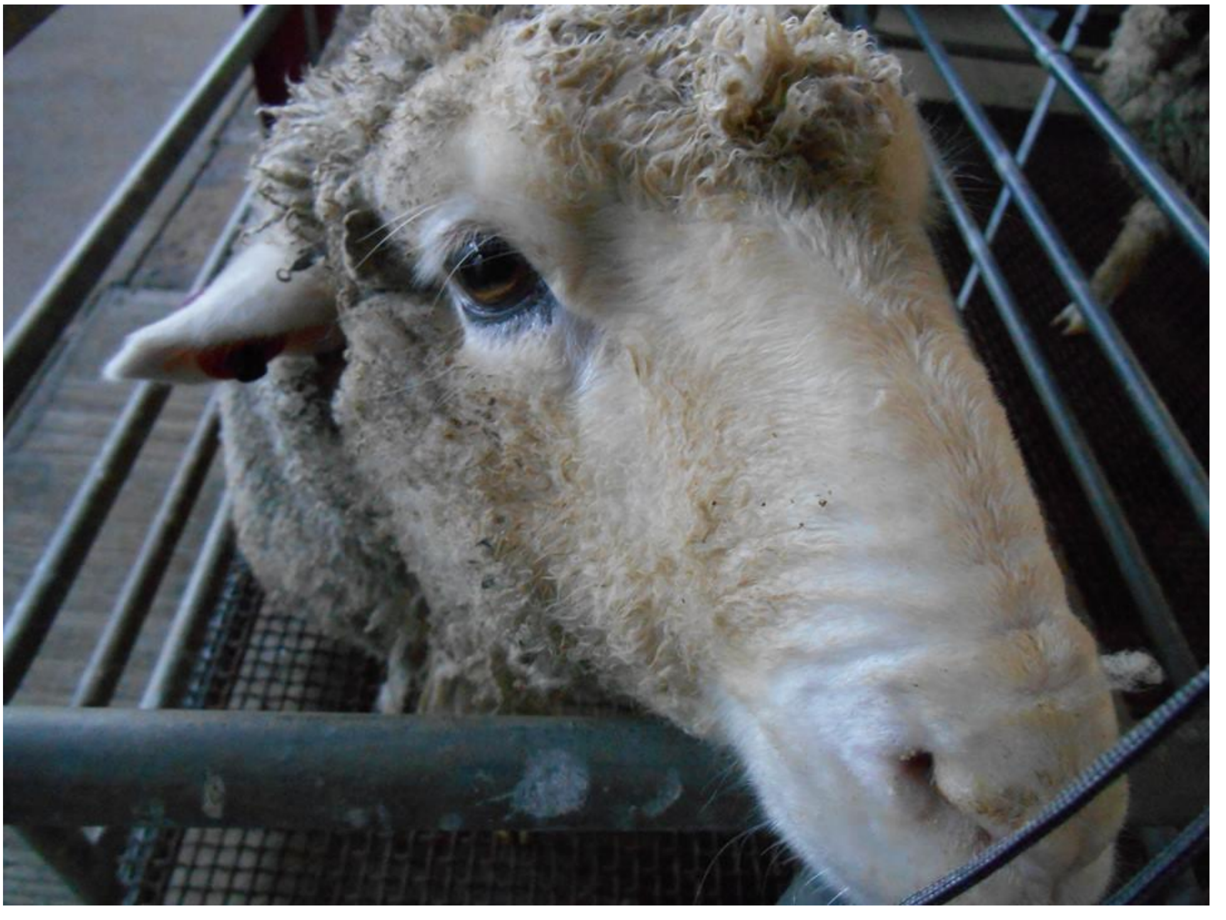
# Chapter 1

# General Introduction



## 1.1. Introduction

It's common practice in parts of the world for lambs to undergo invasive husbandry procedures. Procedures that are part of the yearly routine for many sheep farmers include ear-tagging, castration, tail docking and mulesing (procedure that involves the surgical removal of the breech skin). These procedures have all been shown to cause pain (Lay *et al*., 1992; Dinniss *et al*., 1997; Stafford and Mellor, 2005). The main purpose of conducting these procedures is aid in management; castration prevents indiscriminate breeding (Thornton, 1999; Grant, 2004), tail-docking and mulesing is believed to reduce the risk of animals being fly-struck in the future (Sutherland and Tucker, 2011; Trentini *et al*., 2013). There are several methods that can be used to castrate and tail-dock lambs (Table 1). Castration methods include surgical, clamp castration and ring castration; tail docking methods include use of a docking iron, surgical, ring tail-docking or a mix of clamp and ring or surgical and clamp (Mellor, 2000). In most cases lambs are not provided with pain-relief for these painful husbandry procedures. For example, in the United Kingdom, anaesthetic is only required if lambs are castrated when they are over the age of three months (DEFRA 2003). In Australia, the standards and guidelines for sheep require that adequate pain relief is provided to lambs only when they are over 6 months of age (AHA 2014). However, only the topical formulation Tri-Solfen is available and used by farmers to provide pain relief for mulesing operations. The lack of pain-relief use in lambs and other species of livestock following painful procedures is listed as a priority issue to be addressed by animal rights groups Animals Australia and PETA (Anon 2013a; 2013b; n.d).

In recent years the animal rights groups PETA and Animals Australia have targeted particular husbandry procedures used by the Australian sheep and cattle industry. In 2004, PETA targeted the Australian wool industry over the practice of mulesing sheep, with their protests causing some clothing manufacturers to boycott the Australian wool industry (Sneddon and Rollin, 2010). Mulesing was targeted as it was seen as a highly painful and cruel procedure (Sneddon and Rollin, 2010), which causes considerable stress that negatively impacts the lambs' welfare (Hemsworth, *et al*., 2009).

Because of the consequences of potential direct actions from animal rights groups and the impact on the animal itself, it is important that livestock industries address emerging welfare issues. Therefore, it is a top priority for livestock producers to address the lack of the use of pain relief for animals undergoing painful husbandry procedures. Limited use of pain relief in livestock is the result of many factors, such as, belief that the animal is unable to feel pain, difficulty of administration and particularly in Australia, the lack of registered and approved analgesics for sheep (Coetzee, 2011; Colditz *et al*., 2011; Lizarraga and Chambers, 2012). Pain relief is also difficult to administer to livestock raised in extensive systems due to feasibility of repeated application over several days when the animals are still experiencing pain (Lizarraga and Chambers, 2012). It would require farmers to yard ewes and lambs daily causing unnecessary stress and potentially have an increased risk of mismothering due to repeated separation of lambs from their ewes.

## 1.2. Pain and animal welfare

The International Association for the Study of Pain has defined pain in humans as "*An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage*" with the note that the inability to communicate verbally does not remove the possibility that the individual is experiencing pain (Merskey and Bogduk, 1994). It can be argued that the definition of pain for animals should be the same, as pain in animals encompasses both classifications of animal welfare (the animal's biological state versus its emotional state). This is because pain causes physical stress but it is also seen as a subjective emotional state. Animals are also unable to inform us verbally that they are feeling pain, however, we still assume that they feel pain in the same situations that humans would feel pain (Wall, 1991).

Table 1: Common methods of castrating and tail-docking lambs

| PROCEDURE | METHOD | DESCRIPTION |
|---|---|---|
| CASTRATION | Rubber Ring | Rubber rings are applied to the neck of the scrotum which causes the scrotal sac and testes to atrophy and eventually drop off due to the prevention of blood flow. |
| | Bloodless castrator | A clamp is applied to the scrotal neck and crushes the spermatic cord, this also causes the scrotal sac and testes to atrophy |
| | Surgical | This involves cutting the scrotum and removing the testes via cutting or twisting the spermatic cord, with or without cauterising or clamping. |
| TAIL-DOCKING | Rubber Ring | Rings are applied at the site of attachment of the caudal folds to the tail |
| | Bloodless docking | While clamped the tail is cut off distal to the clamp |
| | Surgical | Tail is cut with a sharp knife |
| | Hot docking-iron | A clamp device is heated using gas and cauterises the tail as it cuts |

When an animal experiences pain, there are two physiological systems that are activated, the sympathoadrenal (SA) system and the hypothalamic-pituitary-adrenal (HPA) axis (Engelmann *et al*., 2004). The SA system is involved in what is known as the “fight or flight” response (Cannon, 1928). Following a challenge, the SA system responds immediately, using adrenaline to alter physiological systems such as the cardiovascular system and enhancing brain function (Jansen *et al*., 1995). This allows the animal to return to homeostasis through an active behavioural response (Wechsler, 1995; Engelmann *et al*., 2004). The activation of the HPA axis and release of cortisol allows the animal to return to homeostasis through a passive response.

Cortisol has a wide range of physiological effects in the body, one of the main functions is to stimulate gluconeogenesis and lipogenisis (Tsigos and Chrousos, 2002; Landys, *et al*., 2006). If the HPA axis is chronically activated and cortisol production is not inhibited (i.e. in the constant presence of a threat or challenging situation such as pain) it can go on to suppress the immune system (Cupps and Fauci, 1982; Glaser and Kiecolt-Glaser, 2005), leading to an increased risk of ill-health. Cortisol can also affect an animal’s growth by inhibiting pituitary growth hormone and gonadotrophin (Dallman *et al*., 1992; Tsigos and Chrousos, 2002) resulting in decreased growth and body size compared to peers.

The assessment of the effect of pain on an animal’s welfare is determined by the approach used; that is looking at the biological state of the animal or the affective state. Assessing welfare using biological state directly looks at an animal's ability to cope within its environment (Broom, 1988), for example it would be a lamb's ability to cope with the pain of castration and tail-docking. Assessing the lamb's ability to cope with pain and determining its welfare state would be done through behavioural and physiological measurements. The lamb's status would then be described on a scale of poor to good welfare based on the measurements taken (Barnett and Hemsworth, 1990; . Mellor and Stafford, 2000). The approach of assessing welfare using affective state looks at the animal's mental or emotional state. It is not possible to directly measure an animal's feelings, however tests such as cognitive bias can be used to help us make inferences as to how an animal may be feeling (Paul ety al., 2005; Mendl *et al*., 2009). In sheep, recent novel indicators of affect have been identified such as ear postures (Boissy *et al*. 2011) and facial expressions (McLennan *et al*., 2016). Facial expressions were used in the pen castration and tail-docking experiment (Chapter 4) to see if it could be used to indicate pain in lambs. However, as it is not within the scope of this Thesis the results are presented in Appendix 1. Overall the assessment of an animal's welfare looks at the animal's wellbeing. Using indicators such as plasma cortisol concentration, the display of abnormal behaviour or the prevalence of illness and impaired growth in an animal can help us determine if that animal it is in a poor welfare state.

It is well known that the resulting pain from common husbandry procedures causes physiological and behavioural changes in lambs that indicates they are in a poor welfare state (Mellor and Murray, 1989; Kent *et al*.1993, 1995; Lester *et al*., 1996; Dinniss *et al*., 1997; Dinniss, *et al*., 1999;

Kent, *et al*., 2000; Kent, *et al*., 2001). Lameness is also a common issue in sheep that causes pain (Bokko *et al*. 2003; Kaler *et al*., 2010). Recognition of lameness uses some different measures of physiological and behavioural changes that will be reported in this review, due to the use of a lameness model in Chapter 1. The changes in physiology and behaviour can be used to recognise if a sheep is in pain or a poor welfare state.

## 1.3. Recognition of pain and poor welfare and its assessment

The use of physiological, behavioural and other measures to indicate if an animal is in a negative state such as pain has been extensively reviewed (Blackshaw, 1986; Duncan, 2005; Fitzpatrick, Scott, and Nolan, 2006). This following section will briefly outline the common methodologies used to recognise pain and assess welfare and highlight the measures used in the experiments described in the chapters of this thesis.

### *1.3.1. Physiological measures*

One of the most commonly used physiological measures is the reaction of the HPA axis in response to a painful stimulus, through the measurement of cortisol concentrations either from the blood or saliva. Surgical trauma is known to activate the HPA axis leading to an increase in plasma cortisol (Desborough, 2000); the cortisol concentration increase can vary with the severity of the surgery (Nicholson *et al*., 1998). Following surgery the presence of cytokines leads to further ACTH and subsequently cortisol release (Desborough, 2000).

Lambs that have undergone painful procedures have increased plasma cortisol levels (Shutt *et al*, 1988), and although the activation of the HPA would be a result of the stress of pain, other components of the procedure such as capture and restraint are also sufficient stressors that lead to HPA activity. Consequently, it can be difficult to determine what level of contribution the pain of the procedure has in activating the HPA. During experimental design the extra stressors need to be taken into account, this is often done by including a handled control group. Comparison of the cortisol increase of control lambs following handling and that of lambs that have undergone castration and/or tail-docking shows that the pain of the procedures, that lead to changes in HPA activity (increase in cortisol concentration of approximately 20 nmol/L vs 40-80 nmol/L respectively) ( Kent *et al*., 1993; Lester *et al*., 1996; Kent *et al*., 1998).

An inflammatory response is also initiated following ring and surgical procedures as well as infectious conditions such as those that cause lameness. The signs of inflammation are commonly measured in relation to a painful stimulus and are often used to determine the effectiveness of NSAIDs due to their anti-inflammatory properties (Cheng *et al*., 1998). Inflammation is often measured through systemic and local responses in the animal. Systemic measurements include reporting white cell count, neutrophil/lymphocyte ratio and haptoglobin (Murata *et al*., 2004; Paull *et al*., 2009; Colditz *et al*., 2011). Local response of inflammation that occurs at the site of injury include measuring wound temperature, circumference of the limb (in relation to lameness), nociceptive threshold testing (such as force plate pressure, palpation, pin-prick testing) and locomotion scoring (Fitzpatrick *et al*., 2006; Colditz *et al*., 2011; Lizarraga and Chambers, 2012). Persistence of inflammation, such as that produced by ring castration, tail docking, footrot and mastitis can lead to chronic pain (Dolan *et al*., 2000; Mellema *et al*.,2006; Melches *et al*., 2007).

### *1.3.2. Behavioural measures*

At the time of a painful procedure the behaviours that lambs display include struggling, kicking and vocalisation ( Grant, 2004; Erber *et al*., 2012). After castration and tail-docking lambs exhibit new abnormal behaviours (Grant, 2004) also known as active pain avoidance behaviours such as rolling, touching the wound site and kicking (Mellor and Murray, 1989; Molony *et al*.,1993; Kent *et al*., 1995) . Postures that have been validated as indicative of lambs in pain include statue standing, lateral lying and abnormal walking (Table 2).

Table 2: Posture and active behaviours indicative of pain as described by Molony (1993); Dinniss *et al*. (1999); Molony *et al*. (2002); Grant (2004)

| **Posture** | **Description** |
|---|---|
| Abnormal lying | Legs extended when lying ventrally or lying with back end lifted off ground. |
| Lateral lying | Lamb lies with shoulder on ground and limbs extended |
| Abnormal standing | Standing or walking unsteadily, walking backwards |
| Statue standing | Standing with limbs apart and stretched away from body for a brief period of time |
| Abnormal Walking | Lamb walking with a stiff gait or walking backwards |
| **Active pain avoidance** | **Description** |
| Rolling | Lying on its side and rolling to the other |
| Restlessness | Amount of times a lamb lies down and rises |
| Foot stamping and kicking | Done with front and hind limbs, lifting and placing down limb with force |

The type of pain, acute or chronic, can also have an effect on an animal's behaviour, with reactions to acute pain perhaps not being seen in animals suffering chronic pain. The behaviours mentioned previously are those often seen in response to acute pain. Behaviours shown when animals are in chronic pain include changes in activity (postures, Table 2), self-administration of analgesics and avoidance of pain-causing stimuli (Chapman *et al*., 1985; Mogil and Crager, 2004).

### *1.3.3 Production measures*

Production measures such as an animal's condition score and growth rate are often used to measure the impact of a painful procedure on welfare. If an animal is subjected to challenging conditions such as pain, this may lead to chronic stress which can have systemic alterations. As mentioned, chronic activation of the HPA induces high circulating concentrations of cortisol, which can inhibit an animal's immune response (Tsigos and Chrousos, 2002; Caroprese *et al*., 2010), leading to the animal becoming more susceptible to disease. High levels of circulating cortisol can also affect an animal's growth by inhibiting pituitary growth hormone and gonadotrophin (Dallman *et al*., 1992; Tsigos and Chrousos, 2002). Therefore, the prevalence of illness or impaired growth in an animal could indicate it is in a poor welfare state. In experimental studies a lamb's growth rate and condition score are often recorded several weeks after a painful procedure to see if there is a long term effect.

## 1.4. Relieving pain

The limited use of pain relief in livestock is the result of many factors, such as a belief that the animal is unable to feel pain, lack of producer knowledge, lack of registered and approved pain relief drugs and difficulty of administration (Coetzee, 2011; Colditz *et al*., 2011; Lizarraga and Chambers, 2012).

One method of pain relief is the use of local anaesthetic just prior to or during the painful procedure. Local anaesthetics work by blocking the sodium channels of nerves, preventing them for conducting, if applied before a surgical procedure they prevent the nerves that are being stimulated from firing signals (Yanagidate, and Strichartz, 2007). A local anaesthetic that has been studied extensively for its efficacy in sheep is lignocaine. Lignocaine injected subcutaneously in lambs is effective at relieving the pain associated with tail docking and castration (Dinniss *et al*., 1997; Dinniss *et al*., 1999; Mellor and Stafford, 2000). The effectiveness of lignocaine depends on the site and the timing of the injection and the method of castration and tail-docking. Injection of lignocaine in the antero-medial surface of the scrotum caused a reduction in abnormal behaviours for clamp plus ring castration in lambs ( Dinniss *et al*., 1999) and for ring castration, injection of lignocaine to the scrotal neck 5 to 10 s before ring application was shown as the most effective method of reducing HPA activity (Sutherland *et al*., 1999). The pain relief provided by lignocaine only lasts a few hours and it can be difficult to administer as an injection (Paull *et al*., 2008; Lomax *et al*., 2010a). Due to the short duration of action of local anaesthetics it has been suggested that a combination of local anaesthetics with longer acting non-steroidal anti-inflammatory drugs (NSAIDs) may be more effective at relieving pain (Paull *et al*., 2007).

NSAIDs reduce pain associated with inflammation by inhibiting cyclooxygenase which leads to the decreased production of prostaglandins (Cheng *et al*., 1998) which are important in promoting inflammation following injury (Ricciotti and FitzGerald, 2011). Currently there are no registered NSAIDs for use in sheep; though, there is a large literature on their efficacy, particularly carprofen and flunixin. Carprofen is a long-acting analgesic that has a half-life of 30+ hours in sheep (Welsh *et al*., 1992). It has been shown to reduce pain-related behaviours in lambs that have undergone mulesing, but it does not reduce physiological measures of pain (Paull *et al*., 2007); Colditz *et al*., 2009. Carprofen has also been shown to reduce the cortisol response to surgical castration and behavioural pain responses to ring castration (Paull *et al*., 2009). Flunixin has been shown to be effective at reducing the thresholds to noxious mechanical stimulation in sheep suffering from footrot (Welsh and Nolan, 1995). NSAIDs are also effective at alleviating other types of pain in sheep such as the pain associated with lameness. Administration of meloxicam has been shown to be effective at reducing lameness associated with a turpentine injection in sheep (Colditz et. al., 2011). However, there is considerable literature on the effectiveness of NSAIDs at alleviating the pain associated with lameness through gait improvement in cattle (Flower, et. al., 2008; Chapinal *et al*., 2010) and horses (Owens *et al*., 1995; Keegan *et al*., 2008; Orsini *et al*., 2012).

Like anaesthetics, NSAIDs are most commonly administered through injection, which can be impractical for producers; however, NSAIDs can also be given orally. Literature on the efficacy of orally administered NSAIDs in sheep is limited, although this route has been examined in cattle (Coetzee *et al*., 2009;Coetzee *et al*., 2012). The efficacy of orally administered NSAIDs have been tested in cattle; when administered orally through a stomach tube, meloxicam had a peak concentration in plasma at 10-12 hours and a half-life of 27 hours (Coetzee *et al*., 2009). Flunixin granules consumed by cattle were found to have a longer retention time than intravenous administration (12 vs

6 hours) and a bioavailability of 60% when compared to intravenous administration (Odensvik, 1995). As sheep and cattle are ruminants, the outcomes seen in cattle could be applicable to sheep, if NSAIDs can be given orally it provides an easy method of administering pain relief.

### *1.4.1. Relieving acute versus chronic pain*

As discussed pain relief can be provided at the time the painful procedure is conducted, however, the pain of the procedures can last for several weeks (Mellema, *et al*., 2006; Melches *et al*., 2007). The pain-relief provided to the animals is dependent on the half-lives of analgesics used, with the time ranging from 1 to 6 h for anaesthetics (Lemke, 2014) and up to 30 h for NSAIDs (Welsh *et al*., 1992). Although this would cover the animal during the procedure and during the acute pain phase it would not cover the animal during the chronic pain phase. Combination use of anaesthetics and NSAIDs post-procedure and has been previously shown to improve the welfare of lambs (Molony *et al*., 1997; Paull *et al*., 2007). However, maintaining pain relief further through repeated administration of medications is impractical within conventional livestock management practices. Not only would it increase the labour of the farmer but it would also expose lambs to excessive mustering and restraint and repeated separation from the ewe which is stressful. There is however potential to provide further pain relief to livestock though administration of NSAIDs through feed or water. If NSAIDs are provided in feed or water, there is also the possibility of teaching livestock to administer feed or water themselves according to their perceived level of pain (known as self-medication).

## 1.5. Potential for sheep to self-medicate for pain

The ability to processes and learn new information is important for farm animals as throughout their life they will experience new locations, equipment, handlers and conspecifics (Wechsler and Lea, 2007). In order for an animal to self-medicate for something like pain they must first be able to learn the association between the feed that contains the medication and the relief from pain that it provides. The mechanisms involved in learning to self-medicate and the methods used to teach animals to self- medicate are covered in Chapter 5.

It is known that sheep are able to discriminate between different sheep and human faces (Kendrick *et al*., 1995; Peirce *et al*., 2001; Ligout and Porter, 2003;) as well as learn to recognise and associate humans with particular interactions. For example, the study by Davis, Norris, and Taylor (1998) found sheep would respond better to a handler with which they have previously received a positive food reward from. It is suggested that visual and olfactory cues help sheep to discriminate between humans (Baldwin and Meese, 1977; Davis *et al*., 1998; Peirce *et al*., 2001).

Learning and memory is important for the role of finding food as well as discriminating between food. Testing has shown that sheep are able to remember the location of where they had found feed within a paddock 24 – 72 hours after they had last located it (Edwards, *et al*., 1996; Dumont and Petit, 1998). In both studies sheep were able to relocate food via spatial location without the presence of extra cues. However, Edwards *et al*. (1996) demonstrated that sheep were able to relocate food faster if extra cues given (in this case a turf of white clover in a container behind the food). An animal learns about the food they consume by processing information about the food's characteristics (odour/flavour/texture) and pairing it with post-ingestive consequences (Provenza *et al*., 1992; Provenza *et al*., 2006). This allows the sheep to remember if feed it has previously consumed has had a harmful or positive effect, so they can make decisions in the future. Through the use of cues and location, it may be possible to help sheep learn the association of a feed that contains pain relief and its pain alleviating effect.

## 1.6. Aims of this thesis

It is well known that castration and tail-docking causes pain to lambs, and as consumer concern for animal welfare is increasing, there is growing interest in the need for use of analgesic agents to control pain associated with invasive husbandry procedures. NSAIDs are attractive candidate drugs for pain relief in sheep as there is considerable literature on their efficacy in these species. This Thesis will explore the potential to administer NSAIDs orally and to train animals to self-administer the drugs in order to provide pain relief over an extended period of several days. This was achieved by conducting the following experiments:

1) Testing the efficacy of several NSAIDs at alleviating pain associated with a lameness model when administered as an oral solution
2) Adding an NSAID to pelleted feed and testing the palatability, pharmacokinetics and the efficacy of it alleviating the pain of castration and tail-docking

3) Testing if lambs can identify, self-select and administer feed containing NSAIDs

## 1.7. References

Animal Health Australia, 2014. Australian Animal Welfare Standards and Guidelines – Sheep. Edition 1, Version: 1.0 January 2016 Endorsed. Retrieved from http://www.animalwelfarestandards.net.au/files/2016/01/Sheep-Standards-and-Guidelines-for-Endorsed-Jan-2016-250116.pdf

Anonymous, 2013a. Cattle: Painful procedures. Retrieved 18th November, 2013, from http://www.animalsaustralia.org/issues/cattle-painful-procedures.php

Anonymous 2013b. Sheep: Painful procedures. Retrieved 18th November, 2013, from http://www.animalsaustralia.org/issues/sheep-painful-procedures.php

Anonymous, n.d. Cows Used for Food. Retrieved 18th November, 2013, from http://www.peta.org/issues/animals-used-for-food/factory-farming/cows/

Baldwin, B. A., Meese, G. B., 1977. The ability of sheep to distinguish between conspecifics by means of olfaction. Physiol Behav 18, 803-808. doi:http://dx.doi.org/10.1016/0031-9384(77)90187-1

Barnett, J. L., Hemsworth, P. H., 1990. The validity of physiological and behavioural measures of animal welfare. Appl Ani Behav Sci 25, 177-187. doi: http://dx.doi.org/10.1016/0168-1591(90)90079-S

Blackshaw, J. K., 1986. Objective measures of welfare in farming environments. Australian Veterinary Journal, 63, 361-364.

Boissy, A., Aubert, A., Désiré, L., Greiveldinger, L., Delval, E., Veissier, I., 2011. Cognitive sciences to relate ear postures to emotions in sheep. Anim Welf, 20, 47-56.

Bokko, B. P., Adamu, S. S., and Mohammed, A., 2003. Limb conditions that predispose sheep to lameness in the arid zone of Nigeria. Small Rumin Res 47, 165-169. doi:http://dx.doi.org/10.1016/S0921-4488(02)00254-7

Broom, D. M., 1988. The scientific assessment of animal welfare. Appl Anim Behav Sci 20, 5-19. doi: http://dx.doi.org/10.1016/0168-1591(88)90122-0

Cannon, W. B., 1928. The mechanism of emotional disturbance of bodily functions. N Engl J Me, 198, 877-884. doi: http://dx.doi.org/10.1056/NEJM192806141981701

Caroprese, M., Albenzio, M., Marzano, A., Schena, L., Annicchiarico, G., Sevi, A,. 2010. Relationship between cortisol response to stress and behavior, immune profile, and production performance of dairy ewes. J Dairy Sci93, 2395-2403. doi: 10.3168/jds.2009-2604

Chapinal, N., de Passillé, A. M., Rushen, J., Wagner, S. A. 2010. Effect of analgesia during hoof trimming on gait, weight distribution, and activity of dairy cattle. J Dairy Sci 93, 3039-3046. doi:http://dx.doi.org/10.3168/jds.2009-2987

Chapman, C. R., Casey, K. L., Dubner, R., Foley, K. M., Gracely, R. H., Reading, A. E., 1985. Pain measurement: an overview. Pain, 22, 1-31. doi:http://dx.doi.org/10.1016/0304-3959(85)90145-9

Cheng, Z., Nolan, A. M., McKellar, Q. A., 1998. Measurement of Cyclooxygenase Inhibition in vivo: A Study of Two Non-Steroidal Anti-Inflammatory Drugs in Sheep. Inflammation, 22, 353-366. doi:10.1023/a:1022364731126

Coetzee, J. F., 2011. A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the United States. Appl Anim Behav Sci 135, 192-213. doi: 10.1016/j.applanim.2011.10.016

Coetzee, J. F., Edwards, L. N., Mosher, R. A., Bello, N. M., O'Connor, A. M., Wang, B., Blasi, D. A., 2012. Effect of oral meloxicam on health and performance of beef steers relative to bulls castrated on arrival at the feedlot. J Anim Sci 90, 1026-1039. doi: 10.2527/jas.2011-4068

Coetzee, J. F., KuKanich, B., Mosher, R. A., Allen, P. S., 2009. Pharmacokinetics of Intravenous and Oral Meloxicam in Ruminant Calves. Vet Therap 10, E1-E8.

Colditz, I. G., Lloyd, J. B., Paull, D. R., Lee, C., Giraudo, A., Pizzatoa, C., Fishera, A. D., 2009. Effect of the non-steroidal anti-inflammatory drug, carprofen, on weaned sheep following non-surgical mulesing by intradermal injection of cetrimide. Aust Vet J 87, 19-26. doi: 10.1111/j.1751-0813.2008.00384.x

Colditz, I. G., Paull, D. R., Hervault, G., Aubriot , D., Lee, C., 2011. Development of a lameness model in sheep for assessing efficacy of analgesics. Aust Vet J 89, 297-304. doi: 10.1111/j.1751-0813.2011.00809.x

Cupps, T. R., Fauci, A. S., 1982. Corticosteroid-mediated immunoregulation in man. Immunol Rev. 65, 133-155.

Dallman, M. F., Akana, S. F., Scribner, K. A., Bradbury, M. J., Walker, C. D., Strack, A. M., Cascio, C. S. ,1992. Stress, Feedback and Facilitation in the Hypothalamo-Pituitary-Adrenal Axis. J Neuroendocrinol 4, 517-526. doi: 10.1111/j.1365-2826.1992.tb00200.x

Davis, H., Norris, C., Taylor, A., 1998. Wether ewe know me or not: the discrimination of individual humans by sheep1. Behav Processes 43, 27-32. doi:http://dx.doi.org/10.1016/S0376-6357(97)00082-X

Department for Environment Food and Rural Affairs.,2003. Code of Recommendations for the Welfare of Livestock: Sheep. London: Defra Publications Retrieved from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/69365/pb5162-sheep-041028.pdf.

Desborough, J. P., 2000. The stress response to trauma and surgery. Br J Anaesth 85, 109-117. doi:10.1093/bja/85.1.109

Dinniss, A. S., Stafford, K. J., Mellor, D. J., Bruce, R. A., Ward, R. N., 1997. Acute cortisol responses of lambs castrated and docked using rubber rings with or without a castration clamp. Aust Vet J 75, 494-496. doi: 10.1111/j.1751-0813.1997.tb14380.x

Dinniss, A. S., Stafford, K. J., Mellort, D. J., Bruce, R. A., Ward, R. N., 1999. The behaviour pattern of lambs after castration using a rubber ring and/ or castrating clamp with or without local anaesthetic. N Z Vet J 47, 198-203. doi: 10.1080/00480169.1999.36143

Dolan, S., Field, L. C., and Nolan, A. M., 2000. The role of nitric oxide and prostaglandin signaling pathways in spinal nociceptive processing in chronic inflammation. Pain, 86, 311-320. doi:http://dx.doi.org/10.1016/S0304-3959(00)00262-1

Duncan, I. J. H., 2005. Science-based assessment of animal welfare: farm animals. Revue Scientifique et Technique-Office International des Epizooties, 24, 483.

Dumont, B., Petit, M., 1998. Spatial memory of sheep at pasture. Appl Anim Beh Sci 60, 43-53. doi:http://dx.doi.org/10.1016/S0168-1591(98)00152-X

Edwards, G. R., Newman, J. A., Parsons, A. J., and Krebs, J. R., 1996. The use of spatial memory by grazing animals to locate food patches in spatially heterogeneous environments: an example with sheep. Appl AnimBehav Sci, 50 147-160. doi:http://dx.doi.org/10.1016/0168-1591(96)01077-5

Engelmann, M., Landgraf, R., Wotjak, C. T., 2004. The hypothalamic-neurohypophysial system regulates the hypothalamic-pituitary-adrenal axis under stress: An old concept revisited. Front Neuroendocrinol 25(3-4), 132-149.

Erber, R., Wulf, M., Becker-Birck, M., Kaps, S., Aurich, J., Möstl, E., and Aurich, C., 2012. Physiological and behavioural responses of young horses to hot iron branding and microchip implantation. Vet J.191, 171-175.

Fitzpatrick, J., Scott, M., Nolan, A., 2006. Assessment of pain and welfare in sheep. Small Rumin Res 62, 55-61. doi:10.1016/j.smallrumres.2005.07.028

Flower, F. C., Sedlbauer, M., Carter, E., von Keyserlingk, M. A. G., Sanderson, D. J., Weary, D. M., 2008. Analgesics Improve the Gait of Lame Dairy Cattle. J Dairy Sci 91, 3010-3014.

Glaser, R., Kiecolt-Glaser, J. K. 2005. Stress-induced immune dysfunction: implications for health. Nat Rev Immunol 5, 243-251.

Grant, C., 2004. Behavioural responses of lambs to common painful husbandry procedures. Appl Anim Behav Sci 87, 255-273. doi: 10.1016/j.applanim.2004.01.011

Hemsworth, P. H,. Barnett, J.L., Karlen, G.M., Fisher, A.D., Butler, K.L., Arnold, N.A., 2009. Effects of mulesing and alternative procedures to mulesing on the behaviour and physiology of lambs. Appl Anim Behav Sci 117, 20-27. doi: 10.1016/j.applanim.2008.12.007

Jansen, A. S. P., Van Nguyen, X., Karpitskiy, V., Mettenleiter, T. C., Loewy, A. D., 1995. Central Command Neurons of the Sympathetic Nervous System: Basis of the Fight-or-Flight Response. Science, 270, 644-646. doi: 10.1126/science.270.5236.644

Kaler, J., Daniels, S. L. S., Wright, J. L., Green, L. E., 2010. Randomized clinical trial of long-acting oxytetracycline, foot trimming, and flunixine meglumine on time to recovery in sheep with footrot. J Vet Intern Med 24, 420-425. doi:10.1111/j.1939-1676.2009.0450.x

Keegan, K. G., Messer, N. T., Reed, S. K., Wilson, D. A., and Kramer, J. 2008. Effectiveness of administration of phenylbutazone alone or concurrent administration of phenylbutazone and flunixin meglumine to alleviate lameness in horses. Am J Vet Res, 69, 167-173. doi:10.2460/ajvr.69.2.167

Kendrick, K. M., Atkins, K., Hinton, M. R., Broad, K. D., Fabre-Nys, C., Keverne, B., 1995. Facial and vocal discrimination in sheep. Anim Behav 49, 1665-1676. doi:http://dx.doi.org/10.1016/0003-3472(95)90088-8

Kent, J. E., Jackson, R. E., Molony, V., Hosie, B. D., 2000. Effects of Acute Pain Reduction Methods on the Chronic Inflammatory Lesions and Behaviour of Lambs Castrated and Tail Docked with Rubber Rings at less than Two Days of Age. Vet J 160, 33-41. doi: http://dx.doi.org/10.1053/tvjl.2000.0465

Kent, J. E., Molony, V., Graham, M. J., 1998. Comparison of methods for the reduction of acute painproduced by rubber ring castration or tail docking of week-old lambs. Vet J 155, 39-51. doi: http://dx.doi.org/10.1016/S1090-0233(98)80033-6

Kent, J. E., Molony, V., Graham, M. J., 2001. The Effect of Different Bloodless Castrators and Different Tail Docking Methods on the Responses of Lambs to the Combined Burdizzo Rubber Ring Method of Castration. Vet J 162, 250-254. doi: http://dx.doi.org/10.1053/tvjl.2001.0598

Kent, J. E., Molony, V., Robertson, I. S., 1993. Changes in plasma cortisol concentration in lambs of three ages after three methods of castration and tail docking. Res Vet Sci 55, 246-251. doi: http://dx.doi.org/10.1016/0034-5288(93)90088-W

Kent, J. E., Molony, V., Robertson, I. S., 1995. Comparison of the burdizzo and rubber ring methods for castrating and tail docking lambs. Vet Rec 136, 192-196.

Landys, M. M., Ramenofsky, M., Wingfield, J. C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen Comp Endocrinol , 148, 132-149.

Lay, D. C., Friend, T. H., Bowers, C. L., Grissom, K. K., Jenkins, O. C., 1992. A comparative physiological and behavioral study of freeze and hot-iron branding using dairy cows. J Anim Sci 70(4), 1121-1125.

Lemke, K.A., 2014. Local Anesthetics, Pain Management in Veterinary Practice, John Wiley and Sons, Ltd, pp. 85-92.

Lester, S. J., Mellor, D. J., Holmes, R. J., Ward, R. N., Stafford, K. J., 1996. Behavioural and cortisol responses of lambs to castration and tailing using different methods. N Z Vet J, 44, 45-54. doi: 10.1080/00480169.1996.35933

Ligout, S., Porter, R. H,. 2003. Social discrimination in lambs: the role of indirect familiarization and methods of assessment. Anim Behav 65, 1109-1115. doi:http://dx.doi.org/10.1006/anbe.2003.2139

Lizarraga, I., Chambers, J. P., 2012. Use of analgesic drugs for pain management in sheep. N Z Vet , 60, 87-94. doi: 10.1080/00480169.2011.642772

Lomax, S., Dickson, H., Sheil, M., Windsor, P. A., 2010. Topical anaesthesia alleviates short-term pain of castration and tail docking in lambs. Aust Vet J 88, 67-74. doi: 10.1111/j.1751-0813.2009.00546.x

McLennan, K. M., Rebelo, C. J. B., Corke, M. J., Holmes, M. A., Leach, M. C., Constantino-Casas, F. Development of a facial expression scale using footrot and mastitis as models of pain in sheep. Appl Ani. Behav.Sci.. doi: http://dx.doi.org/10.1016/j.applanim.2016.01.007

Melches, S., Mellema, S. C., Doherr, M. G., Wechsler, B., Steiner, A., 2007. Castration of lambs: A welfare comparison of different castration techniques in lambs over 10 weeks of age. Vet J 173, 554-563. doi:http://dx.doi.org/10.1016/j.tvjl.2006.01.006

Mellema, S. C., Doherr, M. G., Wechsler, B., Thueer, S., Steiner, A., 2006. Influence of local anaesthesia on pain and distress induced by two bloodless castration methods in young lambs. Vet J 172, 274-283. doi:http://dx.doi.org/10.1016/j.tvjl.2005.06.002

Mellor, D. J., Stafford, K., 2000. Acute castration and/or tailing distress and its alleviation in lambs. N Z Vet J 48, 33-43. doi: 10.1080/00480169.2000.36156

Mellor, D. J., Murray, L., 1989. Effects of tail docking and castration on behaviour and plasma cortisol concentrations in young lambs. Res Vet Sci 46, 387-391.

Mendl, M., Burman, O. H. P., Parker, R. M. A., Paul, E. S., 2009. Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. Appl Anim Behav Sci 118, 161-181. doi: 10.1016/j.applanim.2009.02.023

Merskey, H., Bogduk, N,. 1994. Classification of Chronic Pain: Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms 2nd edition. Seattle, WA.: IASP Press.

Mogil, J. S., and Crager, S. E., 2004. What should we be measuring in behavioral studies of chronic pain in animals? Pain, 112, 12-15. doi:http://dx.doi.org/10.1016/j.pain.2004.09.028

Molony, V. Kent, J. E., Robertson, I. S.,1993. Behavioural responses of lambs of three ages in the first three hours after three methods of castration and tail docking. Res Vet Sci, 55, 236-245. doi: 10.1016/0034-5288(93)90087-v

Molony, V., Kent, J. E., Hosie, B. D., Graham, M. J., 1997. Reduction in pain suffered by lambs at castration. Vet J 153, 205-213. doi:10.1016/s1090-0233(97)80041-x

Molony, V., Kent, J. E., McKendrick, I. J., 2002. Validation of a method for assessment of an acute pain in lambs. Appl Anim Beha. Sci 76, 215-238. doi:10.1016/s0168-1591(02)00014-x

Murata, H., Shimada, N., Yoshioka, M., 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. Vet J 168, 28-40. doi:http://dx.doi.org/10.1016/S1090-0233(03)00119-9

Odensvik, K. 1995. Pharmacokinetics of flunixin and its effect on prostaglandin F metabolite concentrations after oral and intravenous administration in heifers. J Vet Pharmacol Therap 18, 254-259. doi: 10.1111/j.1365-2885.1995.tb00589.x

Orsini, J. A., Ryan, W. G., Carithers, D. S., Boston, R. C., 2012. Evaluation of oral administration of firocoxib for the management of musculoskeletal pain and lameness associated with osteoarthritis in horses. Am J Vet Res 73, 664-671. doi:10.2460/ajvr.73.5.664

Owens, J. G., Kamerling, S. G., Stanton, S. R., Keowen, M. L., 1995. Effects of ketoprofen and phenylbutazone on chronic hoof pain and lameness in the horse. Equine Vet J 27, 296-300. doi:10.1111/j.2042-3306.1995.tb03080.x

Paul, E. S., Harding, E. J., Mendl, M., 2005. Measuring emotional processes in animals: the utility of a cognitive approach. Neurosci Biobehav Rev 29, 469-491. doi: http://dx.doi.org/10.1016/j.neubiorev.2005.01.002

Paull, D. R., Colditz, I. G., Lee, C., Atkinson, S. J., Fisher, A. D., 2008. Effectiveness of non-steroidal anti-inflammatory drugs and epidural anaesthesia in reducing the pain and stress responses to a surgical husbandry procedure (mulesing) in sheep. Aust J Exp Agric 48, 1034-1039.

Paull, D. R., Lee, C., Colditz, I. G., Atkinson, S. J., Fisher, A. D., 2007. The effect of a topical anaesthetic formulation, systemic flunixin and carprofen, singly or in combination, on cortisol and behavioural responses of

Merino lambs to mulesing. Aust Vet J 85(3), 98-106. doi: 10.1111/j.1751-0813.2007.00115.x

Paull, D. R., Lee, C., Colditz, I. G., Fisher, A. D. (2009). Effects of a topical anaesthetic formulation and systemic carprofen, given singly or in combination, on the cortisol and behavioural responses of Merino lambs to castration. Aust Vet J 87(6), 230-237. doi: 10.1111/j.1751-0813.2009.00429.x

Peirce, J. W., Leigh, A. E., daCosta, A. P. C., Kendrick, K. M., 2001. Human face recognition in sheep: lack of configurational coding and right hemisphere advantage. Behav Process 55, 13-26. doi:http://dx.doi.org/10.1016/S0376-6357(01)00158-9

Ricciotti, E., Fitzgerald G.A., 2011 Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol 31, 986-1000. doi: 10.1161/atvbaha.110.207449

Shutt, D. A., Fell, L. R., Connell, R., Bell, A. K.,1988. Stress responses in lambs docked and castrated surgically or by the application of rubber rings. Aust Vet J 65, 5-7. doi: 10.1111/j.1751-0813.1988.tb14920.x

Sneddon, J., Rollin, B,. 2010. Mulesing and Animal Ethics J Agric Environ Ethics 23(4), 371-386. doi: 10.1007/s10806-009-9216-z

Stafford, K. J.,Mellor, D. J.,2005. The welfare significance of the castration of cattle: A review. N Z Vet J 53(5), 271-278.

Sutherland, M. A., Mellor, D. J., Stafford, K. J., Gregory, N. G., Bruce, R. A., Ward, R. N., Todd, S. E., 1999. Acute cortisol responses of lambs to ring castration and docking after the injection of lignocaine into the scrotal neck or testes at the time of ring application. Aust Vet J, 77, 738-741. doi: 10.1111/j.1751-0813.1999.tb12917.x

Sutherland, M. A., Tucker, C. B.,2011. The long and short of it: A review of tail docking in farm animals. Appl Anim Behav Sci 135, 179-191. doi: http://dx.doi.org/10.1016/j.applanim.2011.10.015

Thornton, P. D., 1999. Quantification of the pain and distress responses to castration in young lambs. Res Vet Sci 66, 107-118. doi: 10.1053/rvsc.1998.0252

Trentini, R., Maitino, A. G., Di Fede, E., Iannetti, L., Dalla Villa, P., 2013. Tail docking of sheep and animal welfare: A review. Taglio della coda degli ovini e benessere animale: Revisione della letteratura, 19(1), 21-31.

Tsigos, C., Chrousos, G. P., 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res 53, 865-871.

Wechsler, B., 1995. Coping and coping stratergies: a behavioural view. Appl Anim Behav Sci, 43, 123-134.

Wechsler, B., and Lea, S. E. G. (2007). Adaptation by learning: Its significance for farm animal husbandry. Appl Anim Behav Sci 108, 197-214. doi:http://dx.doi.org/10.1016/j.applanim.2007.03.012

Welsh, E. M., 1995. Effect of flunixin meglumine on the thresholds to mechanical stimulation in healthy and lame sheep. Res Vet Sci 58, 61-66. doi: 10.1016/0034-5288(95)90090-x

Welsh, E. M., Baxter, P., Nolan, A. M., 1992. Pharmacokineties of carprofen administered intravenously to sheep. Res Vet Sci53, 264-266.

Yanagidate, F., Strichartz, G. R., 2007,. Local Anesthetics. In C. Stein (Ed.), Analgesia (pp. 95-127). Berlin, Heidelberg: Springer Berlin Heidelberg.

# Chapter 2

# Randomised trial of the bioavailability and efficacy of orally administered flunixin, carprofen and ketoprofen in a pain model in sheep

D Marini[a,b], J Pippia[c], IG Colditz[a] , G Hinch[b], JC Petherick[d] and C Lee[a]

[a]CSIRO, FD McMaster Laboratory, New England Highway, Armidale, NSW, Australia

[b]School of Environmental and Rural Sciences, The University of New England, Armidale, NSW, Australia

[c] PIA PHARMA Pty Ltd, Unit 3, 46 Buffalo Road, Gladesville, NSW, Australia

[d]QAAFI, The University of Queensland, Brisbane, QLD, Australia



Paper formatted to *Australian Veterinary Journal* 93 (2015) 265-270

This chapter has been removed as it was published as the following journal article:

Marini, D., Pippia, J., Colditz, I., Hinch, G., Petherick, J. and Lee, C. (2015). Randomised trial of the bioavailability and efficacy of orally administered flunixin, carprofen and ketoprofen in a pain model in sheep. *Australian Veterinary Journal*, 93(8), pp.265-270.

http://dx.doi.org/10.1111/avj.12351

# Chapter 3

# Palatability and pharmacokinetics of flunixin when administered to sheep through feed


**D. Marini[1,2,*] J. Pippia[3], I. G. Colditz[1], G. Hinch[2], J. C. Petherick[4] and C. Lee[1]**

[1]CSIRO, Agriculture, Armidale, New South Wales, Australia

[2] The University of New England, Armidale, New South Wales, Australia

[3] PIA PHARMA Pty Ltd, Gladesville, New South Wales, Australia

[4]The University of Queensland, Brisbane, Queensland, Australia

[*]Danila Marini: CSIRO, Agriculture, New England Highway, Armidale, New South Wales, Australia, 2350, (danila.marini@csiro.au)



Paper formatted to *PeerJ* (https://doi.org/10.7717/peerj.1800)

Marini, D., Pippia, J., Colditz, I., Hinch, G., Petherick, C. and Lee, C. (2016). Palatability and pharmacokinetics of flunixin when administered to sheep through feed. *PeerJ*, 4:e1800.

https://dx.doi.org/10.7717/peerj.1800

# Chapter 4

# Self-administration by consumption of flunixin in feed alleviates the pain and inflammation associated with castration and tail docking of lambs

Danila Marini[a, b*], Ian G. Colditz[a], Geoff Hinch[b], J. Carol Petherick[c] and Caroline Lee[a]

[a]CSIRO Agriculture, FD McMaster Laboratory, New England Highway, Armidale, NSW, 2350, Australia

[b]School of Environmental and Rural Sciences, University of New England, Armidale, NSW, 2350, Australia

[c]QAAFI, The University of Queensland, Brisbane St Lucia, QLD, 4072, Australia

* Author for correspondence: danila.marini@csiro.au Ph. +61266761342 Mob. 0422258624, CSIRO Agriculture, FD McMaster Laboratory, New England Highway, Armidale, NSW, 2350, Australia.

Paper formatted to Applied Animal Behaviour Science (Submitted)

Marini, D., Colditz, I., Hinch, G., Petherick, J. and Lee, C. (2017). Self-administration by consumption of flunixin in feed alleviates the pain and inflammation associated with castration and tail docking of lambs. *Applied Animal Behaviour Science,* 188, pp. 26-33.

https://dx.doi.org/10.1016/j.applanim.2016.12.008

# Chapter 5

# Self-medication in animals and its potential role for alleviating pain in livestock

## 5.1. Introduction

Self-medication is considered to occur when animals consume plants or non-vegetable substance (eg soils) that are not part of their normal diet in order to alleviate a negative state. Most evidence for self-medication comes from responses to parasitic infections and there is less experimental evidence for self-medication in the context of pain relief. Several wild animal species have exhibited behaviour that has been interpreted as self-medication including Indian wild boars (*Sus scrofa*), Sumatran rhinoceroses (*Didermocerus sumatrensis*) (Janzen, 1978) and Chimpanzees (*Pan troglodytes*) (Huffman, 1997). Self-medication has also been reported in insects including caterpillars (*Grammia incorrupta*) (Singer *et al*., 2009) and bees (Simone-Finstrom and Spivak, 2012). Animals have been reported to consume plants that contain plant secondary metabolites during periods of high parasitic burdens (Athanasiadou and Kyriazakis, 2004). These metabolites are produced by plants to protect themselves from pathogens and herbivores, giving the plant properties such as a bitter taste, potentially toxic or having insecticide properties (Wink, 1988). Chimpanzees are one of the best documented of these examples as it has been observed that they exhibit self-medication type behaviours in response to parasitic burdens in the wild, by consuming plants that are known to contain secondary metabolites that are natural anthelmintics (Huffman, 1997).

High parasite burdens lead to reduced fitness through diarrhoea and weight loss and can even result in the host's death (Beveridge *et al*., 1985; Dargie, 1987; Vercruysse and Claerebout, 2001). It is thought that the malaise accompanying infection will motivate animals to seek alternative feeds which may have the potential to overcome these negative effects. If this is true then it indicates that the animal is motivated to address a negative state (Duncan and Petherick, 1991). The review which follows largely examines self-medication in response to parasitic infestation. In particular, it examines an animal's ability to learn to self-medicate, examples of self-medication and the potential application of this process for the livestock industries as a pain relief option.

## 5.2. Mechanism to link food eaten with consequences

The idea that animals have nutritional wisdom (are able to adjust intake of nutrients according to need) has been discussed for many years and numerous studies have sought to understand the mechanisms that might allow this to occur. When animals have access to a variety of plants, they have the opportunity to select plants for specific nutrients and they are able to change their preference of plant species based on their current nutritional deficiencies (Provenza, 1995). Animals will also satisfy their nutritional needs by obtaining minerals by either consuming soil or bones (Blair-West *et al*., 1992; Wallisdevries, 1996). Macro and micro nutrient seeking can become a part of an animal's behaviour, for example white tailed deer suffer from sodium deficiency in spring due to their high potassium and water intake (Weeks and Kirkpatrick, 1976) and consequently will visit salt licks every few days during this period (Wiles and Weeks, 1986; Atwood and Weeks, 2002). Other animals have also been observed to have a peak use of natural licks around nutritionally demanding times, such as known seasons of pregnancy and lactation (Ayotte *et al*., 2008). Consumption of soil or natural licks can also have other positive outcomes such as the consumption of clay which helps with the digestion of plant secondary compounds (Gilardi *et al*., 1999; Houston *et al*.,2001). In order for the animal to gain the "nutritional wisdom" they first need to learn what "food" overcomes the deficiency or negative state.

### 5.2.1. Recognition of changed state (positive or negative)

In order for an animal to self-medicate, they must be able to recognise a change of state whether it be positive or negative. They must be able to recognise that they have a deficiency (e.g. mineral or vitamin) or another negative state (e.g. parasitism) which would initiate a searching behaviour. Once finding the substance that would attenuate their state and after sufficient consumption they would then need to recognise that the negative state has been ameliorated to be able to return to normal behaviour. Sheep normally eat a variety of plants but will approach new plants with caution, this neophobia can prevent the sheep from consuming excessive amount of toxin and becoming ill (Provenza *et al*., 1998). However when their internal homeostasis is compromised their neophobia is reduced and they are motivated to select and consume novel feed (Egea *et al*., 2014).

Not only can sheep recognise a change in homeostasis due to a negative state but they are also able to identify different negative states ( Villalba *et al*., 2015) such as mineral deficiencies and select the food that aids in recovery (Villalba *et al*., 2008). Sheep gain the knowledge about the food that overcomes their negative state over time through post-ingestive feedback (Provenza *et al*., 2006).

### 5.2.2. Post-ingestive consequences

In the literature there is demonstrated importance for an animal to learn the effects of a medicinal substance in order to self-medicate therapeutically. For therapeutic self-medication an animal has to first learn that a certain substance can attenuate a state of illness or pain. An individual animal is likely to learn the benefit of a substance through trial-and-error; however, it can be difficult for an animal to make the association if the behaviour and consequence are not paired closely together (Provenza, 1987; Villalba and Provenza, 2007). An animal learns about the food they consume by processing information obtained through the interactions of the affective system (post-ingestive feedback) and the cognitive system (the food's characteristics such as odour and flavour) (Provenza *et al*., 1992; Provenza *et al*., 2006) (Figure 7). This feedback system is considered to be cyclical in its functionality allowing self-regulation of food intake (Provenza *et al*., 2006; Villalba and Provenza, 2009).

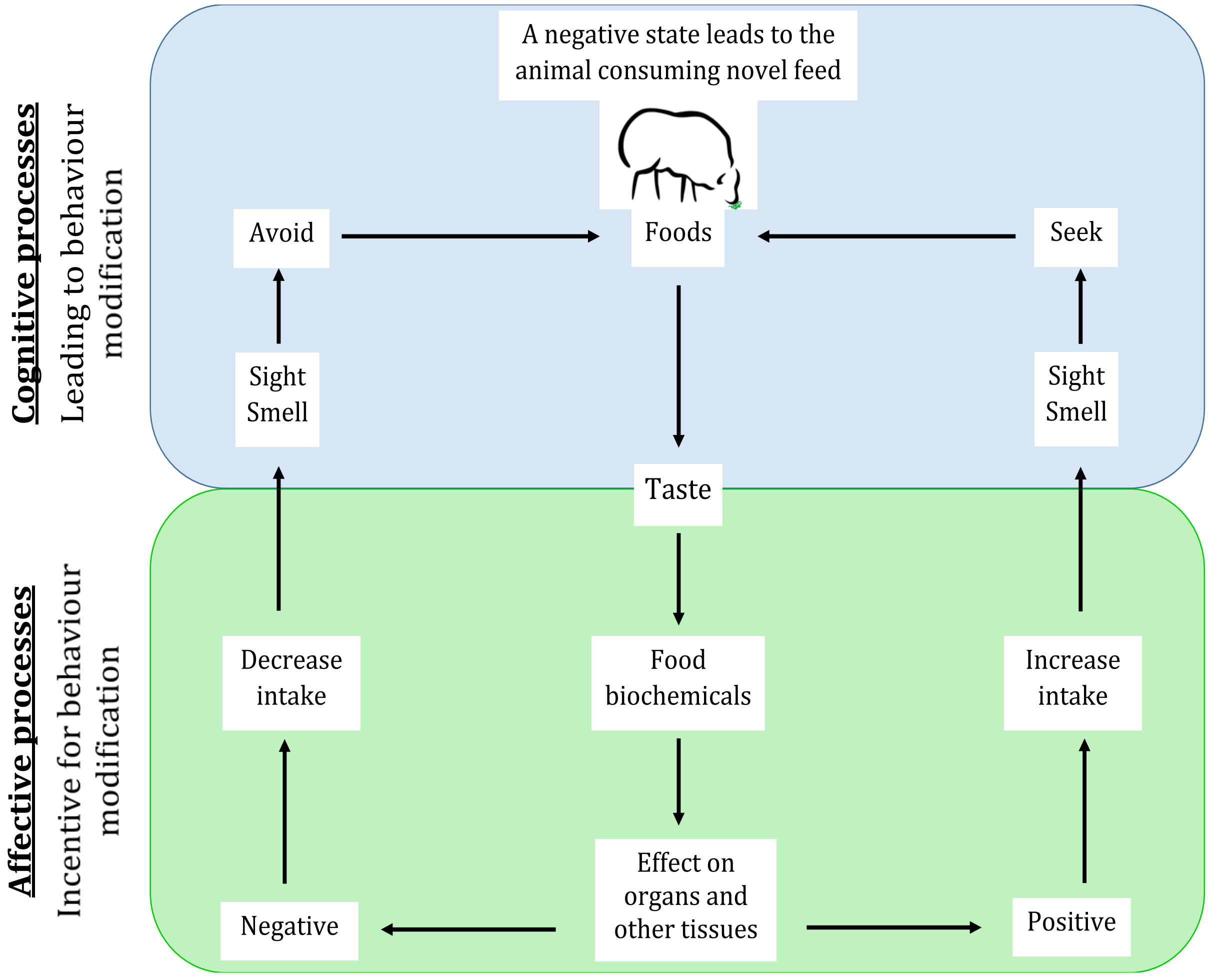



Figure 7: Schematic representation of the processes involved in post-ingestive feedback system modified from Provenza (1992) and Provenza and Villalba (2006). An animal is motivated to consume novel food due to a negative state. Consumption of the food leads to its biochemical constituants having either a positive or negative effect on the animal. Through the affective process animals will either increase or decrease intake of the feed. The cognitive process links the postingestive feedback with the foods' characteristics, leading to an appropriate behavioural response (avoid or seek the food).

### *5.2.3. Learning – trial and error or social?*

Animals often learn by watching and copying close relatives or other animals within their group (Nicol, 1995); however, for them to gain nutritional wisdom they need to sample plants and learn consequences themselves whether positive or negative through post-ingestive feedback (Thorhallsdottir *et al*., 1990; Villalba and Provenza, 2009). For example, in social animals, social facilitation can help an animal overcome a food aversion. The animal with the aversion watches its peers eat the food that they originally had an aversion to. They will resample the food in small amounts, if there are no negative consequences the aversion can be extinguished (Ralphs and Provenza, 1999). Hence on the occasion that an animal has made an association with a medicinal benefit of a specific plant or substance, maternal influences or social-interactions within the group could be important for faster transmission of the learned behaviour, as socialising can increase learning of individuals ( Villalba and Provenza, 2007; Sanga *et al*.,2011). Therefore, if there is genetic variation in capacity to learn then this could be passed to subsequent generations. However, learning the consequences of specific feed still needs to occur in the individual animal. Lambs will often display a preference for food that they have previously seen their mother display a preference for, however they are still willing to sample food that they have seen their mothers avoid (Mirza and Provenza, 1994; Savage *et al*., 2008).

## 5.3. Evidence for self-medication for disease state in grazing animals

A large proportion of the evidence for animal self-medication is anecdotal, where a sick wild animal is observed to consume a plant not normally part of their diet, a plant that  has the capability of improving its health (Huffman, 2003; Lozano, 1998). However, in observations of wild animals, it can be difficult to determine if the behaviour they are exhibiting is deliberate self-medication or a result of the consumption of a wider diversity of feeds. To distinguish whether an animal is exhibiting true self- medication, it has been proposed by Huffman (1997) that the behaviour displayed should pass certain criteria, namely,, that the substance consumed 1) is not part of the animals normal diet; 2) it is of low nutritional value; and 3) is situation-specific and 4) only affected individuals consume it.

### *5.3.1. Wildlife*

In Africa, chimpanzees have been reported to self-medicate, based on the above criteria and control parasitic burdens by leaf swallowing (Huffman, 2001). This leaf-swallowing behaviour is observed during the rainy season when it is known that chimpanzees have an increased risk of developing intestinal parasitic infections (Huffman *et al*., 1997). Infected chimpanzees have been observed to take leaves that have rough or sharp surfaces, they fold these leaves and consume them whole, and consequently are not receiving any nutritional benefits from the leaves. These leaves then increase gut motility, causing diarrhoea (Huffman and Caton, 2001) and subsequently the expulsion of parasitic worms and adult strongyloid nematodes along with the leaves (Huffman and Hirata, 2004; Fowler *et al*., 2007).

The ability to display and capacity to learn the leaf swallowing behaviour has been tested on captive, parasite free, chimpanzees. Huffman and Hirata (2004) offered the captive chimpanzees when in isolation, the leaves of *Helianthus tuberosus* a plant that has a similar textured leaf to those selected by chimpanzees in the wild. The chimpanzees had not been exposed to the leaves prior to the study and of the 11 chimpanzees tested only two exhibited leaf swallowing behaviour on first exposure; the rest either chewed the leaves or avoided them. The chimpanzees were then offered the leaves again and retested in a group, with a chimpanzee that had displayed leaf-swallowing. At the end of the experiment 6 out of the 11 chimpanzees had displayed the leaf-swallowing behaviour (Huffman and Hirata, 2004). As for the chimpanzees who did not display leaf swallowing, they had not directly observed another chimpanzee preform the behaviour (Huffman and Hirata, 2004). Although this study did not demonstrate self-medication as the animals were not infected with parasites it indicated that the animals have the propensity to perform a spontaneous behaviour such a leaf-swallowing as well as demonstrating that social-interaction can help the uptake of a behaviour.

Another observation of self-medication by Huffman (1989) was of a female chimpanzee who was visibly unwell, she moved away from her foraging group until she found the shrub *Vernonia amygdalina*, known as bitter leaf. She then broke of a stem and striped back the bark and chewed and sucked on the inner pith, by the next afternoon her health had started to improve. This behaviour is quite rare and in 10 years after this initial observation only four other chimpanzees with similar symptoms were reported to display this behaviour (Engel, 2002). It is also known that *Vernonia amygdalina* has anthelmintic properties (Adedapo *et al*.,2007; Ademola and Eloff, 2010) and in one of the observed cases in chimpanzees faecal egg count was reported to have dropped within 24 h of consumption (Huffman *et al*., , 1993).

The display of leaf swallowing as well as chewing on bitter leaf pith is considered 'therapeutic' self-medication as the chimpanzees' display the behaviour when they are experiencing sickness. The other type of self-medication is prophylaxis; this is where animals will eat plants all year round that contain plant secondary metabolites to reduce or prevent the risk or burden of infection.

### *5.3.2. Livestock*

Free ranging wild animals graze a variety of plants and may have the opportunity to learn the consequences of ingesting them through post ingestive feedback. Grade, Tabuti, and Van Damme (2009) reported self-medication in livestock with unrestricted access to forage in Uganda. For the behaviour of sheep, cattle and goats to be classified as self-medication, animals had to be observed to self-select and consume plants that they would not normally eat when healthy, then their symptoms needed to improve and their abnormal behaviour ceases. The most common ailment that the livestock were reported to self-medicate for were parasitic diseases (Grade *et al*., 2009). Unfortunately, there have been few such studies, thus a majority of the demonstrations of self-medication in livestock comes from experimental settings. An advantage of these settings is that the role of learning and the medicinal benefits of the consumed forages are easier to quantify.

Most reports of instances of livestock self-medication in experimental contexts are in response to parasitism. A study conducted by Fishpool, Kahn, Tucker, Nolan, and Leng (2012) examined whether sheep could learn to self-medicate with a medicated feed block (MFB) that contained the anthelmintic fenbendazole when they were infected with a gastrointestinal nematode. The sheep underwent a seven-week training period where they were exposed to an un-medicated feed block for five weeks and then a MFB for a total of two weeks. The sheep were then split into two groups, a group infected with the gastrointestinal nematode *Haemonchus contortus* and an uninfected group. Both groups were given access to the MFB for two weeks. During this period, it was found that infected sheep ate more of the MFB in the first four days than uninfected sheep and consumed sufficient fenbendazole to cause worm egg counts to decline by the sixth day. Doubt remained, however, whether self-medication had occurred due to the variability in the amount of the MFB consumed by both infected and un-infected sheep during the one-week period, and the continuing consumption of MFB by infected animals after their worm egg counts had decreased. This experiment may have demonstrated prophylactic self-medication or social facilitation of behaviour as animals were kept as a group, and it is not clearly outlined how the authors identified how much of the block each individual animal consumed.

Similar prophylactic self-medicating behaviour has been observed in free ranging goats that include condensed tannins (CT) in their diet (Cooper and Owen-Smith, 1985; Glasser *et al*., 2012). Tannins are a natural anthelmintic (Min and Hart, 2003), it is known that 2 – 4 % CT to be beneficial in animals (Terrill *et al*., 1992; Wang *et al*., 1996), but higher levels (>5%) can be detrimental to health (Aerts, *et al*., 1999). CT have a low palatability and normally form a low proportion of the diet. However, by consuming plants containing less than 5% CT in their normal diet the goats have been shown to maintain low worm burdens and this provides an example of prophylactically self-medication (Cooper and Owen-Smith 1985; Kabasa, *et al*., 2000; Landau *et al*., 2010; Glasser *et al*., 2012). While initial consumption of CT may be acquired through social learning, it has been shown goats' preferences for plants and even plant parts (i.e. new growth vs old growth) containing lower levels of CT is acquired or learnt through post-ingestive feedback (Provenza *et al*., 1990; Provenza *et al*., 1994).

There have been other demonstrations by Lisonbee *et al*., (2009), Villalba, *et al*., (2010) and Juhnke, *et al*., (2012) that sheep can learn the benefits of CT in their diets and subsequently preferentially consume CT containing feed whilst parasite burdens are high. Similar to the results of Fishpool *et al*. (2012), parasitized lambs preferentially consumed supplements containing CT compared to uninfected sheep (Lisonbee *et al*., 2009). This higher preference for CT feed by parasitized lambs continued for the first 12 days but then reduced as their parasite burden decreased.

Villalba *et al*. (2010) and Juhnke *et al*. (2012) have addressed the role of learning in consumption of CT in response to parasitic burdens. Villalba *et al*. (2010) conditioned a parasitised and a non-parasitised group of lambs to consume feed containing CT. During this conditioning period it was assumed that only parasitized lambs would have the opportunity to learn the post ingestive benefit of CT by associating the consumption of feed containing CT with the relief from their parasitic infection. The control non-parasitized group of lambs did not have the opportunity to experience the relief of CT feed and make the association. Juhnke *et al*. (2012) took a different approach, both groups of lambs were parasitised but only one group was conditioned to a feed that contained CT and the second group was conditioned to a feed that would have no effect on parasitic burden. During conditioning lambs that are parasitised should be able to associate the consumption of feed containing CT with the relief from their parasitic infection. The control group of lambs did not have the opportunity to experience the relief of CT feed and make the association. In both studies by Villalba *et al*. (2010) and Juhnke *et al*. (2012) the lambs that were previously conditioned to the CT feed whilst they were parasitised, consumed more tannin containing feed during subsequent parasitic burdens than the lambs that did not have the opportunity to learn. These results suggest that prior exposure and learning of the benefits of a medicinal substance may be important for an animal's subsequence utilisation of the feed as medication. At the end of both the studies by Villalba *et al*. (2010) and Juhnke *et al*. (2012) the sheep were drenched to remove the parasitic burden and retested. Juhnke *et al*. (2012) found that the sheep in the conditioned group reduced their preference for CT following drenching and intake became similar to the control group. Conversely in Villalba *et al*. (2010) the conditioned group continued to show a preference for the CT containing food. The different results of the two studies may have been due to the time between drenching/retesting and the difference in the percentage of CT in the diets. Juhnke *et al*. (2012) allowed an 8-day period between drenching and retesting for preference. Villalba *et al*. (2010) drenched 6 days before testing and re-drenched the day preference testing began, the whole phase was conducted over 11 days. Having a shorter interval between drenching and testing may have not given the sheep the chance to completely recover from their parasitic burden and they could have still been suffering from parasitic infection symptoms and therefore may have still been motivated to self-medicate. The second difference was the percentage of CT in the diet. Villalba *et al*. (2010) offered a diet containing 2% CT, a quantity which would not cause negative nutritional effects, compared to the provision of 8% CT in the diet in Juhnke *et al*. (2012) study. It is known that diets with over 5% CT can cause negative effects on an animal (Aerts *et al*., 1999; Min and Hart, 2003). So the animals in Juhnke *et al*. (2012) may have no longer been experiencing a positive outcome from consuming CT thus reducing their intake.

A similar conditioning strategy has shown that lambs can learn the medicinal effects of certain substances that can attenuate the aversive effects of malaise-inducing foods (Provenza *et al*.,2000; Villalba and Provenza, 2001; Villalba and Provenza, 2007). The experimental approach is based on exposing sheep to foods that cause sickness, such as high energy diets which cause acidosis (Mackie *et al*., 1978), or a very high tannin diet which can cause lesions in the gut mucosa and thus pain (Reed, 1995). Not only can sheep learn to self-medicate to alleviate negative states, they also have the ability to learn to choose the appropriate feed from several on offer to self-medicate for the particular illness they are experiencing (Villalba *et al*., 2006). Villalba *et al*. (2006) demonstrate this capability to learn an association between malaise and remedy with the malaise inducing feeds grain, tannins, oxalic acid and respective attenuating substances sodium bentonite, polyethylene glycol and dicalcium phosphate. In order for the lambs to choose the appropriate feed for each situation, lambs were offered an illness inducing feed for 5-7 days to acclimatise them to the feed before offering the substance that would attenuate the state of malaise induced by the feed. By using conditioning where lambs were fed on an illness inducing feed and then given a substance that lead to the improvement of their state, lambs were able to pair the three different illness-inducing feeds (grain, tannins, oxalic acid) with the appropriate medicinal substance (sodium bentonite, polyethylene glycol and dicalcium phosphate, respectively).

### *5.3.3. Evidence of self-medicating in response to pain*

Husbandry procedures such as ear-tagging, tail-docking and castration are a major cause of pain in livestock (Fisher, 2011; Grant, 2004; Hodgkinson and Dawson, 2007; Mellor and Stafford, 1999; Stafford and Mellor, 2010). The limited use of pain relief in livestock is often due to registration issues that affect access to drugs, the difficulty of administering pain relief drugs

(Coetzee, 2011; Lizarraga and Chambers, 2012), the difficulty of identifying pain (Weary *et al*., 2006) and duration of pain livestock are experiencing (Lomax *et al*., 2013). Therefore, self-medication could be an interesting option. In such situations, farmers would not need to identify animals in pain knowing that animals would medicate as needed.

Rats have been widely used in biomedical studies of pain relief, and provide one of the few well documented examples of animals self-medicating for pain. Using the learning method of operant conditioning, Martin, *et al*., (2007) and Gutierrez *et al*.,(2011) taught rats to self-administer opioids. In these two studies it was found that rats that had a nerve injury would self-administer drugs more often and the extent of self-administration was correlated with the severity of pain. Pain severity was determined through the rat's reaction to mechanical pressure on their paws. Not only did rats self-medicate in response to pain but they were also willing to work harder to self-medicate by pressing the lever up to 9 times more often to receive their dose, compared to rats not in pain (Gutierrez *et al*., 2011). Rats have also been shown to self-medicate with the opiate fentanyl to alleviate pain associated with adjuvant-induced arthritis (Colpaert *et al*., 2001). Similar to the work with lambs by Villalba *et al*. (2010) and Juhnke *et al*. (2012), both arthritic and non-arthritic rats in Colpaert's *et al*., (2001) study were conditioned to fentanyl by being offered only fentanyl containing water, allowing arthritic rats to experience the positive effect of pain relief. The rat's self-medication was then tested by a preference test, where arthritic and non-arthritic rats had the choice between a sweet water and water containing fentanyl. Arthritic rats self-administered more fentanyl than non-arthritic rats and when arthritic rats were administered fentanyl or dexamethasone subcutaneously their self-administration of fentanyl reduced.

Chickens have also been shown to self-medicate for pain relief by selecting feed containing the non-steroidal anti-inflammatory drug, carprofen in response to the pain associated with naturally occurring lameness (Danbury *et al*., 2000). For this study chickens were conditioned to the medicated feed during a training period and the feed was coloured with bright food dye to help them identify the different feeds. In the first half of the study, group housed chickens were reported to self-medicate in proportion to the severity of lameness and it was shown that self-medication improved lameness scores (indicating it reduced their pain). In the second half of the study, lame and non-lame chickens housed individually were tested for their preference for medicated feed. Although lame chickens self-selected carprofen medicated feed more than non-lame chickens, the average concentration of carprofen in plasma did not significantly different between lame and sound birds (Danbury *et al*., 2000). The lack of difference in carprofen concentration between medicating and non-medicating animals may have been due to the lack of a flush out period between training (where all chickens were only given feed containing carprofen for several days) and the testing period. Carprofen has a long elimination half-life in several species (Welsh *et al*., 1992; Lees *et al*., 1994; Taylor *et al*., 1996) and thus may still have been present in plasma during the self-medication test as a carry-over from the training period.

Conversely another study conducted with chickens that were either beak-trimmed or left intact by Freire *et al.*, (2008) using the same protocol as Danbury *et al.*, (2000) found that chickens that were beak-trimmed did not self-medicate. Throughout the study there was no difference in consumption of medicated feed and non-medicated feed for both beak-trimmed and intact chickens. There was also no evidence that self-medication improved the pain state in the chickens (Freire *et al.*, 2008). The different outcomes seen in the two studies may relate to the relative efficacy of carprofen in different types of pain: Danbury *et al.*, (2000) studied musculoskeletal pain whereas Freire *et al.*, (2008) studied neuropathic pain. While the efficacy of carprofen for alleviating musculoskeletal pain is well established (McGeown *et al.*, 1999; Hocking *et al.*, 2005; Caplen *et al.*, 2013), its efficacy again neuropathic pain is less clear.

In conclusion, experimental studies on parasitic disease, internal malaise and pain indicate that sheep, goats and chickens can learn to self-medicate to alleviate specific ailments, when exposed to a training paradigm that establishes an association between alleviation of malaise and the medicinal substance.

## 5.4. The potential use of self-medication in livestock management

It would seem animals have the ability to self-medicate for different aliments however the most commonly reported self-medication is against internal parasites. This may be due to the ease in identifying animals that suffer from parasites and the non-invasive methods of determining if the animal's behaviour has improved its health. It is clear that livestock have the ability or at least the potential to learn the benefits of particular substances, as observed with free ranging goats and sheep in experimental settings having the ability to select CT when they are suffering from internal parasites. However, in most commercial livestock industries the opportunity to learn are not given to most livestock, particularly those raised in intensive systems. So we may be depriving livestock of the opportunity to learn to self-medicate due to the restricted variety of plants available to them in the pasture feed base or reliance on concentrate diets.

Self-medication has potential in the livestock industries not just as an alternative for parasite control; but also as an alternative method of providing pain-relief if livestock can to learn to medicate for painful situations. Every year sheep and cattle undergo many invasive husbandry procedures that are common practice worldwide. These procedures include castration, tail docking, dehorning and branding, which are all known to cause pain (Lay *et al*., 1992; Dinniss *et al*., 1997; Stafford and Mellor, 2005). Due to the public's increasing concern for animal welfare (Hughes, 1995; Izmirli and Phillips, 2012), it could be considered a priority for livestock producers to provide pain relief in animals undergoing painful husbandry procedures. When pain relief is used, it is administered at the time of injury and animals are usually not given a follow up dose. However a single dose may not provide sufficient pain relief for all animals, as like humans, an animal's sensitivity and experience of pain varies, with factors such as species (Stasiak *et al.*,, 2003), age and sex (Robertson, *et al.*,1994; Guesgen *et al.*, 2011). Providing livestock with the opportunity to learn to self-medicate through food and water could address this need, giving animals a chance to medicate according to the level of pain that they are experiencing and would allow them to return to homeostasis by consuming a substance containing an analgesic. Control by the animal over the amount of medication it consumes differentiates self-medication from the widespread practice of delivering medicines in formulated feeds for disease control and growth promotion.

Learning to self-medicate has important implications in livestock particularly when the animals are not familiar with the potential benefits of the medicinal substance that they need to consume. If given a variety of feed choices, livestock would have the opportunity to learn to self-medicate for their own self-perceived illness. As demonstrated in several studies consumption of CT as part of the diet reduces parasite infection, if given access to feed that contains CT along with an animal's standard diet, livestock could then self-select the CT according to their level of parasitic infection. The implications for self-medicating livestock could be the improvement of animal's welfare as we allow them to take more control over their homeostasis/wellbeing (Boissy and Lee, 2014) as well as reducing a farmer's reliance on chemical interventions. Other benefits of teaching livestock to self-medicate particularly for painful situations include the ease of administration of analgesics as well as reducing the need to identify animals in pain. This could also be of use to animal behavioural science as using self-medication as an approach that lets the animal tell us when it is in pain which reduces the need to solely rely on behavioural responses and physiological measures in pain assessment. However there is no research on livestock's ability to learn to self-medicate for pain such as that caused by painful husbandry procedures and only chickens have been shown to medicate with an analgesic for a painful condition (Danbury *et al*., 2000). It should be noted that training animals to self-medicate in commercial instances could prove difficult and a more pragmatic strategy such as solely providing animals with medicated feed or water during periods of likely pain without the need to choose to self-medicate may be required.

For this thesis, the final experiment will explore the potential to train lambs to self-administer flunixin to provide pain relief over a period of several days. The importance for an animal to learn the consequences of a food before they have the ability to make choices has been outlined in this review. Therefore, lambs will first be conditioned to a medicated feed whilst in a pain state before being offered medicated and un-medicated feed. If sheep can learn to self-medicate, their voluntary choice to ingest medications that are non-addictive when given a choice of a medicated and un-medicated feed, could be indicative that it is experiencing pain. Thus, development of a test procedure to examine the choice of sheep to self-medicate would also provide a valuable indicator of affective state in these animals and help us better understand pain and its impacts in livestock.

## 5.5. References


Adedapo, A. A., Otesile, A. T., Soetan, K. O. 2007. Assessment of the Anthelmintic Efficacy of an Aqueous Crude Extract of Vernonia amygdalina. Pharmaceutical Biology, 45, 564-568. doi:10.1080/13880200701498978

Ademola, I. O., Eloff, J. N., 2010. Anthelminthic activity of acetone extract and fractions of Vernonia amygdalina against Haemonchus contortus eggs and larvae. Trop Anim Health Prod 43, 521-527. doi:10.1007/s11250-010-9727-7

Aerts, R. J., Barry, T. N., McNabb, W. C., 1999. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. Agric Ecosyst Environ 75, 1-12. doi:http://dx.doi.org/10.1016/S0167-8809(99)00062-6

Athanasiadou, S., Kyriazakis, I., 2004. Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. Proc Nutr Soc 63, 631-639.

Atwood, T. C., Weeks, H. P., 2002. Sex- and Age-specific Patterns of Mineral Lick Use by White-tailed Deer (Odocoileus virginianus). Am Midl Nat 148, 289-296. doi:10.1674/0003-0031(2002)148[0289:SAASPO]2.0.CO;2

Ayotte, J. B., Parker, K. L., Gillingham, M. P.,2008. Use of Natural Licks by Four Species of Ungulates in Northern British Columbia. J Mammal, 89, 1041-1050.

Beveridge, I., Brown, T., Fitzsimons, S., Ford, G., Judson, G., Martin, R.,Miller, D., 1985. Mortality in weaner sheep in South Australia under different regimes of anthelmintic treatment. Aust J Agric Res, 36, 857-865. doi:http://dx.doi.org/10.1071/AR9850857

Blair-West, J. R., Denton, D. A., McKinley, M. J., Radden, B. G., Ramshaw, E. H., and Wark, J. D., 1992. Behavioral and tissue responses to severe phosphorus depletion in cattle. Am J Physiol Regul Integr Comp Physiol 263, R656-R663.

Boissy, A., and Lee, C.,2014. How assessing relationships between emotions and cognition can improve farm animal welfare. Scientific and

Technical Review of the Office International des Epizooties, 33, 103-110.

Caplen, G., Baker, L., Hothersall, B., McKeegan, D. E. F., Sandilands, V., Sparks, N. H. C., Murrell, J. C., 2013. Thermal nociception as a measure of non-steroidal anti-inflammatory drug effectiveness in broiler chickens with articular pain. Vet J 198(3), 616-619. doi:http://dx.doi.org/10.1016/j.tvjl.2013.09.013

Coetzee, J. F., 2011. A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the United States. Appl Anim Behav Sci, 135, 192-213. doi:10.1016/j.applanim.2011.10.016

Colpaert, F. C., Tarayre, J. P., Alliaga, M., Bruins Slot, L. A., Attal, N., and Koek, W., 2001. Opiate self-administration as a measure of chronic nociceptive pain in arthritic rats. Pain, 91, 33-45. doi:http://dx.doi.org/10.1016/S0304-3959(00)00413-9

Cooper, S. M., Owen-Smith, N., 1985. Condensed tannins deter feeding by browsing ruminants in a South African savanna. Oecologia, 67, 142-146. doi:10.1007/bf00378466

Danbury, T. C., Weeks, C. A., Waterman-Pearson, A. E., Kestin, S. C., Chambers, J. P., 2000. Self-selection of the analgesic drug carprofen by lame broiler chickens. Vet Rec, 146, 307-311.

Dargie, J. D. (1987). The impact on production and mechanisms of pathogenesis of trematode infections in cattle and sheep. Int J Parasitol, 17, 453-463. doi:http://dx.doi.org/10.1016/0020-7519(87)90121-4

Dinniss, A. S., Stafford, K. J., Mellor, D. J., Bruce, R. A., Ward, R. N., 1997. Acute cortisol responses of lambs castrated and docked using rubber rings with or without a castration clamp. Aust Vet J 75, 494-496. doi:10.1111/j.1751-0813.1997.tb14380.x

Duncan, I. J. H., Petherick, J. C., 1991. The implications of cognitive processes for animal welfare. J Anim Sci, 69(, 5017-5022.

Egea, A. V., Hall, J. O., Miller, J., Spackman, C., Villalba, J. J., 2014. Reduced neophobia: A potential mechanism explaining the emergence of self-medicative behavior in sheep. Physiol Behav 135, 189-197.

Engel, C. (2002). Wild health: lessons in natural wellness from the animal kingdom. New York: Houghton Mifflin Harcourt.

Fisher, A. D., 2011. Addressing pain caused by mulesing in sheep. Applied Anim BehavSci, 135, 232-240. doi:10.1016/j.applanim.2011.10.019

Fishpool, F. J., Kahn, L. P., Tucker, D. J., Nolan, J. V., Leng, R. A., 2012. Voluntary intake of a medicated feed block by grazing sheep is increased by gastrointestinal nematode infection. Anim Prod Sci, 52, 1136-1141. doi:10.1071/an12104

Fowler, A., Koutsioni, Y., Sommer, V., 2007. Leaf-swallowing in Nigerian chimpanzees: evidence for assumed self-medication. Primates 48, 73-76.

Freire, R., Glatz, P., Hinch, G., 2008. Self-administration of an analgesic does not alleviate pain in beak trimmed chickens. AsianAustralasJ Anim Sci, 21, 443-448.

Gilardi, J. D., Duffey, S. S., Munn, C. A., Tell, L. A., 1999. Biochemical Functions of Geophagy in Parrots: Detoxification of Dietary Toxins and Cytoprotective Effects. J Chem Ecol 25, 897-922. doi:10.1023/a:1020857120217

Glasser, T. A., Landau, S. Y., Ungar, E. D., Perevolotsky, A., Dvash, L., Muklada, H., Walker, J. W., 2012. Foraging selectivity of three goat breeds in a Mediterranean shrubland. Small Rumint Res 102, 7-12. doi:http://dx.doi.org/10.1016/j.smallrumres.2011.09.009

Grade, J. T., Tabuti, J. R. S., Van Damme, P.,2009. Four Footed Pharmacists: Indications of Self-Medicating Livestock in Karamoja, Uganda. Econ Bot 63, 29-42.

Grant, C. ,2004. Behavioural responses of lambs to common painful husbandry procedures. Appl Anim Behav Sci, 87, 255-273. doi:10.1016/j.applanim.2004.01.011

Guesgen, M. J., Beausoleil, N. J., Minot, E. O., Stewart, M., Jones, G., Stafford, K. J., 2011. The effects of age and sex on pain sensitivity in young lambs. Appl Anim Behav Sci 135, 51-56. doi:http://dx.doi.org/10.1016/j.applanim.2011.09.008

Gutierrez, T., Crystal, J. D., Zvonok, A. M., Makriyannis, A., Hohmann, A. G. 2011. Self-medication of a cannabinoid CB2 agonist in an animal model of neuropathic pain. Pain 15), 1976-1987. doi:http://dx.doi.org/10.1016/j.pain.2011.03.038

Hart, B. L., 1992. Behavioral Adaptations to Parasites: An Ethological Approach. J Parasitol, 78, 256-265. doi:10.2307/3283472

Hocking, P. M., Robertson, G. W., Gentle, M. J., 2005. Effects of non-steroidal anti-inflammatory drugs on pain-related behaviour in a model of articular pain in the domestic fowl. Res Vet Sci, 78, 69-75. doi:http://dx.doi.org/10.1016/j.rvsc.2004.05.005

Hodgkinson, O., Dawson, L.,2007. Practical anaesthesia and analgesia in sheep, goats and calves. In Practice 29, 596-603. doi:10.1136/inpract.29.10.596

Houston, D. C., Gilardi, J. D., Hall, A. J., 2001. Soil consumption by Elephants might help to minimize the toxic effects of plant secondary compounds in forest browse. Mammal Rev, 31, 249-254. doi:10.1111/j.1365-2907.2001.00091.x

Huffman, M. A., 1989. Observations on the illness and consuption of a possibly medicinal plant Vernonia amygdalina (Del.), by a wild chimpanzee in the Mahale mountains national park, Tanzania. Primates, 30, 51-63.

Huffman, M. A., 1997. Current evidence for self-medication in primates: a multidisciplinary perspective. Yearbook of Physical Anthropology, 40, 171-200.

Huffman, M. A., 2001. Self-medicative behavior in the African great apes: an evolutionary perspective into the origins of human traditional medicine. Bioscience, 51, 651-661.

Huffman, M. A., 2003. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. Proc Nutr Soc 62), 371-381.

Huffman, M. A., Caton, J. M., 2001. Self-induced Increase of Gut Motility and the Control of Parasitic Infections in Wild Chimpanzees. International Journal of Primatology, 22(3), 329-346. doi:10.1023/A:1010734310002

Huffman, M. A., Gotoh, S., Izutsu, D., Koshimizu, K., Kalunde, M. S., 1993. Further obervations on the use of the medicinal plant, Vernonia amygdalina (Del), by a wild chimpanzee, its possible effect on parasite load, and its phytochemistry. Afr Stud Monogr , 14, 227-240.

Huffman, M. A., Gotoh, S., Turner, L. A., Hamai, M., Yoshida, K., 1997. Seasonal trends in intestinal nematode infection and medicinal plant use among chimpanzees in the Mahale Mountains, Tanzania. Primates, 38, 111-125.

Huffman, M. A., Hirata, S., 2004. An experimental study of leaf swallowing in captive chimpanzees: insights into the origin of a self-medicative behavior and the role of social learning. Primates, 45, 113-118.

Hughes, D., 1995. Animal welfare: the consumer and the food industry. Bri Food J 97, 3-7. doi:doi:10.1108/00070709510104529

Izmirli, S., Phillips, C. J. C., 2012. Attitudes to animal welfare and rights throughout the world in the modern era: a review. Eurasian J Vet Sci, 28, 65-68.

Janzen, D. H., 1978. Complications in interpreting the chemical defenses of trees against tropical arboreal plant-eating vertebrates (pp. 73-84): Smithsonian Institution Press, Washington, D.C.

Juhnke, J., Miller, J., Hall, J. O., Provenza, F. D., Villalba, J. J.,2012. Preference for condensed tannins by sheep in response to challenge infection with Haemonchus contortus. VetParasitol188, 104-114. doi:http://dx.doi.org/10.1016/j.vetpar.2012.02.015

Kabasa, J. D., Opuda-Asibo, J., ter Meulen, U., 2000. The Effect of Oral Administration of Polyethylene Glycol on Faecal Helminth Egg Counts in Pregnant Goats Grazed on Browse Containing Condensed Tannins. rop Anim Health Prod, 32, 73-86. doi:10.1023/A:1005274502184

Landau, S., Azaizeh, H., Muklada, H., Glasser, T., Ungar, E. D., Baram, H. Markovics, A., 2010. Anthelmintic activity of Pistacia lentiscus foliage in two Middle Eastern breeds of goats differing in their propensity to consume tannin-rich browse. VetParasitol, 173, 280-286. doi:http://dx.doi.org/10.1016/j.vetpar.2010.07.006

Lay, D. C., Friend, T. H., Bowers, C. L., Grissom, K. K., Jenkins, O. C.,1992. A comparative physiological and behavioral study of freeze and hot-iron branding using dairy cows. J Anim Scie, 70, 1121-1125.

Lees, P., McKellar, Q., May, S. A. Ludwig, B. 1994. Pharmacodynamics and pharmacokinetics of carprofen in the horse. Equine VetJ 26, 203-208. doi:10.1111/j.2042-3306.1994.tb04370.x

Lisonbee, L. D., Villalba, J. J., Provenza, F. D., and Hall, J. O. (2009). Tannins and self-medication: Implications for sustainable parasite control in herbivores. Behavioural Processes, 82(2), 184-189. doi:10.1016/j.beproc.2009.06.009

Lizarraga, I.,Chambers, J. P., 2012. Use of analgesic drugs for pain management in sheep. N Z Vet J 60, 87-94. doi:10.1080/00480169.2011.642772

Lomax, S., Sheil, M., Windsor, P. A. 2013. Duration of action of a topical anaesthetic formulation for pain management of mulesing in sheep. Aust Vet J, 91), 160-167. doi:10.1111/avj.12031

Lozano, G. A., 1998. Parasitic stress and self-medication in wild animals. Advances in the Study of Behavior, 27, 291-317.

Mackie, R. I., Gilchrist, F. M. C., Robberts, A. M., Hannah, P. E., Schwartz, H. M.,1978. Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. J Agric Sci, 90, 241-254. doi:doi:10.1017/S0021859600055313

Martin, T. J., Kim, S. A., Buechler, N. L., Porreca, F., and Eisenach, J. C. (2007). Opioid Self-administration in the Nerve-injured Rat: Relevance of Antiallodynic Effects to Drug Consumption and Effects of Intrathecal Analgesics. Anesthesiology, 106(2), 312-322.

McGeown, D., Danbury, T. C., Waterman-Pearson, A. E., Kestin, S. C.,1999. Effect of carprofen on lameness in broiler chickens. Vet Rec, 144, 668-671.

Mellor, D., Stafford, K., 1999. Assessing and minimising the distress caused by painful husbandry procedures in ruminants. In Practice, 21, 436-446. doi:10.1136/inpract.21.8.436

Min, B. R., Hart, S. P. 2003. Tannins for suppression of internal parasites. J Anim Sci, 81, E102-E109.

Mirza, S. N., Provenza, F. D.,1994. Socially induced food avoidance in lambs: direct or indirect maternal influence? J Anim Sci, 72, 899-902. doi:/1994.724899x

Nicol, C. J., 1995. The social transmission of information and behaviour. Appl Anim Behav Sci, 44, 79-98. doi:http://dx.doi.org/10.1016/0168-1591(95)00607-T

Poulin, R. (1995). "Adaptive" changes in the behaviour of parasitized animals: A critical review. International Journal for Parasitology, 25(12), 1371-1383. doi:http://dx.doi.org/10.1016/0020-7519(95)00100-X

Provenza, F. D., 1987. Diet learning by domestic ruminants: Theory, evidence and practical implications. Appl Anim Behav Science 18, 211-232. doi:10.1016/0168-1591(87)90218-8

Provenza, F. D. (1995). Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J Range Manage, 48, 2-17.

Provenza, F. D., Burritt, E. A., Clausen, T. P., Bryant, J. P., Reichardt, P. B., Distel, R. A., 1990. Conditioned Flavor Aversion: A Mechanism for Goats to Avoid Condensed Tannins in Blackbrush. Am Nat, 13), 810-828.

Provenza, F. D., Burritt, E. A., Perevolotsky, A., Silanikove, N.,2000. Self-regulation of intake of polyethylene glycol by sheep fed diets varying in tannin concentrations. J Anim Sci, 7, 1206-1212.

Provenza, F. D., Lynch, J. J., Burritt, E. A., Scott, C. B., 1994. How goats learn to distinguish between novel foods that differ in postingestive consequences. J Chem Ecol, 20, 609-624. doi:10.1007/bf02059601

Provenza, F. D., Pfister, J. A., Cheney, C. D., 1992. Mechanisms of Learning in Diet Selection with Reference to Phytotoxicosis in Herbivores. J Range Manage, 45, 36-45. doi:10.2307/4002523

Provenza, F. D., Villalba, J. J., Bels, V., 2006. Foraging in domestic herbivores: linking the internal and external milieux. Feeding in domestic vertebrates: from structure to behaviour, 210-240.

Provenza, F. D., Villalba, J. J., Cheney, C. D., Werner, S. J., 1998. Self-organization of foraging behaviour: From simplicity to complexity without goals. Nutr Res Rev 11, 199-222.

Ralphs, M. H., Provenza, F. D., 1999. Conditioned food aversions: principles and practices, with special reference to social facilitation. Proceedings of the Nutrition Society, 58(04), 813-820. doi:doi:10.1017/S002966519900110X

Reed, J. D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes.J Anim Sci 73, 1516–1528.

Robertson, I. S., Kent, J. E., Molony, V., 1994. Effect of different methods of castration on behaviour and plasma cortisol in calves of three ages. Res Vet Sci 56(1), 8-17. doi:http://dx.doi.org/10.1016/0034-5288(94)90189-9

Savage, D. B., Ferguson, D. M., Fisher, A. D., Hinch, G. N., Mayer, D. G., Duflou, E., Raue, M., 2008. Preweaning feed exposure and different feed delivery systems to enhance feed acceptance of sheep. Aust J Exp Agric, 48, 1040-1043. doi:http://dx.doi.org/10.1071/EA08055

Simone-Finstrom, M. D.,Spivak, M.,2012. Increased Resin Collection after Parasite Challenge: A Case of Self-Medication in Honey Bees? PloS One, 7, 1-7. doi:10.1371/journal.pone.0034601

Singer, M. S., Mace, K. C., Bernays, E. A., 2009. Self-Medication as Adaptive Plasticity: Increased Ingestion of Plant Toxins by Parasitized Caterpillars. PloS One, 4, 1-8. doi:10.1371/journal.pone.0004796

Stafford, K. J., Mellor, D. J. 2005. The welfare significance of the castration of cattle: A review. N Z Vet J 53, 271-278.

Stafford, K. J., Mellor, D. J., 2010. Painful husbandry procedures in livestock and poultry. In T. Grandin (Ed.), Improving Animal Welfare: A Practical Approach (pp. 88-124): CABI.

Stasiak, K. L., Maul, D., French, E., Hellyer, P. W., Vandewoude, S., 2003. Species-Specific Assessment of Pain in Laboratory Animals. J Am Assoc Lab Anim Sci, 42, 13-20.

Taylor, P. M., Delatour, P., Landont, F. M., Deal, C., Pickett, C., Aliabadi, F. S., Lees, P., 1996. Pharmacodynamics and enantioselective pharmacokinetics of carprofen in the cat. Res Vet Sci, 60, 144-151. doi:http://dx.doi.org/10.1016/S0034-5288(96)90009-0

Terrill, T. H., Douglas, G. B., Foote, A. G., Purchas, R. W., Wilson, G. F., Barry, T. N., 1992. Effect of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla ( Hedysarum coronarium) and perennial pasture. J Agric Sci, 119, 265-273. doi:doi:10.1017/S0021859600014192

Thorhallsdottir, A. G., Provenza, F. D., Balph, D. F., 1990. Ability of lambs to learn about novel foods while observing or participating with social models. Appl Anim Behav Sci, 25, 25-33. doi:10.1016/0168-1591(90)90066-m

Vercruysse, J., Claerebout, E., 2001. Treatment vs non-treatment of helminth infections in cattle: defining the threshold. VetParasitol, 98, 195-214.

Villalba, J. J., Provenza, F. D., 2001. Preference for polyethylene glycol by sheep fed a quebracho tannin diet. J Anim Sci 79, 2066-2074.

Villalba, J. J., Provenza, F. D., 2007. Self-medication and homeostatic behaviour in herbivores: learning about the benefits of nature's pharmacy. Animal, 1(9), 1360-1370.

Villalba, J. J., Provenza, F. D., 2009. Learning and Dietary Choice in Herbivores. Rangeland Ecology and Management, 62(5), 399-406. doi:http://dx.doi.org/10.2111/08-076.1

Villalba, J. J., Provenza, F. D., Catanese, F., Distel, R. A., 2015. Understanding and manipulating diet choice in grazing animals. Animal Production Science, 55, 261-271. doi:http://dx.doi.org/10.1071/AN14449

Villalba, J. J., Provenza, F. D., Hall, J. O., 2008. Learned appetites for calcium, phosphorus, and sodium in sheep. J Anim Sci, 86, 738-747.

Villalba, J. J., Provenza, F. D., Hall, J. O., Lisonbee, L. D., (2010). Selection of tannins by sheep in response to gastrointestinal nematode infection. Journal of Animal Science, 88, 2189-2198. doi:10.2527/jas.2009-2272

Villalba, J. J., Provenza, F. D., Shaw, R., 2006. Sheep self-medicate when challenged with illness-inducing foods. Anim Behav, 71, 1131-1139. doi:10.1016/j.anbehav.2005.09.012

Wallisdevries, M. F., 1996. Nutritional Limitations of Free-Ranging Cattle: The Importance of Habitat Quality. J Appl Ecol 33, 688-702. doi:10.2307/2404940

Wang, Y., Douglas, G. B., Waghorn, G. C., Barry, T. N., Foote, A. G., 1996. Effect of condensed tannins in Lotus corniculatus upon lactation performance in ewes. J Ag Sci 126, 353-362. doi:doi:10.1017/S0021859600074918

Weary, D. M., Niel, L., Flower, F. C., Fraser, D., 2006. Identifying and preventing pain in animals. Appl Anim Behav Sci 100, 64-76. doi:http://dx.doi.org/10.1016/j.applanim.2006.04.013

Weeks, H. P., Kirkpatrick, C. M., 1976. Adaptations of White-Tailed Deer to Naturally Occurring Sodium Deficiencies. J Wildl Manag 40(4), 610-625. doi:10.2307/3800555

Welsh, E. M., Baxter, P., Nolan, A. M., 1992. Pharmacokinetics of carprofen administered intravenously to sheep. Res Vet.Sci 53, 264-266. doi:http://dx.doi.org/10.1016/0034-5288(92)90123-J

Wiles, G. J., Weeks, H. P., 1986. Movements and Use Patterns of White-Tailed Deer Visiting Natural Licks. J. Wildl. Manag 50, 487-496. doi:10.2307/3801111

Wink, M., 1988. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theor Appl Genet 75, 225-233. doi:10.1007/BF00303957

# Chapter 6

# Can lambs in pain identify medicated feed?

## 6.1. Introduction

In experimental settings sheep have been shown to have the ability to learn to self-medicate (Chapter 5). There has been extensive research conducted previously with sheep on their ability to learn to self-medicate for parasitic infection (Villalba *et al.*, 2010; Fishpool *et al.*, 2012; Juhnke *et al.*, 2012) as well as other internal pain not caused by parasites (Provenza *et al.*, 2000; Villalba and Provenza, 2001; Villalba *et al.*, 2006). If sheep can learn to self-medicate, then their choice to ingest medications that are non-addictive is a strong indicator that the animal is motivated to alleviate a negative affective state. One of the methods used to determine if an animal has learnt to self-medicate is through preference testing. In preference testing, animals are offered choices and their relative choices indicate preferences. For example, the animal is provided with a specific situation/resource (bedding type) and given a variety of options (bare flooring, hay or woodchips) and they are allowed to essentially "vote with their feet" as to which of these they prefer (Duncan, 1992). In this case their preference would be the bedding type that they spend the most time on or interacting with.

Preference testing could also potentially be used to detect pain in an animal if the animal can associate the preference with pain relief. For instance, in a scenario where an animal selects a non-addictive analgesic when it is given a choice of a normal feed and a feed containing an analgesic, this could be indicative that the animal is in pain. For the animal to have the ability to make such a choice, it must first experience the consequences (whether positive or negative) of the feed options available to it. An individual animal is likely to learn the benefit of a substance through trial-and-error and by pairing the association of the behaviour and consequence together (Provenza, 1987; Villalba and Provenza, 2007). The trial-and-error experience can be created by including a conditioning period prior to the preference test and there is evidence that this conditioning is effective in rats suffering from chronic pain (Colpaert *et al*., 2001). There is little work to see if conditioning is effective in situations of chronic pain in lambs following castration and tail-docking (Rennie, 2004).

Chronic pain is common in young lambs that are castrated and tail-docked using rubber rings. The use of rubber rings for castration and tail-docking and have been shown to cause an acute pain phase lasting 4 h (Lester *et al*., 1996; Kent *et al*., 2000) as well as causing a chronic pain phase that can last for up to 35 days (Mellema *et al*., 2006; Melches *et al*., 2007). Several analgesics have been shown to alleviate pain associated with castration and tail-docking including bupivacaine (Graham *et al*., 1997), naloxone (Wood *et al*., 1991) and non-steroidal anti-inflammatory drugs (NSAID) (Graham *et al*., 1997; Paull *et al*., 2012) such as flunixin.

Flunixin is a potent NSAID and has been shown to reduce pain-related behaviours in lambs that have undergone mulesing (Paull *et al*., 2007) as well as reducing inflammation and pain in lambs that have undergone surgical castration and tail-docking (Chapter 4). It is commonly used in veterinary medicine for its anti-inflammatory, analgesic and antipyretic properties and, like other NSAIDs it reduces inflammation by inhibiting cyclooxygenase and, in turn, decreasing the production of prostaglandins

(Cheng *et al*., 1998). Flunixin is reported to be more potent as an analgesic than codeine in rats and has been shown to be a comparable analgesic to morphine in primates; however, animals receiving flunixin do not develop a tolerance to the analgesic action like they do to codeine and morphine (Ciofalo *et al*., 1977).

The objectives of the present study were to identify if lambs that experience acute and chronic pain after ring castration and ring tail-docking could identify, as indicated through preference testing, a flunixin medicated food. Further, we predict that that the preference should disappear when the animal is no longer in pain.

## 6.2. Methods and Materials

A method of castrating and tail-docking lambs on separate days to produce two acute pain phases as well as demonstrate chronic pain was previously tested as part of developing the methods for this self-medication experiment (Appendix 2; ARA 14/31). The current experiment was undertaken at CSIRO's FD McMaster Laboratory, Armidale, New South Wales (NSW), Australia. The protocol and conduct of the experiment was approved by The CSIRO Chiswick Animal Ethics Committee under the NSW Animal Research Act, 1985 (approval ARA 15/09). Lambs were ear tagged at birth and ewe-lamb pairs allocated to two cohorts, cohort being based on birthdate. Prior to the study ewes and lambs were exposed to the food pellets that that were to be used later in the experiment, through daily feeding for 1 week whilst in the paddock.

### 6.2.1. Self-medication method

#### *Acclimation period*

At 7-8 weeks of age, 36 male Merino lambs were checked for health, drenched with 2 mL of Zolvix (Novartis Animal Health, Australia), 2 mL of Flukazole C (Virbac, Australia) and vaccinated with Glanvac 6S B12 (Zoetis, Australia) prior to being moved into an animal house with their mothers. In the animal house the animals became accustomed to indoor housing and to a standard pelleted ration (Ridley Agriproducts, Australia; 17% crude protein dry matter; 9.04 MJ/kg dry matter) *ad libitum*. Throughout the experiment water was available *ad libitum*. At 9 - 10 weeks old, ewe-lamb pairs were moved into individual pens (2.5m x 1.5m), which were in close proximity to allow visual and social interaction with other experimental animals (Figure 8). Lambs were exposed to two artificial liquid odours, strawberry and banana (IMCD, Australia, diluted with water to 0.15%) that were later used as cues. The odours were sprayed onto the pellets (10 mL per kg of feed), the odour that was added to the feed was alternated between days. These odours had been previously identified in a separate preference test (Appendix 3). The lambs were weaned from their mothers at 10-11 weeks of age.

#### *Odour preference*

After weaning, lambs were tested for their preference of the two odours during the week prior to experimental treatments; this was done to determine their base preference to be used for comparison of their choice during the self-medication test. Feed was placed into an 11 L feed bucket;

lambs were given two buckets each containing 800 g of pellets and 200 g of chaff. The feed was sprayed with a 10 mL volume of an allocated odour (diluted with water to 0.15%) (Figure 9). Lambs were offered both feed buckets simultaneously between 08:00 and 09:00 h for four days. The location (left or right) of the odours (strawberry and banana) were alternated each day, and the feed was removed 12 h after offer and weighed. During this period lambs were also handled for 2 min once a day to reduce subsequent handling stress. Four of the lambs were removed from the study due to ill health and having reactive temperaments, hence 32 of the 36 lambs from the odour preference test were used the self-medication training.


Figure 8: Lambs were placed in individual pens next to other lambs, where they were able to interact with each other.


Figure 9: A lamb in its individual pen, selecting feed from one of the two feed buckets on offer.

### *Treatment allocation*

Lambs were blocked by weight and randomly allocated within block randomly allocated to a treatment. The two treatments were:

- Ring castrated day 0 and tail-docked day 7 (Ring)
- Sham castrated day 0 and sham and tail-docked day 7 (Sham)

The treatments were then allocated to subsequent sub groups, ensuring that the odour used for the medicated feed was evenly divided between the treatment groups (Figure 10).

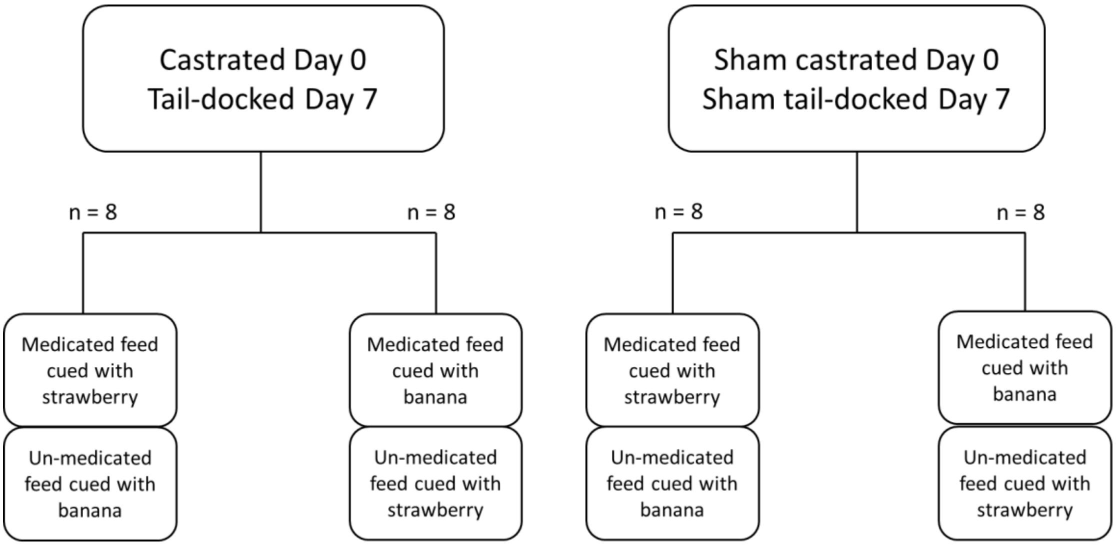



Figure 10: Diagrammatic representation of the experimental design indicating the allocation of groups and subgroups during the experiment.

*Conditioning period*

Lambs were either ring castrated or sham handled as if they were to be castrated on day 0, and the treatments were spaced to occur every 2 min. During the conditioning phase lambs were only presented with one bucket of feed which contained the medicated feed with their assigned odour cue. Feed was prepared in the bucket with which the animals were presented and contained; 600 g of pellets and 200 g of chaff mixed. The amount of feed on offer was just below recommended requirements to ensure lambs could consume all feed on offer. Lambs were weighed the day before castration to calculate a correct medication dosage of flunixin based on 4.0 mg/kg. Liquid flunixin (10 mg/ml, BOVA, Australia) was then applied directly to the pellets using a syringe and mixed through the feed, incorporation being identified by the change in pellet colour. The odour corresponding to the allocated treatment (Fluxinin + cue odour) was then added to the feed; using a spray bottle, 10 mL was applied and also mixed through. At 30 min after castration lambs were offered the bucket of feed. This procedure occurred also on days 1 to 3 post-treatment. The feed bucket was placed in the same location each day, with location (L or R) alternated between pens. On days 4 to 6, lambs were given one bucket with pellets and chaff without odour or flunixin to ensure that they would not have therapeutic levels of flunixin (Chapter 2 and 3) when they were tail-docked.

### *Self-medication period*

On the treatment day for tail-docking (day 7), lambs were weighed so the dosage of flunixin could be adjusted. Lambs that were not to be tail docked (Sham) were handled as if they were to be tail-docked and the treated lambs had rings placed on the 3$^{rd}$ palpable joint of the tail (Ring), treatments were again spaced to occur every 2 min.

Thirty minutes following treatment all lambs were then offered a choice of both the feeds with the odours (medicated 1200g and non-medicated 1200g). The amount of feed made available in each container was above the lambs' full daily allowance and the amount offered had to be more than recommended requirements as lambs readily consumed 1000g of feed. The bucket containing the medicated feed was placed in the same location in the pen as during the conditioning phase.

To receive their full dose of flunixin, lambs had to consume all of their allowance from the medicated feed container. Feed was prepared as described for the conditioning period, making sure the medicated feed contained the odour to which the lambs had been conditioned. For 5 consecutive days following tail-docking, the residual feed was weighed at 1 h and at 12 h after offering to obtain individual intakes of both feeds. Both feed troughs were removed at 12 h, nothing else was offered.

After the self-medication test, lambs were treated with CLiKZiN (Novartis, Australia) for fly control and returned to the paddock. After 5 weeks lambs re-entered the animal house and were retested for their preference of each odour (without flunixin) for five days using the same method as in the odour preference phase. The amount of feed offered to lambs was increased to 1400 g per bucket (1200 g of pellets + 200 g of chaff) to account for their higher bodyweight. The location of the feed with the cue for medicated feed was again placed in the same location as during conditioning and the self-medication test. A summary of the different experimental phases is presented in Figure 11.

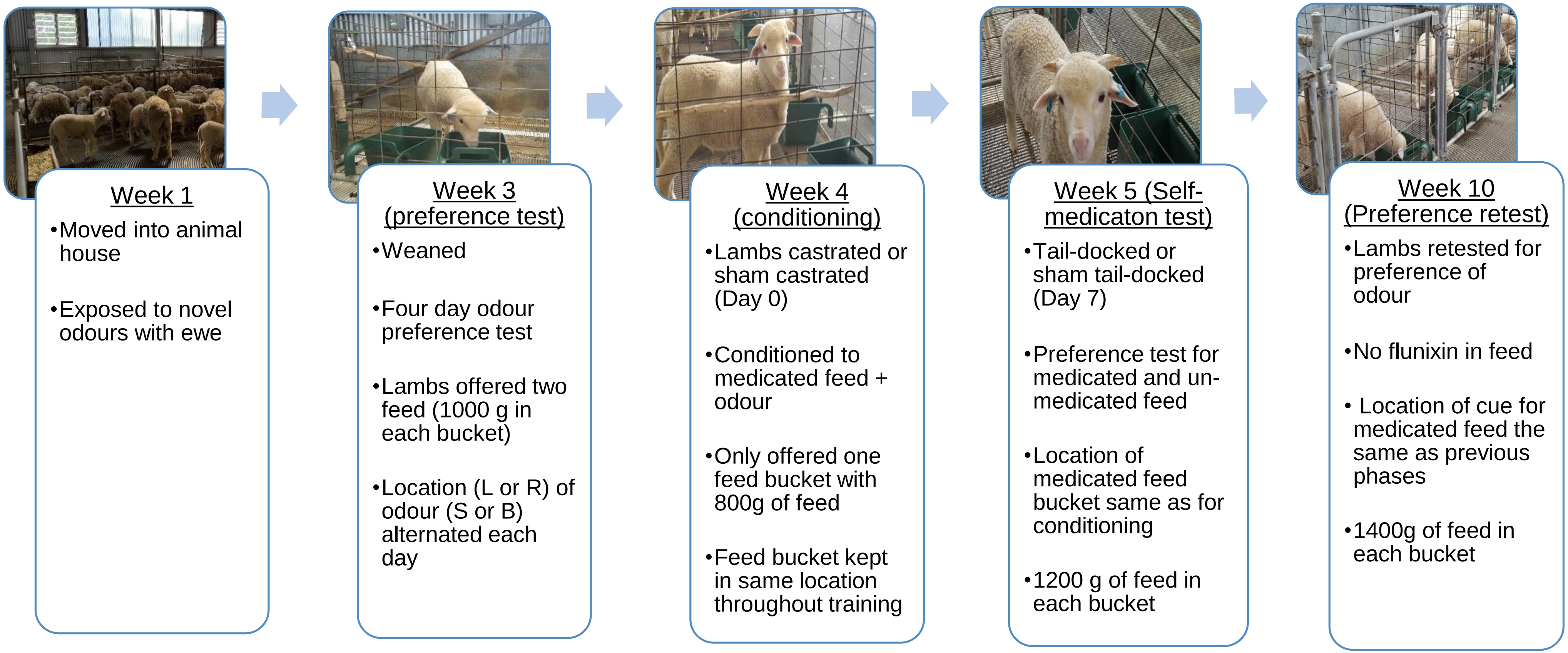



Figure 11: Summary of the experimental phases lambs underwent during the experiment

### *6.2.2. Measurements*

#### *Behaviours*

Video cameras were used to continuously record the behaviour of lambs in the study. Five cameras were mounted on roofing rafters to record 3 or 4 of the pens. Each camera provided a view of the entire area available to the lambs. The cameras were connected to digital video recorders and captured by IVMS4200 software from Hangzhou Hikvision Digital Technology Co., Ltd (Hangzhou, China). The behaviour of the lambs in their pens on day 0 to 3 and day 7 to 10 were collated from the digital video records by observation of a replay of the video using The Observer software (Noldus, The Netherlands). The behaviours recorded were the same as that for the pen castration (Chapter 4, Table 7). Behavioural assessment was undertaken by a single operator, who was blinded to the animals' treatment. The pain avoidance behaviours were measured continuously for 1 min and took place every 5 min during the first 90 min post treatment (day 0 and day 7). Postural behaviours were scan sampled every 15 min for 12 h on the days of treatment as well as on days 1 to 3 and days 8 to 10. Observation time points were synchronized to each lamb's individual treatment time.

#### *Blood parameters*

Blood was collected via the jugular vein using 21 gauge needles into 10 mL vacutainers containing EDTA. Individual blood samples were collected at 0 h, 30 min, 6 h and 12 h on the day of treatment (day 0 and day 7) and then every morning (08:00-09:00 h) up to 72 h post-treatment. Neutrophil and lymphocyte counts in whole blood were determined with an automated haematology analyser (Cell Dyn 3500R, Abbott Diagnostics, Illinois U.S.A). The blood samples were then centrifuged at 2000 × g for 15 min at 5°C and plasma were separated into three aliquots which were then stored at -20°C until assayed for cortisol concentration. Plasma cortisol concentrations were determined using a commercial radioimmunoassay (Plasma Cortisol RIA, MP Biomedical, Australia) which has been previously adapted and validated for ovine plasma (Paull *et al*., 2007). Coefficients of variation on the quality control plasma samples (50.3, 101.1, 211.7 nmol/L cortisol) were 8.48, 9.85, 7.28% for intra-assay and 11.2, 10.46 and 10.35% for inter-assay, respectively.

At the time of blood sampling (from 30 min onwards) the lambs also had their wounds palpated as previously described (Melches *et al*., 2007) and their behavioural response recorded as a score from 0 = no response, 1 = wincing to 2 = struggling, attempt at escape. The lambs were weighed on Day -1, 7, 14, 21, 28 and 35 relative to day 0 (day of castration).

### *6.2.3. Statistics*

All data were analysed using R (R Development Core Team, Boston, Massachusetts) and the packages *nlme* (Pinheiro *et al*., 2015) and *pscl* (Zeileis *et al*., 2008) were used. Data were tested for normality using the Shapiro-Wilk test and visual inspection of residual plots. In all instances $P < 0.05$ was considered statistically significant and $0.1 > P > 0.05$ was considered a statistical tendency, results are presented as mean ± SEM. Lamb 8454 from the Ring treatment group was removed from analysis for the self-medication test and excluded from week 5 preference testing as he was removed during the self-medication test due to being lethargic and not eating.

### *Behaviours*

Active pain avoidance behaviour had to be combined and required the use of a zero inflated Poisson model due to a high number of zeros in the data. Postural behaviour (as a proportion) was analysed using a repeated measures analysis, fitting the main effects of treatment, time and potential interactions and fitting lamb as a random variable. Postural data analysed following castration were normal lying, abnormal lying and total standing. Total standing was analysed using a Kruskal-Wallis test. For tail docking, postural behaviour was analysed for abnormal lying, normal lying and normal standing.

### *Blood parameters and additional measurements*

Bodyweight, cortisol and neutrophil/lymphocyte ratio were analysed using a repeated measures analysis, fitting the main effects of treatment, time and potential interactions. The bodyweight of castrated animals was also analysed separately to test the effect of medicated feed preference on weight gain. For neutrophil/lymphocyte ratio and cortisol, pre-treatment values (time 0 h) were fitted as a covariate when significant and animal was fitted as a random effect. Wound palpation scores were analysed using a general linear model with a Poisson distribution with the frequency counts as the outcome. Neutrophil/lymphocyte ratio required a log transformation and cortisol required a square root transformation.

*Feed preference analysis*

Feed intake was recorded daily at 12 h (20:00-21:00 h) after lambs were first offered their feed and during the self-medication phase feed intake was also recorded at 1 h after being offered (9:00 - 10:00 h). The calculation, for feed preference used in this study has been as described by Bell (1959). Preference Indices are more sensitive than the individual intake of lambs (as intake can vary across days) and preference index have been used in several studies (Mirza and Provenza, 1990; Provenza *et al*., 1990; Ngwa *et al*., 2000; Omokanye *et al*., 2001). However, the animal's intake (g) was also analysed. To allow for calculation, 5 g of feed was included to the preference index calculation for instances where lambs did not consume any of the feed, 5 g was chosen as it was lower than the lowest intake recorded (12 g). The preference was calculated by:

$$\frac{(Intake\ of\ medicated\ feed + 5)}{(Intake\ of\ medicated\ feed + 5) + (Intake\ of\ un-\ medicated\ feed + 5)}$$

Lamb feed choice (tested as feed intake and preference index) were analysed using a repeated measure. The main effects that were fitted, where appropriate were treatment (Ring or Sham), feed type (medicated or un-medicated) the location of the feed bucket (left or right), odour cue (strawberry or banana), and day. When significant the initial preference for the medicated cue was fitted as a covariate for the self-medication phase and week 5 data and lamb was fitted as a random effect. Feed intake data obtained during the self-medication phase in the first hour and for week 5 was not able to be normalised and was analysed using a Kruskal-Wallis test. Intake of flunixin (mg) was also calculated for each gram of feed consumed.

## 6.3. Results

### 6.3.1. Week 1 Odour preference

When analysing the feed intake of all animals, there was no odour (banana vs strawberry, $P$ = 0.58) or location (left or right, $P$ = 0.76) or day ($P$ = 0.96) effect on the consumption of feed (Figure 12). When analysing the preference index of the cue to be used for medicated feed there was no effect of odour ($P$ = 0.63), location ($P$ = 0.65) or day ($P$ = 0.43, Figure 13).

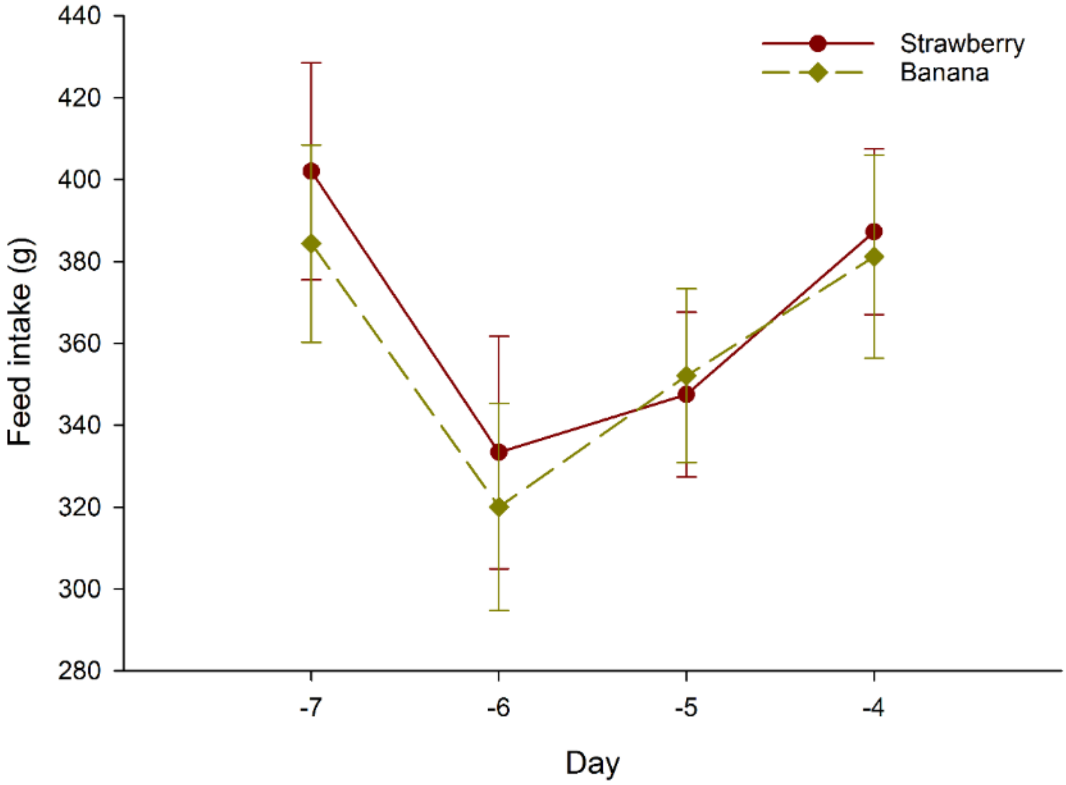



Figure 12: The mean intake of the feed containing the two odours (strawberry and banana) calculated from the lambs that were included in the conditioning and self-medication phases (n = 32). Days are in relation to the first treatment day (castration at day 0).

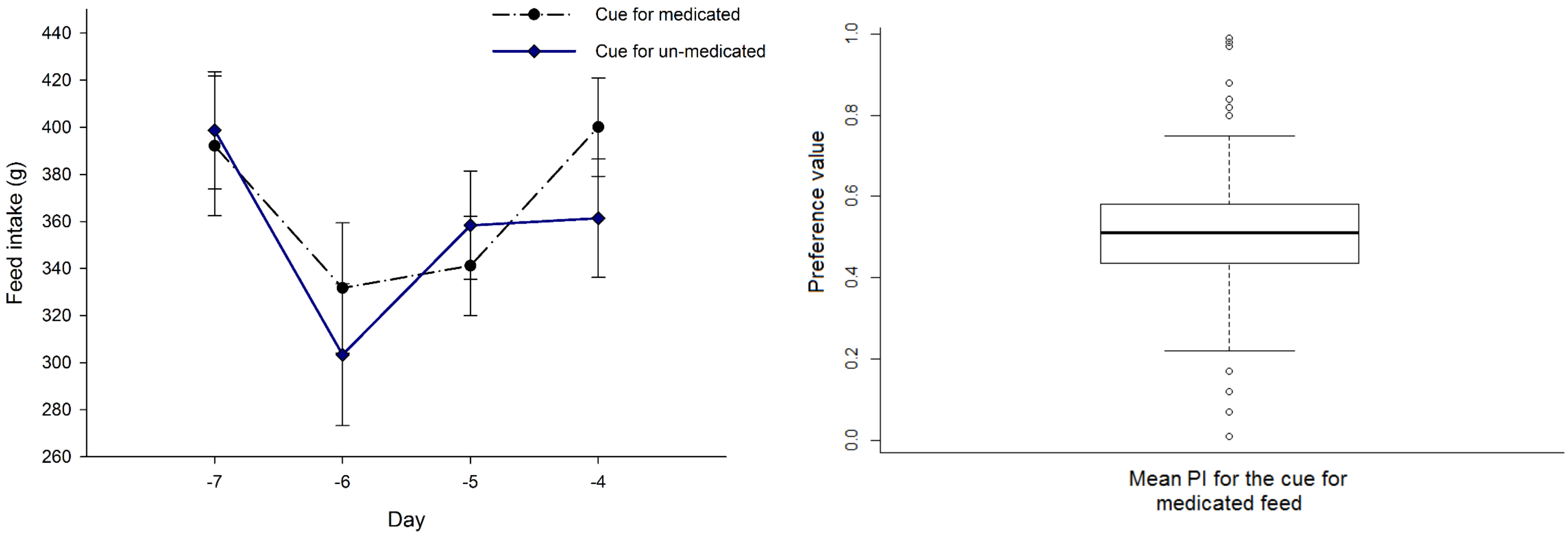



Figure 13: The mean intake of feed containing what was assigned as the cue for medicated feed and cue for un-medicated cue (left) as well as the preference index (PI) (median, 95 % CI and outliers), for the cue for medicated feed (right), calculated from the lambs that were included in the conditioning and self-medication phase (n = 32). Days are in relation to the first treatment day (castration at day 0)..

### *6.3.2. Conditioning Phase*

All lambs consumed all of the feed on offer each day throughout the week, except for one lamb who on day 2 only consumed 200 g. In the 90 min following treatment, lambs that were castrated displayed more active pain behaviours (mean =15.1) than sham lambs (mean = 0.4, $P$ < 0.05). For abnormal lying, Ring lambs displayed a higher proportion of abnormal lying on the day of treatment (day 0, 34 ± 3 %) compared with Sham lambs (24 ± 4 %, $P$ = 0.02). The display of abnormal lying was also affected by day ($P$ = 0.001), with ring lambs displaying less abnormal lying on day 2 and 3 (17 ± 4%, $P$ < 0.05) compared with treatment day. Sham lambs also displayed a lower proportion of abnormal lying on days 1 and 3 (17 and 16 ± 3%, $P$ = 0.04) compared with treatment day. There was no interaction between time and treatment ($P$ = 0.23) on abnormal lying. For normal lying only a day effect was seen ($P$ <0.001), Ring lambs exhibited normal lying more often on days 1 (27 ± 4 %), 2 (35 ± 4 %), and 3 (32 ± 4 %) compared with the treatment day (16 ± 3 %). Sham lambs exhibited more normal lying on days 2 (32 ± 3 %) and 3 (28 ± 3 %) compared with the treatment day (20 ± 3 %). Treatment had an effect on the display of normal standing ($P$ = 0.04). Lambs in the Ring group stood less ($H$ (1) = 4.01, $P$ = 0.04) than Sham lambs (*difference* = 13.0), the critical difference was 13.09.

Following castration there was a time by treatment effect ($P$ = 0.02) for the neutrophil/lymphocyte ratio between Ring and Sham lambs. There was also a time effect, with Ring lambs having a higher neutrophil/lymphocyte ratio at 6, 24, 48 and 72 h compared to their baseline

($P < 0.05$). Sham lambs also had an increase in neutrophil/lymphocyte ratio at 12 and 48 h compared to baseline ($P < 0.05$). At 72 h post treatment Ring lambs still had a higher mean neutrophil/lymphocyte ratio than Sham lambs (Figure 14).

There was an interaction between time and treatment on cortisol concentration ($P = 0.053$) in addition to a significant time effect ($P < 0.001$). Thirty minutes following treatment Ring lambs had a significant increase in cortisol concentration (transformed mean = 3.5, back transformed = 12.25 nmol/L, $P < 0.001$) at 30 min compare to baseline. Ring lambs had higher cortisol concentrations at 30 min compared to Sham lambs (transformed mean = 8.3 back transformed = 68.89 vs transformed mean = 7.25 back transformed = 56.64 nmol/L respectively, $P <0.001$). At 6, 12, 24 h Ring lamb's cortisol concentrations returned to baseline but they experienced a significant rise again which was higher than baseline concentrations at 48 h ($P = 0.02$) and 72 h ($P = 0.001$). Sham lambs experienced no change in cortisol concentration (Figure 15).

Treatment had an effect on lambs' reaction to palpation ($P = 0.001$) with more Ring lambs reacting (mean = 5) compared to Sham lambs (mean = 2). There was no time effect ($P = 0.16$).

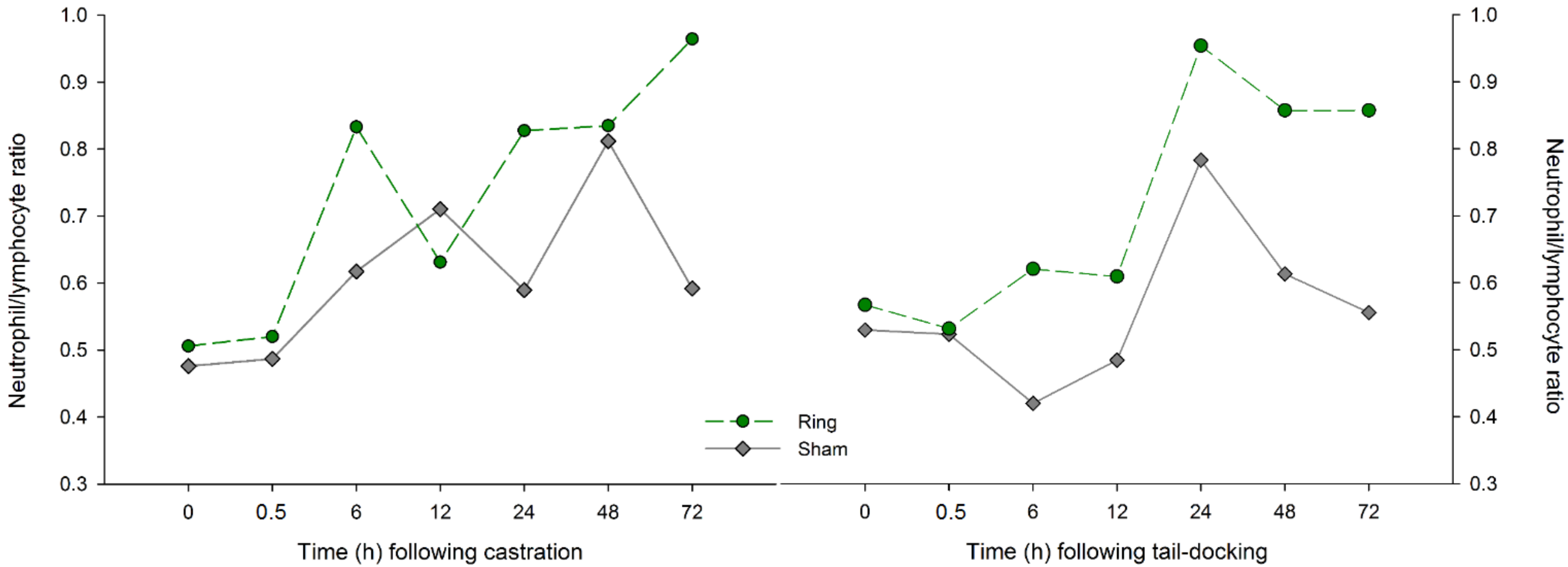



Figure 14: Raw data of the mean neutrophil lymphocyte ratios (10*6/mL) for lambs in the Sham (n =16) and Ring (n =16) treatment groups following castration and tail-docking

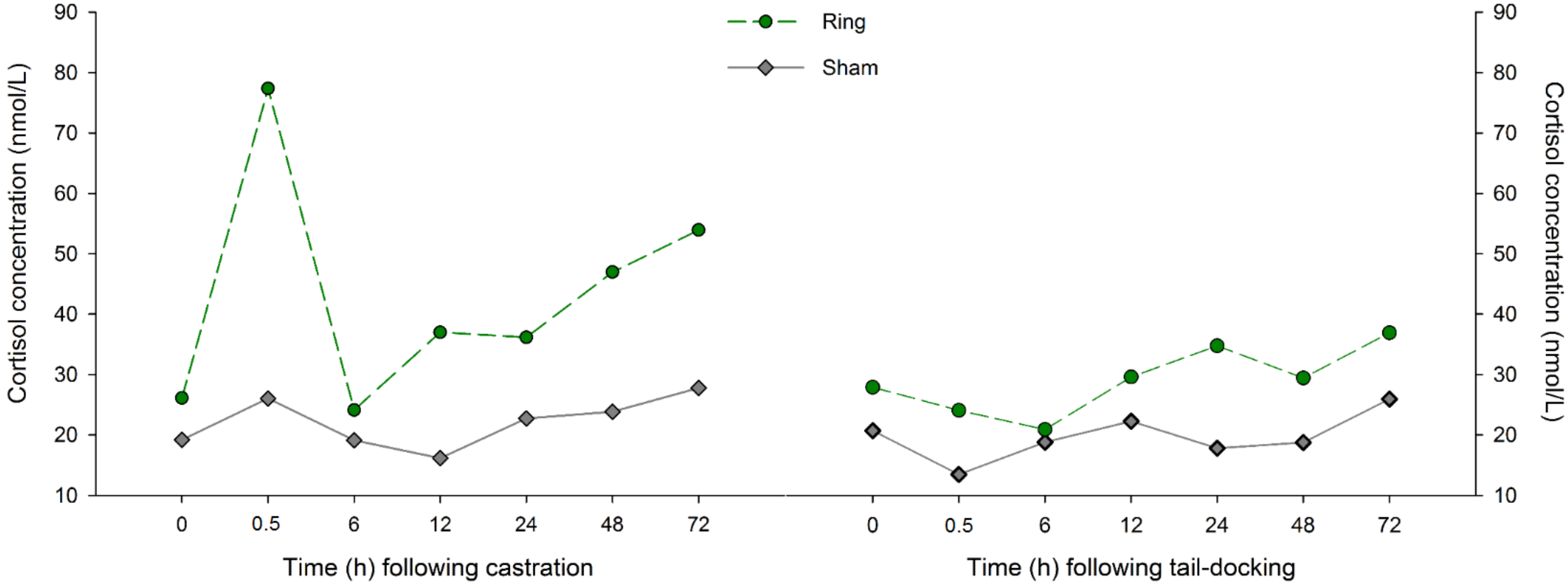



Figure 15: Raw data of the mean plasma cortisol concentrations (nmol/L) for lambs in the Sham (n =16) and Ring(n =16) treatment groups following castration and tail-docking

### 6.3.3. Self – medication phase

In the 90 min following tail-docking Ring lambs showed significantly more active pain behaviours (mean = 5.9) than sham lambs (mean = 0.4, $P < 0.001$). There was no overall treatment difference in the display of abnormal lying ($P = 0.44$), however, there was a significant time by treatment effect on abnormal lying ($P = 0.02$), with lambs in the Sham group displaying more abnormal lying on day 8 (24 %) and 9 (23 %) compared with day 7 (13%). There was no difference between treatment groups for normal lying ($P = 0.20$) and normal standing ($P = 0.12$).

In the first hour of being offered the feed, Ring lambs consumed on average 121.3 ± 13.5 g and Sham lambs consumed 109.5 ± 16.5 g of medicated feed. Ring lambs consumed 161.6 ± 19.9 g and Sham lambs 168.3 ± 15.9 g of un-medicated feed. The mean intake of medicated feed in the first hour in Ring lambs corresponded to an intake of approximately 10 ± 1% of the lambs flunixin dose. Only one lamb consumed 1% of its flunixin dose on average in the first hour (Table 11). There was no difference in PI between Ring and Sham lambs in the first hour ($P = 0.18$).

For the 12 h feed intake there was no effect of feed location ($P = 0.75$), or day ($P = 0.75$) and there was no difference between Ring and Sham lambs' intake of feed ($P = 0.61$). The cue odour strawberry influenced feed intake ($P < 0.05$), with strawberry odoured feed having 149.2 ± 25.5 g extra pellets consumed. At 12 h Ring lambs consumed on average 516.7 ± 44.6 g and Sham lambs consumed 489.7 ± 48.9 g of medicated feed. Ring lambs consumed 469.7 ± 48.6 g and Sham lambs 518.5 ± 40.0g of un-medicated feed. Ring lambs on average consumed 43 ± 3% of their flunixin dose (Table 11). There was no effect of day ($P$ = 0.88), or treatment ($P$ = 0.98) or an interaction of day by treatment ($P$ = 0.84) for PI (Figure 16). Location approached significance ($P$ = 0.07) and there was a location by treatment effect with PI increasing by 0.14 for Sham lambs that had medicated feed located on the right. The covariate was also significant ($P$ = 0.001).

Table 11: Mean approximate percentage dose of flunixin consumed by lambs in the Ring group (n = 15) at 1 h and 12 h during the self-medication phase.

| Variable | 1 h | 12 h |
|---|---|---|
| Mean intake (%) ± S.D. | 10 ± 1 % | 43 ± 3 % |
| Median (%) | 10 % | 49 % |
| Range (5) | 1 – 18 % | 12 – 66 % |

For neutrophil/lymphocyte ratio following tail-docking there was a significant time by treatment interaction ($P$ = 0.02) and treatments approached significance ($P$ = 0.064). Sham lambs experienced an increase in neutrophil/lymphocyte (transformed = 3.49, back-transformed=1.25 ($10^{*}6$/mL), P = 0.02) at 24 h compared with baseline, while Ring lambs had a significantly higher ($P$ < 0.05) neutrophil/lymphocyte ratio compared with baseline at 24, 48 and 72 h following treatment (Figure 14).

There was no effect of time ($P$ = 0.37), treatment ($P$ = 0.33) or their interactions ($P$ = 0.75) on cortisol concentrations for both Ring and Sham lambs (Figure 15). For the palpation scores there was a significant treatment effect ($P < 0.001$), more lambs in the Ring group reacted to palpation (mean = 8.3) compared to Sham (mean = 1.5).

### 6.3.4. Week 5 preference

Overall there was no difference in the intake of medicate or un-medicated feed ($P = 0.96$), there was no treatment ($P = 0.41$), odour ($P = 0.80$) or location effect ($P = 0.45$) on lamb's feed intake. There was a day effect with lambs consuming less feed on day 46 compared to days 47 to 50 ($\alpha = 0.05$, *difference* = 40.20). There was no difference between treatment groups and PI of the medicated odour cue ($P = 0.95$, Figure 17).

### 6.3.5. Weight gain

The lambs' weight gain was not affected by treatment (Ring or Sham, $P = 0.28$). For the animals that were castrated there was no effect of their PI for medicated feed on weight gain ($P = 0.50$).

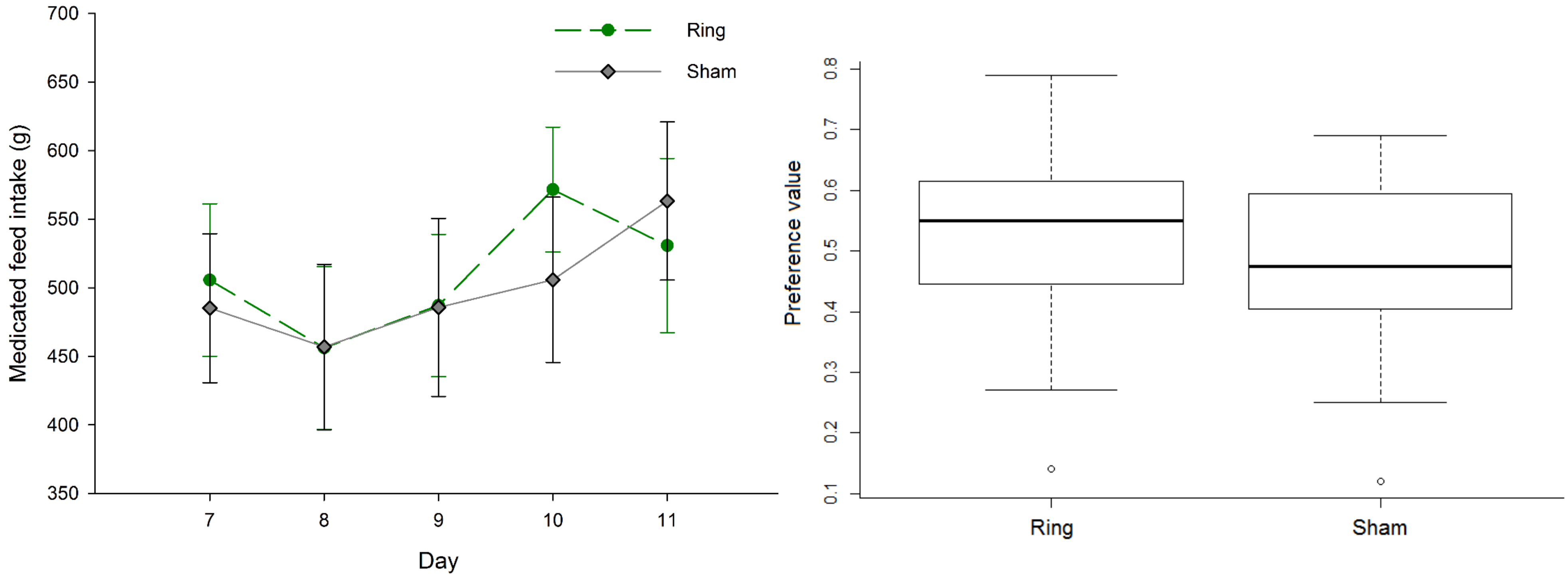



Figure 16: The mean medicated feed intake (± SEM) (left) as well as the PI for medicated feed (right; median, 95% CI and outliers) for Ring (n = 15) and Sham lambs (n = 16). Days are in relation to the first treatment day (castration at day 0) tail-docking occurred on day 7.

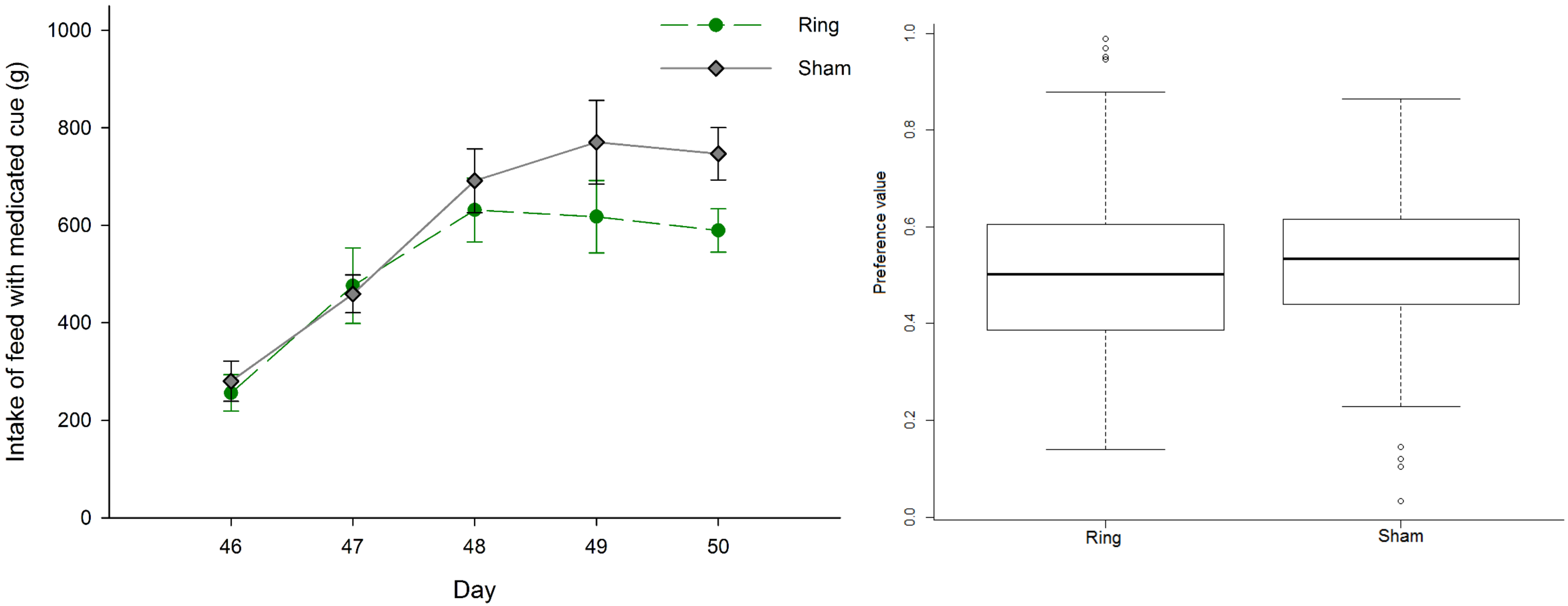



Figure 17: The mean intake (± SEM) of feed with the medicated cue (left) as well as the PI for medicated cue (left) for Ring (n = 15) and Sham lambs (n = 16). Days are in relation to the first treatment day (castration at day 0).

## 6.4. Discussion

Ring animals experienced pain in response to both castration and tail-docking as evidenced by the increased amount of active pain avoidance behaviours and increased reaction to wound palpation in comparison to Sham lambs. Neutrophil/lymphocyte ratio increased for both treatment groups but tended to be higher in castrated and tail-docked lambs. However, Ring lambs did not show an increase in abnormal postures following ring tail-docking and did not experience a significant increase in cortisol concentration following treatment as they did for castration. There was also no indication that the lambs in the Ring group were able to self-medicate their pain through preference for medicated feed.

As lambs that were in the Ring group displayed more active pain behaviours than Sham lambs following both castration and tail-docking, it can be assumed that they were experiencing pain before they were given access to feed containing flunixin. During the conditioning phase all lambs consumed their entire dose of flunixin (except for one lamb on day 2) so we would have expected that all lambs would have experienced pain alleviation.

Previous work (Chapter 3) has indicated that lambs would be required to eat at least 22 g of the medicated feed in 10 min for flunixin to become first detectable in their blood and continued consumption of medicated feed would lead to therapeutic concentrations in their blood within 2 h (Marini *et al.*, 2016). During the self-medication phase lambs consumed on average just over 100 g in the first hour (approximately 10% of their flunixin dose) and so there may have been a delay in obtaining pain relief. To reach and to maintain therapeutic levels lambs would have had to have eaten most of their medicated feed in the 8-12 h following tail-docking (Marini *et al*., 2016). In the study Marini *et al*., (2016) ewes that consumed their full dose of flunixin (4.0 mg/kg) over 12 h reached slightly higher than inferred therapeutic concentrations (1.78 ± 0.48 μg/mL). There is no work on the therapeutic concentration of flunixin in sheep however therapeutic effects are seen in horses when plasma concentrations reach 0.2 - 0.9 μg/mL (Toutain *et al*., 1994). Lambs in the Ring group consumed on average 43% of their flunixin dose in the 12 h that they had access to the medicated feed; this may suggest that lambs may not have reached therapeutic concentrations of flunixin. While plasma was collected in this study, the drug concentrations in the plasma were not analysed, the information from the pharmacokinetics study (Chapter 3, Marini *et al*., 2016), should be applicable to these lambs, as the lambs would have functioning rumens (Boda *et al*., 1962).

Lambs in the Ring group displayed active pain avoidance behaviours and increased reaction to wound palpation after both castration and tail docking. However, following tail-docking Ring lambs did not display postural behaviours indicative of pain. The procedure of tail-docking with rings is known to cause pain in lambs, with lambs displaying more postural behaviours indicative of pain than those who have received pain-relief (Graham *et al*., 1997; Kent *et al*., 1998). The difference seen in the display of abnormal postures in the current study may have been due to age, with the studies by Graham *et al*., (1997) and Kent *et al*., (1998) using lambs aged 5 to 8 days and 3 weeks old, respectively. Lambs in the current study were aged between 12 and 13 weeks when tail-docked. A previous study by McCracken *et al*. (2010) that tail-docked lambs at a separate time to castration reported that the age at which lambs were castrated had an effect on the reaction to tail-docking, with lambs castrated at 1 day old reacting more to subsequent tail-docking than lambs castrated at 10 days of age. It has been suggested that that early life pain experiences increases hyperalgesia in later life (Pattinson and Fitzgerald, 2004). Although lambs in this study experienced ear-tagging within a few hours of birth, the level of pain produced by the procedure is low and not as severe as castration (Grant, 2004). The reduced response to tail-docking in the current study is possibly due to castration and tail-docking occurring at an older age in these lambs and lambs having no prior exposure to severe pain.

Following tail-docking, the lambs in the current study also did not show a cortisol peak. Tail-docking alone has been shown to result in cortisol peaks, with cortisol concentrations remaining elevated for 1 -2 h (Mellor and Murray, 1989; Graham *et al*., 1997; Kent *et al*., 1998). Kent *et al*., (1998) did report a lower cortisol peak in lambs that were only tail-docked (60 nmol/L) compared with lambs that were only castrated (120 nmol/L). In this present study lambs did not experience a peak in cortisol after tail-docking, with Ring lambs having a mean cortisol concentration below 25 nmol/L at 30 min following treatment, which was no different to Sham lambs. It is also possible that the excessive handling experienced by lambs by the time they were tail-docked could have reduced their cortisol response to tail-docking. Gentle handling of lambs is often used in experiments that look at the effects of pain to reduce the lamb's cortisol response being influenced by capture and restraint (Paul, Small, Lee, Palladin, and Colditz, 2012). This reduction of cortisol response in lambs following habituation to handling was demonstrated in Sham lambs in Chapter 4.

Sex has also been shown to effect lambs' response to tail-docking, with male lambs reported to have lower cortisol response to tail-docking at 8 weeks of age compared with female lambs of the same age (Turner *et al*., 2006). It is known that the response to pain in lambs can vary depending on factors such as age and sex (Molony *et al*., 1993; Turner *et al*., 2006; Guesgen *et al*., 2011). The reduced response to the pain of tail-docking compared with that from castration in Ring lambs may have affected the lambs' choice to consume medicated feed.

A majority of the studies that have reported self-medication in animals including sheep is in response to a parasitic infection (Villalba *et al*., 2010; Fishpool *et al*., 2012; Juhnke *et al*., 2012). It is suggested that the animals learn which substance will improve a negative state (whether it be caused by parasitic infection or pain) by trial and error, the animal develops preferences through the interactions of the foods characteristics (odour, flavour and texture) and post-ingestive feedback (Provenza *et al*., 1992; Provenza *et al*., 2006). In the current study, we ensured lambs were experiencing pain induced by ring castration before giving them the opportunity to learn the benefits of a medicated feed. To help the animals learn through post-ingestive feedback the medicated feed was cued with an odour, and the location of the feed bucket which contained the medicated feed was kept in the same location throughout the conditioning period and the self-medication phase. However, the lambs in this study did not display self-medication in response to pain. Perhaps the mechanisms for learning to self-medicate for pain may be different to other negative states such as nutritional deficit and parasitism. The information that an animal receives from post-ingestive feedback goes through the limbic system, where it is processed through the hypothalamus, modifying drive and behaviour to maintain internal homeostasis (Provenza *et al*., 1992; Provenza *et al*., 1998). Whereas information that would be received from a painful stimulus such as tail-docking is processed in the pons, medulla, thalamus (Basbaum and Fields, 1978; Cross, 1994; Steeds, 2013) and the cortex, which is involved in cognitive processing, learning and memory (LeDoux, 1993; Bechara *et al*., 2000; Davidson, 2002; Wiltgen *et al*., 2004). The differences in the feedback system and the difference in the locality of the feedback may impact on the animal's ability to learn that feed they consume containing medication is linked to the relief from pain that they experience.

It has been previously shown that sheep have the ability to learn self-medicate for parasitism, but this study was not able to demonstrate that lambs can self-medicate for a state of pain. There may have been several possibilities as to why lambs were unable to learn to medicate for pain. The learning mechanism to link the experience of pain relief following the consumption of a medicated feed may be far more complicated than the post-ingestive feedback system that helps animal gain nutritional wisdom. Perhaps the feedback between consuming the feed and pain relief may have been to slow for the lambs to make the connection. There may also be the possibility that lambs in the Ring group may not have been experiencing a sufficient level of pain, as the lambs response to tail-docking was less than what has been seen in previous studies. There may have been several factors that contributed to the lambs reduced perception and display of pain following tail-docking. As the lambs in the current study were 12-13 weeks old, they may have had a better capacity to tolerate pain compared with younger lambs. Excessive handling could have reduced the lambs cortisol response to tail-docking or they were possibly receiving mild benefit from flunixin, which would have effected their motivation to preferentially select the medicated feed.

## 6.5. References


Bechara, A., Damasio, H., Damasio, A.R., 2000. Emotion, decision making and the orbitofrontal cortex. Cereb Cortex 10, 295-307.

Bell, R., 1959. Preference thresholds for taste discrimination in goats. J. AgSci 52, 125-128.

Boda, J.M., Riley, P., Wegner, T., 1962. Tissue glycogen levels in relation to age and some parameters of rumen development in lambs. J Anim Sci 21, 252-257.

Cheng, Z., Nolan, A.M., McKellar, Q.A., 1998. Measurement of cyclooxygenase inhibition in vivo: a study of two non-steroidal anti-inflammatory drugs in sheep. Inflammation 22, 353-366.

Ciofalo, V.B., Latranyi, M.B., Patel, J.B., Taber, R.I., 1977. Flunixin meglumine: a non-narcotic analgesic. J Pharmacol Exp Ther 200, 501-507.

Colpaert, F.C., Tarayre, J.P., Alliaga, M., Bruins Slot, L.A., Attal, N., Koek, W., 2001. Opiate self-administration as a measure of chronic nociceptive pain in arthritic rats. Pain 91, 33-45.

Davidson, R.J., 2002. Anxiety and affective style: role of prefrontal cortex and amygdala. Biol Psychiatry 51, 68-80.

Duncan, I.J.H., 1992. Measuring Preferences and the Strength of Preferences. Poult Sci 71, 658-663.

Fishpool, F.J., Kahn, L.P., Tucker, D.J., Nolan, J.V., Leng, R.A., 2012. Voluntary intake of a medicated feed block by grazing sheep is increased by gastrointestinal nematode infection. Anim Prod Sci 52, 1136-1141.

Graham, M.J., Kent, J.E., Molony, V., 1997. Effects of four analgesic treatments on the behaviouraland cortisol responses of 3-week-old lambs to tail docking. Vet J153, 87-97.

Grant, C., 2004. Behavioural responses of lambs to common painful husbandry procedures. Appl Anim Behav Sci 87, 255-273.

Guesgen, M.J., Beausoleil, N.J., Minot, E.O., Stewart, M., Jones, G., Stafford, K.J., 2011. The effects of age and sex on pain sensitivity in young lambs. Appl Ani. Behav Sc. 135, 51-56.

Juhnke, J., Miller, J., Hall, J.O., Provenza, F.D., Villalba, J.J., 2012. Preference for condensed tannins by sheep in response to challenge infection with Haemonchus contortus. Vet Parasitol 188, 104-114.

Kent, J.E., Jackson, R.E., Molony, V., Hosie, B.D., 2000. Effects of Acute Pain Reduction Methods on the Chronic Inflammatory Lesions and Behaviour of Lambs Castrated and Tail Docked with Rubber Rings at less than Two Days of Age. Vet J 160, 33-41.

Kent, J.E., Molony, V., Graham, M.J., 1998. Comparison of methods for the reduction of acute painproduced by rubber ring castration or tail docking of week-old lambs. Vet J 155, 39-51.

LeDoux, J.E., 1993. Emotional memory systems in the brain. Behav Brain Res 58, 69-79.

Lester, S.J., Mellor, D.J., Holmes, R.J., Ward, R.N., Stafford, K.J., 1996. Behavioural and cortisol responses of lambs to castration and tailing using different methods. N Z Vet J 44, 45-54.

Marini, D., Pippia, J., Colditz, I.G., Hinch, G.N., Petherick, J.C., Lee, C., 2016. Palatability and pharmacokinetics of flunixin when administered to sheep through feed. PeerJ 4:e1800.

McCracken, L., Waran, N., Mitchinson, S., Johnson, C.B., 2010. Effect of age at castration on behavioural response to subsequent tail docking in lambs. Vet Anaesth Analg 37, 375-381.

Melches, S., Mellema, S.C., Doherr, M.G., Wechsler, B., Steiner, A., 2007. Castration of lambs: A welfare comparison of different castration techniques in lambs over 10 weeks of age. Vet J 173, 554-563.

Mellema, S.C., Doherr, M.G., Wechsler, B., Thueer, S., Steiner, A., 2006. Influence of local anaesthesia on pain and distress induced by two bloodless castration methods in young lambs. Vet J 172, 274-283.

Mellor, D.J., Murray, L., 1989. Effects of tail docking and castration on behaviour and plasma cortisol concentrations in young lambs. Res Vet Sci 46, 387-391.

Mirza, S.N., Provenza, F.D., 1990. Preference of the mother affects selection and avoidance of foods by lambs differing in age. Appl Anim Behav Sci 28, 255-263.

Molony, V., Kent, J.E., Robertson, I.S., 1993. Behavioural responses of lambs of three ages in the first three hours after three methods of castration and tail docking. Res Vet Sci 55, 236-245.

Ngwa, A.T., Pone, D.K., Mafeni, J.M., 2000. Feed selection and dietary preferences of forage by small ruminants grazing natural pastures in the Sahelian zone of Cameroon. Ani Feed Sci Tech 88, 253-266.

Omokanye, A.T., Balogun, R.O., Onifade, O.S., Afolayan, R.A., Olayemi, M.E., 2001. Assessment of preference and intake of browse species by Yankasa sheep at Shika, Nigeria. Small Rumin Res 42, 201-208.

Pattinson, D., Fitzgerald, M., 2004. The neurobiology of infant pain: development of excitatory and inhibitory neurotransmission in the spinal dorsal horn. Reg Anesth Pain Med 29, 36-44.

Paull, D.R., Lee, C., Colditz, I.G., Atkinson, S.J., Fisher, A.D., 2007. The effect of a topical anaesthetic formulation, systemic flunixin and carprofen, singly or in combination, on cortisol and behavioural responses of Merino lambs to mulesing. Aust Vet J 85, 98-106.

Paull, D.R., Small, A.H., Lee, C., Palladin, P., Colditz, I.G., 2012. Evaluating a novel analgesic strategy for ring castration of ram lambs. Vet Anaesth Analg 39, 539-549.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2015. nlme: linear and nonlinear mixed effects models. R package version 3.1-121, URL: http://CRAN.R-project.org/package=nlme.

Provenza, F.D., 1987. Diet learning by domestic ruminants: Theory, evidence and practical implications. Appl Anim Behav Sci 18, 211-232.

Provenza, F.D., Burritt, E.A., Clausen, T.P., Bryant, J.P., Reichardt, P.B., Distel, R.A., 1990. Conditioned Flavor Aversion: A Mechanism for Goats to Avoid Condensed Tannins in Blackbrush. Am Nat 136, 810-828.

Provenza, F.D., Burritt, E.A., Perevolotsky, A., Silanikove, N., 2000. Self-regulation of intake of polyethylene glycol by sheep fed diets varying in tannin concentrations. J Anim Sci 78, 1206-1212.

Provenza, F.D., Pfister, J.A., Cheney, C.D., 1992. Mechanisms of Learning in Diet Selection with Reference to Phytotoxicosis in Herbivores. J.Range Manage45, 36-45.

Provenza, F.D., Villalba, J.J., Bels, V., 2006. Foraging in domestic herbivores: linking the internal and external milieux. Feeding in domestic vertebrates: from structure to behaviour, 210-240.

Provenza, F.D., Villalba, J.J., Cheney, C.D., Werner, S.J., 1998. Self-organization of foraging behaviour: From simplicity to complexity without goals. Nutr Res Rev 11, 199-222.

Rennie, A.E., 2004. Studies of Chronic Inflammatory Pain in Lambs after Rubber Ring Castration and Tail-docking: self-administration of analgesic and neurohistochemistry to validate behavioural assessment, University of Edinburgh, p. 305.

Toutain, P.L., Autefage, A., Legrand, C., Alvinerie, M., 1994. Plasma concentrations and therapeutic efficacy of phenylbutazone and flunixin meglumine in the horse: pharmacokinetic/pharmacodynamic modelling. J Vet Pharmacol Therap. 17, 459-469.

Turner, A.I., Hosking, B.J., Parr, R.A., Tilbrook, A.J., 2006. A sex difference in the cortisol response to tail docking and ACTH develops between 1 and 8 weeks of age in lambs. J Endocrinol 188, 443-449.

Villalba, J.J., Provenza, F.D., 2001. Preference for polyethylene glycol by sheep fed a quebracho tannin diet. J Anim Sci 79, 2066-2074.

Villalba, J.J., Provenza, F.D., 2007. Self-medication and homeostatic behaviour in herbivores: learning about the benefits of nature's pharmacy. Animal 1, 1360-1370.

Villalba, J.J., Provenza, F.D., Hall, J.O., Lisonbee, L.D., 2010. Selection of tannins by sheep in response to gastrointestinal nematode infection. J Anim Sci 88, 2189-2198.

Villalba, J.J., Provenza, F.D., Shaw, R., 2006. Sheep self-medicate when challenged with illness-inducing foods. Anim Behav 71, 1131-1139.

Wiltgen, B.J., Brown, R.A.M., Talton, L.E., Silva, A.J., 2004. New Circuits for Old Memories: The Role of the Neocortex in Consolidation. Neuron 44, 101-108.

Wood, G.N., Molony, V., Fleetwood-Walker, S.M., Hodgson, J.C., Mellor, D.J., 1991. Effects of local anaesthesia and intravenous naloxone on the changes in behaviour and plasma concentrations of cortisol produced by castration and tail docking with tight rubber rings in young lambs. Res Vet Sci 51, 193-199.

Zeileis, A., Kleiber, C., Jackman, S., 2008. Regression Models for Count Data in R. Journal of Statistical Software 27(8). URL http://www.jstatsoft.org/v27/i08/.

# Chapter 7

# Overall Conclusion

Every year lambs undergo several husbandry procedures and it is well accepted that the procedures such as castration, tail-docking and mulesing cause pain to lambs (Wood *et al*., 1991; Molony *et al*., 1993; Molony, *et al*.,1997; Melches *et al*., 2007; Paull *et al*., 2008; Lomax *et al*., 2010; Small, Belson, Holm, and Colditz, 2014). Due to increasing concern for animal welfare (Hughes, 1995; Izmirli and Phillips, 2012), it could be considered a priority that lambs are provided pain relief following painful husbandry procedures.

When pain-relief is provided to lambs it would be done so at the time of the procedure. However, the pain associated with most painful husbandry procedures in sheep may last for several days to weeks (Mellema *et al*.,2006; Melches *et al*., 2007; Tucker *et al*. 2014) and the effectiveness of analgesics used at the time the procedure is performed is limited by their half-lives. Therefore, repeated administration of medications to livestock still experiencing pain would be required to maintain adequate pain relief. There are limitations that effect the provision of pain relief to sheep such as lack of registered analgesics and ease of administration, as anaesthetics and NSAIDs are commonly administered parenterally and can require veterinary involvement. To combat some of the issues of administering pain relief to lambs, administration of analgesics through feed was explored as a potentially practical method for farmers to provide pain relief to lambs over several days.

For this Thesis, flunixin, a non-steroidal anti-inflammatory drug (NSAID), was selected as an appropriate candidate to be used throughout the experiments, due to the well documented use as a pain relief for a variety of species including horses (Toutain *et al*.,1994; Pellegrini-Masini *et al*.,2004; Valverde and Gunkel, 2005), cattle (Anderson *et al*., 1986; Webster *et al*., 2013) and sheep(Welsh and Nolan, 1995; Paull *et al*., 2007). Flunixin also has the ability to be administered orally, it is water soluble and is stable under normal temperatures and pressures. Flunixin is currently registered for use in livestock in the USA, Europe and Australia.

Flunixin was tested for palatability and pharmacokinetics when administered through feed, this was required to:

- Identify if sheep may have an aversion to flunixin;
- Determine how long it would take for sheep to obtain levels of flunixin in their blood that would be sufficient to provide pain relief.

It was found that flunixin when consumed with pellets is rapidly absorbed into sheep plasma and therapeutic concentrations could be reached within a few hours of ingestion. Although neophobia was displayed in one ewe who did not eat feed with flunixin on both days, the possible mild aversion displayed to feed supplemented with flunixin on day 1 in the remaining sheep did not persist on day 2 indicating that the medicated feed is readily accepted by most sheep.

The display of neophobia by the one ewe may have been a result of being housed individually. In a group house setting it is possible to reduce neophobia and increase the uptake of the feed with an NSAID due to social facilitation (Thorhallsdottir *et al.*,1990), as discussed the self-medication literature review in Chapter 5. It would also still be possible to allow all animals receive their dose of a NSAIDS through feed when group housed. Using a similar method to the one used for the castration experiment in Chapter 4. The dose of the NSAID provided in the feed would be calculated on the heaviest ewe in the flock and on the average amount of feed eaten by ewes. It is at least known from the experiment in Chapter 3 that if the ewes were to eat variable amounts of the feed throughout the day they would still receive an adequate dose of NSAID.

Following the palatability and pharmacokinetics experiment, flunixin was tested for efficacy at alleviating the pain of surgical castration and tail-docking in lambs when administered through pellets (Chapter 4). Flunixin was effective at providing some pain relief and ameliorating the inflammatory response (reduced neutrophil/lymphocyte ratios and reduced wound swelling and improved wound appearance) to lambs when administered through feed. Lambs receiving flunixin in feed exhibited fewer pain related behaviours and reduced abnormal postures following castration and tail-docking than lambs with access to un-medicated feed. This experiment demonstrated the potential in providing pain relief to lambs through feed. Furthermore, if we can teach lambs to self-select and self-administer feed containing analgesics, it can provide us with an insight into animal pain states.

The final experiment attempted to create a model to train lambs to identify a feed that contained an analgesic and to determine if lambs could then self-medicate for chronic pain. If the lambs were able to display a preference for medicated feed, it could have given better understanding more about the lamb's pain perception. For the lambs to have the ability to choose between medicated and un-medicated feed they must first experience the consequences (whether positive or negative) of the feed options in question. This required the use of a pain model that would provide acute and chronic pain, allowing lambs sufficient time to learn the effects of flunixin. Currently there are limited pain models for use in sheep, one model used in the thesis was the induced lameness model (Chapter 2). This pain model used an oil of turpentine injection, which elicits an immediate acute pain response as well as a longer inflammatory response. The effects of the injection only last for up to 72 h (Colditz *et al*., 2011). It was determined that this would not have been sufficient time to allow the lambs to learn an association between feed and pain relief and also provided them the opportunity to self-medicate, unless it was turpentine was reinjected. However, the toxicity of turpentine is unknown and it was considered unethical to re-administer. Another pain model that may have been suitable to use due to its chronic pain was foot rot (McLennan, *et al*. 2016). However, foot rot is not common in our area (Armidale, New South Wales, Australia) and it would have been unethical and uneconomical to induce these conditions in sheep. Another potential chronic pain model was the peroneal nerve injury (PNI) model created by Wilkes *et al*., (2012). This model of pain is an irreversible surgery that involves nerve ligation, this was not possible to do and also considered unethical for the requirements of the experiment.

Due to the limitations of these pain models the use of a common husbandry procedure, ring castration and tail-docking, was considered. To determine the effectiveness of the use of ring castration and tail-docking as a model, a pilot study was conducted to develop to determine if it would provide lambs with a sufficient timeframe to be able to learn to self-medicate (Appendix 2). The model looked at separating the events of ring castration and tail-docking, considering that both ringing events may lead to a more prolonged experience of pain. In substance this model does not change the treatment animals would receive on farm for lamb marking and was considered less invasive than the other chronic pain models listed.

To test if lambs could learn to self-medicate, lambs were castrated and offered medicated feed with a cue during a training period. The lambs were then tail-docked a week later and offered medicated and non-medicated feed to see if they would have a preference. If lambs had learnt to associate medicated feed with pain relief, they should display preference for the medicated feed. Even though lambs appeared to be experiencing pain from tail-docking there was no indication that the lambs were able to self-medicate for their pain through preference for medicated feed.

There may have been several possibilities as to why lambs were unable to learn to medicate for pain. Although we ensured lambs were experiencing pain before giving them the opportunity to learn the benefits of a medicated feed, with the assistance of cues and location, it may have been difficult for lambs to link the experience of pain relief following the consumption of a medicated feed. This may be due to the way that an animal receives information from post-ingestive feedback and a painful stimulus. Another possibility was that the feedback received from the pain relief may have occurred too slowly and they may not have made that link between feed ingestion and pain-relief. It can be difficult for an animal to make the association if the behaviour and consequence are not paired closely together (Provenza, 1987; Villalba and Provenza, 2007). There is potential to retest the self-medication method using the pain model we used, however, instead of providing the pain-relief through the feed during the training period, an intra ruminal infusion of flunixin could be provided instead. As soon as the lamb has started consuming the pellets the infusion of flunixin could be provided. This may allow feedback to occur faster. Similar methods have previously been used to train sheep to associate a particular feed and cue with a nutritional consequence. Using intraruminal infusion Villalba and Provenza (1999) were able to teach lambs to associate between poorly nutritious foods (paired with intraruminal infusion of energy and flavour cue or paired with water and a different flavour cue). Lambs in this study showed a preference for the flavour cue linked with the intraruminal infusion of energy.

There may also be the possibility that lambs in the Ring group may not have been experiencing a sufficient level of pain, as the lambs response to tail-docking was less than what has been seen in previous studies, which could have effected their motivation to preferentially select the medicated feed. The lambs may also have found a different way to cope with the pain and so the measurements of pain that were used may not have been sufficient enough to detect this chronic pain. Similar to what was seen in the Chapter 1 experiment where sheep appeared to be able to cope with the pain of a turpentine injection in a different way to what was previously recorded and so we were not able to adequately measure their pain response using the standard methods.

Using our self-medication experiment method, we were not able to establish whether lambs could identify a benefit of medicated over un-medicated feed. However, we were able to demonstrate the potential for delivering pain relief to lambs in medicated feed. The supplementation of both ewes and lambs with medicated feed following painful procedures as demonstrated in Chapter 4 could be a suitable practical method for farmers to provide pain relief to lambs over several days without the need to train lambs to associate medicated feed with pain relief.**Key Findings**

- For practical provision of pain relief to lambs for painful husbandry procedures such as castration and tail-docking, flunixin can be administered as a drench or incorporated into feed.
- Oral or in-feed administration of flunixin 1-2 h prior to painful husbandry procedures would ensure that lambs have therapeutic

concentrations in their blood at the time the procedures are performed.

- Medicated feed could be offered before and on the days following castration and tail-docking to provide pain relief for the duration of the pain responses to castration and tail-docking with minimal disturbance to the animals.
- There is potential for flunixin to be incorporated into pellets in its powdered form.
- As a generic drug, flunixin could be taken to market by more than one pharmaceutic company or feed manufacturer. Registration issues for infeed delivery of flunixin have not been addressed in this Thesis.

Although it is recommended throughout the Thesis that pain relief should be provided to lambs for several days following painful husbandry procedures more work needs to be done to determine any toxicity issues. The longest period lambs had access to a the NSAID flunixin was 2 weeks, which included a rest period of 3 days and lambs did not consume their complete dose every day (Chapter 6). During this experiment lambs were monitored for any signs of adverse effects but none were observed. In Chapter 2 ewes were given a complete dose of their assigned NSAID for 6 days, some adverse effects (diarrhoea) were reported for ewes that received ketoprofen. It is known that NSAIDs can cause gastrointestinal issues, such as ulceration, vomiting and diarrhoea, when given to cats and dogs over long periods of time (Gaynor, and Muir, 2014). Currently there are no toxicity data for flunixin in sheep, further study should be conducted to determine toxicity levels and effects in sheep.

## Conclusion

Using our self-medication experiment method, we were not able to establish whether lambs could identify a benefit of medicated over un-medicated feed. Further research needs to be conducted to determine if self-medicating for pain is achievable in livestock. There are a variety of methods that could be tested to teach animals the link between feedback from feed containing pain relief with the sensation of pain alleviation. This could include using different models of chronic pain, such as foot rot, or attempting to use intraruminal infusion to reduce the time between medicated feed ingestion and feedback. Further work also still needs be conducted to determine the therapeutic levels of flunixin, as well as the toxicity in sheep. The provision of pain relief was shown to be an effective and practical method of pain administration to lambs following castration and tail-docking. Providing pain relief through feed would allow farmers to give livestock access to pain relief over a longer period of time and avoid excessive mustering, restraint, the use of injections and stress of ewe-lamb separation.

## References

Anderson, K. L., Smith, A. R., Shanks, R. D., Davis, L. E., Gustafsson, B. K., 1986. Efficacy of flunixin meglumine for the treatment of endotoxin-induced bovine mastitis. Am J Vet Res, 47, 1366-1372.

Colditz, I. G., Paull, D. R., Hervault, G., Aubriot , D., Lee, C., 2011. Development of a lameness model in sheep for assessing efficacy of analgesics. Australian Veterinary Journal, 89(8), 297-304. doi:10.1111/j.1751-0813.2011.00809.x

Gaynor, J. S., Muir III, W. W.,2014. Handbook of veterinary pain management. Elsevier Health Sciences.

Hughes, D.,1995. Animal welfare: the consumer and the food industry. Bri Food J 97, 3-7. doi:doi:10.1108/00070709510104529

Izmirli, S., and Phillips, C. J. C., 2012. Attitudes to animal welfare and rights throughout the world in the modern era: a review. Eurasian J Vet Sci, 28, 65-68.

Lomax, S., Dickson, H., Sheil, M., and Windsor, P. A., 2010. Topical anaesthesia alleviates short-term pain of castration and tail docking in lambs. Aust Vet J 88, 67-74. doi:10.1111/j.1751-0813.2009.00546.x

Melches, S., Mellema, S. C., Doherr, M. G., Wechsler, B., Steiner, A., 2007. Castration of lambs: A welfare comparison of different castration techniques in lambs over 10 weeks of age. Vet J, 173, 554-563. doi:http://dx.doi.org/10.1016/j.tvjl.2006.01.006

Mellema, S. C., Doherr, M. G., Wechsler, B., Thueer, S., Steiner, A., 2006. Influence of local anaesthesia on pain and distress induced by two bloodless castration methods in young lambs. Vet J, 172, 274-283. doi:http://dx.doi.org/10.1016/j.tvjl.2005.06.002

Molony, V., Kent, J. E., Hosie, B. D., Graham, M. J.,1997. Reduction in pain suffered by lambs at castration. Vet J, 153,205-213. doi:10.1016/s1090-0233(97)80041-x

Molony, V., Kent, J. E., Robertson, I. S.,1993. Behavioural responses of lambs of three ages in the first three hours after three methods of castration and tail docking. Res Vet Sc 55, 236-245. doi:10.1016/0034-5288(93)90087-v

Paull, D. R., Lee, C., Atkinson, S. J., Fisher, A. D.,2008. Effects of meloxicam or tolfenamic acid administration on the pain and stress responses of Merino lambs to mulesing. Aust Vet J 86, 303-311. doi:10.1111/j.1751-0813.2008.00325.x

Paull, D. R., Lee, C., Colditz, I. G., Atkinson, S. J., Fisher, A. D., 2007. The effect of a topical anaesthetic formulation, systemic flunixin and carprofen, singly or in combination, on cortisol and behavioural responses of Merino lambs to mulesing. Aust Vet J 85, 98-106. doi:10.1111/j.1751-0813.2007.00115.x

Pellegrini-Masini, A., Poppenga, R. H., Sweeney, R. W.,2004. Disposition of flunixin meglumine injectable preparation administered orally to healthy horses. J Veterinary Pharmacol Therap 27, 183-186. doi:10.1111/j.1365-2885.2004.00575.x

Provenza, F. D., 1987. Diet learning by domestic ruminants: Theory, evidence and practical implications. Appl Anim Behav Sci, 18, 211-232. doi:10.1016/0168-1591(87)90218-8

Small, A. H., Belson, S., Holm, M., Colditz, I. G.,2014. Efficacy of a buccal meloxicam formulation for pain relief in Merino lambs undergoing knife castration and tail docking in a randomised field trial. Aust Vet J 92, 381-388. doi:10.1111/avj.12241

Toutain, P. L., Autefage, A., Legrand, C., Alvinerie, M.,1994. Plasma concentrations and therapeutic efficacy of phenylbutazone and flunixin meglumine in the horse: pharmacokinetic/pharmacodynamic modelling. J Vet Pharmacol and Therap, 1, 459-469. doi:10.1111/j.1365-2885.1994.tb00278.x

Thorhallsdottir, A. G., Provenza, F. D., Balph, D. F., 1990. Ability of lambs to learn about novel foods while observing or participating with social models. Appl Anim Behav Sci 25, 25-33. doi:10.1016/0168-1591(90)90066-m

Tucker, C. B., Mintline, E. M., Banuelos, J., Walker, K. A., Hoar, B., Varga, A.,Weary, D. M., 2014)=. Pain sensitivity and healing of hot-iron cattle brands.J Anim Sci 92, 5674-5682. doi:10.2527/jas.2014-7887

Valverde, A., Gunkel, C. I., 2005. Pain management in horses and farm animals. J Vet Emerg Crit Care 15, 295-307. doi:10.1111/j.1476-4431.2005.00168.x

Villalba, J. J., and Provenza, F. D., 2007. Self-medication and homeostatic behaviour in herbivores: learning about the benefits of nature's pharmacy. Animal, 1, 1360-1370.

Webster, H. B., Morin, D., Jarrell, V., Shipley, C., Brown, L., Green, A., Constable, P. D., 2013. Effects of local anesthesia and flunixin meglumine on the acute cortisol response, behavior, and performance of young dairy calves undergoing surgical castration. J Dairy Sci 96, 6285-6300. doi:http://dx.doi.org/10.3168/jds.2012-6238

Welsh, E. M., and Nolan, A., 1995. Effect of flunixin meglumine on the thresholds to mechanical stimulation in healthy and lame sheep. Res Vet Sci58,61-66. doi:10.1016/0034-5288(95)90090-x

Wood, G. N., Molony, V., Fleetwood-Walker, S. M., Hodgson, J. C., Mellor, D. J., 1991. Effects of local anaesthesia and intravenous naloxone on the changes in behaviour and plasma concentrations of cortisol produced by castration and tail docking with tight rubber rings in young lambs. Res Vet Sci 51, 193-199. doi:http://dx.doi.org/10.1016/0034-5288(91)90013-E

# 8 Appendix

## 8.1. Appendix 1: Using a grimace score to identify pain in lambs

Grimace scales have been previously developed for rats, rabbits and horses. They are an alternative non-invasive method that has been validated to identify and assess pain in these animals. The lamb grimace scale used to identify pain in lambs in this study was developed by Guesgen (2014). Using methods similar to those used to develop mouse and rat grimace scales, Guesgen (2014) identified five facial action units that change in lambs following painful procedures. These were the nose, mouth, cheek, eyes and ear posture, the facial units were scored on a 3-point scale, 0 (not present), 1 (moderate), 2 (obvious). The aim of this study was to assess Merino lambs for pain following surgical castration and tail-docking by gas knife using the lamb grimace scale.

**Methods**

This grimace scale was tested on the lambs in Chapter 4 and compared to the behavioural measurements. The groups as described in Chapter 4 were Sham handling (S), Castration and Tail-docking (C), Flunixin in feed + Castration and Tail-docking (CF) and Flunixin injected + Castration and Tail-docking (CI). Lambs were recorded, in their pens (free) and when restrained (held) at 0, 0.5, 6, 24 and 48 h relative to castration and tail-docking. For analysis the scores given for each facial unit were averaged to give an overall score. Ears were not included as they could not be scored with confidence. Lambs were first assessed prior to castration and tail-docking; this was done to see if the stress of being held may affect the facial expression of the lamb. Data at time 0 h were analysed by Chi-square test and data post-marking were analysed as proportions using a binomial test.

Figure 18: Examples of the changes and scores given to lambs using the 3 point scale 0 (not present), 1 (moderate), 2 (obvious). When in pain, eyes become more closed, nostrils become tighter and flatter, the mouths normal appearance of a curved "smile" becomes a flattened line, cheeks appear hollow, ears are rotated backwards and faced downwards

## Results

Prior to castration and tail-docking, there was a significant association between free vs. held and incorrect pain assessment $\chi^2$ (1) = 12.70, $P < 0.001$, with 62% of held lambs and 18% of free lambs incorrectly identified as being in pain. After the procedures, a free vs held effect ($P = 0.03$) was observed for some treatments, with lambs in the S and CF and CI groups being scored as in pain more when held than free. A treatment effect ($P < 0.001$) and treatment x position effect (held vs free) were also observed. When assessed in the pen, lambs in the C group were classified as in pain more often compared to lambs in the other groups ($P < 0.05$ for all). CF and CI lambs were classed as in pain more than S lambs ($P < 0.05$). When held C lambs were classified as in more pain more often than held S and CI lambs ($P < 0.05$).

**Conclusions:**

The grimace scale shows potential as a rapid and practical method to assess pain in lambs, but lambs should be observed without interference, as restraint may change aspects of facial expression. Using the grimace scale we were able to correctly identify lambs in pain, when scores were based on photos of lambs in their pen (free). Ear postures were difficult to determine, as lambs would orientate to the observer and so ears were excluded from the analysis. Hollowing of cheeks were infrequently observed, this could have been due to lambs having wrinkled, woolly faces, which affected the ability to detect changes in the cheeks.

Guesgen, M (2014) The Social Function of Pain-Related Behaviour and Novel Techniques for the Assessment of Pain in Lambs. Ph.D. Thesis. Massey University: NZ

## 8.2. Appendix 2 Develop methodology for self-selection and self-administration of analgesics to lambs

**Investigation of a model of pain to allow lambs to learn to self-medicate**

**Objective**

Lambs were monitored for up to 35 days after ring castration and tail-docking to assess chronic pain that may be associated with this procedure. Ring castration and ring tail-docking were performed on separate days to produce two events rather than one, so that the pain effect was more similar to chronic pain. The objective of this study was to establish a chronic pain model through extending the duration of pain with the application of rings for castration and tail docking on separate days to be used for a self-medication experiment.

**Methods and Materials**

There were 30 lambs involved in this experiment. Lambs were castrated at approximately 6-12 weeks of age. Lambs were weighed the day before treatment, individually side branded, and assigned to treatment groups on the basis of weight:

There were three treatment groups, 10 lambs in each treatment group.

- Group 1: Sham control
- Group 2: Elastrator castration day 0 and tail-docking on day 3
- Group 3: Elastrator castration and tail-docking on day 0 as per farm protocol (day 0)

Lambs were kept with their mothers, in a paddock situation. They were separated from their mothers on the morning of treatment, for a maximum of 3 h. The ewes were released into a small paddock (approximately 40 m X 60 m) beside the lamb marking pen. Lambs were caught, restrained in a marking cradle, and treated according to assigned treatment. After castration, the lamb was released into the small paddock containing their mothers.

Behaviour of the lambs was monitored for 2 h by personnel blinded to treatments. A team of 2 observers undertook scan sampling of lamb behaviour every 15 minutes. Behaviour was classified as

- Standing (normal standing, hunched standing, grazing)
- Lying –ventral and lateral
- Suckling
- Walking
- Running/ playing

At day 1, 2, 3, 7, 10, 14, 17 21, 28 and 35 post-castration, behaviours were assessed by 5-min continuous focal animal sampling. Counts of all events were summed for each 5-min observation period. On day 3 lambs had their behaviour observed after group 2 lambs were tail-docked.

On days -1, 7, 14, 21 and 28, ewes and lambs were mustered and lambs weighed.

Following blood sampling each lamb had their wound palpated day 1, 2, 3, 7, 10, 14, 17 21, 28 and 35 post-castration. Their response was recorded as a score from 0=no response to 2= struggling.

Observations ceased after day 35.

### *Results*

Lamb weight was not affected by treatment, all lambs gained weight following treatment day. On the day of treatment, there was a significant difference ($P < 0.05$) in the amount of pain related postures exhibited by the three groups. Control animal exhibited less pain related postures than both castrated groups. Lambs in the castrated and docked group exhibited more pain related postures ($P = 0.03$, $1.6 \pm 0.71$) than lambs that were only castrated on day 0.

On day three which was the day of tail-docking for the castrated only group, this group of lambs exhibited more pain related behaviour then they did on day 1 ($P = 0.04$). They also exhibited more pain related behaviour than the group that was castrated and tail-docked on day 0 ($P < 0.05$). However, overall there was no difference in the cumulative amount of pain related behaviour exhibited by the groups over the 35-day period.

Lambs that were castrated and tail-docked still exhibited a response to the palpation of their wound at 35 days' post-castration (Figure 19). Both groups that were castrated and tail-docked had more lambs react to the palpation of their wound site than the control group ($P < 0.001$). There was no difference between the two castration groups in the number of lambs that reacted to palpation.

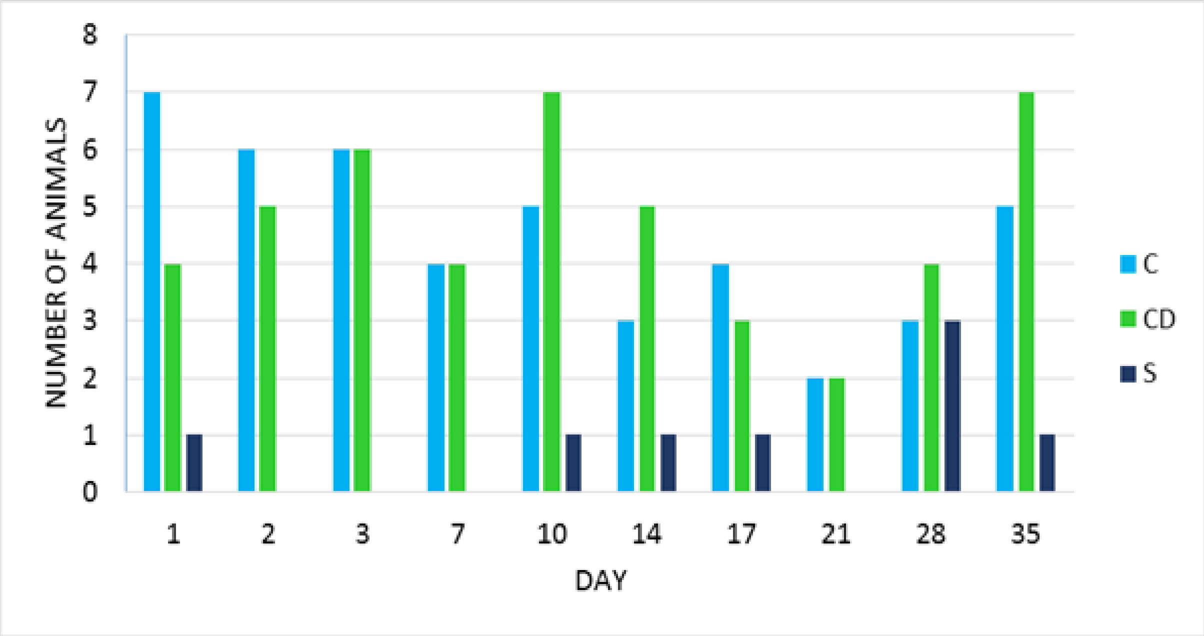



Figure 19: Number of lambs in each group (n=10/group) that exhibited a response to palpating the "wound" site following castration and tail-docking. C is lambs castrated and tail-docking on day 0, CD is lambs that were castrated on day 0 and tail-docking on day 3, S is control lambs that did not undergo the procedures.

***Conclusion***

Lambs were monitored for up to 35 days after ring castration and tail-docking to assess chronic pain that may be associated with this procedure. Ring castration and ring tail-docking were performed on separate days to produce two acute pain events and potential increase the length of time chronic pain was experienced. The objective of this study was to establish a chronic pain model through extending the duration of pain with the application of rings for castration and tail docking on separate days to be used for a self-medication experiment.

Overall lambs that were castrated and tail-docked didn't show a significant amount of observable signs of pain (pain related behaviour and postures) after the treatment day. This may have been due to limitations of observing the lambs in the field.

However, lambs still exhibited discomfort of the wound site up to 35 days following the procedure. This suggests that ring castration and tail-docking can be a suitable chronic pain model for use in the self-medication experiment. Lambs that were tail-docked 3 days after castration, again displayed pain related behaviour on that day of treatment, indicating that a secondary acute pain can be achieved.

## 8.3. Appendix 3 Determining an odour cue

To determine which odours to use in the self-medication experiment and to test for any aversions in the sheep, we conducted a pilot trial where five odours: banana, apple, strawberry, coconut and green tea were tested in pelleted feed.

To test the odours, eight sheep were acclimated to the mating yards and reintroduced to eating pellets for a week, after which they were split into two groups of four in two of the yards. They then had the option of 6 feeds placed in separate feed troughs (2.5 kg of each). One feed contained only normal pellets and the remaining five contained one of the odours (banana, apple, strawberry, coconut or green tea). They were offered these feeds over a period of 5 days and their intake of each was recorded 24 h after they were first offered.

The results of this pilot trial indicated that sheep had a higher preference for both banana and strawberry over the other odours.

# 9. Publications and conference abstracts

## Publications

Danila Marini, Joe Pippia, Ian Colditz, Geoff Hinch, Carol Petherick and Caroline Lee (2014). Randomised trial of the bioavailability and efficacy of orally administered flunixin, carprofen and ketoprofen in a pain model in sheep. *Australian Veterinary Journal*, Volume **93**, Issue 8, pages 265–270

Danila Marini, Joe Pippia, Ian Colditz, Geoff Hinch, Carol Petherick and Caroline Lee (2016). Palatability and pharmacokinetics of flunixin when administered to sheep through feed. *PeerJ*; DOI 10.7717/peerj.1800

Danila Marini, Ian Colditz, Geoff Hinch, Carol Petherick and Caroline Lee (nd). Self-administration through consumption of flunixin in a total mixed ration can alleviate the pain and inflammation associated with castration and tail docking lambs (*Submitted to Applied Animal Behaviour Science*)

## Conferences

### *Oral presentations:*

Danila Marini, Ian Colditz, Geoff Hinch, Carol Petherick and Caroline Lee (2016) *Can lambs learn to self-medicate with feed containing flunixin?* University of New England Postgraduate conference.

Danila Marini, Ian Colditz, Geoff Hinch, Carol Petherick and Caroline Lee (2015) *Can flunixin in feed alleviate the pain associated with castration and tail docking?* ISAE 49th international Congress in Japan.

Danila Marini, Ian Colditz, Geoff Hinch, Carol Petherick and Caroline Lee (2014) *Developing a self-medication method for sheep.* In ISAE Australia, New Zealand, Philippines and Africa Regional Meeting: Forward thinking Applying ethology to solve behaviour and welfare questions. Presentation given in Sydney at the ISAE regional meeting.

Danila Marini, Ian Colditz, Geoff Hinch, Carol Petherick and Caroline Lee (2013) *Developing a self-medication method for sheep and cattle.* Presented at the AARHUS University, Pain course.

### *Poster Presentations:*

Danila Marini, Joe Pippia, Ian Colditz, Geoff Hinch, Carol Petherick and Caroline Lee (2015) *Assessing pain in lambs using a grimace scale*. IEC Behaviour Conference in Cairns

Danila Marini, Joe Pippia, Ian Colditz, Geoff Hinch, Carol Petherick and Caroline Lee (2014) *Bioavailability and efficacy of orally administered flunixin, carprofen and ketoprofen in a pain model in sheep*. In I. Estevez, X. Manteca, R.H. Marin and X. Averos (Eds.), International Society for Applied Ethology: Proceedings of the 48th international congress of the ISAE (pp.220) The Netherlands, Wageningen Academic Publishers.