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Automated measurement of disease and pain in New Zealand group-housed calves

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Abstract

Exposure to disease and pain will prolong animal ‘suffering’, and as such, diminishes welfare. Identifying behaviours indicative of these conditions can facilitate early detection, timely treatment and improved welfare. Contributing to the problem of disease and pain detection in calves is the innate tendency of these animals to mask behavioural signs of vulnerability (stoicism), and the extensive use of group-housing systems. Existing technology was used in my research to obtain objective measures of behaviour in response to disease and pain. The goal of this thesis was to explore these key aspects of calf welfare:

The work reported in Chapter 2 investigated a naturally occurring disease (neonatal calf diarrhoea complex) in pre-weaned calves to assess whether changes in milk-feeding and lying behaviours could be used for early disease detection. Calves were observed for a three week period, starting when they were four days old. Data on milk feeding and lying behaviours were obtained using automated milk-feeders and HOBO data loggers respectively. Lying postures were analysed from daily video footage at five minute intervals between 10:00-14:00 (seven days per week). For statistical analysis, calves were classified as ‘sick’ (n=21), or ‘not sick’ (n=91). This thesis identified three feeding measures of interest for disease detection: 1) reduced milk consumption, 2) increased duration of visits to the milk feeder, and 3) sick calves were less likely to receive a rewarded visit compared to calves that were not sick. Sick calves increased the duration of lying bouts nearing time of illness; however, no difference was observed between sick calves and those that were not sick. Postural observations were not effective at predicting illness. The use of automated milk-feeders to detect disease in calves has been studied extensively overseas; to my knowledge, this is the first New Zealand study to use automated feeders for this purpose. The results of this study indicate that aspects of milk feeding behaviour can be used to detect diseased calves in group housing systems.

The work reported in Chapter 3 used hot iron disbudding as a pain model to determine whether changes in milk-feeding and lying behaviours could be used to identify pain in calves less than 4 weeks of age. Data was obtained using automated feeders and HOBO data loggers respectively over three observation periods (pre-treatment, treatment day and post-treatment). Fifty-three calves (26.5

\pm 3.5 days of age) were allocated to one of five treatment groups: hot iron disbudded with no analgesia (n=11), disbudded with a local anaesthetic (LA, n=11), disbudded with a non-steroidal anti-inflammatory drug (NSAID; n=11), disbudded with NSAID and LA (n=10), and SHAM calves (n=11). Analysis of feeding behaviour revealed only one difference between all treatment groups; SHAM calves showed a greater number of visits to the milk feeder during the recovery period compared to disbudded animals. Feeding and lying behaviours in this study were considered to be insufficient measures of pain. The use of automated milk-feeders to detect pain in calves is limited, thus necessitating this research.

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1 Introduction

The longer an animal is exposed to pain or disease, the more it is thought to “suffer”, or have a diminished state of welfare; thus, early detection of these conditions is important if we are to facilitate good welfare. This thesis attempts to identify behavioural measures that can be obtained using readily available technology and used for the early detection of disease and pain on-farm. A general overview of animal welfare and its associated measures is given as a means to provide context for this research.

1.1 Animal welfare

As a result of increasing societal concerns regarding the treatment and wellbeing of intensively farmed species, the UK government commissioned an investigation into the welfare of production animals in 1965 (McCulloch, 2013; Hemsworth et al., 2015). This work, lead by Professor Roger Brambell, gave rise to two important concepts: 1) that animal suffering should be a primary concern in the assessment of welfare, and 2) that animals have behavioural ‘needs’ that could lead to welfare concerns in the form of negative mental states (e.g. frustration; Mench, 1998) if deprived of the opportunity to perform them. In 1975, the Farm Animal Welfare Council itemised and further developed Brambell’s conclusions, leading to what we now recognise as the “Five Freedoms” of animal welfare (McCulloch, 2013). These “freedoms” have provided the foundations for welfare assessment, legislation, and research in many countries including New Zealand.

1. **Freedom from hunger and thirst** - by ready access to fresh water and a diet to maintain full health and vigour.
2. **Freedom from discomfort** - by providing an appropriate environment including shelter and a comfortable resting area.
3. **Freedom from pain, injury and disease** - by prevention or rapid diagnosis and treatment.
4. **Freedom to express (most) normal behaviour** - by providing sufficient space, proper facilities, and company of the animal’s own kind.
5. **Freedom from fear and distress** - by ensuring conditions and treatment which avoid mental suffering (Farm Animal Welfare Committee, 2013, p.2).

Whilst the construct of animal welfare is one that is inherently difficult to define, it is often thought to describe an animal's 'quality of life' (Duncan, 2005; Bracke & Hopster, 2006), with both physical and mental health contributing to overall welfare (Hemsworth et al., 2015). No matter what definition is used, it is important to recognise that welfare occurs on a progressive scale that ranges from very poor to very good depending on multiple factors, and should not be viewed as a simple bilateral concept (Broom, 1991a,b).

Three ethical concerns central to the issue of animal welfare have been identified by Fraser et al. (1997): 1) the ability for animals to display natural behaviours, 2) the ability for animals to feel "well", and 3) the ability for animals to function normally. Whilst these ethical views of welfare have been described independently, when an animal's welfare is compromised, it is likely that more than one of these factors will be simultaneously affected. For example, in the case of neonatal calf diarrhoea, the animal will experience physiological changes (e.g. activation of immune responses), and negative emotional states (e.g. discomfort and depression). Behaviour may also be affected (e.g. motivation to feed and perform social interactions tends to decline during periods of disease).

1.1.1 Measurements of welfare

The scientific assessment of animal welfare embraces a multidisciplinary approach with contributions coming from several biological fields such as: neuroscience, behavioural ecology, animal behaviour, evolution, and genetics (Dawkins, 2006). This approach has greatly improved our knowledge of animal welfare from various scientific perspectives; however, it has also given rise to a myriad of measures available for the assessment of animal welfare. The assessment of animal welfare necessitates the use of several indicators (e.g. physiological, genetic, reproductive, immunological and behavioural measures); however, the challenge lies in which measures to use over others, and how much weight should be assigned to each (Dawkins, 2004). In response to this, Dawkins (2003; 2004) argues that there are only two elements fundamental to the assessment of animal welfare: the animal's physical health, and whether or not the animal has what it wants.

To answer the question of physical health, combinations of physiological and behavioural measures are used. Physiological measures offer the distinct

advantages of being quantifiable and objective; they allow us to identify how the physiology of an animal changes during its attempts to adapt to a situation or stimulus (Barnett & Hemsworth, 1990). These measures have been used extensively for the clinical assessment of animal health (Dawkins, 2006), and studies of animal stress (Smith & Vale, 2006). For example, horses have been shown to experience an increased heart rate, and elevated levels of salivary cortisol in response to travel induced stress. Further, the extent of changes in these measurements was affected by duration of transport, with longer journeys producing higher changes in salivary cortisol (Schmidt et al., 2010). Behavioural measures are also used in the assessment of physical health. For example, gait assessments in cattle (Flower & Weary, 2009) and pigs (Grégoire et al., 2013) have proven effective in the identification of lameness; further, observations of lethargy, depression and reduced feeding have been commonly noted in animals succumbing to illness (Hart, 1988).

The second of Dawkin's (2003; 2004) two questions, "does the animal have what it wants", deals with affective states that cannot be measured directly; however, the use of behavioural indicators can provide indirect information on how an animal 'feels' about a particular situation or stimulus (Duncan, 2005). For example, piglets produce different vocalisations when experiencing feelings of hunger, pain, and cold distress, thus vocalisations may be used to determine conditions piglets find unpleasant (Cordeiro et al., 2013). Simply observing natural behaviours can also provide a useful indication of welfare (see Bracke & Hopster, 2006).

The preference for one resource over another, and the importance placed on these resources from an animals' perspective, can also be gauged using preference tests and consumer demand studies (for review see Kirkden & Pajor, 2006). These studies are commonly used to assess specific aspects of living and social conditions, from the animals' perspective. For example, preference testing of 3 week old calves using a Y-maze design revealed that individuals choose to spend significantly more time with a familiar calf than an unfamiliar calf. An additional separation test showed that calves were less reactive to separation in the company of another calf, and even less reactive still, if the accompanying calf was familiar to it (Færevik et al., 2006).

The importance of behaviour for welfare assessments has been recognised by many; however, numerous on-farm assessments continue to focus heavily on the animals' physical health. Rushen et al. (2012) suggests that this could be attributed to the time and costs involved in obtaining behavioural measures of interest, and the often irregular nature of behaviour patterns which can necessitate extended periods of observation. One way to encourage the use of behavioural measures for on-farm assessments would be to use information gathered by existing technology and on-farm automation (e.g. automated milk feeders) in conjunction with the existing measures of on-farm welfare assessments (Rushen, et al., 2012; Cornou, 2009). This approach may provide four key benefits: 1) reduce the need for lengthy, manual observation of behaviour, 2) minimise the need to specifically train observers whilst potentially improving reliability of behavioural findings, 3) provide early warning signs of poor health and/or pain, prior to the emergence of clinical symptoms, and 4) ongoing monitoring of behaviour and welfare may be possible due to data storage options that are often available (Rushen et al., 2012).

1.2 Sickness behaviour

The initial identification of diseased animals is often based on a synchronised series of behavioural responses to infection (or inflammation) and fever, collectively known as “sickness behaviour” (Hart, 1988; Millman, 2007). Sickness behaviour is mediated by pro-inflammatory cytokines (produced by activated immune cells) and entails several non-specific behavioural and symptomatic responses, including: fever, increased periods of sleep, reduced social exploration and grooming, adipsia, hypophagia, and feelings of lethargy and malaise (Hart, 1988; Aubert, 1999; Johnson, 2002; Millman, 2007). Sickness behaviour, once thought to be an adverse effect of sickness-induced debilitation, was later proposed to be an adaptive behavioural strategy used by the host to overcome infection and improve chances of survival (Hart, 1988). For example, cytokine-induced hypophagia in mice during infection with *Listeria monocytogenes* (Murray & Murray, 1979) could be interpreted as a means to reduce the intake of certain micronutrients required for pathogen growth (Aubert, 1999).

Increased resting behaviours and reduced activity during periods of disease is thought to facilitate energy conservation and reduce heat loss (Hart, 1988). This is considered to play an important role in meeting the metabolic demands required to produce a febrile response (body temperature of 38-40°C), which in turn increases the odds of host survival by way of potentiating immune cell activity, and reducing growth of certain bacterial and viral pathogens (as summarised by Auberts, 1999; see Vaughn et al., 1974, and Kluger, 1979, for fever studies on iguanas and mammals respectively). Further, Hart (1988) suggests that some animals may alter their lying postures in response to sickness as a means to further regulate body temperature and conserve energy. For example, a tightly curled position (short lying position) may serve to minimise heat loss by reducing the area of body surface exposed to the environment. Studies of postural thermoregulation in cattle as a response to variable environmental conditions have produced mixed results (cattle; see Hänninen, 2007 for review); however, postural thermoregulation as a disease response appears to be an area less well explored.

Aubert (1999) further proposed that sickness behaviour illustrates motivational changes that occur as a result of immune system activation. This argument is strengthened by studies that show sickness behaviour can be temporarily interrupted if the need to perform another activity is judged by the animal to be more pressing than self-recovery (i.e. reorganisation of motivations). For example, lactating female mice challenged with lipopolysaccharide (LPS) in a warmer environment (22°C) continued to retrieve stray pups, however, they did not engage in nest building activities. Nest building and pup retrieval increased when the temperature was reduced to 6°C. This suggests that maternal motivations to ensure pup survival outweighed the motivation to perform sickness behaviours when a real thermal threat to the poikilothermic offspring was perceived (Aubert et al., 1997). Many other studies have demonstrated that physiological and environmental triggers can change an animal's motivational state during illness, temporarily altering the expression of sickness behaviour (for reviews see Aubert, 1999; Larson, 2002).

The expression of sickness behaviour has also been reported to be influenced by other factors such as the evolutionary role of particular species (Hart, 1988; Weary et al., 2006). For example, prey species such as cattle and sheep have been described as 'stoic' due to their tendency to mask behaviours indicative of pain

and disease, presumably as a means to avoid predatory attention (Stafford & Mellor, 1993; Weary et al., 2009). Whilst this particular theory has not been formally examined, it does emphasise the need to further explore species-specific expression of sickness behaviour for the purposes of disease detection, and how these behaviours may be altered by individual differences, and changing motivations.

1.2.1 Disease in the calf: Neonatal calf diarrhoea complex

The prevalence of disease in pre-weaned calves presents several challenges for farmers with regards to animal welfare, animal management, and economic loss. The potential for economic loss is not restricted to disease treatment and calf mortality losses; major secondary costs may also be incurred in the form of increased labour, reduced growth rates, and poor production following clinical disease (Chi et al., 2002; Lorenz et al., 2011; Uetake, 2013; Al Mawly et al., 2015a). Disease occurs as a result of interactions between several factors: the pathogen of interest, health status of the animal (immunological and nutritional status), animal management, and environmental factors (Izzo et al., 2011).

Calves under the age of 5 weeks do not have active immunity, and thus rely solely on the passive transfer of colostral immunoglobulins (Ig) in the first hours of life to protect them against infectious disease (Beam et al., 2009; Uetake, 2013). A survey of US dairy operations revealed approximately 19% of heifer calves suffered from failure of passive transfer (FPT = serum concentration of IgG <10 mg/ mL) of colostral antibodies (Beam et al., 2009). A New Zealand study found that approximately 45% of the dairy calves involved showed low serum gamma glutamyltransferase (GGT) activity (< 200 U / l GG), indicative of low serum concentrations of Ig (Wesselink et al., 1999). FPT in dairy calves have been linked to higher morbidity and mortality rates among neonatal calves, and a long term reduction in productivity (Beam et al., 2009). Three key factors have been credited with contributing to successful passive transfer: 1) feeding high quality colostrum, with high immunoglobulin concentrations (IgG >50 mg / mL), 2) ensuring calves receive a sufficient volume of colostrum soon after birth, and 3) limiting bacterial contamination of colostrum (Beam et al., 2009).

Neonatal calf diarrhoea complex (NCDC) is generally recognised as one of the largest health challenges facing cattle industries worldwide. Appropriate calf

management, both before, and following observations of diarrhoea, is extremely important for farmers to avoid further financial, and welfare pressures (Lorenz et al., 2011). Enteropathogens that are most commonly associated with NCDC include: *Salmonella*, *Escherichia coli*, bovine coronavirus, bovine rotavirus and the protozoan parasite *Cryptosporidium parvum* (Lorenz et al., 2011; Al Mawly et al., 2015b). Al Mawly et al. (2015a) reported that 31% of 1-5 day old calves involved in a New Zealand study, tested positive for at least one enteropathogen; this increased to 39% when looking at 9-21 day old calves. However, it is important to recognise that these pathogens can also be found in faecal samples obtained from healthy animals, and that infection occurs when infectious pressures outweigh the animals' resistance to disease (Lorenz et al., 2011). Infectious pressures can be minimised by means of general hygiene practices at the site of housing and feeding, and during general handling procedures (Lorenz et al., 2011).

Enteropathogens associated with NCDC are known to damage the intestinal mucosa of the calf; consequently, animals show signs of nutritional malabsorption and secretory diarrhoea (Lorenz et al., 2011). Fluid replacement therapy continues to be the most significant treatment measure for NCDC, with the aim of replacing fluids and electrolytes lost through the intestines (Lorenz et al., 2011). Of course, for this to be effective, the rehydration solution must be readily absorbed with the goal of regulating extracellular fluid volume and correcting acidosis (Lorenz et al., 2011). Efficacy of oral rehydration will be much improved if administered at the onset of diarrhoea, thus early detection is paramount. It is important to note that fluid replacement therapy should not be substituted for the animal's normal milk diet, but rather, administered as an additional feed thus avoiding further malnutrition (Michell, 2005).

1.2.2 Early disease detection on-farm

Poor physical health is relatively simple to identify once clinical symptoms have manifested (Dawkins, 2006), and can often be quantitatively measured (e.g. size of foot-rot lesions in sheep (Conington et al., 2008), and scoring of the animals' gait (for review on gait scoring in dairy cows see Flower & Weary, 2009)). However, other more obscure measures indicative of poor health prior to the expression of clinical symptoms, have also been identified (e.g. reduction in feed

intake, and lethargy; Hart, (1988)), thus allowing animal handlers to act promptly to minimise spread of disease, and reduce 'suffering' by means of early identification and treatment.

Given the immunological vulnerability of neonatal calves, early disease detection is extremely important in minimising the negative welfare and economic impacts brought about by illness. In accordance with this, several studies have been conducted to assess the viability of using automated technology to monitor behaviours and symptoms indicative of early disease (e.g. Schaefer et al., 2007; Svensson & Jensen, 2007; Borderas et al., 2009). For example, fever has an important role in defending the host against many bacterial and viral infections, thus identifying animals with a fever seems to be a logical means of contributing to early disease detection. Infrared thermography has been identified as a particularly useful tool for this purpose. Schaefer et al. (2007) showed that infrared thermography could be used for the early detection of bovine respiratory disease (BRD) in weaned calves; importantly, infected individuals were often identified days before clinical symptoms of BRD were observed. Similarly, a recent study used a vaccination regime as a model for febrile disease in weaned piglets (Cook et al., 2015). The authors concluded that infrared thermography could be used to identify febrile-inducing disease by means of assessing the mean temperature radiating from a group of animals. Vaccinated animals showed increased huddling behaviours and an associated increase in mean radiating temperature when compared to sham (injected with 0.9% saline) and untreated animals (Cook et al., 2015).

Changes in feeding behaviour is often the first behavioural indication that an animal is sick, and as such, automated feeders have been used successfully to identify changes in the milk-feeding behaviour of morbid pre-weaned calves. A study by Svensson and Jensen (2007) reported no effect of disease on total milk consumption when calves were fed restrictively; however, the number of unrewarded visits became significantly reduced in diseased calves.

Correspondingly, Borderas and associates (2009) found total milk consumption to be an insufficient indicator of disease in calves that were fed restricted milk volumes (4 L per day). Instead, the most sensitive measure of disease was found to be the duration of visits to the feeder, with sick animals showing significant reductions in time spent at the feeder. On the other hand, sick calves fed a higher

milk allowance (12 L per day) not only showed a reduction in total milk consumption, but also reduced their frequency of visits to the milk feeder. The results of this study suggest that the volume of daily milk rations influence which features of milk feeding behaviour are changed as a result of illness (Borderas et al., 2009).

Activity levels and time spent lying represent yet another set of behaviours commonly influenced by illness (Aubert, 1999), and as such, may provide another complimentary approach for disease detection. Many studies involving the assessment of lying behaviours have utilised direct, or video observation, methods that are rather time consuming and not very practical for on-farm use. Nonetheless, many of these studies have demonstrated changes in lying behaviour and activity levels during sickness (e.g. pigs subjected to LPS challenge increased total lying time (Johnson & von Borell, 1994); calves subjected to *Escherichia coli* LPS challenge were shown to decrease the frequency and duration of bouts standing inactive, while time spent lying showed no change (Borderas et al., 2008). The attachment of small data loggers to animals, is gaining traction in the field of welfare research, and has previously been validated for use on cows (see Ledgerwood et al., 2010) and calves (see Bonk et al., 2013). A study by Cyples et al. (2012) assessed the lying behaviours of 21 cows that were subjected to experimentally-induced clinical mastitis. A reduction in the time spent lying was observed on the day of intra-mammary infusion with *Escherichia coli* LPS compared to the 2 day period preceding infection. These results appear to contradict the notion that lying and resting behaviours increase as an adaptive response to illness; however, it is also possible that typical sickness behaviour was interrupted due motivational reorganisation i.e. cows stood more to avoid pain or discomfort of the udder when lying (Cyples et al., 2012).

1.3 Pain in animals

As is the case with the term ‘animal welfare’, pain is also difficult to define. The International Association for the Study of Pain (IASP) has identified three key factors that should be considered in any attempt to formulate a definition: 1) pain involves the presence of negative emotional and sensory experiences resulting from actual or potential tissue damage, 2) pain is always considered to be a subjective and personal experience, and 3) identification of pain in the absence of

actual tissue damage, means that any working definition of pain should avoid linking pain to “an external eliciting stimulus” (as cited in Rose et al., 2014, p. 99). The capacity of different animals to experience pain, rather than just the automatic nociceptive response to noxious stimuli, is a source of continued debate for some within the science community, particularly because for an animal to experience pain, it must first be acknowledged as being ‘conscious’ (see Sneddon et al., 2014 for review of animal pain). For the purposes of this thesis, it is assumed that animals are in fact conscious, and thus possess the capacity to experience the negative affective states of pain and suffering.

1.3.1 Physiological measures of pain

Stimuli that are known to induce pain in humans have been found to provoke similar changes in the physiology and behaviour of other mammals. Many of the physiological alterations that occur as a result of pain are mediated by the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis, and as such, direct physiological measurement of changes in these systems can be obtained (Sneddon et al., 2014). Pain-induced changes in the SNS are immediate (involved in the ‘fight or flight response’) and can be assessed using direct measurements of circulating catecholamine (e.g. adrenalin and noradrenalin; Mellor et al., 2002), or indirectly by means of measuring the subsequent autonomic changes (e.g. elevated heart rate and body temperature; Stuart et al., 2008). The delayed, but longer lasting, pain-induced changes of the HPA axis are most frequently assessed using measures of glucocorticoid (e.g. cortisol) production (Mellor et al., 2002; Sneddon et al., 2014). Together, these physiological measures are generally considered to be a good indicator of negative affective states such as pain, fear and distress (Sneddon et al., 2014). For example, Robertson et al. (1994) used plasma cortisol (together with behavioural responses) as a means to assess pain in calves of different ages (6, 21, and 42 days of age) in response to three methods of castration (Burdizzo, surgical and rubber ring methods). This study found that the largest cortisol response was displayed by 42 day old calves following surgical castration, which may be indicative of a more painful/distressing method of castration in older calves (Robertson et al., 1994).

1.3.2 Behavioural measures of pain

Deviations from normal behaviour will often be the first indicator that an animal is experiencing pain, and as such, has an important role to play in pain assessments. The identification and interpretation of these sometimes minor deviations, however, is often problematic and can lead to detection and treatment delays (Stafford & Mellor, 1993; O'Callaghan et al., 2003). It is for this reason that a combination of measures is often used for pain assessment in animals (Weary et al., 2006). Pain assessment of farm animals in particular, may be complicated by several factors including: 1) limited opportunities for farmers/handlers to assess individual animals, particularly those in group housing systems (Fitzpatrick et al., 2006), 2) the stoic nature of prey species such as cattle and sheep – where behavioural signs of vulnerability are often masked (Stafford and Mellor, 1993; Weary et al., 2006), and 3) differences of opinion, and perhaps a breakdown in communication, between farmers and veterinarians leading to confusion over whether the use of analgesics is required or advised in any given case (Thomsen et al., 2012).

The assessment of behaviour in relation to pain assessment can be divided into two main areas. The first consists of more generalised changes in 'normal' behaviour (e.g. feed consumption and activity levels). These behaviours may be similar to some of those described earlier under 'sickness behaviour', and as such, can be assessed using similar methods. For example, a recent study showed that automated milk feeders could be used to assess changes in the feeding behaviour of young calves (3-6 weeks) disbudded without (controls) or with a sedative-local anaesthetic (LA) combination (Bates et al., 2015). Control calves disbudded with no analgesia were found to have a lower milk intake over the subsequent 11 days when compared to those of the sedation-LA treatment group (Bates et al., 2015). Another study demonstrated the use of accelerometers (attached to the animal's hind leg) and a remote triangulation device to monitor calf activity within the pen following thermocautery dehorning. The two treatment groups were controls (no analgesia provided), and calves who received a non-steroidal anti-inflammatory drug (NSAID). Calves in the NSAID group spent more time around the grain station on two non-consecutive days post-dehorning (day 2 and day 6 after disbudding), and spent more time lying than control calves during the first four days following the procedure (Theurer et al., 2012). In both of these studies, use

of technology and automation allowed for objective measures of pain-related behaviour changes.

The second area of interest is the presence of abnormal behaviours. For example, Weary et al. (1998) found that piglets undergoing castration without analgesia produced more high frequency calls (> 1000 Hz) when compared to SHAM handled piglets (identically restrained but not physically castrated). Further, severing of the somatic cords was found to produce the largest difference between vocalisations of castrated and SHAM animals, with lesser differences observed during the phases of scrotal incision and testicle extrusion. This would suggest that the severing of the somatic cords is the most painful phase of castration in piglets, and that the rate of high frequency calls could be used as a reliable measure of pain in piglets (Weary et al., 1998). Gait (e.g. dairy cows; Flower and Weary, 2009) and postural abnormalities (e.g. lambs; Molony et al., 2002) have also been commonly used to identify animals in pain.

1.4 Conclusions and thesis objectives

In conclusion, caring for a large number of animals poses a number of welfare challenges; one priority among these, is the early identification of animals suffering from disease and pain. The immunological vulnerability of neonatal calves to disease, and the use of group housing systems in New Zealand, necessitates further research into behavioural measures that can be used to identify these conditions. The goal of this thesis was to explore two key areas important to calf welfare. The first study used naturally occurring disease (NCDC) in pre-weaned calves to assess whether changes in milk-feeding and lying behaviours could be used for early disease detection (chapter 2). The second study used thermal disbudding as pain model to determine whether changes in milk-feeding and lying behaviours could be used to identify pain in calves less than 4 weeks of age (chapter 3). Key results from this research are discussed in Chapter 4 and future opportunities identified.

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2 Lying and milk-feeding behaviour as measures of disease in New Zealand group-housed calves

2.1 Abstract

This study examined whether feeding and lying behaviours could be used for the early detection of neonatal calf diarrhoea (NCDC) in calves under 4 weeks of age. The milk feeding (total and proportion of milk consumed, frequency and duration of visits to the feeder, and the number of rewarded visits) and lying (number of lying bouts and proportion of time spent lying) behaviours of 112 calves (4 days of age at induction) were measured over a three week period (four replicates: 30, 28, 27 and 27 calves respectively). Data was collected via automated feeders and HOBO data loggers respectively. Postural observations (lying only) were also analysed (daily) from video footage at five minute intervals between 10:00 and 14:00. For statistical analysis, calves were considered ‘sick’ (i.e. animal showed clinical symptoms of NCDC, n=21), or ‘not sick’ (n=91) as a result of daily health checks and observation of clinical symptoms (diarrhoea, lethargy, dehydration, and/or fever). Analysis of feeding behaviour revealed four measures indicative of disease: 1) reduced total milk consumption, 2) increased duration of visits to the milk feeder, and 3) fewer rewarded visits compared to calves that were not sick. No difference in the number of lying bouts or proportion of time spent lying were detected between sick calves and calves that were not sick; however, sick calves increased the duration of lying bouts nearing the time of illness. Postural observations were not effective at predicting illness.

2.2 Introduction

Group housing of dairy calves in the first 6 to 8 weeks of life is common practice in New Zealand; in this context, early detection of diseased animals has long been identified as an important welfare and economic goal (de la Fuente et al., 1998; Svensson & Jensen, 2007). Certain animal management and environmental factors have been identified as contributing to the spread of disease among farm animals, with an increased risk of infection occurring within communal/group housing systems (de Graaf et al., 1999; Svensson & Jensen, 2007).

Neonatal calf diarrhoea complex (NCDC) has been recognised as the leading cause of poor health and mortality in neonatal calves worldwide (de la Fuente et

al., 1998; Hepola, 2003; Izzo et al., 2011; Al Mawly et al., 2015b). Among the most common enteropathogens involved in NCDC are: *Salmonella*, *Escherichia coli*, bovine coronavirus, bovine rotavirus and the protozoan parasite *Cryptosporidium parvum* (de la Fuente et al., 1998; Izzo et al., 2011; Al Mawly et al., 2015b). Each of these could affect the animal as an isolated pathogen or concurrently with other infections (de la Fuente et al., 1998; Izzo et al., 2011; Al Mawly et al., 2015b). A recent New Zealand study found 31% of 1 to 5 day old calves tested positive for at least one enteropathogen; this increased to 39% in calves 9 to 21 days old (Al Mawly et al., 2015a). Infected calves may present with a range of symptoms, from no symptoms at all (asymptomatic) to severe watery diarrhoea, fever, lethargy and dehydration (de la Fuente et al., 1999).

Aside from the obvious welfare implications of disease, potentially significant economic losses are also taken on by farmers. Beyond the direct treatment costs associated with neonatal diarrhoea, farmers also face increased labour costs, and mortality losses (resulting in fewer replacement cows). Major secondary economic consequences in the form of growth retardation and poor production following clinical disease may also be incurred (de la Fuente et al., 1998; Al Mawly et al., 2015a).

During the initial stages of infection with a pathogen, the host animal will often present with a fever and a reduction in plasma iron levels (Hart, 1988). This serves to inhibit growth of certain bacterial and viral pathogens, and to stimulate aspects of the immune system to assist in fending off the invading pathogen (Hart, 1988; Johnson, 2002). Activated cells of the immune system synthesise endogenous pyrogens which operate to increase the animals' thermoregulatory set point (regulated by the hypothalamus), thus producing a febrile response – maintenance of a body temperature higher than normal (Hart, 1988; Kluger et al., 1998).

Hart (1988) reviewed common behaviour patterns displayed by sickly animals at the onset of infectious disease, including: depression and lethargy, reduced appetite, and a reduction in grooming and exploratory behaviours (collectively termed 'sickness behaviour'). An increase in resting behaviours and reduction in social exploration has also been noted (Millman, 2007; Proudfoot et al., 2014). Due to the increased metabolic cost of initiating and maintaining the febrile

response, Hart (1988) argued that these common behavioural responses to illness are a means to promote energy conservation whilst maintaining an increased body temperature. Aubert (1999) further proposed that these behavioural alterations represented motivational changes in response to activation of the immune system, as a means to promote recovery. Such activities have long been used as a means to assess and/or predict disease and physical ailments by animal handlers, clinicians and scientists (Johnson, 2002; Weary et al., 2009).

A study by Stanton (2011) hypothesised that dairy calves, during disease, would alter their lying posture as a means to conserve energy or minimise pain/discomfort brought on by illness. They showed that 12% of calves suffering from calf diarrhoea were observed in a short lying position (calf in a tightly curled position with head tucked into the flank), compared to only 2% of healthy calves. By reducing the surface area exposed to the elements, animals observed in a short lying position were assumed to be conserving energy. Stanton (2011) predicted that lateral lying would be displayed by calves experiencing abdominal pain or discomfort due to diarrhoea, whilst lying in a ventral position with the neck extended was predicted to increase oxygen transfer in calves with respiratory illness. Observations of these final two postures were too few to draw any conclusions in the Stanton (2011) study.

Illness is often associated with a reduced appetite (Hart, 1988), and as such, several studies have reported that milk-feeding behaviours of young calves are influenced by disease (e.g. Svensson & Jensen, 2007; Borderas et al., 2008; Borderas et al., 2009). Methodological, animal management and calf demographic (age and breed) differences between studies, however, have produced varied results. For example, a study by Maatje et al. (1993) evaluated the milk intake of sick calves produced for veal and found that feeding behaviour alone was an inadequate indicator of illness; whilst a study by Borderas et al. (2009) found that the most sensitive milk-feeding measures of disease in dairy calves were dependent on the volume of daily milk allowance. High allowance calves (12 L) reduced milk intake and frequency of visits to the feeder, while visit duration increased. Low allowance (4 L) calves only reduced the duration of visits to the feeder, with no impact on total milk consumption. Measures previously assessed by automated feeders in relation to illness in calves have included: total milk intake, rate of milk intake (Svensson & Jensen, 2007; Borderas et al., 2008;

Borderas et al., 2009), frequency of visits to the milk-feeder, duration of these visits (Borderas et al., 2009), and the frequency of rewarded/unrewarded visits (Svensson & Jensen, 2007).

Advances in technology have provided a non-invasive means to objectively measure certain behaviours (Svensson & Jensen, 2007; Weary et al., 2009; Bonk et al., 2013). Studies that have utilised existing technology (e.g., Maatje et al., 1993; Svensson & Jensen, 2007; Borderas et al., 2009) to assess behavioural changes in response to disease have produced varying results. Furthermore, there is limited information regarding the effects of calf breed, animal management practices, and climatic differences on the disease-induced behavioural changes of New Zealand calves.

The objective of this study was to assess whether milk-feeding and lying behaviours could be used for the early detection of disease in New Zealand group-housed calves. Based on previous studies, it was hypothesised that morbid calves, compared to their healthy counterparts, would: a) reduce total milk consumption), b) show an increase in the average duration of lying bouts, and c) display specific lying postures such as lateral and short lying positions in response to active diarrhoea.

2.3 Material and Methods

2.3.1 Animals

Data was collected in accordance with protocols approved by the AgResearch Ruakura Animal Ethics Committee, Hamilton, New Zealand and the University of Waikato Animal Ethics Committee, Hamilton, New Zealand (Protocol 13283 and Protocol 941 respectively). A total of 112 animals were used and consisted of 71 dairy calves (Jersey, Friesian and dairy cross breeds - heifer calves only) and 41 New Zealand Herefords (18 heifers and 24 bull calves). Calves were allocated to one of four groups (30, 28, and two replicates of 27 calves respectively) at 4 days of age according to their order of birth, with one pen filled at a time - this ensured pen mates were all of similar size and stage of development. Age-matched Hereford calves were added to the final two groups, 14 and 27 calves respectively, due to a shortage of 4-day-old dairy heifers. Calves were returned to normal farm

management at the conclusion of the trial when they were an average of 3.5 weeks of age.

Identification of individual calves was four tiered. Numbered ear tags were used on all calves for identification purposes during daily observations and physical handling. EID tags were used by the automated feeders to quantify feeding activity. To ensure easy identification of individuals from a distance, calves were also allocated one of two coloured collars and one of four coloured symbols which were painted (Tell Tail, Farmers Industries (New Zealand) Limited; Mt Maunganui South, New Zealand) on each animal's back. Finally, photographs were used to document the unique coat patterns of each calf to ensure accurate identification in video footage, particularly in cases where painted markings had faded or were difficult to see.

2.3.2 Housing and feeding

Calves were housed in one of two adjoining indoor pens (Figure 1), located inside a commercial calf rearing shed. Flooring material consisted of wood chips. Post and rail fencing surrounded the outer perimeter of the two pens, while the shared partition consisted of two large steel gates. Pens were each equipped with four Sony handy cams (DCR-SX65E, Sony Corp., Tokyo, Japan) to record behaviours 4 hours per day (1000-1400 h), 7 days per week. Camera positioning differed slightly between pens due to minor differences in enclosure features (Figure 1).

Each pen contained three water troughs, one large hay container, and two narrow, elongated feed troughs (see Figure 1). Hay, pellets (Calf-pro1 20%, Seales Winslow., Morrinsville, New Zealand) and fresh water were available to calves *ad libitum* for the duration of the trial. Pens were fitted with an automated calf feeder (rEID Calf Feeder, A&D Reid, Temuka, New Zealand) that used an electronic identification device (EID) to identify individual animals as they approached to feed (Figure 2). Each feeder had a single teat to deliver whole milk (at approximately 22-25°C) on the following schedule: 2 L, three times per day with a minimum 400 minute withholding period between each feed. If a feed was not completed, calves could return at any point to consume what remained of that two litre allowance. Calf feeders recorded data pertaining to the frequency and duration of visits by each calf, in addition to the amount of milk consumed at each visit.

All calves were trained over a 3 to 4 day period to use the automated feeder from day one of the trial. To do this, each calf was directed to the feeder by an experienced handler and using the calves' natural instinct to suckle, handlers allowed the calf to suckle their fingers while gently guiding the calve's mouth over the teat. A padded lever mechanism directly above the teat required calves to press down on the lever with their muzzle as they suckled to release the milk. Calves that had trouble with this were first aided by the handler pushing the lever down before being encouraged to move further forward so that pressure was applied to the lever. This procedure was conducted twice daily for all animals. Once calves began to suckle reliably the handler stopped providing assistance and simply observed. Data collected by the automated feeder was then used to identify animals that needed further training.

Upon completion of the trial for the first two groups, the experimental pens were cleaned in preparation for the final replicates (groups 3 and 4). The top layer of woodchips was removed and replaced with clean chips. All surfaces were thoroughly sprayed using a broad spectrum disinfectant (Halamid, Axcentive; Bouc-Bel-Air, France). All water troughs and feed containers were also thoroughly cleaned and refilled.

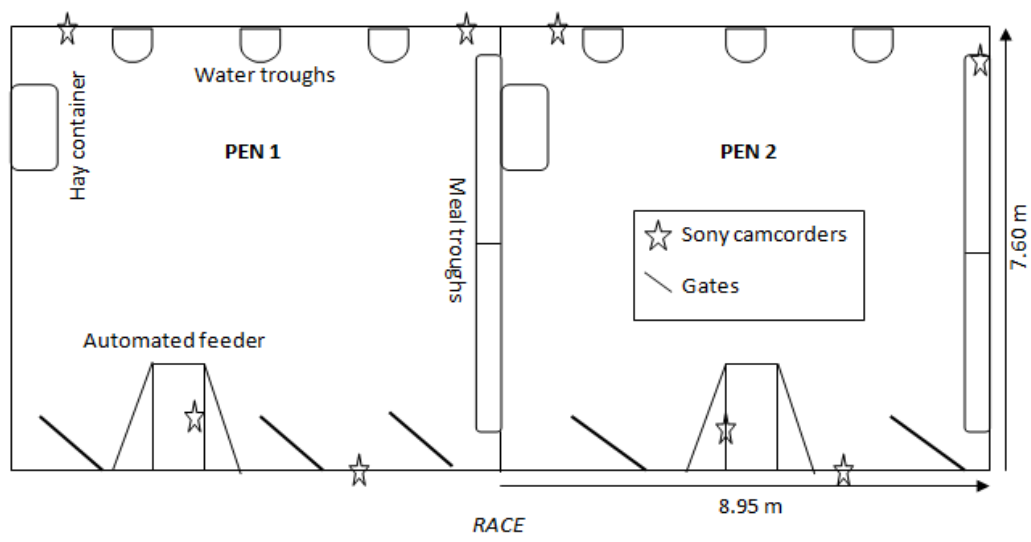


Figure 1 Layout of experimental pens and camera placement. Groups 1 and 2 were removed from the experimental pens upon completion of their trial and replaced with groups 3 and 4 respectively.

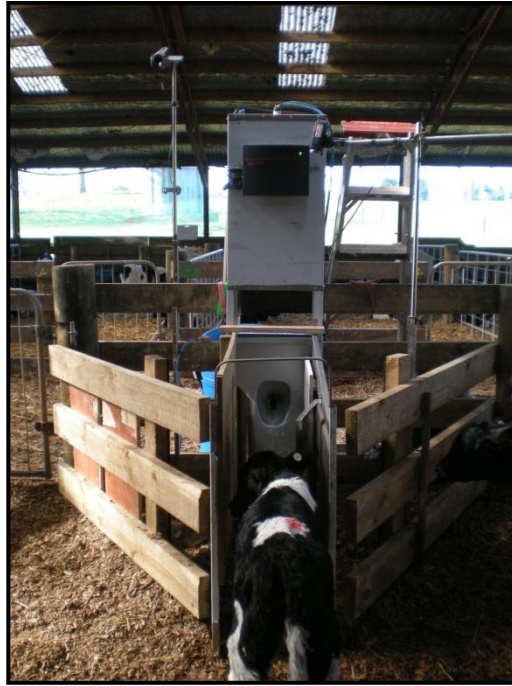


Figure 2 Calf approaching the single teat of the automated calf feeder

2.3.3 Data collection

2.3.3.1 Health status

Daily health checks and instantaneous postural observations were performed on each calf for the purpose of assessing general health and wellness, and the early detection of clinical illness, such as scours, high rectal temperature (39.5°C or higher), or signs of dehydration (sunken eyes, or poor skin elasticity – assessed using the skin tent test). Among other measurements, five illness indicators were used during health checks: coat condition (shiny and smooth vs. dry and rough), position of the ears (erect vs droopy; Figure 3A , B), eye appearance (bright and alert vs sunken and dull; Figure 3A, B), whether the calf presented as apathetic (Figure 3A, B) and whether the gut was visually observed as full or empty (Figure 3C, D).

During the physical exam, calves were also checked for signs of navel ill (inflammation, swelling and/or discharge from the navel) and other ailments such as nasal/ocular discharge, swelling, abscesses, and injury. Farm staff were instructed to treat any animals displaying signs of illness as per standard farm protocols. Calf lying/standing postures were recorded daily using the scan sampling method, prior to any physical handling, and calf weights were obtained on a weekly basis.



Figure 3 A) Calf presenting with bright eyes, erect ears and alert disposition. B) Calf presenting with dull, sunken eyes, droopy ears and apathetic disposition. C) Calf with “full” gut shows a filling out of the flank region (circled in red). D) “Empty” gut displays: pronounced hollowing of the flank region.

As a result of these daily health checks, calves were allocated to one of four general health scores, or a combination of these (Table 1).

Table 1 Daily health score based on daily observations and basic health check

| Health score/category | Description |
|------------------------------|---|
| 1. Healthy | No recorded signs of illness |
| 2. Intermediate | Calf presented with three or more of the five “illness indicators” (droopy ears, dull or sunken eyes, dull coat, gut is empty on visual inspection, or apathetic) and/or the calf has a rectal temperature higher than 39.5°C |
| 3. Sick | Any calf that was identified by farm staff or experimenters as clinically ill and blood and faecal samples taken (e.g. severe scours, signs of dehydration, fever) |
| 4. Other | Calf appeared to be healthy, but had signs of other abnormalities (e.g. swelling, lameness, clear ocular or nasal discharge) |

2.3.3.2 Lying behaviour








During allocation to groups, HOBO Pendant G data loggers (Onset Computer Corp., Bourne, MA) were fitted to the right hind leg just above the fetlock joint. The device was used to record lying behaviour at one minute intervals, and was removed from the calf at 3.5 weeks of age.

Observations of posture that were obtained from daily checks and video footage are described in Table 2. A single observer analysed video footage and recorded the lying postures using instantaneous sampling at five minute intervals between 10:00-14:00 h. This was done for all calves that received a health score of “2” or “3” (Table 1), and 10 healthy calves (health score of 1) from each group.

Observations of “intermediate/sick” calves were stopped the day prior to their last health score of interest (“2” or “3”). Observations of healthy calves ceased at the same time as those of the last “intermediate/sick” calf for each group.

Intra-observer reliability was conducted at three pre-determined time points for each of the four groups during video observations: 1) start of the trial, 2) near the group’s mid-way point, and 3) towards the end of video observations. Due to time constraints, the observer waited no longer than two weeks before repeating observations for up to 25% of calves. Initial observations were compared to secondary observations to determine reliability (%).

Table 2 Ethogram of postures observed during daily checks and examination of video footage

| Behaviour | Definition | |
|------------------|--|---|
| Lateral lying | Calf recumbent with one shoulder in full contact with the ground; all four legs often extended to the side. Head may be up or down. |  |
| Ventral lying | Calf lying with one or both front legs tucked under the body; hind legs may be tucked beneath or close to the body or positioned outward. Head positioned in a relaxed, up-right position, not touching the ground |  |
| Short lying | Calf in a tightly curled position with both forelegs tucked beneath or close to the body with the hind legs positioned close to the body and the head tucked tight to the body |  |
| Midway | Similar to short lying position, with the head positioned toward the body but not tightly tucked against the side |  |
| Neck extended | Neck outstretched in front of the calf with the ventral surface of the mandible in full contact with the ground while calf is in a ventral lying position |  |
| Natural standing | All four hooves are weight bearing and in contact with the ground; body is relaxed |  |
| Tucked standing | Calf standing stiff and motionless with the abdomen noticeably tucked up and the tail tucked tight between the hind legs |  |

2.3.4 Blood analysis (plasma and serum)

Trained staff obtained two, 4 mL blood samples (serum and plasma) from each calf at 4, 11 and 18 days of age by means of jugular venipuncture. Calves were manually restrained for the procedure by a skilled handler. Blood samples were stored at approximately 4°C until they were delivered to the New Zealand Veterinary Pathology (NZVP; Hamilton, New Zealand) laboratory for analysis

(same day as collection for samples collected Monday to Friday; see below for weekend samples). Additional blood and faecal (manual collection via gentle palpation of the rectum and stored at 4°C) samples were obtained from any calves that were considered to be clinically ill. Calves were considered to be clinically ill if they presented with one or a combination of the following symptoms: severe scours, high rectal temperature (39.5°C or higher), signs of dehydration, apathetic appearance. These samples were stored in the same manner as routine samples.

Weekend samples were handled as follows: serum samples were centrifuged (Heraeus Multifuge X1R, Thermo Fisher Scientific; Waltham, MA) at 1,610 g for 10 minutes to separate red blood cells (RBCs) from plasma. The top plasma layer was aliquotted into eppendorf tubes and refrigerated at 4°C along with any faecal samples. Whole blood samples collected in EDTA tubes were used to perform a blood smear, with the remaining sample placed in the refrigerator. Blood smear slides were housed in slide covers and stored at room temperature. All samples were then taken to the NZVP laboratory on the following Monday for analysis.

All laboratory analyses were conducted by technicians at New Zealand Veterinary Pathology laboratory. The following analyses were performed on plasma and serum samples collected during routine sampling (4, 11 and 18 days old) and those obtained from clinically ill calves.

2.3.4.1 Haematology analysis

Total white blood cell count and neutrophil:lymphocyte ratios were measured from whole blood samples. Total white blood cell counts were measured by means of hydro dynamic focusing and flow cytometry using a Sysmex XT-2000iV Haematology Analyser (Sesmex Corporation, Kobe, Japan). The neutrophil:lymphocyte ratio was calculated (% neutrophils / % lymphocytes) following a 200 cell manual differential on Leishman stained blood smears (Leishman stain, Milton Adams Ltd., Auckland, New Zealand).

2.3.4.2 Chemistry analysis

Haptoglobin concentrations were measured from serum samples. A commercially available colorimetric assay (“PHASE”TM Haptoglobin Assay Cat. No. TP-801; Tridelta Development Limited, Maynooth, County Kildare) was used, and the automated method performed in accordance with the manufacturer’s instructions. The analytical sensitivity of this assay was 0.005mg/ml haptoglobin.

2.3.5 Faecal analysis

Faecal samples were obtained from diarrhoeic calves and were analysed for the presence of Cryptosporidium, Rotavirus, Coronavirus and Salmonella as follows:

2.3.5.1 Cryptosporidium

An acid fast stain was performed to visually assess the presence of cryptosporidium which could be seen as round bodies measuring 4-5 μ in diameter and dark red or pink in colour. If more lightly stained, the parasites showed internal bodies that were darker blue or brownish in colour.

2.3.5.2 Rotavirus and coronavirus (ELISA)

Faecal samples were analysed for the presence of rotavirus and coronavirus using a commercially available ELISA kit (Pourquier® ELISA Calves Diarrhoea; Institut Pourquier®, Montpellier, France). The five-step, naked eye reading method was conducted in accordance with the manufacturer's instructions.

2.3.5.3 Salmonella

Selective enrichment faecal cultures were used for the detection of salmonella. Suspect colonies underwent slide agglutination using polyvalent antisera for confirmation purposes.

2.3.6 Statistical analysis

Due to the small number of clinically diseased calves (actively diarrhoeic plus signs of dehydration and/or fever), animals were grouped for data analysis based on health scores received throughout the trial (i.e. "sick" calves received a health score of 3 at some point during the trial whereas "not sick" calves never received a health score of 3). Sick calves were then analysed separately from the time they started the trial until the day prior to showing signs of illness. This showed changes in behaviour over time as they became diseased. Data was removed from analysis if the calf was not allocated a health score on a particular date; these animals were most likely observed to be clinically ill (active diarrhoea, fever, lethargic) and removed from the experimental pen, leading to the missing data. All data was analysed using R (version 3.02; package mgcv).

Total milk consumption, proportion of milk consumed (amount consumed / amount permitted), frequency of visits to the feeder, and the duration of visits

across the two health categories (“sick” and “not sick”) were all analysed using linear mixed models. The fixed component for all of these models was “health category” (sick/not sick), whilst the random components consisted of date, calf identification number and replication number. These analyses were repeated for only “sick” calves to assess any changes in these behaviours as animals near a time of illness. The fixed component for these models was changed to “days before illness” (the day calves received a health score of 3 = 0, one day before this = -1, two days before = -2 etc). Random components remained the same as previous models.

A generalized linear mixed model assuming binomial data with a logit link was used to analyse the proportion of rewarded visits to the feeder across the two health categories. The number of rewarded visits to the feeder was used as the response variate, whilst the total number of visits to the feeder (regardless of whether they were rewarded or not) made up the binomial totals. Again, “health category” was used as the fixed component, while random components included the replicate number, calf identification number, and date. This analysis was repeated for “sick” calves only and “days before illness” replaced “health category” as the fixed component (random components remained the same).

The total number of lying bouts and proportion of time spent lying was compared across health categories; this was analysed using linear mixed models. In each model the fixed component and random components remained the same as previous models. A second analysis assessed changes in the number of lying bouts and proportion of time spent lying by sick calves as they approached illness and “days before illness” was used as the fixed component. Random components remained the same as previous models. Data was removed if HOBOT data loggers showed that the animal had not moved for 24 hours as this was very unlikely to have occurred and probably indicated malfunction.

Again, lying postures of the two health categories were analysed using linear mixed models. Data was log transformed due to all posture counts being positive, and some very high counts having been obtained. Lying postures thought to be associated with illness (lateral lying, short lying and neck extended lying) were grouped for analysis due to the low frequency in which they were observed. “Health category” was used as the fixed component, whilst random components

remained the same: replicate number, calf identification number and date. This process was repeated for only sick calves to assess posture changes nearing the time of disease and “days before illness” replaced “health category” as the fixed component for this model.

Statistical significance for all results was set at $P \leq 0.05$.

2.4 Results

Of the 112 calves used in the current study, 21 were identified as clinically sick (i.e. severe scours plus signs of dehydration and/or fever). Only four of these animals tested positive for enteropathogens (*Cryptosporidium*).

2.4.1 Feeding data

Total milk consumption and proportion of milk consumed was found to be lower for “sick” calves compared to “not sick”, however, no difference in the frequency of visits were detected between these two health categories. Group averages for the proportion of milk consumed by calves in each health category are shown in Figure 4. “Sick” calves were less likely to receive a rewarded visit when compared to calves that were “not sick”, and visit duration was found to be significantly higher (see Figure 5). Analysis of “sick” calf feeding data in the days leading up to illness (date they were considered clinically ill and got a health score of “3”) showed no trends or changes in milk-feeding behaviour for any of the factors described above. All test statistics are presented in Table 3.

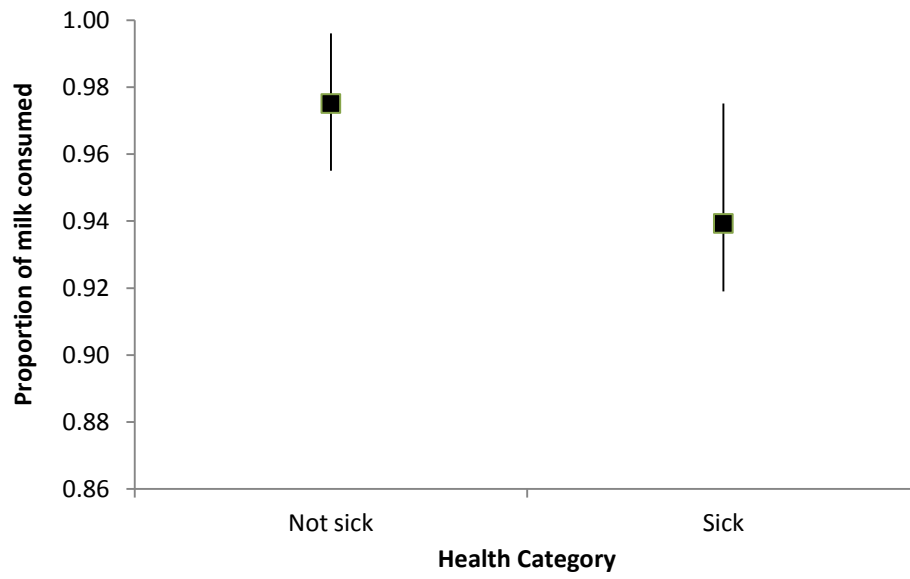


Figure 4 Average proportion of daily milk allowance consumed by "sick" (n=21) and "not sick" (n=91) calves - upper and lower CI included (P<0.001; see Table 3).

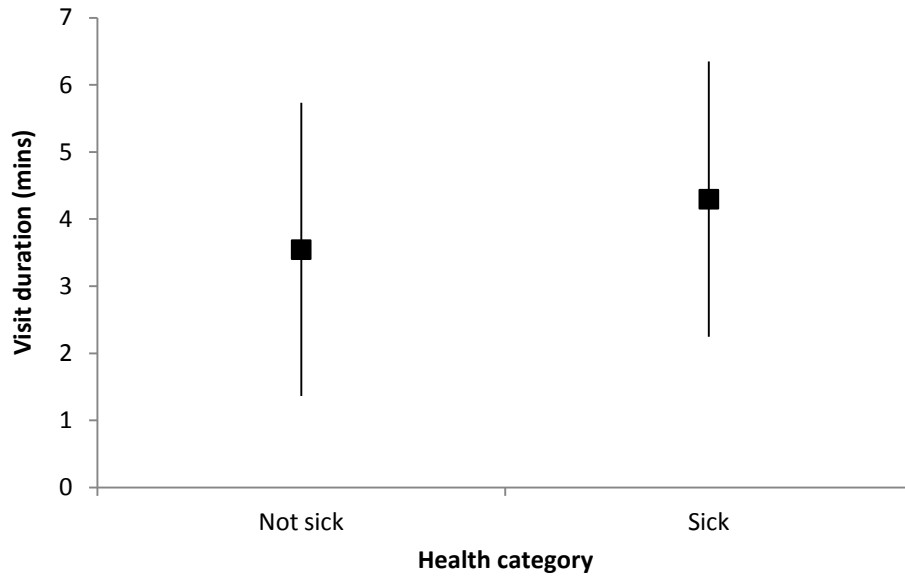


Figure 5 Average visit duration (mins) for "sick" (n=21) and "not sick" (n=91) calves - upper and lower CI included (P = 0.04; see Table 3).

Table 3 Test statistics relating to the feeding activities of "sick" (n=21) and "not sick" (n=91) calves, and feeding activities of sick calves in the days leading up to illness

| “Sick” vs “Not sick” calves | | | | | |
|------------------------------------|-----------------|-------------------|-------------|----------------|----------------|
| MILK CONSUMPTION | Estimate | Std. error | d.f. | T value | P-value |
| Intercept | 1938.9 | 14.9 | 3.5 | 130.5 | <0.001 |
| Sick / Not sick | -67.1 | 12.7 | 170.5 | -5.3 | <0.001 |
| PROP. CONSUMED | | | | | |
| Intercept | 0.98 | 0.007 | 3.4 | 142.8 | <0.001 |
| Sick / Not sick | -0.04 | 0.007 | 174.3 | -5.9 | <0.001 |
| FREQ. OF VISITS | | | | | |
| Intercept | 5.3 | 0.5 | 4.2 | 11.6 | <0.001 |
| Sick / Not sick | -0.7 | 0.4 | 430.4 | -1.8 | 0.08 |
| VISIT DURATION | | | | | |
| Intercept | 213.1 | 43.0 | 3.2 | 5.0 | 0.01 |
| Sick / Not sick | 44.4 | 21.6 | 191.8 | 2.1 | 0.04 |
| PROP. OF REWARDED VISITS | | | | Z value | P-value |
| Intercept | 1.3 | 0.2 | - | 6.3 | <0.001 |
| Sick / Not sick | 0.7 | 0.2 | - | 3.9 | <0.001 |
| Sick calf analysis | | | | | |
| MILK CONSUMPTION | Estimate | Std. error | d.f. | T value | P-value |
| Intercept | 1860.2 | 36.7 | 3.4 | 50.7 | <0.001 |
| Days until sick | 0.5 | 3.2 | 83.3 | 0.2 | 0.9 |
| PROP. CONSUMED | | | | | |
| Intercept | 0.9 | 0.02 | 3.4 | 50.7 | <0.001 |
| Days until sick | 0.0003 | 0.002 | 83.3 | 0.2 | 0.9 |
| FREQ. OF VISITS | | | | | |
| Intercept | 4.4 | 0.8 | 2.7 | 5.8 | 0.01 |
| Days until sick | -0.05 | 0.04 | 52.9 | -1.2 | 0.2 |
| VISIT DURATION | | | | | |
| Intercept | 318.1 | 38.7 | 29.0 | 8.2 | <0.001 |
| Days until sick | -1.4 | 3.2 | 29.3 | -0.4 | 0.7 |
| PROP. OF REWARDED VISITS | | | | Z value | P-value |
| Intercept | 1.6 | 0.3 | - | 5.4 | <0.001 |
| Days until sick | -0.02 | 0.02 | - | -0.9 | 0.4 |

2.4.2 Lying data

2.4.2.1 Lying bouts and proportion of time spent lying (HOBO data)

No difference in lying bouts or proportion of time spent lying were detected between sick calves and those that were not sick; however, sick calves showed a reduced number of lying bouts as they approached “day of illness”. No change in

the proportion of time spent lying was observed. All test statistics for lying bouts and proportion of time spent lying are presented in Table 4.

Table 4 Test statistics relating to lying behaviours of "sick" (n=21) and "not sick" (n=91) calves, and lying behaviours of sick calves in the days leading up to illness

| “Sick” vs “Not sick” calves | | | | | |
|------------------------------------|-----------------|-------------------|-------------|----------------|----------------|
| NO. OF LYING BOUTS | Estimate | Std. Error | d.f. | T value | P-value |
| Intercept | 23.4 | 0.06 | 4.5 | 367.9 | <0.001 |
| Sick / Not sick | -0.009 | 0.1 | 106.6 | -0.07 | 0.9 |
| PROP. LYING | | | | | |
| Intercept | 0.7 | 0.01 | 3.7 | 68.6 | <0.001 |
| Sick / Not sick | -0.003 | 0.02 | 93.0 | -0.2 | 0.9 |
| Sick calf analysis | | | | | |
| NO. OF LYING BOUTS | Estimate | Std. Error | d.f. | T value | P-value |
| Intercept | 23.0 | 0.3 | 2.5 | 83.5 | <0.001 |
| Days until sick | 0.05 | 0.02 | 75.7 | 3.0 | 0.004 |
| PROP. LYING | | | | | |
| Intercept | 0.7 | 0.01 | 49.3 | 61.2 | <0.001 |
| Days until sick | 0.002 | 0.001 | 70.2 | 1.6 | 0.1 |

2.4.2.2 Lying posture (video data)

Results show that both “health category” and “days leading up to illness” influenced lying positions. The frequency of lateral, short and neck extended lying (grouped for analysis), and ventral lying, was found to be higher for calves that were not sick when compared to sick calves (Figure 6); however, these positions were seen to increase in sick calves during the days leading up to illness (Figure 7). Conversely, midway lying was found to be higher in sick animals, yet this position declined in the days leading up to clinical disease.

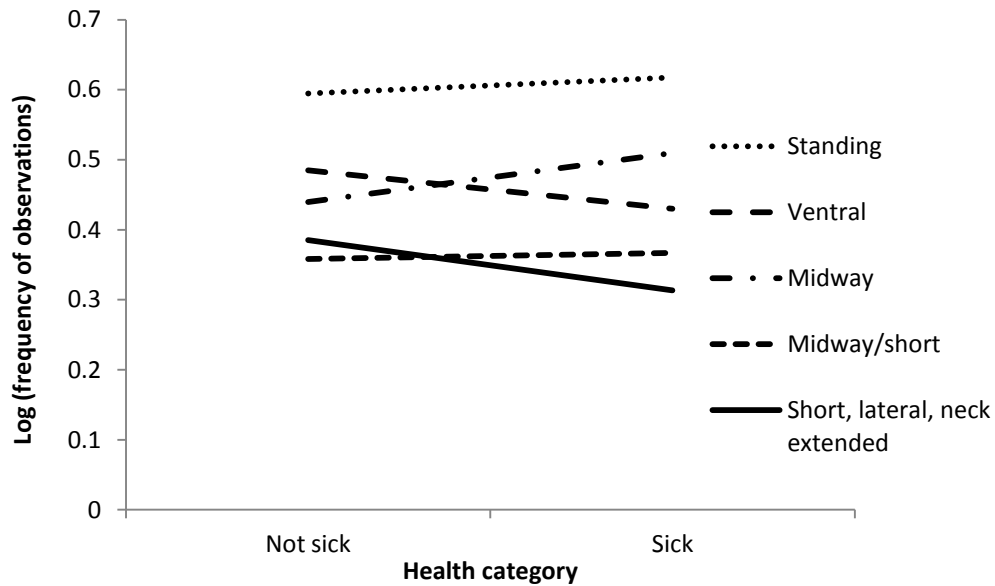


Figure 6 Logged frequency of postural observations between the two health categories (“sick” (n = 12) and “not sick” (n=40)). Average standard error across both health categories = 0.03.

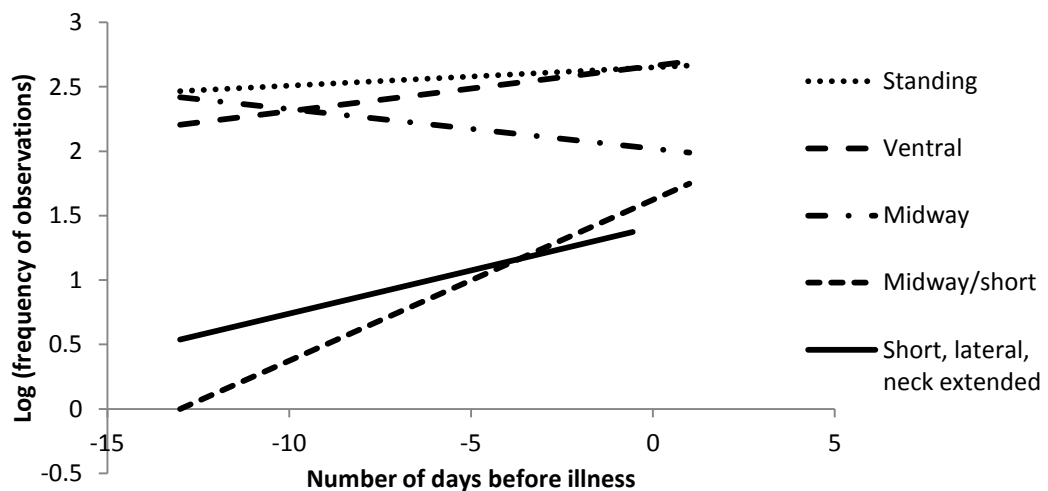


Figure 7 Logged frequency of postural observations for sick calves (n=12) in the days leading up to illness (0 = day of illness, -1 = one day before illness etc). Average standard error for all lying positions = 0.27.

The average observer reliability for video data (lying postures) across all four repetitions was 94.4% (range 85.7 – 98.0%).

2.5 Discussion

The current study showed that calves later identified as diseased, displayed significant differences in milk-feeding behaviours when compared to calves that were deemed “not sick” over the duration of the trial. Milk consumption was found to be significantly lower for sick calves; these animals were also less likely to receive rewarded visits, and the duration of visits was longer than those of

calves deemed “not sick”. Interestingly, a second analysis of only sick calves revealed no significant changes in milk feeding behaviours as they approached day of illness. This would suggest that group means for total and proportion of milk consumed, number of rewarded visits, and duration of visits was either influenced by a dramatic change in feeding behaviour after disease was identified, or that at least some calves showed longer-term milk-feeding deficits, originating well before symptoms of disease were observed. In the latter case, it may be possible that milk-feeding deficits impacted on, or were indicative of, the animals’ future susceptibility to disease.

Initial results appear to correspond well with those of Borderas et al. (2008) who found that in the two days preceding illness (LPS challenge), sick calves fed on a high milk allowance reduced their total milk consumption, and increased duration of visits to the feeder when compared to healthy counterparts. Borderas et al. (2008) also reported that sick calves reduced their frequency of visits to the feeder, whilst the current study showed no such change. Calves in the Borderas et al. (2008) study could be seen as conserving energy by limiting the number of feeder visits, and drinking more at each visit (longer visit duration) to maintain the elevated metabolic costs of producing a febrile response (Hart, 1988). The reduced milk intake of these sick calves should not be surprising since the high milk allowance of 12 L per day, is far beyond that of a conventional calf feeding allowance (volume based on 10% of the animal’s total bodyweight; Jasper & Weary, 2002); however, my study also showed a reduction in total milk consumption when calves were receiving only 6 L of milk per day. This could be explained by a stronger behavioural response to disease in my study, compared to the mild response produced by an LPS challenge (Borderas et al., 2008). Svensson and Jensen (2007) reported that diseased calves fed on restricted milk rations (volume was breed dependent) showed no change in the volume of milk consumed; however, unrewarded visits declined. These results may indicate that calves were receiving enough milk to maintain metabolic homeostasis during disease, while the reduced number of unrewarded visits may be viewed as a means to conserve energy. While direct comparisons between these three studies cannot be made due to methodological differences, it is clear that the milk-feeding behaviour of sick calves differ from that of healthy calves. All of these studies

support the idea of sickness-induced changes in behaviour (Hart, 1988), and motivational reorganisation (Aubert, 1999) to facilitate recovery.

Sick calves in the current study showed no difference in the number of lying bouts or proportion of time spent lying when compared to calves deemed “not sick”. Analysis of sick calves in the days leading up to illness, however, showed a significant decline in the number of lying bouts during this period. No difference in the proportion of time spent lying was detected which indicates that the duration of individual lying bouts had increased. Similarly, Borderas et al. (2008) reported that calves exposed to varying levels of bacterial lipopolysaccharide (LPS) showed no changes in the overall time spent lying, but longer periods of lying inactive were observed. Similarities in the number of lying bouts and proportion of time spent lying between the two health categories in the current study indicates that any changes in lying activities as a result of disease are short-lived. These results further support Hart’s (1988) argument that an increase in resting behaviours encourages energy conservation and aids in the facilitation of a febrile response in the early stages of disease.

Evidence indicates that lying postures in the current study were influenced by health category (“sick” or “not sick”), and days leading up to illness in sick calves. Interestingly, positions that were observed more frequently in calves deemed “not sick” over the duration of the study, were found to steadily increase in sick calves nearing the day of illness (e.g. short, lateral and neck extended (grouped), ventral lying positions). One possible explanation for this could be that lying positions were also influenced by environmental factors (e.g. ambient temperature, wind speed and level of solar radiation) leading to a thermoregulatory response in the form of different lying positions (Hänninen, 2007). Further complicating the results of lying posture in response to disease was the need to group lying positions that were of particular interest due to the small number of observations (short, lateral and neck extended lying). This has meant that the significance of these individual postures in relation to diseased calves could not be assessed. Future studies should include abnormal standing postures, and stomping or kicking activities as indicators of abdominal discomfort. These behaviours have been noted following castration in young calves (Molony et al., 1995).

The current study utilised naturally occurring disease to assess changes in the milk-feeding and lying behaviours of diarrhoeic, pre-weaned calves. While this method permitted investigation of naturally induced changes in behaviour, it also resulted in a very small sample size of clinically diseased calves. This necessitated the grouping of all other calves into a single health category (“not sick”) for comparison against sick calves. This newly formed group consisted of healthy calves with no sign of disease or injury (“healthy”), calves that showed a general deterioration of condition with no other apparent signs of disease (“intermediate”), and calves that were showing other concerns such as ocular discharge, abscesses, lameness etc (“other”). This may have confounded differences in milk-feeding and lying behaviours that would have otherwise been clear if diseased calves were compared to only healthy calves.

In conclusion, milk-feeding activities differed between sick calves and calves that were deemed “not sick”. Any deficits in these feeding behaviours, as displayed by sick calves over the duration of the trial, could be indicative of a calves’ susceptibility for future disease, and may thus be an area worthy of further exploration. Several measures of milk-feeding behaviour were considered to be useful for disease detection: 1) total and proportion of milk consumed, 2) longer duration of visits to the feeder, and 3) reduced probability of receiving a rewarded visit. Lying activities (as measured by HOBO data loggers) showed that a reduced number of lying bouts may be a useful indicator for early disease detection in pre-weaned calves. Lying positions were found to be influenced by the animals’ health status and days preceding illness; however, the potential for lying positions to be used as a means of early detection of disease could not be determined due to methodological issues.

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3 Lying and milk-feeding behaviour as measures of pain in New Zealand group-housed calves

3.1 Abstract

This study examined whether feeding and lying behaviours could be used to identify calves in pain after hot iron disbudding (pain model), and how the expression of these behaviours differed with the use of various analgesic regimes. Milk feeding (total and proportion of milk consumed, frequency and duration of visits to the feeder, and the number of rewarded visits) and lying (number of lying bouts and proportion of time spent lying) behaviours of 53 calves (26.5 ± 3.5 days of age) were measured over three observation periods (pre-treatment -120 h–0 h, treatment 0-24 h, and recovery 24-72 h). Calves were randomly allocated to one of five treatment groups: hot iron disbudded with no analgesia (DB, n=11), disbudded with a local anaesthetic (DB+LA, n=11), disbudded with a non-steroidal anti-inflammatory drug (DB+NSAID, n=11), disbudded with NSAID and LA (DB+NSAID+LA, n=10), and SHAM (identical handling but no physical DB; n=11). Data was obtained using automated feeders and HOBO data loggers respectively. Analysis of feeding behaviour revealed only one difference between all treatment groups; SHAM calves showed a greater number of visits to the milk feeder during the recovery period compared to disbudded animals. All calves (including SHAM animals) showed a significant reduction in the number of lying bouts and proportion of time spent lying on the day of treatment. Feeding and lying behaviours in this study were considered to be an insufficient measure of pain.

3.2 Introduction

Freedom from pain by means of prevention, or prompt identification and treatment, is important to maintain animal welfare (Fitzpatrick et al., 2006; Thomsen et al., 2012). Behavioural alterations will often be the first sign that an animal is in pain. In cases where these changes are subtle (e.g. stoicism of cattle), detection and interpretation may be hindered leading to treatment delays and negative welfare implications (Stafford & Mellor, 1993; O'Callaghan et al., 2003). Further adding to the difficulty of pain assessment of farm animals is the use of group-housing systems which may limit the ability of farmers to assess individual

animals (Fitzpatrick, et al., 2006), and a difference of opinion (or communication breakdown) between farmers and veterinarians over the best course of treatment in any given case (Thomsen et al., 2012). On-farm pain assessment has garnered a lot of attention in recent years from veterinarians, animal researchers and farmers, particularly with a surge in public concern and awareness of production animal welfare (Fraser et al., 2001; Mench, 2008).

At the same time, automated practices have been implemented on-farm to improve labour efficiency (e.g. automated feeders, robotic milking systems). Together with the extensive use of group-housing systems in New Zealand, this could increase delays in identifying individuals experiencing pain due to reduced opportunities for animal handling and direct observation. Identification of how automated technology could be used to assist in the early detection of animals experiencing pain, and perhaps its severity, could lead to improved animal welfare.

Standard on-farm husbandry practices such as disbudding/dehorning, castration, and tail docking are considered “essential” procedures, required to maintain and/or improve the health and safety of both animals and handlers (Molony & Kent, 1997; Stafford & Mellor, 2005; Gottardo et al., 2011). Each of these procedures involves trauma to the animal’s tissues, and have been reported to induce varying degrees of pain and distress (Stafford & Mellor, 1993; Molony & Kent, 1997; Grant, 2004; Lomax et al., 2009). Pain severity is likely to be affected by factors including the method employed to conduct the procedure, age of the animal, and use of pain relief (i.e. analgesics and/or anaesthetics; Stafford & Mellor, 1993; Molony et al., 1995; Stilwell et al., 2009). Bufalari et al. (2007) also suggests species, breed and health status can affect pain thresholds and responses of individual animals.

Calf disbudding studies involving caustic paste and hot iron methods (Vickers et al., 2005; Stewart et al., 2009; Stilwell et al., 2009) have shown that physiological and behavioural responses to pain can be observed for several hours post-disbudding. Further, alleviation or reduction of these responses can be achieved with the administration of local anaesthetics (LA) and nonsteroidal anti-inflammatory drugs (NSAIDs) (Stewart et al., 2009; Stilwell et al., 2009; Stafford & Mellor, 2011). Lidocaine, a commonly used LA, reduces the immediate physiological and behavioural pain responses to disbudding; the affect is short-

lived, lasting approximately 2-3 hours from the time of administration (Faulkner & Weary, 2000; Stafford & Mellor, 2005). Complimentary to the action of LA, NSAIDs may reduce longer lasting inflammatory responses of disbudding, but has little effect on the initial spike in plasma cortisol concentration, observed immediately following the procedure (Stafford & Mellor, 2005). A combination of LA and NSAIDs, administered prior to disbudding, may enable the alleviation of acute and chronic pain and distress following the procedure (McMeekan et al., 1999; Stafford & Mellor, 2005).

Physiological measures associated with detecting disbudding pain have included those related to the activity of the hypothalamus-pituitary-adrenal-axis (HPA) (Morisse et al., 1995; Stilwell et al., 2009; Graf & Senn, 1999), and responses of the autonomic nervous system (Stewart et al., 2008; Stewart et al., 2009; Stewart et al., 2010). The collection of physiological measures is often invasive and time consuming, and requires specific training to obtain. Whilst these measures are useful in a clinical or research setting, for day-to-day use on-farm, their practicality may be limited.

Behavioural measures associated with disbudding pain in calves have included: increased head shaking/jerking, head rubbing (Vickers et al., 2005), ear flicking (Faulkner & Weary, 2000; Stilwell et al., 2009), tail wagging, abnormal backward movements (Graf & Senn, 1999), kicking/stomping (Grøndahl-Nielsen et al., 1999), and a reduction in social interactions (Morisse et al, 1995) and play behaviours (Mintline et al., 2013). Due to the specificity of some of these activities and the location of pain after disbudding (residual head wounds), the assessment value of many of these behaviours will be limited to head pain (e.g. increased head movements and ear flicking).

Reductions in feed intake (Fisher et al., 1996; Weary et al., 2006; Bates et al., 2015) and mobility (or increased lying times) (Stilwell et al., 2009) have been described following painful procedures (e.g. castration and disbudding) and during periods of chronic pain (e.g. lameness) in many species (McGlone et al., 1993; Prunier & Leterrier, 2014). The close relationship between various pain types and feed intake and lying behaviours, along with the ability to easily quantify these activities in a non-invasive manner using technology, made these behaviours ideal for use in my research.

The current study utilises the hot iron method of disbudding, with various analgesic regimes, on calves under 4 weeks of age. The aim was to assess whether milk-feeding and lying behaviours can be used to identify calves experiencing pain, and its severity. Justification for this decision was three-fold: first, disbudding is a procedure that calves are routinely subjected to on New Zealand dairy farms as an “essential” husbandry procedure, and thus offers the opportunity to assess pain measurement and mitigation. Second, milk-feeding and lying behaviours of calves may offer a simple measure of pain that could be obtained non-invasively using existing technology. Finally, the use of control animals, and various combinations of LA and NSAIDs should allow for assessments of pain mitigation strategies.

I predicted that calves disbudded with no LA or NSAIDs would experience intense pain following disbudding and would thus show a significant increase in the duration of lying, and a reduction in the number of visits to a milk feeder (leading to reduced milk consumption). Conversely, calves that were SHAM disbudded, or received both LA and NSAIDs, were expected to show little to no change in lying and feeding behaviours due to the absence of pain, or effective pain management. Calves receiving either LA or NSAIDs alone were expected to show changes in feeding and lying behaviours intermediary to those described above.

3.3 Material and Methods

3.3.1 Animals

This study was conducted at AgResearch New Zealand’s Tokanui Dairy Research Farm in South Waikato, New Zealand (-38.071947, 175.327592). A total of 53 animals were used across three replicates (20, 18 and 15 animals respectively); 50 were dairy calves (Fresian, Jersey and dairy crossbreeds - heifers only) and three were New Zealand Herefords (one heifer and two bull calves – used in the third replicate) at 26.5 ± 3.5 days of age. All calves were part of three larger groups (30, 28 and 27 respectively) that were used in a previous trial assessing the viability of using non-invasive behavioural measures for early disease detection in calves up to 3 weeks of age (see previous chapter). Data was collected in accordance with protocols approved by the AgResearch Ruakura Animal Ethics Committee,

Hamilton, New Zealand and the University of Waikato Animal Ethics Committee, Hamilton, New Zealand (Protocol 13283 and Protocol 941 respectively).

The three-tiered approach used to identify individual calves in the current study is described in Table 6.

Table 5 Methods for identification of individual calves

| Identification type | When it was used |
|--|---|
| <i>Numbered ear tags (left ear)</i> | Used to identify calves for daily health observations, physical handling and disbudding |
| <i>EID (right ear)</i> | Used by automated feeders to log feeding data and milk allocation for individual calves |
| <i>Coloured collars and painted symbols on the animal's back</i> | Used to identify subjects from a distance for the behavioural observations used in the previous chapter |

3.3.2 Housing and feeding

The subset of calves from the previous trial were randomly selected as subjects for the current study. These animals remained in their original (larger) groups from the first trial, and adjoining indoor pens (Figure 1). By maintaining group stability, I ensured minimal interference with the calves' daily routines and social relationships. The outer boundary of the experimental pens was comprised of post and rail fencing, with large steel gates making up the shared division between pens. A thick layer of wood chips covered the pen floor. Fresh water, hay and pellets (Calf-pro1 20%, Seales Winslow, Morrinsville, New Zealand) were available to calves *ad libitum* for the duration of the trial. Groups one and two were subjected to the trial simultaneously, with group three replacing group one in the experimental pen upon completion of the initial trial (hygiene procedures described in previous chapter).

An automated calf feeder (rEID Calf Feeder, A&D Reid, Temuka, New Zealand) was fitted to each pen (Figures 8 and 9); an Electronic Identification Device (EID) was used to identify individual calves as they approached the teat. Feeders were equipped with a single teat positioned between two barriers to prevent access by more than one calf at a time (Figure 9). Whole milk was delivered to calves via the automated feeder at a temperature of 22-25°C. Each calf was permitted a total allowance of 6 litres per day on the following schedule: 2 litres, three times daily

with an enforced minimum withholding period of 400 minutes between each completed feed. Duration and frequency of rewarded and unrewarded visits (i.e. those occurring during withholding periods) to the feeder by individual calves was automatically recorded, along with the volume of milk consumed at each rewarded visit.

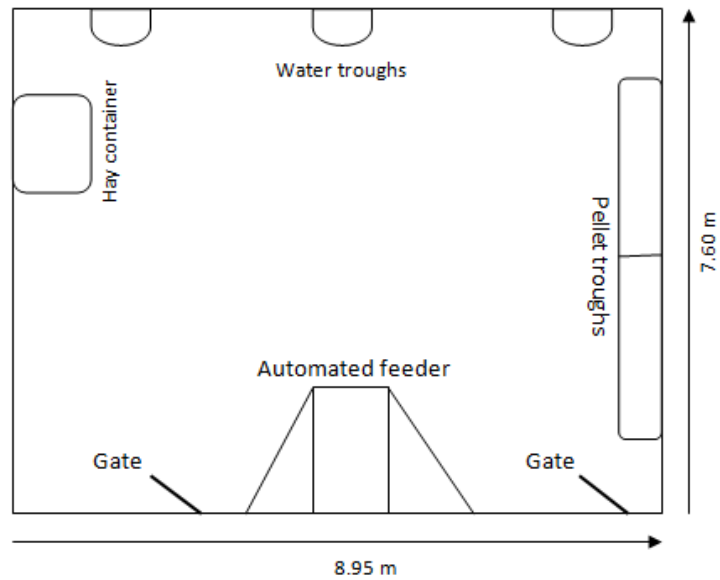


Figure 8 Experimental pen (one of two adjoining pens used in the study). The second pen was size-matched to the one depicted, however, minor differences in the positions of gates at the front of the pen resulted in small adjustments to the exact position of the automated feeder



Figure 9 Calf approaches the automated feeder between two barriers designed to ensure that only one calf could access the single teat at any one time

3.3.3 Treatments

Subsets of calves in each replicate were randomly allocated to one of five treatments (see Table 5). On the morning of disbudding (or SHAM treatment), portable gates were used to section off treatment calves from the rest of their group in the observation pen. This was done approximately one hour prior to treatments. Calves were restrained using a manually operated calf crush with a head bale (Front Opening Calf Bail, Te Pari, Oamaru, New Zealand). Once the head was restrained, any required drugs (determined by the treatment group, and described in Table 5) were administered.

Drug administration (where applicable) was followed by a five minute baseline period (Table 5). The concave tip of an electric cautery iron (Electric soldering iron LI 230b (Figure 10), Lister GmbH; Lüdenscheid, Germany), was then placed over the horn buds of subjects in the first four treatments in Table 5. Residual wounds were treated with a broad spectrum antibacterial aerosol spray (Aerotet, Virbac New Zealand Ltd; Hamilton, New Zealand) to reduce the possibility of infection (Figure 11). A non-operational cautery iron was used to apply pressure to the horn buds of sham animals. Animals were then released back into their main group and observed for 72 hours before being returned to normal farm conditions. All five treatments were administered by the same experienced veterinarian across replicates.

Table 6 Treatments and associated protocols

| Treatment and number of animals | Description |
|---|--|
| Disbudding (DB) N=11 | An electric cautery iron was used to remove the horn buds and surrounding tissue, with no drugs administered. |
| Disbudding + local anaesthetic (DB+LA) N=11 | 5 ml of 2% lignocaine hydrochloride was used to administer corneal (3 ml) and ring nerve blocks (2 ml spread over approximately five injection sites around the base of the horn bud) (Lopain, Ethical Agents Ltd., Auckland, New Zealand) five minutes prior to disbudding (Figures 12 and 13). |
| Disbudding + non-steroidal anti-inflammatory drug (DB+NSAID) N=10 | A subcutaneous injection of meloxicam (Metacam, Boehringer Ingelheim Ltd., Auckland, New Zealand) was administered five minutes prior to disbudding. |
| Disbudding + local anaesthetic + non-steroidal anti-inflammatory drug (DB+LA+NSAID) N=10 | In addition to the corneal and ring nerve blocks described above, a subcutaneous injection of meloxicam was administered five minutes prior to disbudding. |
| SHAM N=11 | Calves were subjected to handling procedures, and pressure applied to the horn buds using an unheated device. Actual disbudding did not occur and no injections were administered. |

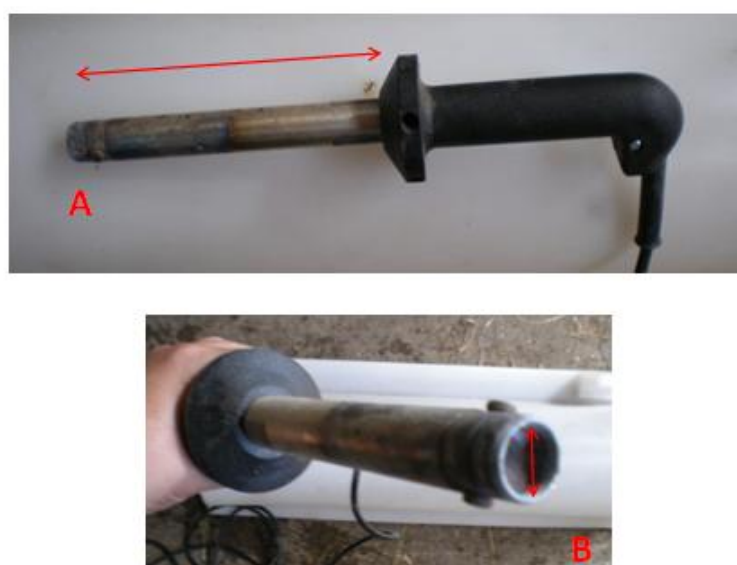


Figure 10 Electric hot iron disbudding tool: A) heated metal shaft 160 mm in length and B) concave disbudding tip 20 mm in diameter



Figure 11 Disbudded calf with residual head wound from where the horn bud was removed – the purple colour is a result of the coloured antibacterial spray

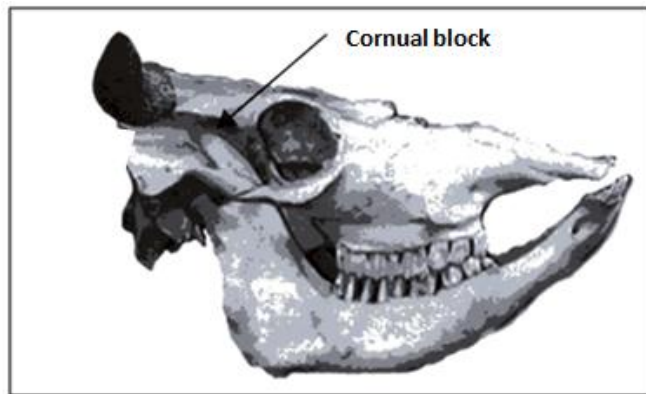


Figure 12 Injection site used to achieve a cornual block in calves prior to disbudding (Reproduced with permission from the July 2000, revised DCV 2010 Standard Operating Procedure for the Process of Humane Disbudding of Calves © National Quality Veterinary Services Limited)

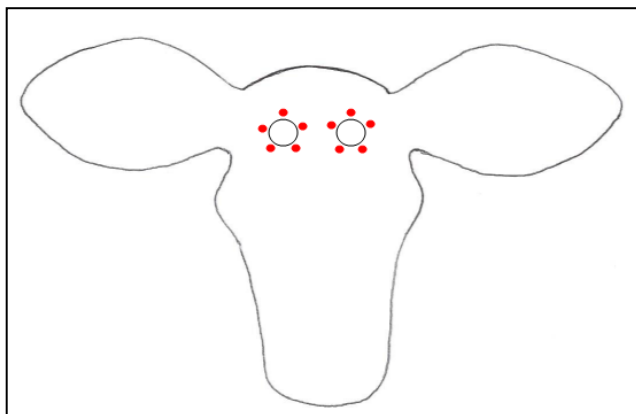


Figure 13 Approximate injection sites required to achieve an effective ring nerve block around the base of the horn buds prior to the disbudding of calves (shown as red dots)

3.3.4 Data collection

In addition to data collected by the automated feeders (total volume and proportion of milk consumed, frequency of visits, number of rewarded visits, and visit duration), lying behaviours (number of lying bouts and proportion of time spent lying) were recorded at one minute intervals by HOBO Pendant G data loggers (Onset Computer Corp., Bourne, MA). Loggers (Figure 14) were strapped to the lower right hind leg (above the fetlock joint) in a Velcro fastened pouch during the daily health check, approximately one hour prior to treatment administration. The device was removed 72 hours after disbudding.

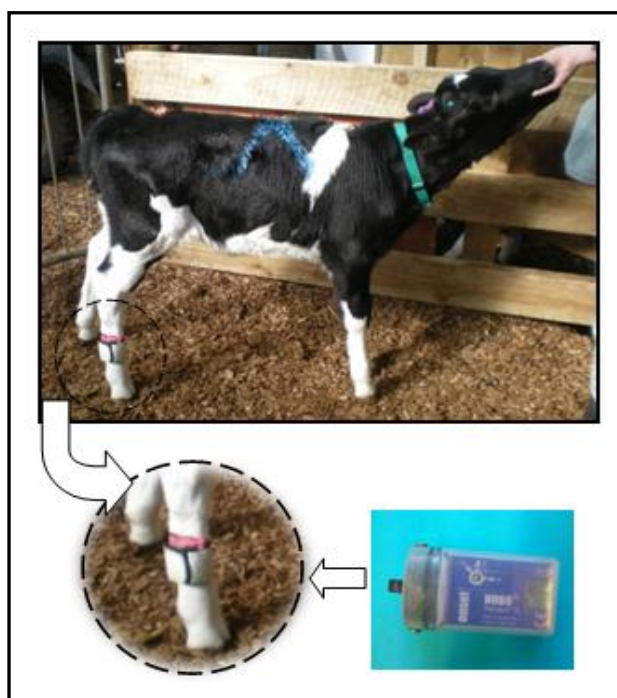


Figure 14 The HOBO recording device (bottom right) was placed in a custom designed pouch (AgResearch Ltd, Ruakura, Hamilton) and strapped to the right hind leg of the calf (top) above the fetlock joint using Velcro straps (bottom left – enlarged view)

Daily health checks (see previous chapter for details) from the previous trial continued for the duration of the current trial. These health checks ensured daily routines remained consistent, and gave handlers an opportunity to check for signs of inflammation and infection at the site of disbudding following treatments (thus ensuring early treatment by farm staff as required).

3.3.5 Statistical analysis

All data was analysed using Genstat (16th ed.). A mixed effects model was used to analyse both the total milk consumption and the proportion of milk consumed (amount consumed / amount permitted) by calves in each of the five treatments

(DB, DB+LA, DB+NSAID, DB+LA+NSAID, and SHAM; Table 5) during the pre-treatment (120 hours immediately preceding the time of treatment), treatment (24 hours from the time of treatment) and recovery (48 hours immediately following the first 24 hours after treatment) periods. Fixed components for each of these analyses consisted of treatment and time (pre-treatment, treatment, and recovery), whilst the random variables consisted of replicate number (1-3) and calf identification numbers.

Total frequency of daily visits to the milk feeder for each treatment group were analysed using a linear mixed model. Proportion of rewarded visits was analysed using a generalized linear mixed model assuming binomial data with a logit link. The response variate used in the later analysis was the number of rewarded visits to the feeder, and the binomial totals consisted of the total number of visits (rewarded and unrewarded) to the feeder. Again, fixed components for each of these analyses consisted of the treatment and time (pre-treatment, treatment and recovery), while random components included the replicate number and calf identification numbers.

The total number of lying bouts and proportion of time spent lying during the pre-treatment, treatment and recovery periods was analysed for calves in each treatment group using mixed effects models. In each model, fixed components consisted of treatment group and time, whilst random components included the replicate number and calf identification numbers.

Statistical significance for all results was set at $P \leq 0.05$.

3.4 Results

3.4.1 Feeding data

Total milk consumption and proportion of milk consumed were not influenced by treatment or time period - test statistics are displayed in Table 7. There was no evidence of an interaction between treatment and time period for either total milk consumption or proportion of milk consumed. Average proportion of milk consumed by each treatment group can be seen in Table 8.

Calves visited the automated feeder between 1 and 25 times daily over the eight days of observation (pre-treatment to recovery time periods); however, not all visits were rewarded (i.e. if the allocated amount had already been consumed by

an individual and the required time had not elapsed before the next permitted feed). The average frequency of visits to the feeder was influenced both by treatment, and time period (Table 7). SHAM calves showed an increase in the average frequency of daily visits to the feeder during the recovery period (not all of these visits were rewarded), whilst calves in the other treatment groups showed no change.

The proportion of rewarded visits was also influenced by treatment and time period (Table 7), with SHAM calves showing a lower proportion of rewarded visits compared to other treatments, particularly during the recovery period. Average frequency of total visits and average proportion of rewarded visits to the automated feeder for all groups over the pre-treatment, treatment, and recovery periods are illustrated in Figures 15 and 16 respectively.

Table 7 Test statistics for fixed effects relating to: 1) total milk consumption, 2) proportion of milk consumed, 3) total frequency of visits, and 4) proportion of rewarded visits

| MILK CONSUMPTION | Wald statistic | n.d.f | F statistic | d.d.f | P-value |
|--------------------------------------|-----------------------|--------------|--------------------|--------------|----------------|
| Treatment | 4.15 | 4 | 1.04 | 48 | 0.40 |
| Time period | 3.21 | 2 | 1.60 | 3941.4 | 0.20 |
| Interaction | 3.16 | 8 | 0.39 | 3944.4 | 0.92 |
| PROPORTION CONSUMED | | | | | |
| Treatment | 3.12 | 4 | 0.78 | 47.7 | 0.54 |
| Time period | 3.24 | 2 | 1.62 | 3931.3 | 0.20 |
| Interaction | 3.61 | 8 | 0.45 | 3934.5 | 0.89 |
| FREQUENCY OF VISITS | | | | | |
| Treatment | 13.35 | 4 | 3.34 | 519.5 | 0.01 |
| Time period | 14.65 | 2 | 7.32 | 1485.4 | <0.001 |
| Interaction | 10.90 | 8 | 1.36 | 1485.2 | 0.21 |
| PROPORTION OF REWARDED VISITS | | | | | |
| Treatment | 13.75 | 4 | 3.44 | 353.9 | 0.009 |
| Time period | 46.37 | 2 | 23.19 | 1493 | <0.001 |
| Interaction | 13.69 | 8 | 1.71 | 1484.8 | 0.09 |

Table 8 Average proportion of milk consumed by each of the five treatment groups (average standard error of difference for all groups = 0.014)

| | DB | DB+LA | DB+LA+NSAID | DB+NSAID | SHAM | TOTAL |
|----------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Pre-treatment | 0.98 | 0.98 | 0.98 | 0.97 | 0.98 | 0.98 |
| Treatment | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| Recovery | 0.99 | 0.97 | 0.97 | 0.97 | 0.98 | 0.98 |
| TOTAL | 0.98 | 0.98 | 0.98 | 0.97 | 0.98 | 0.98 |

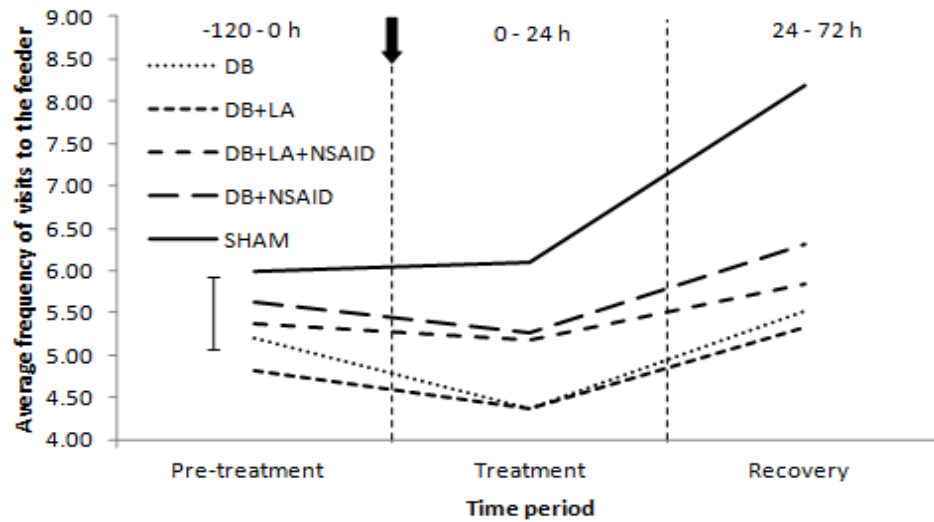


Figure 15 Average frequency of visits to the automated feeder by five treatment groups (DB, DB+LA, DB+LA+NSAID, DB+NSAID, SHAM) across three time periods. Time of disbudding or sham procedures is indicated by a solid black arrow. Average standard error of difference for all groups is also shown to the left of the data lines for reference purposes

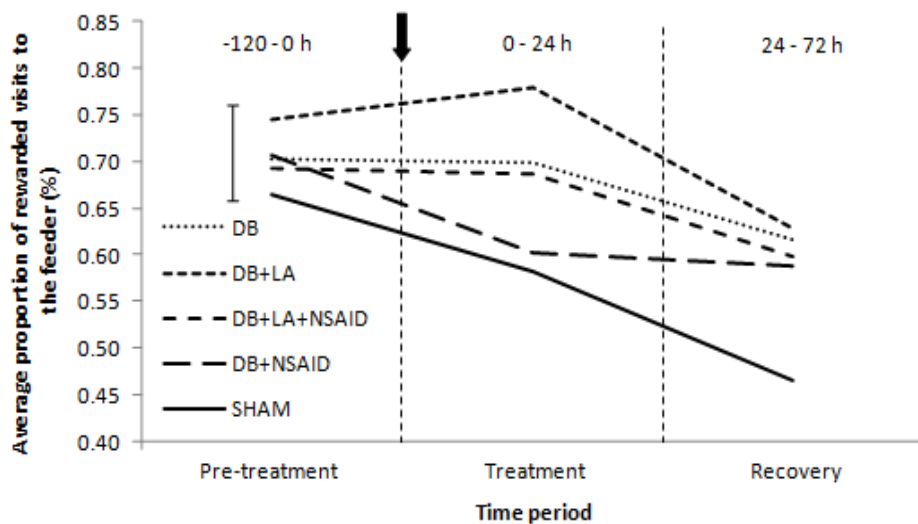


Figure 16 Average proportion of rewarded visits made to the automated feeder by each of the five treatment groups (DB, DB+LA, DB+LA+NSAID, DB+NSAID, SHAM) across the three time periods. Time of disbudding or sham procedures is indicated by a solid black arrow. Average standard error of difference for all groups is shown to the left of the data lines for reference purposes

3.4.2 Lying data

The average number of lying bouts and proportion of time spent lying differed between observation periods (Figures 17 and 18; Table 9); however, no difference was detected across treatments (Table 9). The number of lying bouts and time spent lying was found to be lowest on the day of treatment (treatment period) compared to pre-treatment and recovery periods (Figures 17 and 18). The number of lying bouts was also found to be highest during the recovery period, whilst no significant difference was detected in the proportion of time spent lying between pre-treatment and recovery periods. No interaction between treatment and time period for either of these lying factors was found (Table 9).

Table 9 Test statistics for fixed effects relating to the number of lying bouts and proportion of time spent lying

| LYING BOUTS | Wald statistic | n.d.f | F statistic | d.d.f | P-value |
|--------------------------------|----------------|-------|-------------|-------|---------|
| Treatment | 2.27 | 4 | 0.56 | 237.4 | 0.69 |
| Time period | 59.93 | 2 | 29.96 | 391.6 | <0.001 |
| Interaction | 11.44 | 8 | 1.43 | 391.6 | 0.182 |
| PROPORTION OF TIME SPENT LYING | | | | | |
| Treatment | 3.77 | 4 | - | - | 0.44 |
| Time period | 29.24 | 2 | - | - | <0.001 |
| Interaction | 6.98 | 8 | - | - | 0.54 |

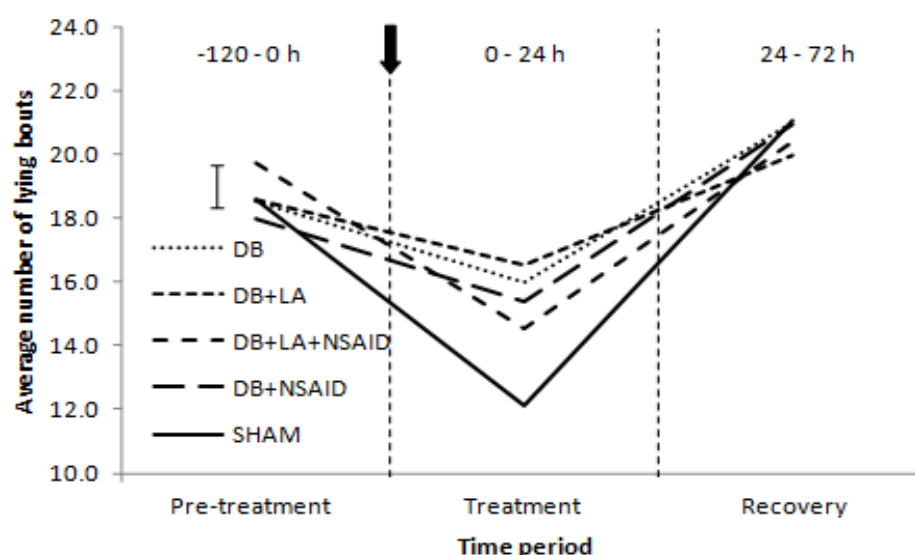


Figure 17 Average number of lying bouts for each of the five treatment groups (DB, DB+LA, DB+LA+NSAID, DB+NSAID, SHAM) over three time periods. Time of disbudding or sham procedures is indicated by a solid black arrow. Average standard error of difference for all groups is shown to the left of the data lines for reference purposes

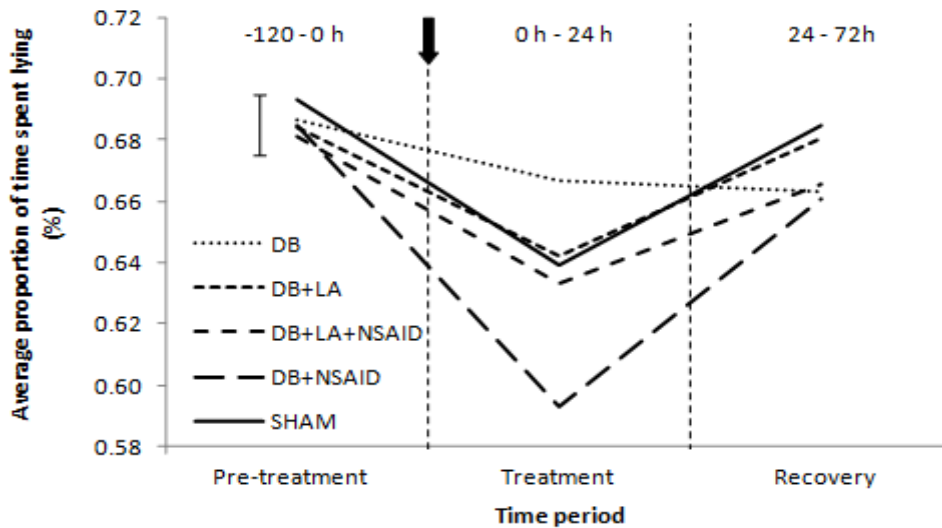


Figure 18 Average proportion of time spent lying by calves belonging to each of the five treatment groups. Time of disbudding or sham procedures is indicated by a solid black arrow. Average standard error of difference for all groups is also shown to the left of the data lines for reference purposes

3.5 Discussion

Assessment of milk feeding or lying behaviours alone in the current study proved to be inadequate indicators of pain and its severity in disbudded calves under the age of four weeks. The combination of measures, however, did highlight one behavioural difference displayed by animals that had undergone the painful disbudding procedure and those that were allocated to the SHAM treatment. Disbudded animals displayed similar feeding and lying behaviours during the pre-treatment and recovery periods, irrespective of treatment group. This indicates little apprehension from disbudded calves to enter the feeder during the recovery period. SHAM animals, however, showed a significantly greater frequency of visits to the milk-feeder during the recovery period resulting in an increased number of unrewarded visits. This leads me to propose that disbudded calves were less likely to participate in agonistic interactions to gain access to the feeder (due to inflammatory pain of residual head wounds), where there is an increased risk of direct head contact with other calves. This would allow SHAM animals, regardless of hierarchical status or size, to enter the feeder with little competition from disbudded animals. To my knowledge, this is the first study to explore “frequency of visits” (rewarded and unrewarded) to an automated milk feeder as a possible pain response variable.

A recent study by Bates et al. (2015) found calves disbudded without sedation and pain relief displayed a reduced milk intake in the 11 days subsequent to the

procedure. Conversely, the current study showed no difference in cumulative milk consumption between disbudded treatment groups or SHAM animals (all groups consumed no less than 97% of their daily milk allowance). This disparity in results could possibly be explained by methodological factors such as: 1) the reduced number of animals used in the current study (200 animals used by Bates et al. (2015), compared to 53 animals in the current study), or 2) the shorter recovery period (2 days vs. 11 days used by Bates et al (2015)) from which feeding data was obtained for the current study.

Interestingly, a study by Svensson and Jensen (2007) looking at disease in calves, found reduced frequencies of unrewarded visits to an automated feeder to be the most effective indicator of illness when calves were fed restricted milk volumes - not actual milk consumption. Thus, it is possible that the best (milk-feeding) indicators of pain will also be dependent upon the volume of daily milk rations. A study by McMeekan et al. (1999) found 3-4 month old calves that were dehorned (using the scoop method) without pain relief, were slower than controls to return to grazing. Given the results of McMeekan et al. (1999), other factors to consider in feeding-related pain assessment may include how quickly animals from each treatment group return to the automated feeder (rewarded or not), and the volume of hard feed consumed (hay, pellets etc). These measures were not explored in the current study.

Calves from all treatment groups (including SHAM animals) in the current study showed a significant reduction in the number of lying bouts and proportion of time spent lying on the day of treatment. This does not support my prediction that calves experiencing pain would increase the proportion of time spent lying. It is possible that lying activities were disrupted by the double handling of calves on the day of treatment, first with the daily health check and replacement of the HOBO data logger, then by further restraint in the calf crush for drug administration and the treatment procedure. The setting up and dismantling of temporary fencing within the experimental pen to isolate calves undergoing treatment may have also impacted lying behaviours.

The average number of lying bouts was found to be highest during the recovery period; however, when compared to the pre-treatment period these differences were marginal. No differences in the proportion of time spent lying were detected

between pre-treatment and recovery periods for any of the treatment groups. It is likely that pain or discomfort experienced during the recovery period had reduced sufficiently to allow lying activities to resume to almost pre-treatment levels. With a half life of 26 hours (Stewart et al., 2009), meloxicam concentrations would be expected to have declined by approximately 50% by the onset of the recovery period. This leads to a second possibility, that inflammatory pain caused some restlessness in disbudded animals (as shown by an increase in lying bouts during the recovery period); the lying activities of SHAM calves may have then been disrupted by the increased activity of these animals.

Frequency of visits (and proportion of rewarded visits) to the automated milk feeder appears to be the most sensitive indicator of pain in the current study. Pain severity was not able to be determined using the various drug combinations and behavioural measures chosen for this study. This could be due to the prolonged disruption of activities on the day of treatment causing a heightened state of arousal or stress, and the dissipation of NSAID action during the recovery period. Alternatively, individual variation in drug sensitivity (Muralidharan & Smith, 2011), or differences in pain sensitivity (Nielsen et al., 2009; Muralidharan & Smith, 2011) could account for any disparities in the level of pain relief achieved. Future research should look to identify and broaden our knowledge of other behavioural measures of pain that can be obtained non-invasively using existing technology, and maybe more sensitive to pain severity.

In conclusion, lying activities were found to be an insufficient measure of pain in the current study. The use of milk-feeding behaviours for pain detection was also limited. The potential value of automated feeders in pain detection is likely to be dependent on several factors: 1) what measures are used (e.g. frequency of rewarded/unrewarded visits, time to first visit, duration of visits, total milk volume consumed), 2) daily milk allowance per animal, 3) other dietary provisions (i.e. hard feed – pellets, hay etc), 4) health status of the animal (i.e. the presence of disease is likely to interfere with results), and 5) severity and duration (acute vs. chronic) of pain.

3.6 References

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4 General Discussion

Exposure to disease and pain will prolong animal ‘suffering’, and as such, clearly diminishes welfare. Identifying behaviours indicative of disease and pain in animals can facilitate early detection of these conditions, prompting early treatment and improved welfare. The immunological vulnerability of pre-weaned calves (Beam et al., 2009; Uetake, 2013), coupled with the stoicism of cattle in general (Stafford & Mellor, 1993; Weary et al., 2009), calls for further species-specific research into how the behaviour of these animals is altered by illness and pain. Additionally, group housing systems, can make observation of individual animals increasingly difficult; however, use of existing technology and on-farm automation offers opportunities to monitor behaviour (e.g. feeding and lying) automatically and objectively. This thesis explored two key areas of interest with regards to the welfare of pre-weaned calves: 1) the use of feeding, lying, and postural behaviours for early disease detection (Chapter 2), and 2) the use of feeding and lying behaviours for the identification of animals in pain (Chapter 3).

Neonatal calf diarrhoea (NCDC) is recognised as the leading health concern in pre-weaned calves’ worldwide (Lorenz et al., 2011). Beyond the obvious welfare implications of NCDC, farmers also face potential economic losses in the form of calf mortality (fewer replacement cows), treatment expenses, increased labour costs and potentially long-term impacts on productivity (Chi et al., 2002; Lorenz et al., 2011). Early detection of diseased calves permits timely intervention by farmers by way of treatment, thus minimising negative impacts on calf welfare. Previous studies (e.g. Borderas et al., 2009; Svensson & Jensen, 2007) have successfully demonstrated the use of automated milk feeders to monitor disease-induced changes in calf feeding behaviour; however, the sensitivity of individual measures differs between studies. HOBO data loggers have also been validated (Bonk et al., 2013) for use on calves, to monitor lying behaviour.

My thesis identified three milk-feeding variables that were indicative of illness in pre-weaned calves, using automated milk feeders: 1) reduced total volume (and proportion) of milk consumed, 2) longer duration of visits to the feeder, and 3) reduced probability of receiving a rewarded visit, compared to calves that were not sick. The reduction in milk consumption supports the prediction that sick will calves consume less milk than healthy calves. Further analysis of sick calves in

the days preceding illness revealed no changes in feeding behaviour during this period. This could indicate a dramatic reduction in milk consumption after illness occurred, or that at least some calves were showing milk feeding deficits for the duration of the trial. Previous studies (Beam et al., 2009) have linked failure of passive transfer (FPT) of colostral immunoglobulins with higher morbidity and mortality rates in dairy calves; however, further research is required to determine whether general milk feeding deficits (after initial colostrum intake), in otherwise healthy calves, could influence, or predict, susceptibility to disease.

Analysis of lying data (obtained from HOBO data loggers) showed no difference between the lying behaviours (number of lying bouts and proportion of time spent lying) of sick calves compared to those that were not sick; however, further analysis of sick calves revealed a reduction in the number of lying bouts nearing time of illness. No change in the proportion of time spent lying during this period was found which suggests that the duration of lying bouts had increased during the early stages of illness, and may be a useful indicator of diseased animals. These results supported the prediction that sick calves would increase duration of lying bouts, although this did not equate to an increased proportion of time spent lying. The increased duration of lying bouts and decreased milk intake also support the widely reported occurrence of common 'sickness behaviour' and energy conservation strategies (Hart, 1988; Aubert, 1999) employed by animals as a means to facilitate recovery.

Abnormal postures have been reported in cattle as a result of pain and discomfort (e.g. Molony et al 1995; Poursaberi et al., 2010). I found evidence to suggest that lying postures were influenced by illness, and time nearing disease; however, postures more frequently observed in calves that were not sick, were found to increase in sick animals nearing time of illness. These results were likely confounded by the grouping of particular postures (short, lateral and neck extended lying), and health categories ('healthy', 'intermediate', and 'other' were pooled for comparisons with sick calves), for statistical analysis. Pooling was necessitated by low frequency of observations for these particular postures, and insufficient numbers of sick calves, respectively. Results were ultimately considered inconclusive. It is recommended that future studies include abnormal standing postures and stomping/kicking activities as a means to assess the occurrence of abdominal discomfort in calves with NCDC. These behaviours have

been reported in young calves following castration (Molony et al., 1995), and may be a better indicator of abdominal or visceral discomfort over lying postures.

The greatest limitation of the work reported in Chapter 2 was the low number of 'sick' calves (n = 21); further, only four of these animals tested positive for enteropathogens (*Cryptosporidium*). It is likely that the 17 remaining calves suffered from some mild form of nutritional- or stress-induced diarrhoea (Kumaresan et al., 2012); these cases would not be expected to produce the same febrile response or damage to the intestines as pathogen-induced diarrhoea (Kumaresan et al., 2012), and thus may also lead to less pronounced behavioural changes (e.g. absence or reduced frequency of abnormal postures). Some calves were removed (temporarily) from the observation pen upon detection of moderate clinical symptoms of NCDC (diarrhoea, dehydration, and/or fever, and lethargy), and were returned when diarrhoea ceased and general condition improved (as judged by farm staff). During this period of isolation, calves did not have access to automated feeders. This limited the subsequent analysis of feeding behaviour to 'pre-disease detection' and 'post-recovery' periods for these particular animals. Consequently, analysis of feeding behaviour during the presumably 'peak disease' period, was not possible for the more severely affected calves, potentially diluting actual changes in feeding behaviour associated with illness.

Failure to recognise an animal in pain could lead to prolonged suffering and worsening of painful conditions. Therefore, early identification is important for a quick recovery and maintaining calf welfare. Disbudding is known to be a painful practice (Stafford & Mellor, 1993; Molony & Kent, 1997) that dairy calves are subjected to as a routine husbandry procedure. The research reported in Chapter 3 involved the use of a hot-iron method of disbudding as a means to assess pain-induced changes in the feeding and lying behaviours of calves less than four weeks of age. Changes in these behaviours have been reported in cattle (Stilwell et al., 2009; Bates et al., 2015) following painful procedures (e.g. disbudding and castration), and as a result of conditions causing chronic pain (e.g. lameness). Young animals are particularly vulnerable if nutrient/water intake is reduced for prolonged periods (e.g. anorexia/adipsia; Millman, 2007) and could lead to additional deterioration of calf condition and welfare.

SHAM calves showed a significant increase in the frequency of visits to the milk feeder during the recovery period, resulting in a reduced number of rewarded visits compared to disbudded calves. Disbudded animals, regardless of treatment group, showed similar frequencies of feeder visits during pre-treatment and recovery periods. This suggests little hesitation to enter the feeder during the first 24 hours following the procedure. Frequency of visits to the milk feeder was found to be lowest for all calves (including SHAM animals) on the day of treatment; however, the average proportion of milk consumed by all groups remained high (almost 100%). It was concluded that feeding data was an inadequate detector of pain in this study. These results did not support the hypothesis that calves disbudded without pain relief would show a marked decline in milk consumption compared to SHAM, or other disbudding treatment groups, as a result of intense pain following disbudding.

Lying behaviours (proportion of time spent lying and number of lying bouts) were found to be affected by observation period only, no difference between treatment groups were identified. Increased frequency of lying bouts during the recovery period was not reflected by a change in the proportion of time spent lying for any of the treatment groups, and may indicate restlessness of disbudded calves (any effects of pain relief would have largely worn off by the recovery period). SHAM calves produced similar changes in lying behaviour, and it is suggested that this may have occurred as a result of disruption by the increased activity (restlessness) of disbudded animals. Again, the hypothesis that calves disbudded without pain relief would spend a greater proportion of time spent lying following the painful procedure was not supported by this study.

Changes in milk-feeding (reduced frequency of visits) and lying (reduced number of lying bouts and proportion of time spent lying) behaviours on the day of treatment are proposed to be the result of increased disruption within the observation pen. Calves were disbudded within a sectioned part of the observation pen. Double handling of animals and the setting up/dismantling of portable fences were likely to have been major contributing factors, thus representing an important limitation of this study. In the future, study design could be improved to ensure minimal handling of calves prior to treatment. The disbudding procedure should also be done away from the main observation pen to ensure minimal disturbance of pen mates. Morisse et al. (1995) reported that acute pain, identified

by changes in social behaviour and the appearance of other behaviours indicative of pain or distress (restlessness, shaking of the head, scratching site of injury), lasted approximately 4 hours after disbudding (hot iron and caustic paste methods). Therefore, short-term changes in milk-feeding and lying behaviours may have been masked by the extended 'treatment' period of observation (24 hours). Shortening observation periods in future studies, should facilitate a greater analysis of acute pain-related behaviours.

The results of this thesis have successfully demonstrated that aspects of milk-feeding behaviour (recorded by automated feeders) can be used to assist in the early identification of sick calves in New Zealand group housing systems. Regular, direct observation of calves is crucial to ensure good health and disease management; however, this thesis supports current evidence that existing on-farm automation can be used to supplement farmer observations, and perhaps alert farmers to animals that may need further attention. The practical use of HOBO data loggers to obtain lying data appears to be limited to increased duration of lying bouts, which may be indicative of impending illness in young calves. The use of automated feeders and data loggers to assess pain-related changes in milk-feeding and lying behaviours after disbudding were less successful due to aforementioned study design issues and limitations. To maintain and improve calf welfare, future research should aim to identify and further refine species-specific behaviours indicative of pain and disease in pre-weaned calves. Knowledge is particularly limited with regards to social interactions under these adverse conditions.

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