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The effects of early handling on dairy calves' physiological and behavioural responses to routine husbandry procedures

A thesis submitted in partial fulfilment

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Haley Shepherd

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This thesis is dedicated to my parents

Alton and Theresa Shepherd

for all their love and support and their constant belief in me

Abstract

The quality and quantity of human-animal interactions are crucial to animal welfare, productivity and management of livestock on-farm. Forty Holstein Friesian calves, from one week of age, were exposed to experimental handling for five minutes twice daily for five weeks. Calves were allocated to either positive or negative handling treatments (n=20 per treatment). Positive handling required handlers to slowly approach calves whilst using soft voices to encourage voluntary friendly interactions such as gentle pats. Negative handling consisted of continuous 45 second cycles of direct and indirect handling to discourage friendly interactions. Direct handling required handlers to use fast movements and harsh voices whilst forcibly moving animals around the pen. Indirect handling required handlers to stand in the pen, stare at the animals and tap a polyurethane pipe to make noise to maintain disturbance. Two other novel objects, a plastic bag and an empty water bottle filled with stones, were alternatively used each week to prevent habituation to the negative stimulus. At six weeks of age, all animals were subjected to three routine management procedures: restraint, ear tagging and disbudding, which occurred in stated order over a week period. There were no significant treatment differences between positive and negative groups for heart rate or heart rate variability (measured using Polar heart rate watches), eye temperature (measured using infrared thermography), respiration rates (measured visually), struggling behaviour, and plasma cortisol levels (measured during disbudding only). There were however within treatment differences in response to ear tagging, with an increase in heart rate ($p<0.01$) post-ear tagging, and in response to disbudding with an increase in heart rate ($p<0.001$), tail flicking

($p<0.001$) and cortisol levels ($p<0.001$). It was concluded that, under the conditions of this experiment, early handling does not affect the behavioural and physiological responses of calves to routine management procedures.

In a follow up trial at three months of age, the initial 40 animals and 20 additional three month old minimally handled animals (controls) were assessed for ease of handling using a force test, which ranked the time and effort required to move animals individually into a crush, and an exit speed test which recorded the animals speed exiting the crush, after two minutes of restraint. There were no significant differences between positive, negative and minimally handled treatment groups for heart rate, respiration rates or behaviour in the crush. However, the minimally handled group did appear to be more fearful of humans, with a significantly quicker entry time ($p<0.05$) into the crush than positive and negative treatment groups. There were no differences in entry scores for effort required to move the animals during the force test or for exit speeds. It was concluded that, under the conditions of the present experiment, initial early handling does not appear to cause long lasting effects on calves' behavioural and physiological responses to routine farm management procedures, but minimal contact with humans early in life may lead to a fear of humans later in life.

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CHAPTER 1

General Introduction

This general introduction provides a detailed background on human-animal interactions, by summarising current research and knowledge in this area; I also highlight the gaps in knowledge that led to my research. Animals' fear and stress responses and the effect of these on animal welfare and productivity are discussed, as are current methods to assess these responses; I also provide a detailed summary of each behavioural and physiological measure used in this research and the benefits and limitations of each. This chapter concludes with a summary of the relevance and aims of the research.

HUMAN-ANIMAL INTERACTIONS

Human-animal interactions occur on a daily basis in most farming situations. The frequency and quality of these interactions are therefore important factors contributing to the welfare and productivity of animals (Rushen et al., 1999b; Hemsworth & Barnett, 2001). Previous research has shown that some species of animals can distinguish between humans based on previous experiences (Munksgaard et al., 1997; Boivin et al., 1998; Munksgaard et al., 2001; Waiblinger et al., 2006). Research with veal calves found that providing additional human contact improved

handling, animal performance and welfare compared to minimally handled control calves (Lensink et al., 2000). Research has shown that dairy calves in particular can distinguish between different people based on how they are handled; aversive handling such as pushing and yelling results in a generalised fear of people (de Passillé et al., 1996; Munksgaard et al., 1997), while positive handling such as petting and calm approach can overcome this fear (de Passillé et al., 1996). Research on the effects of human-animal interactions and different handling techniques in pigs has shown that aversive handling can lead to decreased growth and pregnancy rates, avoidance behaviour and also increased levels of free corticosteroid concentrations (Hemsworth et al., 1981a, 1981b; Gonyou et al., 1986; Hemsworth et al., 1986, 1987; Hemsworth & Barnett, 1991).

Paterson & Pearce (1992) investigated the effects of two handling treatments on the behaviour, growth and free corticosteroid concentrations of 16 juvenile female pigs. The treatments consisted of pleasant handling where experimenters gently stroked the pigs on approach, and unpleasant handling where experimenters randomly either lightly slapped, attempted to place a snout noose on the animal or using an electric prod on the approaching animal. Treatments were imposed for two minutes, three times per week for a ten week period. Results showed acute and chronic stress responses with pigs in the unpleasant handling treatment having slower growth rates than those in pleasant treatments; they also spent less time near the experimenter and exhibited fewer behavioural interactions with the experimenter and had higher corticosteroid concentrations at rest and in response to the presence of the experimenter. This research indicates that previous handling can cause acute and chronic stress responses in pigs, which shows that human influence can affect the wellbeing of animals.

A large body of literature has been published by Hemsworth and associates on the effects of previous handling on commercial pig farms. This research collectively shows that human-animal interactions can have substantial effects on the behaviour, physiology and productivity of pigs (Hemsworth et al., 1981a, 1981b; Hemsworth et al., 1986, 1987; Hemsworth & Barnett, 1991).

Of particular, interest is the research by Hemsworth et al. (1987) that investigated the effects of four handling treatments on the behaviour, growth and free corticosteroid concentrations of 32 young female pigs over a six week period. The treatments consisted of experimenters handling the pigs in (a) a pleasant manner involving stroking whenever the pig approached the experimenter, (b) an unpleasant manner involving forcing the pig away whenever it approached the experimenter and (c) an inconsistent manner involving a combination (1:5 ratio) of unpleasant and pleasant treatment responses. A fourth treatment consisted of (d) minimal handling involving no contact with humans apart from feeding and cleaning. These treatments were imposed for three minutes at a time, three times per week. Findings indicated that the unpleasant and inconsistent handling treatments resulted in pigs being more fearful of humans in an approach test than pleasant and minimal treatment groups. The concentration of free corticosteroids were also higher in unpleasant (19.5 $ng.ml^{-1}$) and inconsistent (18.6 $ng.ml^{-1}$) treatment groups than minimal (12.8 $ng.ml^{-1}$) and pleasant (12.6 $ng.ml^{-1}$) treatments. Minimal and pleasant treatment pigs were quicker to enter an area with a human and quicker to interact with a human; these pigs also spent more time within 0.5m of the human and had more interactions with the human.

Further research by Hemsworth & Barnett (1991) investigated the effects of three handling treatments on the behaviour, growth, and free corticosteroid concentrations of 60 young female pigs over a ten week period. The treatments

consisted of experimenters handling the pigs individually in a pleasant manner, using gentle stroking, and two unpleasant handling situations, on an individual and group level, with brief shocking or slapping of the pig if it failed to withdraw from the experimenter. Individual treatments were imposed for 30 seconds a day for five days a week, and the group handling treatment was imposed for 2.5 minutes a day for five days a week. Results showed that the pigs handled in both unpleasant treatments were more fearful of humans, shown by an approach test with a stationary experimenter at 20 weeks of age. Individually handled pigs in the unpleasant condition had a greater increase in free corticosteroid concentrations in response to humans at 24 weeks of age than those in the pleasant treatment group. There were no differences in growth rate between treatments for the ten week period. The two studies show that relatively short handling treatments can have significant effects on welfare.

Similar research has also been conducted in dairy cattle. Breuer, Hemsworth, & Coleman (2003) investigated the effects of positive and negative handling on the behavioural and physiological responses of 48 non-lactating heifers, over a five week period. Positive handling consisted of slow and deliberate tactile contact while negative handling consisted of sudden, fast aversive tactile contact. Treatments were imposed twice daily for two to five minutes per session. Animals were tested at five weeks for fear responses to humans; tests included approach and avoidance tests including cortisol responses and an ease of movement test which required an unfamiliar human to move the animals individually along a route to a crush. Animals were timed and graded on their resistance to move through this area into the crush and agitation levels were graded while the animal was held in the crush in the presence of an unfamiliar human (with 0 indicating no movement or sound, 1 indicating quiet with slight movement, 2 was moderate movement and 3 indicated vigorous

movement). Results showed that negatively handled heifers took less time to move to the crush than positive heifers and were significantly more agitated while in the crush. Negatively handled heifers also took longer to approach humans in an approach test and had greater increases in total cortisol concentrations due to exposure to humans compared to positively handled heifers. It was concluded that the type of handling affects the subsequent behavioural responses of heifers to humans and that this response may be generalised to other humans, with negative handling resulting in acute and later to chronic stress responses in the presence of humans.

Waiblinger et al. (2004) investigated the effects of previous gentle handling on the heart rate and behavioural parameters of 20 adult dairy cows' stress responses during rectal palpation with sham insemination. Ten animals received gentle handling from one handler which involved feeding, stroking the neck and head of animal and speaking in a soothing voice for 5 minutes a day for 10 days over a four week period; the other ten control animals were managed under routine farm procedure with different caretakers. Rectal palpation tests were carried out the week after the handling period ended on four successive days with each test lasting nine minutes. These nine minutes included four minutes rectal palpation under four situations, one with the cow being alone during the test, one with the handler, one with a usual caretaker and one with an unknown person. Results showed that previously handled animals had lower heart rates during the tests, kicked less when alone and tended to show less restless behaviour. Cows showed significantly less restless behaviour when gentled by the handler, but there were no differences in behaviour or heart rate when cows were gentled by a usual caretaker or an unknown person during the procedure. It was concluded that the stress responses of cows during this procedure could be

reduced by previous positive handling as well as by a handler providing positive gentle interactions during the procedure.

Petherick et al. (2009a, 2009b) investigated the effects of quality versus quantity handling/yarding on the stress, productivity, flight speed and fear of humans in 144 beef cattle. Three treatments, good handling/yarding, minimal handling/yarding and poor handling/yarding were imposed on six occasions over a 12 month period. Good handling/yarding was designed to provide cattle with a neutral or positive experience. It consisted of cattle being drafted, by humans, into a yard containing food. The animal was then put into a race and held with a group mate, to minimise stress from isolation, for three minutes and then moved into a crush individually where they were held for a further three minutes. Whilst in the crush the animal was exposed to humans in a neutral setting (2-3 humans stood 1-2m adjacent to the crush a talked quietly without making physical contact with the animal) and then release and scored for flight speed. The animal was held in the initial yard with food for a further 30 minutes with group mates and a human walked slowly and quietly amongst them. All animals were then released to walk back to their home paddock. Minimal handling/yarding was designed to provide cattle with a standard yarding experience. It consisted of cattle being separated, by humans, from other group mates in a yard and then being immediately released with access to water and being allowed to walk back to their home paddock. Poor handling/yarding was designed to provide cattle with a negative experience. It consisted of cattle being drafted, by humans, into a bare yard without food or water and being held for three to four hours. The animal was then moved into a race and held with a group mate for three minutes before being moved into a crush individually where they were held for a further three minutes. Whilst in the crush the animal was exposed to humans in a

negative setting (2-3 humans made loud noises banging gates, hitting rails and shouting; cattle were also intermittently slapped and prodded with a pipe and had their heads pushed and pulled) and then released and scored for flight speed. The animal was held in the initial yard until all testing was done. All animals were then released to walk back to their home paddock. Overall results showed good handling/yarding reduced fearfulness of humans, with flight speed decreasing quickest compared to other treatments. The poor treatment negatively impacted on weight gain and the minimal treatment appeared to cause animal's stress, which was attributed to the novelty of being handled by humans and confined during testing. This research is significant as it highlights the effects of quality versus quantity handling, showing that increased levels of positive human contact decreases human fear, where as poor and minimal treatment can cause stress and increased fear of humans.

Table 1.1 provides a summary of the current knowledge of different handling treatments on the responses of cattle and pigs; this research collectively shows that handling does have an effect on behavioural and physiological responses of these animals to humans.

Table 1.1 Summary of research on pigs and cattle with positive and negative handling treatments.

Species used and authors of research	Handling Treatment			Resulted in significant differences?
	Positive	*Negative*	*Minimal*	
Non-lactating heifer cows – Breuer et al. (2003)	Slow deliberate tactile contact	Fast, aversive tactile contact	N/A	Yes – negative treatment had ↑ fear of humans and ↑ cortisol levels
Dairy cows – Waiblinger et al. (2004)	Feeding, stroking & use of soft voices	N/A	Routine handling only	Yes - previous gentle handling had ↓ heart rate and fear behaviour
Dairy cows – de Passillé et al. (1996)	Petted and offered milk	Cattle prod and nose tongs	Routine handling only	Yes – calves avoided negative handler
Dairy heifers – Boissy & Bouissou (1988)	Brushing and leading with a halter	N/A	Routine handling only	Yes – positive heifers had a ↓ flight distance
Dairy heifers – Bertenshaw & Rowlinson (2008)	Stroking and brushing	N/A	Routine handling only	Yes – positive heifers had a ↓ flight distance and ↑ levels of voluntary approach
Beef calves – Boivin et al (1998)	Extensive positive handling, stroking	N/A	Routine handling only	Yes - extensively handled calves allowed contact with humans quicker than minimally handled calves
Pigs - Hemsworth	Gentle stroking	Brief shock or slapping in both	N/A	Yes - aversive (both group and

& Barnett (1991)	individually on approach	group and individual situation		individual) had ↓ growth, ↑ fear of humans and individual only had ↑ cortisol levels
Pigs – Hemsworth et al. (1981a)	Gentle stroking on approach	Lightly slapped or fitted snout ring	N/A	Yes – negatively handled pigs had ↑ fear of humans and ↑ cortisol levels
Hemsworth et al. (1987)	Gentle stroking on approach	Forcing away on approach and inconsistent 1:5 ratio of pleasant/unpleasant	Routine handling only	Yes – pleasant pigs had ↑ growth, unpleasant and inconsistent ↑ cortisol and ↑ fear of humans
Pigs – Gonyou et al. (1986)	Knelt in pen and scratched the pig when receptive (did not move away from touch)	Aversive – electric shock Negative – standing and approaching with gloved hand	Routine handling only	Yes – negative and aversive had less weight gain, aversive had ↑ adrenal cortex, and avoided humans

ANIMAL WELFARE, FEAR AND STRESS RESPONSES

In 1965 the Farm Animal Welfare Committee (FAWC) established the 'five freedoms' which lead to an increased interest in the study of animal welfare (Gonyou, 1994; Stafford et al., 2002; Hristov et al., 2008). Welfare is a broad term which implies both the physical and mental wellbeing of an animal (Kilgour & Dalton, 1984; Phillips, 1993). When an animal has compromised welfare it is often said to be in a

state of stress. There are various opinions among researchers regarding a definition for stress in animals. Fraser, Ritchie, & Fraser (1975, p. 659) defined stress as a state that occurs when an animal is 'required to make abnormal or extreme adjustments in its physiology or behaviour in order to cope with adverse aspects of its environment and management'. A stressor can be any external stimulus which challenges an animal's homeostasis; this challenge causes the animal to undergo a stress response making physiological and/or behavioural changes in an attempt to maintain this homeostasis (Moberg, 1985). Individual animals will respond differently to different stressors and these responses will vary considerably with an animal's age, sex, species, physiological and emotional state, as well as with previous experience of the situation (Hemsworth et al., 1981a; Moberg, 2000). This broad range of factors means that there is no easy way to assess an animal's stress response, but it is generally agreed that an animal's stress response can be measured by assessing a combination of an animal's behavioural and physiological adaptations (Ewbank, 1985; Moberg, 1985, 2000). An animal's stress response begins when its central nervous system perceives a potential threat to homeostasis, the animal's reaction then consists of a combination of three responses: behavioural, autonomic nervous system (ANS) and neuroendocrine (Moberg, 2000). An animal's behavioural response is usually immediate and easy to observe (Broom & Johnson, 1993), for example a calf struggling and pulling in response to being restrained by a head bail in a crush. An animal's ANS response, often referred to as a 'fight or flight' response, is the body's primary stress response and has relatively short term effects on the body (Dantzer & Mormede, 1983; Herd, 1991; Maule & VanderKooi, 1999). This response involves changes in the cardiovascular system, gastrointestinal activity, exocrine gland and adrenal medulla activity allowing the animal to reallocate energy to enable the animal

to cope with the perceived threat (Moberg, 2000); for example, a restrained animal often has increased heart rate, blood pressure, respiratory rates and suppressed gastrointestinal activity. An animal's neuroendocrine response has broad, longer lasting effects on the body and involves the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Minton, 1994; Maule & VanderKooi, 1999; Moberg, 2000; Tsigos & Chrousos, 2002). Activation of the HPA axis results in the secretion of glucocorticoids (e.g., cortisol) from the adrenal gland (Matteri et al., 2000). Elevated cortisol levels are often considered to be a key indicator of the level of stress an animal is experiencing, with increased plasma cortisol concentrations indicating that an animal's homeostasis is unbalanced (Minton, 1994; Matteri et al., 2000; Tsigos & Chrousos, 2002). Prolonged exposure to the stressor can cause an animal to experience chronic side effects, which may include suppressed immunity, suppressed reproduction and decreases in weight and general health (Matteri et al., 2000; Moberg, 2000).

BENEFITS AND LIMITATIONS OF MEASURES USED TO ASSESS STRESS RESPONSES

In order to assess an animal's stress response to a stressor, it is important to measure a wide range of behavioural and physiological variables to get an overview of the behavioural, ANS and neuroendocrine stress responses. There are however, some benefits and limitations involved in using these measures.

Physiological measures

Heart rate

Measuring an animal's heart rate is a traditional method, used in human and veterinary medicine, which is based on the assumption that heart rate reflects the activity of the sympathetic nervous system, it is used to assess the short term effects of stress (Hopster & Blokhuis, 1994; Hagen et al., 2005). Measurement of heart rate alone has been used as an indicator of stress; however this has limitations as heart rate reflects the separate effects of the sympathetic and parasympathetic branches of the ANS. These branches of the ANS are complex and do not necessarily function on a continuum (von Borell et al., 2007), heart rate may therefore vary beat-to-beat as a result of activity from either branch or a combination of both, making the cause of change difficult to define (Hainsworth, 1995; Hagen et al., 2005; von Borell et al., 2007). Changes in heart rate can also be reflective of different emotional states, but still result in the same response (von Borell et al., 2007). For example an increased heart rate can occur in a state of pleasure or fear or in response to a negative stimulus. Heart rate can be measured using a number of different methods which differ in invasiveness and overall effectiveness. Gluing or surgically attaching electrodes onto the skin is a common method to record heart rate, but attachment can be stressful for the animal (Lay et al., 1992a, 1992b; Mitchell et al., 2004). Polar heart rate watches (S810i^{TM}, Polar Electro Oy, Helsinki, Finland) were used in this research because once animals are accustom to wearing a heart rate strap, they provide a non invasive method for recording heart rate and require minimal contact with the animal. This allows more concise data to be obtained as results are not confounded by discomfort from electrode attachment or human contact, which may cause a stress response itself, and thus perturb the results. (Hopster & Blokhuis, 1994).

Heart rate variability (HRV)

Healthy cardiac function is characterised by irregular time intervals between consecutive heartbeats (von Borell et al., 2007). Heart rate variability (HRV) is a tool used to describe the temporal variations between these consecutive heartbeats (R-R intervals, the time between R waves of the electrocardiogram) and assess the balance between the sympathetic (fight or flight) and parasympathetic (house keeping) divisions of the ANS, thereby providing a more accurate assessment of stress than heart rate alone (Porges, 1995; Mohr et al., 2002; Marchant-Forde & Marchant-Forde, 2004; Stewart et al., 2008). HRV was first documented by Hales in the 18th century and has since been used in pigs, cattle, horses, sheep, goats, poultry and humans (Berntson et al., 1997; Terkelsen et al., 2005; von Borell et al., 2007). Research has investigated changes in HRV in response to painful husbandry procedures (e.g., disbudding and castration of calves), often causing depressed parasympathetic activity and increased sympathetic activity, resulting in reduced HRV (Niskanen et al., 2004; Hagen et al., 2005; Stewart et al., 2008). Time and frequency domain parameters of HRV are used to assess the balance of the ANS (Mohr et al., 2002). Methods of HRV analysis use the cardiac inter-beat interval (R-R interval), which is calculated as the time interval between successive R waves of the electrocardiograph. In the time domain, the root mean square of successive differences (RMSSD) is used to estimate the high frequency beat-to-beat variations which represent vagal (parasympathetic) regulatory activity (von Borell et al., 2007). Research has shown that a decrease in RMSSD is indicative of increased stress load in calves (Mohr et al., 2002). In the frequency domain, high frequency (HF) power bands (0.26-0.86 Hz), indicate vagal activity, low frequency (LF) power bands (0.04-0.26 Hz), indicate sympathetic activity and the LF/HF ratio is a measure of the sympathovagal balance. Both HF and

LF power are measured in normalised units (n.u.) to remove differences in overall variance. Research has shown that the LF/HF ratio increases with sympathetic dominance, indicating a stress response (von Borell et al., 2007). Respiration rates affect HF and LF power bands, so the average respiration rate is calculated and used to set the HF power limits for HRV analysis (Mohr et al., 2002; Hagen et al., 2005; von Borell et al., 2007). There are however issues regarding the interpretation of HRV parameters; debate exists over whether certain frequency parameters can accurately measure sympathetic tone. Després, Veissier, & Boissy (2002) used autonomic pharmacological stimulants and blockades and found that sympathetic activity was not accurately portrayed by HRV parameters, however, other research has found that HRV parameters, such as LF/HF ratios, are reliable indicators of increased sympathetic activity (Yamamoto et al., 1991; Marchant-Forde & Marchant-Forde, 2004; Terkelsen et al., 2005). There is also debate regarding the accuracy of LF, with research suggesting that LF rhythms actually reflect activity of both sympathetic and parasympathetic divisions, not just sympathetic activity alone (Berntson et al., 1997). Therefore, further research is required to investigate the underlying mechanisms driving the changes in some of these different parameters of HRV.

Respiration rate

As a general rule, respiration rate will increase in correspondence with increasing heart rate. This automatic response occurs to increase the level of oxygen provided to the body during increased activity or a 'fight or flight' reaction (Mellor & Stafford, 1999). Respiration rates have been used in human and veterinary medicine and can be measured in a number of ways including flank movements (Gaughan et al.,

2000), nostril movements, air flow (Keyhani et al., 1995), pressure sensors (Eigenberg et al., 2000), or via a stethoscope (Graf & Petersen, 1953). Flank movements were recorded in this research over a 15sec period and calculated as breaths per minute. Measuring respiration rates in the field can often be difficult, as the animal needs to be still, (not feeding, ruminating, moving or defecating) and relatively close to obtain an accurate count. Gaughan et al., (2000) investigated the effects of thermal stress on respiration rates in beef cattle, results showed that respiration rates were a reliable indicator of heat stress, providing ambient conditions were taken into account. Respiration rate was used in this research to primarily assess the calves' stress reactions and secondly to provide additional information for the HRV analysis.

Eye temperature

When an animal is stressed, activation of the HPA axis causes heat production as a result of increases in catechloamines and cortisol concentrations and also heat changes in response to blood flow changes (Schaefer, 2002). An infrared camera can be used to accurately measure radiated electromagnetic energy which is emitted from the body, due to changes in blood flow during stress or pain. (Stewart et al., 2005; Stewart et al., 2007). Infrared thermography (IRT) has been used in human and veterinary medicine (Yang & Yang, 1992; McCafferty, 2007), with a numerous number of body sites being used (Scott et al., 2000; Cook & Schaefer, 2002). Research on the facial surface temperature patterns of Rhesus monkeys has shown that IRT is capable of detecting fear in response to the threat of capture (Nakayama et al., 2005). In this study, IRT was used to measure temperature changes around the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle (as described in Stewart et al. 2005, Figure 2.8). Previous research has shown that

temperatures emitted from this specific area of the eye will decrease in response to pain, due to sympathetically-mediated vasoconstriction in the extremities of the body (Stewart et al., 2008). There are limitations associated with this method, as IRT images must be recorded out of direct sunlight and wind drafts, animals' coats need to be free of dirt, moisture and foreign material, and measurements need to be recorded in reasonably close proximity to the animal to ensure accurate temperature readings (Stewart et al., 2005; McCafferty, 2007).

Plasma cortisol concentrations

An animal undergoing a prolonged stress response will produce elevated levels of free corticosteroids indicative of chronic stress (Hemsworth et al., 1986; Morisse et al., 1995; Sylvester et al., 1998). Corticosteriod hormones, mainly cortisol in mammals, are released from the cortex of the adrenal gland following stimulation from the hypothalamus and pituitary gland. This response is part of the animals' neuroendocrine response and involves the activation of the HPA axis (Matteri et al., 2000). These hormones are valuable indicators of stress because the activity of the HPA axis generally increases in a graded fashion in response to a negative experience (Mellor & Stafford, 1999). Blood samples were taken in this research for analysis of cortisol to assess the effects of disbudding on the calves' stress response. The use of cortisol levels to estimate welfare has some limitations; the collection of blood from an animal itself is often stressful and therefore may confound the interpretation of the results (Moberg, 1985). Alam & Dobson (1986) investigated the effects of blood sampling techniques on dairy cows. They found that a single blood sample, via jugular venipuncture, caused an increase in cortisol concentrations for up to 60 minutes. However, Hopster et al. (1999) found that the stress associated with blood

sampling was less in cows which were accustomed to handling and restraint procedures. Calves in this research will have experienced handling and restraint methods numerous times before blood collection, therefore, stress caused by this procedure should be minimal.

Exit speed test

Exit speed tests are traditionally used in beef cattle as an 'ease of handling' test to assess temperament. (Burrow & Dillon, 1997; Müller & von Keyserlingk, 2006). Temperament of animals is thought to have innate and acquired elements, having a genetic element to their behaviour and also behaviours that are shaped by experience (Petherick et al., 2009). Previous research has suggested that an animal held in a crush will exit, when released, at a faster speed if it is more stressed than if it is calm in the crush (Fisher et al., 2000; Petherick et al., 2002; Müller & von Keyserlingk, 2006). Exit speed tests were used in this research as a fear test and it was predicted that quicker exit times would be an indication of increased fearfulness of the crush and the restraint situation. Exit speed tests are quick, repeatable and easily performed (Fisher et al., 2000).

Force test

Force tests have been used to assess 'ease of movement' in dairy cows and also the effort required by humans to move cows through a given space and then into a crush (Breuer et al., 2003). Breuer et al. (2003) investigated the effects of previous positive and negative handling on the level of graded behavioural response needed by a human handler to move heifers along a race; this response depended on the level of resistance shown by heifers. Moderate waving and vocalisations were used if the

animal was moving, if stationary, the handler would use a positive interaction (hand placed on the animals back) to move the animal. If no movement follows, a negative interaction (moderate hit of hind quarters with plastic pipe) would be used, a second negative interaction (forceful hit with pipe) would be used, if necessary, to move the animal. The animal's ease of movement was quantified by the time taken and number of positive or negative interactions needed to move the animal. Results showed that negatively handled heifers took less time to move to the crush than positively handled heifers. There are some limitations associated with force tests; graded responses obtained are based on reactions to the animal's behaviour and therefore can be difficult to interpret. For example, one animal may have a low force score (moves freely) because it is fearless of the race situation, while another animal may have a low score because it is fearful of the human behind. Therefore, caution is required with interpretation of the results from these tests.

Behavioural measures

Movement in the crush

A wide variety of behavioural tests and behavioural movement definitions have been developed and used over the last century to assess animal's responses to stressful or emotional situations (Ramos & Mormède, 1997). Behavioural reactions to noxious stimulus/situations give immediate and measurable indications of an animal's fear levels. Stewart et al. (2008) used a range of physical movement behaviours to assess pain levels of calves during disbudding procedures. They found that disbudded calves had elevated levels of physical movement, when compared to baseline levels, than control calves. However, due to the complexity of mechanisms underlying fear responses, it is difficult to allocate a behavioural response to any single emotion such

as fear (Rushen, 2000; Forkman et al., 2007) and often the usefulness of recorded behavioural responses are debated (Van Reenen et al., 2005). Care is therefore needed with interpretation of results as behavioural responses to a stressor can vary between animals due to individual characteristics, different coping styles, previous experiences, breed and sex of the animal (Stewart et al., 2005; Van Reenen et al., 2005).

JUSTIFICATION AND AIMS OF THE RESEARCH

Public awareness and interest in animal welfare and the management of farm animals has increased in recent years (Wells et al., 2004). This awareness has resulted in significant pressure from society on livestock industries to provide 'welfare friendly' products to keep with New Zealand's 'clean, green, animal friendly' image (Gregory, 2000; Morris, 2000). This image is crucial to New Zealand's export industry and negative connotations regarding animal welfare could seriously affect market access. Stockmanship has been identified in the livestock industries as an animal welfare issue that has the potential to jeopardise overseas market access and one which has recently been given media attention. This therefore makes research into the effects of handling and stockmanship extremely important. This research will therefore investigate the effects of early positive and negative handling on behavioural and physiological responses to restraint, ear tagging and disbudding procedures in 5 week-old heifer calves. An additional follow up trial will investigate the effects of this early handling on behavioural and physiological responses to a force, approach, and exit speed tests at 3 months of age, in the same heifer calves.

Research on the effects of these types of handling techniques have not been extensively explored in calves; therefore research is needed to investigate whether handling affects the welfare and productivity of these animals both early and later on in life. Fearful animals have already been shown to be more difficult to handle and more unpredictable in farming situations, putting stock and farm staff at risk (de Passillé et al., 1996; Breuer et al., 2003). Similar research in other species, summarised above, has shown that positive and negative handling in juvenile animals affects their welfare and productivity, both early and later on in life. Previous research in adult dairy cows has shown that positive and aversive handling affects physiological and behavioural responses during sham rectal insemination (Waiblinger et al., 2004). The effects of human-animal interactions and different handling techniques are therefore important to assess, to help minimise this risk and provide reliable information on the handling effects on calves, and the possible implications of this handling on calves behaviour and physiological responses later in life.

The aims of this research are to assess the behavioural and physiological effects of early handling on calves' responses to routine farm procedures. This will assess any possible differences caused by positive and negative handling treatments on heart rate, heart rate variability, eye temperature, respiration rates and behaviour in response to restraint, ear tagging and disbudding procedures. At three months of age, the potential of lasting effects of early handling will be assessed by measuring calves' ease of movement through a force test, behaviour and heart rate in a crush, and their exit speed from the crush. It is hypothesised that positively handled calves will show less struggling behaviour to the routine farm procedures, have lower heart rates and respiration rates, less change in heart rate variability parameters and eye temperature responses, and during disbudding have lower concentrations of plasma cortisol levels.

At three months of age the positively handled calves and control calves are predicted to show less fear and avoidance behaviour compared to negatively handled calves. It is hypothesised that positively handled animals will have quicker entry times, struggle less in the crush, have lower heart rates and respiration rates, and exit the crush slower than negatively handled and control calves.

This research has implications for both dairy and beef cattle industries in New Zealand. Results may be used to improve animal management systems, provide guidelines on early rearing and stockmanship procedures. There is also potential to reduce stress during routine husbandry procedures commonly used on-farm and improve also to improve general on-farm animal welfare.

CHAPTER 2

The effects of positive and negative handling on dairy calves' physiological and behavioural responses to routine farm procedures

INTRODUCTION

Human-animal interactions are a common daily occurrence in most farming situations. The frequency and quality of these interactions are therefore important factors contributing to the welfare and productivity of animals. Routine husbandry procedures commonly used on-farm such as restraint, ear tagging and disbudding can result in an aversive experience for animals, and lead to physiological and behavioural stress responses, and a general fear towards humans (Rushen et al., 1999b; Forkman et al., 2007). This fear can decrease the welfare and productivity of farmed animals and also increase handling times and risk of injury to the animals and their handlers (Rushen et al., 1999a, 1999b; Hemsworth, 2003; Waiblinger et al., 2004). Research has shown that animals will discriminate between humans based on previous positive and negative experiences. (Munksgaard et al., 1997; Taylor & Davis, 1998; Grandin, 2004). A study by de Passillé et al. (1996) investigated the effects of positive (petted and offered milk), neutral (no interaction) and aversive (use of an electric cattle prod and nose tongs) handling on dairy calves and found that

calves were able to discriminate between individual humans, but not the gender of humans, based on previous handling experiences. Rushen et al. (1999a) studied the ability of dairy cows to recognise individual humans and the effects that these humans had on the cow's behavioural fear response at milking. Results showed that cows were able to distinguish between handlers with the presence of the aversive handler causing increased movement and higher heart rates during milking; this indicated an increased fear in comparison to cows in the control and gentle handling situations. Waiblinger et al. (2004) investigated the effects of previous gentle handling (feeding, stroking at neck and head of animal and speaking in soothing voice) on the heart rate and behavioural responses of dairy cows during rectal palpation. Results showed that the presence of a handler gentling the animal during the procedure reduced the stress of that animal in this aversive situation, quantified by lowered heart rates, reduced kicking and less restless behaviour; this research is important as it shows the behavioural and physiological effects previous experience with humans can have on dairy cows.

When an animal is subjected to an aversive environmental situation, its natural response is to enter a state of 'fight or flight' to allow the animal to cope with the situation (Dantzer & Mormede, 1983). This acute physiological response is typified by an increase in heart rate, blood pressure, respiration rate and muscle tonus as well as the release of stress hormones (Moberg, 2000). Behavioural and physiological parameters are traditionally used by scientists to assess this stress response. Assessing an animal's behavioural response to a situation allows an insight into the underlying stress levels of that animal; however, although behaviour is an immediate and measurable indicator, care is needed with interpretation as it can be misleading as behavioural responses to a stressor may often vary between animals due to individual

characteristics, previous experiences, breed and sex of the animal (Stewart et al., 2005; Van Reenen et al., 2005). A range of physiological measures are also commonly used to assess stress responses. A summary of the physiological measures used in this research is as follows: (a) Respiration rates measured in breathes per minute. Mellor & Stafford (1999) stated that both the rate and depth of respiration rates can be used as physiological indices of an animal's distress response to noxious stimuli. (b) Infrared thermography (IRT) is another non-invasive technique for detecting pain and fear in animals, which measures changes in radiated heat, using an infrared camera (Stewart et al., 2005; Stewart et al., 2007; McCafferty, 2007; Stewart et al., 2008). Previous research has shown that the change in eye temperature, specifically the area around the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle, is a consistent measure of pain-induced stress in cattle (Stewart et al., 2008). (c) Heart rate is a common measure of physiological stress in animals and has been used to assess welfare in cattle (Rushen et al., 1999a; Waiblinger et al., 2004; Van Reenen et al., 2005). Waiblinger et al. (2004) suggested that heart rate is a reliable tool for assessing stress responses; however, there are several limitations to assessing heart rate alone as it can only provide information on the net effects of the autonomic nervous system, and therefore cannot accurately assess the stress response of sympathovagal regulation (von Borell et al., 2007). (d) Heart rate variability provides more detail of a stress response by measuring the inter-beat interval (R-R interval) and calculating parameters in time, frequency and non-linear domains which assesses the balance between the sympathetic and parasympathetic divisions of the autonomic nervous system (von Borell et al., 2007; Stewart et al., 2008). (e) Changes in plasma cortisol concentrations over time is the most commonly used technique to assess pain responses to procedures such as

disbudding. This technique is accurate but is limited by the practicality of sampling as the collection of blood itself may cause distress and alter cortisol levels (Stafford & Mellor, 2005). Petrie et al. (1995) investigated cortisol responses in young calves which had undergone cautery iron disbudding with and without local anaesthetic. They showed a significant rise from baseline levels within the first hour for both treatments, this rise returned to baseline levels within three hours post-disbudding, which indicated distress and pain caused by the disbudding procedure.

In the present study, cortisol concentrations, behavioural responses, heart rate, heart rate variability, respiration rates and eye temperature were used to investigate the effects of early handling on the responses of dairy calves to restraint, ear tagging and disbudding procedures. The hypothesis was that calves handled positively would be calmer around humans and in novel testing situations and therefore show overall lower levels of behavioural and physiological responses in the testing procedures in comparison to negatively handled calves. This research addresses a gap in current knowledge and will allow an insight into the effects of early handling on calf welfare.

MATERIALS AND METHODS

The experiment described below was approved by the University of Waikato Animal Ethics Committee (Protocol No. 732) and the Ruakura Animal Ethics Committee (Protocol No. 11576).

ANIMALS, HOUSING, HUSBANDRY AND LOCATION

Forty Holstein-Friesian heifers, approximately 1 week of age (range 3-7 days old) were brought from stock sales yards to be used in this study. Calves were randomly allocated into two treatment groups, positive and negative handling, and each treatment group was split further into four groups of five animals. Calves arrived at the study site (AgResearch Tokanui farm, located in the Waikato region of New Zealand; latitude: -38 03' 00", longitude: 175 18' 00") in groups of 10 animals (five positive and five negative calves) on the 9th, 16th, 18th and 25th of September 2008. This staggered arrival allowed time to perform all treatments and tests on the animals at the same age. All calves were weighed (average positive weight: 41 kg, range: 36-54.5 kg; average negative weight: 40.5 kg, range: 33-48.5 kg) and ear-tagged on arrival. Calves were also assigned a colour (red, pink, green, blue or yellow) and spray painted to allow easy identification during the trial. Animals were housed in identical indoor home pens (Fig. 2.1) (4.8m length x 3.5m width) which were filled with straw bedding to approximately 10cm deep; all pens were completely cleaned out and sprayed with Virkon® S (Antec International, New Market, Auckland, New Zealand) disinfectant once a week for hygiene purposes. Calves faecal matter was collected each day and surplus matter was covered with lime for hygiene purposes and to control odour in the barn; faecal samples were later analysed and presented in another student's thesis. Vetpak ® Rotagen "combo" powder (Vetpak Limited, Te Awamutu, New Zealand) was given to all calves for the first 5 days after arrival to protect against rota virus and salmonella. Scourban Plus suspension (Bomac Laboratories Ltd, Manukau City, Auckland, New Zealand) was used to treat scours during this trial and was administered to calves in tablet form. All other health problems were addressed under the advice of the farm staff and/or veterinarian

(Appendix II). All calves were fed two litres of Ancalf ™ calf milk replacer (Fonterra Ltd, Auckland, New Zealand) twice daily (morning 0830h and afternoon 1430h) and offered water and meal *ad libitum*. At three weeks of age Fiberpro® (Fibre Fresh Feeds, Reporoa, New Zealand) was supplemented *ad libitum* into their diet. This trial was under taken in the spring calving season (Sept/Oct).

Figure 2.1. One group from the negative handling treatment in their home pen.

HANDLING PROCEDURE

The positive handling treatment required handlers to quietly enter the home pen and approach the calves slowly whilst using calm, soft voices to encourage voluntary friendly interactions such as pats and stroking. The handler attempted to interact equally with all calves in the allocated time but would not force interactions if a calf resisted. The negative handling treatment involved continuous 45 second cycles of direct and indirect handling to discourage any friendly interactions. Direct handling

required handlers to use fast, sharp movements and short, harsh voices whilst forcibly moving animals around. Calves were forcibly unsettled by the handler changing the calves' direction of movement and splitting the group up by singling out animals. Indirect handling required handlers to stand in the pen starring at the animals and tap a polyurethane pipe (novel object A) to make a banging noise to maintain disturbance. Any animal that approached during this time was pushed away and deterred by the handler. Two additional novel objects, a plastic bag (novel object B) and an empty water bottle filled with stones (novel object C) were used during negative handling to prevent the animals from habituating to one type of handling aid. One novel object was introduced (in the stated order) weekly, and used for the whole week on every second day. During the fourth week the handler used one of the three objects every second day, rotating between the three items so all were used for one handling day per week. These handling procedures occurred twice daily, once after morning feeding at 0830h and again after afternoon feeding at 1430h for the entire duration of the trial (Table 2.1). Handlers were one of six staff members, depending on staff scheduling. Handling duration for the first five days after arrival was 10 minutes; after these initial five days, handling was reduced to five minute periods as it was difficult for all the handlers to maintain a constant energy level for 10 minutes during negative handling and treatments needed to be consistent. Each handling session followed a pre-determined order across groups which ensured that the youngest group of calves were fed and handled first (for their first week in the barn only), followed by the group which were to be tested on that day. If no testing was scheduled for that day then feeding and handling was randomly assigned between groups. Staff that performed handling treatments were not used in any of the routine farm management procedures and testing staff avoided contact with the calves to ensure that animals' behavioural

and physiological responses were not affected by the presence of a familiar human. Table 2.1 outlines the handling treatments used and the timing of routine husbandry procedures.

Table 2.1 Handling and routine farm procedure timeline.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wk 0							Calf arrives at farm
Wk 1	Handling am & pm	Handling am & pm with plastic bag	Handling am & pm	Handling am & pm with plastic bag	Handling am & pm	Handling am & pm with plastic bag	Handling am & pm
Wk 2	Handling am & pm with bottle	Handling am & pm	Handling am & pm with bottle	Handling am & pm	Handling am & pm with bottle	Handling am & pm	Handling am & pm with bottle
Wk 3	Handling am & pm	Handling am & pm with stick	Handling am & pm	Handling am & pm with stick	Handling am & pm	Handling am & pm with stick	Handling am & pm
Wk 4	Handling am & pm with plastic bag	Handling am & pm	Handling am & pm with bottle	Handling am & pm	Handling am & pm with stick	Handling am & pm	Handling am & pm with plastic bag
Wk 5	Handling am & pm	Handling am **Restraint** Handling pm	Handling am & pm with bottle	Handling am **Ear Tagging** Handling pm	Handling am & pm with stick	No handling **Disbudding**	Testing finished

FARM MANAGEMENT PROCEDURES

RESTRAINT PROCEDURE

At four weeks of age all calves were individually subjected to a 15 minute restraint test in a calf crush with a head bail (Cattlemaster, Te Pari Products, Oamaru, New Zealand, Fig. 2.2). On treatment days, all calves were fed and handled at 0830h

as normal and then moved to the testing area where they were held in treatment pens (5.1m length x 2.3m width, with access to water and food *ad libitum*). The order of testing was randomised for all groups, with the order of positive and negative calves being tested differing across all routine farm procedures (Appendix III). All calves were secured in the crush with a head bail and a chain which attached across the back of the crush behind the animal to prevent it backing out. Respiration rates, heart rate, heart rate variability, eye temperature and behaviour recordings were taken as described below and the timing of the events are shown in Fig 2.3. All testing was completed between morning and afternoon feedings, and calves were returned to their home pens and fed as usual in the afternoon.

Figure 2.2. The general set-up in the testing area showing crush location, front camera and IRT camera set up in background.

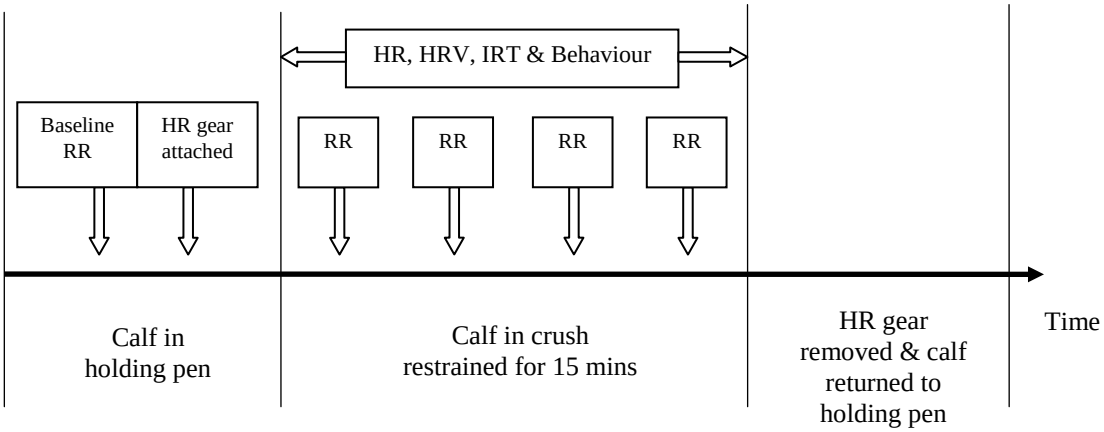

Figure. 2.3. A timeline showing the schedule of events during the restraint procedure RR= respiration rate, HR= heart rate, HRV=heart rate variability, IRT=infrared thermography.

EAR TAGGING PROCEDURE

Approximately two days after the restraint test, calves were individually subjected to a second test (20 min in duration) involving an ear tagging procedure. As in the restraint test, animals were fed, handled and then moved up to the test area. After 10 minutes of being secured in the crush, the calves were ear tagged using an applicator (Allflex® Universal applicator) with either a pink (positive) or blue (negative) button ear tag in the middle of their left ear between the two main cartilage ridges (Fig 2.4). The time of first contact to the ear and the exact time of tagging were recorded (average time to perform the procedure was 6.7 seconds). Respiration rates, heart rate, heart rate variability, eye temperature and behavioural recordings were taken during this procedure, the timing of these events are shown in Fig 2.5.

Figure 2.4. Calf restrained in the crush while having an ear tag inserted with applicator.

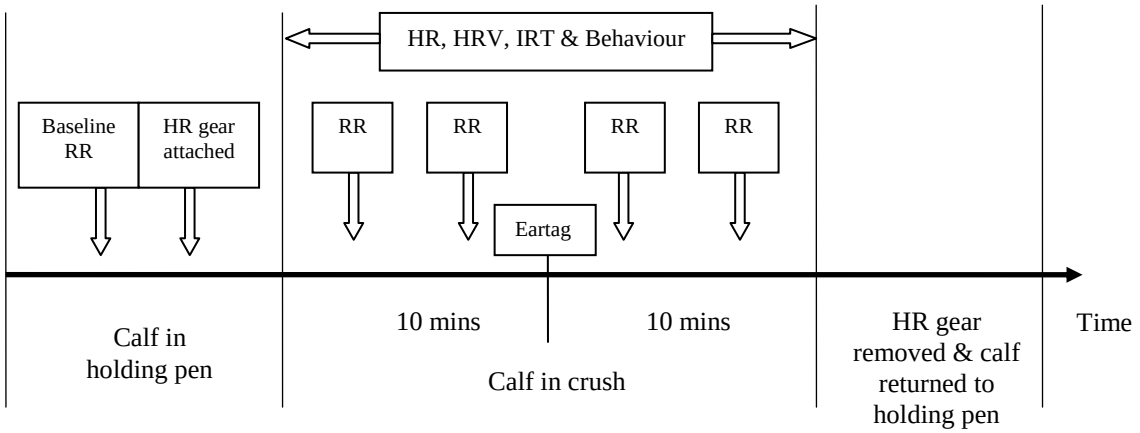

Figure 2.5. A timeline showing the schedule of events during the ear tagging procedure RR= respiration rate, HR= heart rate, HRV=heart rate variability, IRT=infrared thermography.

DISBUDDING PROCEDURE

Approximately two days after the ear-tagging test, calves were individually subjected to a third test (10 minutes in duration) involving routine disbudding. Calves were fed as usual at 0830h but were not handled and therefore moved straight to the testing area. Blood samples (10ml) for plasma cortisol concentrations were taken in the holding pen via vena puncture of the jugular vein at -20, 20 and 40 minutes in relation to disbudding (time 0) by the same veterinary technician (no blood samples were taken in the crush). Calves were restrained in the crush and immediately given a local anaesthetic (LA) injection (6 ml of 2% lignocaine hydrochloride: Lopaine, Ethical Agents Ltd, Auckland, New Zealand) into the corneal notch around each horn bud (average time to perform procedure was 11.2 seconds). Calves were left for five minutes to allow the local anaesthetic to take effect; then both horn buds were removed (average time to perform procedure was 98.2 seconds) using a standard gas powered cautery iron (ABER LPG debudder, Shoof International Ltd, Cambridge, New Zealand) heated to approximately 700ºC (Fig 2.6.). The wound was sprayed with Aerotet Forte antibacterial spray after both horns were removed to help avoid infection. The exact start time and length of the disbudding procedure was recorded and calves remained in the crush for five minutes after the procedure had finished. Respiration rates, heart rate, heart rate variability, and behaviour recordings were taken during this procedure; the timing of these events are shown in Fig 2.7.

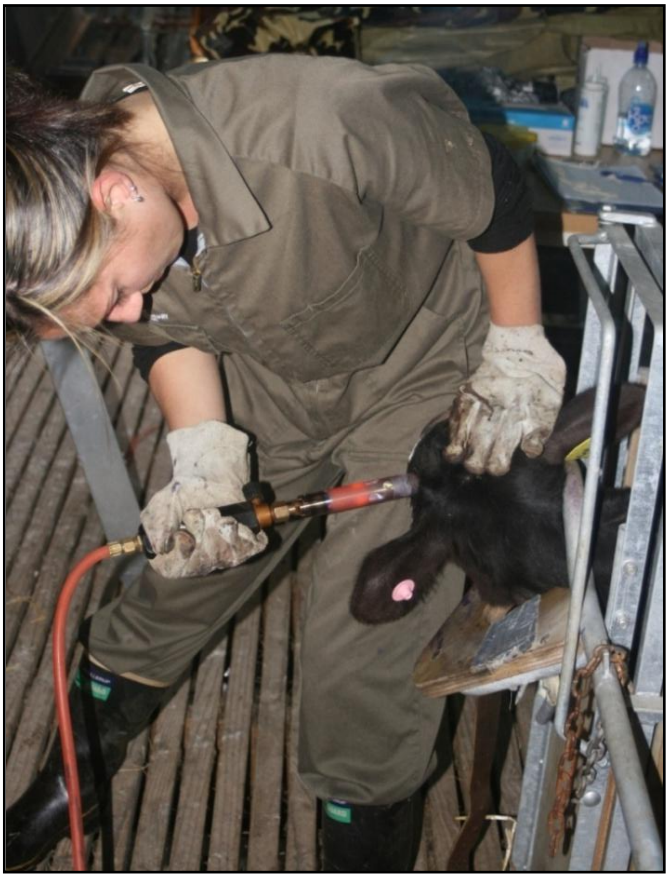

Figure 2.6. Calf being disbudded by applying heated cautery iron to the horn buds.

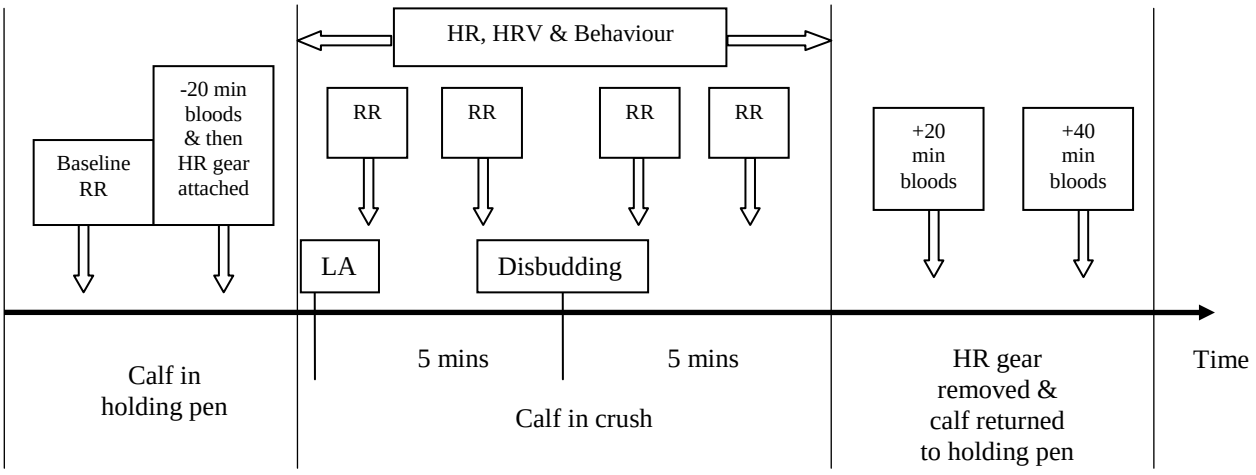

Figure 2.7. A timeline showing the schedule of events during the disbudding procedure RR= respiration rate, HR= heart rate, HRV=heart rate variability, IRT=infrared thermography, LA=local anaesthetic.

MEASUREMENTS RECORDED DURING PROCEDURES

RESPIRATION RATE

For all routine procedures a 15 second respiration rate was obtained before the calf was removed from the test holding pen as a baseline reading approximately 10-15 minutes before testing. Respiration was taken by counting flank movements on the animal caused by breathing. This baseline reading was usually taken whilst the calf was lying down (60% of recordings) but if this was not possible a reading was taken while the animal was standing (40% of recordings). All 15 second measurements were later calculated in breaths per minute. Four further respiration rates were recorded during each test procedure while the animal was in the crush. In the ear tagging and disbudding procedures, two readings were taken before the ear tag/disbudding event and two were taken after the event, all four restraint readings were taken evenly throughout the 15 minute period.

HEART RATE

One day before their restraint test all calves had a 5cm wide strip shaved into their coat, starting slightly to the left of the shoulder blade and ending at the underside of the girth close to the foreleg to be used for HR monitor attachment. Before any testing began, the times on all heart rate equipment was synchronised with a master clock for precise timing of events. The test calf was then fitted with a Polar heart rate monitor (S810i^{TM}, Polar Electro Oy, Helsinki, Finland). Ultrasound transmission gel was applied to the calf's shaved coat area at each electrode site to optimise conductivity; approximately 2 minutes later the calf was moved from the holding pen to the crush for testing. Heart rate was continuously recorded whilst in the crush and

all heart rate recordings were downloaded at the end of the testing day onto a laptop (HP Compac nc6400 notebook PC). Data was later analysed using Polar software (Polar Precision Performance Software; Version 5.0).Thirty minute baseline heart rate readings were also taken from home pens from four randomly chosen calves from each group of five.

HEART RATE VARIABILITY

Heart rate variability was calculated from the heart rate recordings using Polar software (Polar Precision Performance Software; Version 5.0). The 15 minutes of restraint, ten minutes pre- and post-ear tagging, and five minutes pre- and post-disbudding was analysed. The continuous recording of heart rate was analysed in R-R inter-beat intervals in sections of 512 beats. Restraint was analysed in four blocks, ear tagging had three blocks of 512 beats in both pre- and post- treatment and disbudding had two blocks of 512 beats in both pre- and post- treatment. All R-R interval data were corrected before analysis for errors in the data, using the error correction function in the Polar software. An average error rate of five percent was accepted and included in the analysis. This means that data with more errors, possibly caused by bad connections during heart rate recordings, were excluded. The time domain parameters analysed included heart rate, the R-R interval and the root mean square of successive R-R interval differences (RMSSD). The frequency domain parameters analysed included high frequency (HF) power, low frequency (LF) power and the LF/HF ratio. HF and LF are presented in normalised units (nu) to account for inter-individual differences (von Borell et al., 2007). All parameters were calculated using advanced HRV software (Niskanen et al., 2004).

EYE TEMPERATURE USING INFRARED THERMOGRAPHY

The maximum eye temperature (°C) within the area of the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle (Fig 2.8) was recorded during restraint and ear tagging procedures approximately every 30 seconds using an infrared camera (ThermaCam S60, FLIR Systems AB, Danderyd, Sweden) and entered directly into an Excel spreadsheet. All measurements were scanned from the left side of the animal at a 90° angle at a distance of approximately 0.5m. Ambient temperature and the relative humidity in the test area were measured before each calf entered the crush; these values were used during IRT analysis to allow for any atmospheric changes. Continuous recordings were also collected using an interface connection between the camera and laptop as a backup to the excel data. Eye temperature was not recorded during the disbudding procedure.

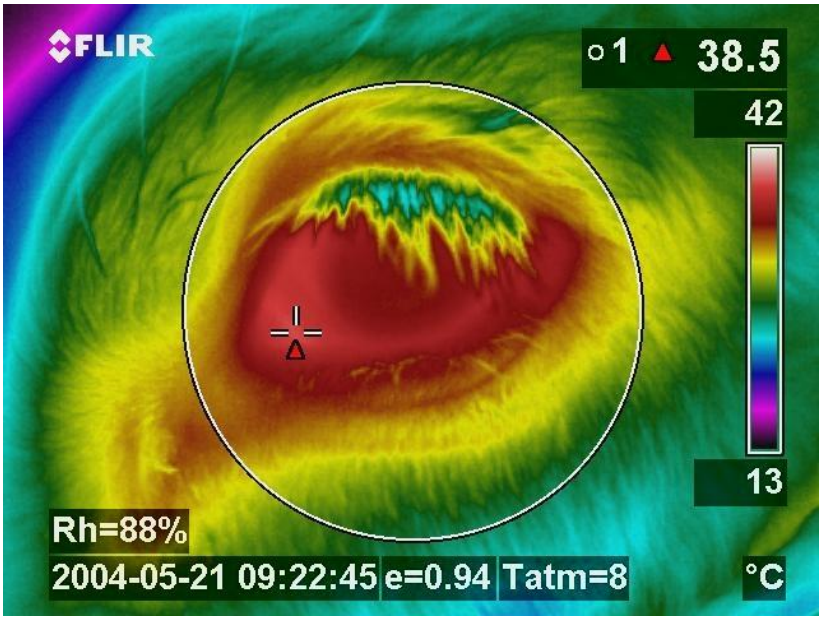

Figure 2.8. Infrared image of the eye region of a calf. The cross indicates the position of the maximum temperature within the area of the eye used for analysis, the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle. Taken from Stewart et al. (2008).

BEHAVIOUR

Prior to testing, the times on all camera equipment, including IRT, was synchronised for precise timing of events. Three video cameras (DCR-TRV355E, Sony, Japan) were mounted to continuously record all behaviours (Table 2.2). One camera was mounted directly behind the crush to capture all body and leg movements, one camera was directly above the crush to capture any struggling behaviour, and one camera was mounted directly in front of the animal to capture head movements.

ANALYSIS OF BLOOD CORTISOL SAMPLES

Blood samples (10 ml) were collected via venipuncture of the jugular vein into vacutainers containing EDTA (Vacuette ®, greiner bio-one, Germany) and were chilled on ice immediately. Samples were then centrifuged at 7000 cycles/min and 3mls of plasma was extracted and frozen at -20 ^{o}C. Plasma cortisol concentrations were processed by Dairy NZ using a double-antibody radioimmunoassay as described previously (Fisher et al., 2002). The minimum detectable level was 0.47 ng/ml. The inter-assay coefficient of variation for plasma pools measuring 70.5, 28.7 and 6.9 ng/ml were 3.6, 8.8 and 13% respectively.

Table 2.2. Behaviours recorded during all three routine "on farm" procedures.

Behaviour	**Definition**
Leg lift hind/front	Any foot raised off the ground and then replaced, often in a rapid movement (within 2 sec)
Rear	One or both front legs are raised off the ground in a forward pawing action at the front wall
Back leg lunge	Both back legs move rapidly either forward or backwards to land simultaneously
Rump squat	When the top of the tail reaches to or the tail lowers below the escutcheon (*escutcheon – the part of a cow that extends upward just above and back of the udder where the hair turns upward in contrast to the normal downward direction of hair; also called milk mirror*)
Fall	The calf collapses to the ground onto both knees and/or hocks
Slip	Hind leg is extended backwards or stretched forwards as it slides along the floor
Elimination	When the calf urinates or defecates – recorded as separate events
Tail flick	When any part of the tail moves from a central body position distally to the outer leg line. One flick is counted when the tail returns back to the central body position; multiple tail flicks are common in either distal direction. Flicks are often combined with tail arching.
Lateral body movements	When either the hip or shoulder (or both simultaneously) hit one side of the crush followed by the other hip or shoulder (or both simultaneously) hitting the other side of the crush within 2 sec.

STATISTICAL ANALYSIS

Data was interpreted with the assistance of Neil Cox, Ruakura statistician. Data was analysed using Genstat (version 10.2) statistics program and Microsoft Excel 2007. Heart rate data collected from all three routine tests was initially analysed using Polar Precision Performance software. All obvious outlier readings were removed from data and treatment effects were explored using a restricted maximum likelihood (REML) analysis. A t-distribution test was used to look at within treatment change for ear tagging and disbudding and to compare within treatment differences for restraint. Heart rate variability data was also initially analysed using Polar Precision Performance software; data was then transformed to log and analysed using an ANOVA. Respiration rates were analysed using REML to analyse within treatment effects and compare treatment groups. Post treatment responses were also compared to baselines using REML analysis. Eye temperatures were analysed using REML to analyse within treatment effects and to compare treatment groups. Cortisol samples were compared at all sampling periods (-20, 20 and 40 minutes in relation to disbudding) using an ANOVA. All behaviour data was log transformed prior to analysis and counts of behavioural events were normalised to a frequency per minute. Restraint data was assessed over the entire 15 minute period, ear tagging data was compared pre- and post- tagging and disbudding data was compared pre- and post-disbudding using REML analysis. Front and hind leg movements, leg slips and tail flicks were analysed and all other struggling behaviours were omitted as incidences were not frequent enough for significant analysis. Data was further analysed to assess actual movement per minute of the testing period using an ANOVA test and t-distribution tests on treatment groups. Total activity data was determined by adding all incidences of back leg lunges, rump squats, rears, falls, lateral body movements,

leg slips, tail flicks and hind and front leg movements together as one unit, data was then further analysed as normal behaviour data above. Data are presented as the mean ± the standard error of the mean (s.e.m) or the standard error of the difference (s.e.d). One calf was excluded from this trial due to ill health and two calves were polled (did not grow horn buds) and were therefore excluded from disbudding (Appendix II).

RESULTS

RESTRAINT PROCEDURE

HEART RATE

There were no significant differences in heart rate at any stage of the 15 minute restraint procedure between positive and negative treatment groups ($F_{1,32}$=0.011, p=0.91, Fig 2.9). The average heart rate for the 15 minute period was 80.5±3.9 and 81.2±3.4 beats/min for positive and negative respectively. There was however a significant change over time within treatment groups; heart rate was significantly lower during the final ten minutes within each treatment compared to the first five minutes, with a reduction of 4.3±1.7 and 4.1±1.8 beats/minute, for negative (p=0.016) and positive (p=0.029) groups respectively.

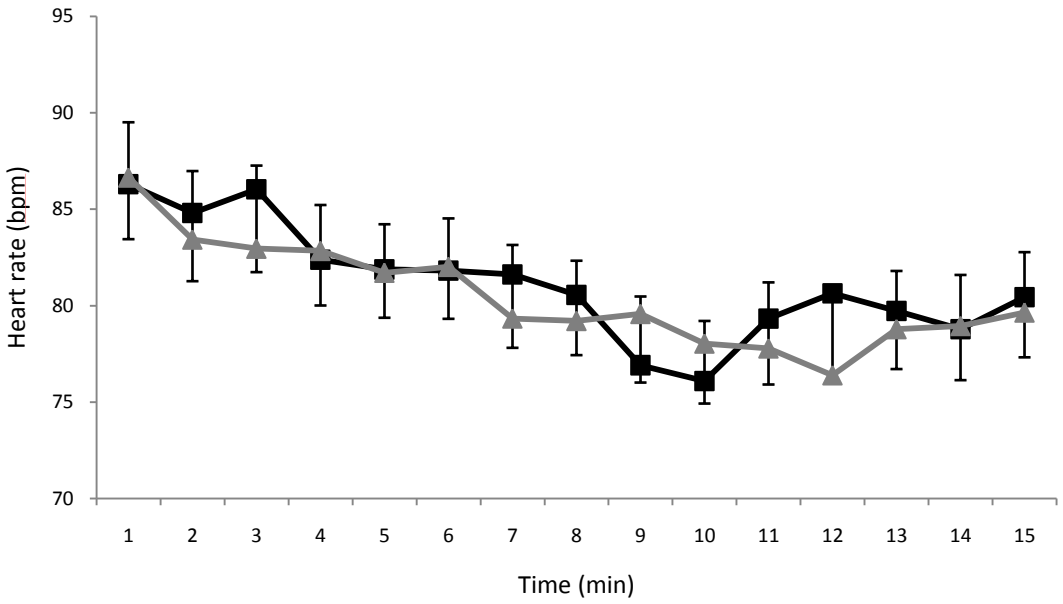

Figure 2.9. Heart rates of negative (■, n =20) and positive (▲, n=19) treatment groups over 15 minute restraint period. Mean ± standard error of the difference.

HEART RATE VARIABILITY

There were no significant differences in heart rate variability at any stage of the restraint procedure between treatment groups (Table 2.3).

Table 2.3. Heart rate variability (HRV) parameters (± s.e.m) in time domain, the root mean square of successive R-R interval differences (RMSSD), and frequency domain, high frequency (HF) and low frequency (LF) power and the LF/HF ratio comparing the first two 512 inter-beat sections during restraint (n=39).

HRV parameter	Positive	Negative	ANOVA	F Value
HF (n.u.)	1.20±0.24	1.15±0.23	p=0.488 ns	$F_{1,27}$=0.495
LF (n.u.)	1.03±0.05	1.00±0.04	p=0.458 ns	$F_{1,27}$=0.567
HF/LF ratio	1.29±0.99	2.76±0.96	p=0.671 ns	$F_{1,27}$=1.84
RMSSD (ms)	8.30±6.40	-3.33±6.18	p=0.202 ns	$F_{1,27}$=1.71

ns = non significant n.u. = Hz in normalised units

RESPIRATION RATES

There were no significant treatment differences in respiration rates with the average respiration rate being 26.6± 2.5 and 27.6± 2.5 breaths/min for positive and negative treatments respectively ($F_{1, 34}$=0.18, p=0.674). Compared to holding pen baseline rates (restraint-baseline), the positively treated calves had an increase in respiration rate by 2.1 breaths/min ± 1.6, and the negatively treated calves had a decrease in respiration rate by 1 breath/min ± 1.6 ($F_{1,32}$=1.834, p=0.185) when restrained.

EYE TEMPERATURE

There were no significant differences in eye temperature at any stage of the restraint procedure between positive and negative treatment groups (p=0.793). The mean temperature for positive and negative treatment groups was 37.4± 0.1 and 37.4± 0.1 respectively (Fig 2.10).

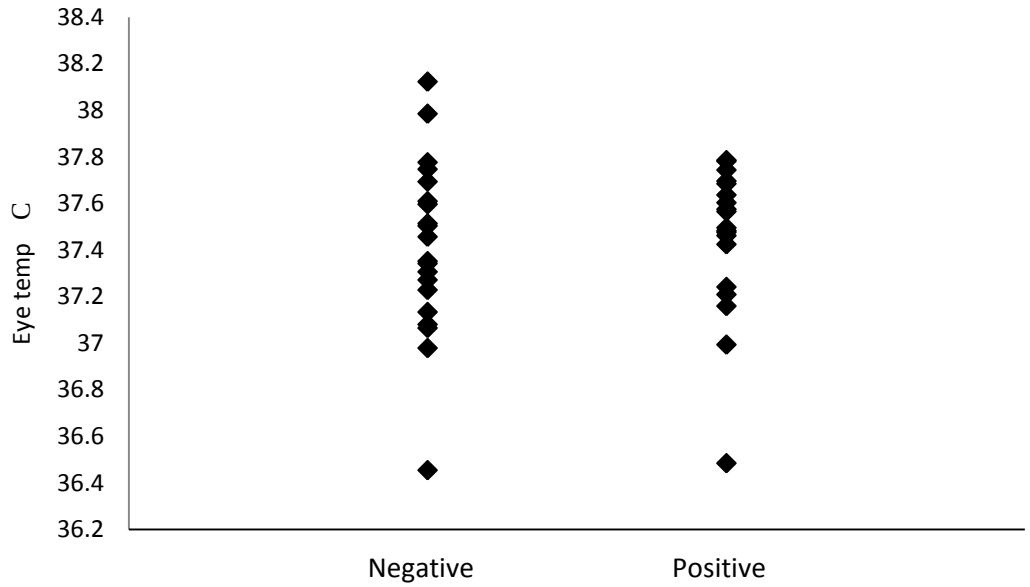

Figure 2.10. Mean eye temperature (°C) for each individual animal in negative (n=20) and positive (n=19) treatment groups over the 15 minute restraint period.

BEHAVIOUR

There were no significant differences in struggling behaviour between or within the positive and negative treatments with a log average of 3.2±0.2 and 3.2±0.2 movements/minute, for negative and positive treated groups respectively ($F_{1,37}$=0.001, p=0.970). Back leg lunges, rump squats, eliminations, rears, falls and lateral body movements were not performed frequently enough during the 15 minute period to be analysed statistically. There were no significant differences between the average frequency of hind and front leg movements, leg slips and tail flicks for positive and negative treatment groups (Fig 2.11.).

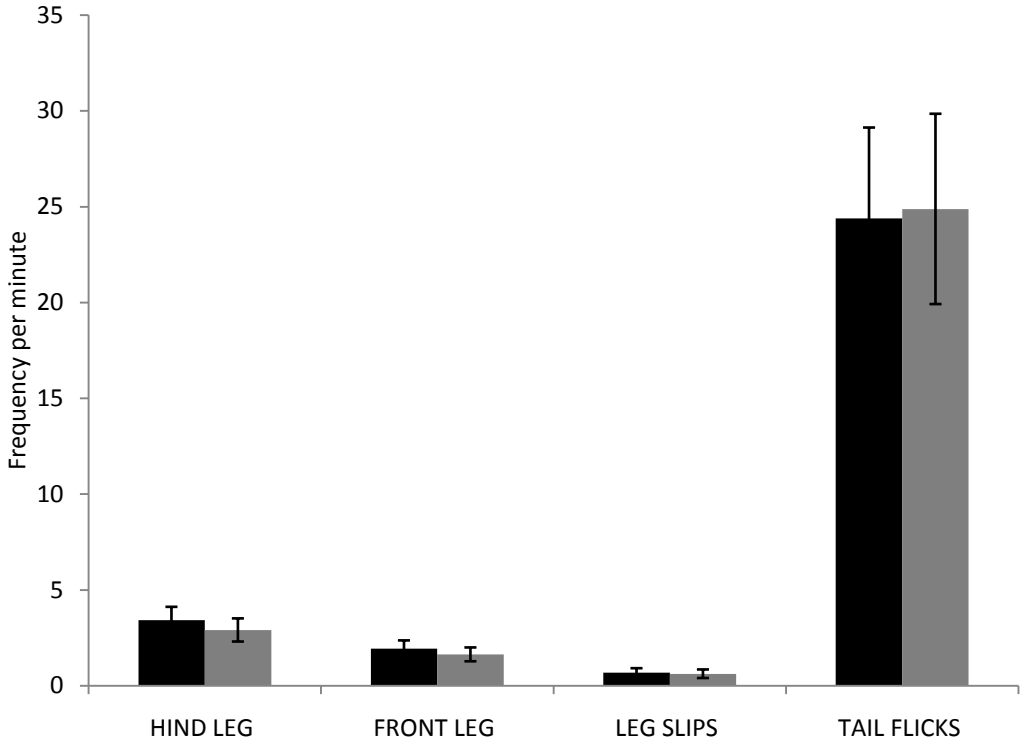

Figure 2.11. Average frequency of hind leg, front leg, leg slip and tail flick behaviours for negative (■, n=20) and positive (■, n=19) treatment groups over the 15 minute restraint period. Mean ± standard error of the difference.

Total behavioural activity, which included rearing, back leg lunges, rump squats, falls, lateral body movement, hind and front leg movements, leg slips and tail flicks, were not significantly different between positive and negative treatment groups ($F_{1,37}$=0.265, p=0.610). There was also no significant change in behavioural activity over time ($F_{1,37}$= 0.00, p=0.990) (Fig 2.12).

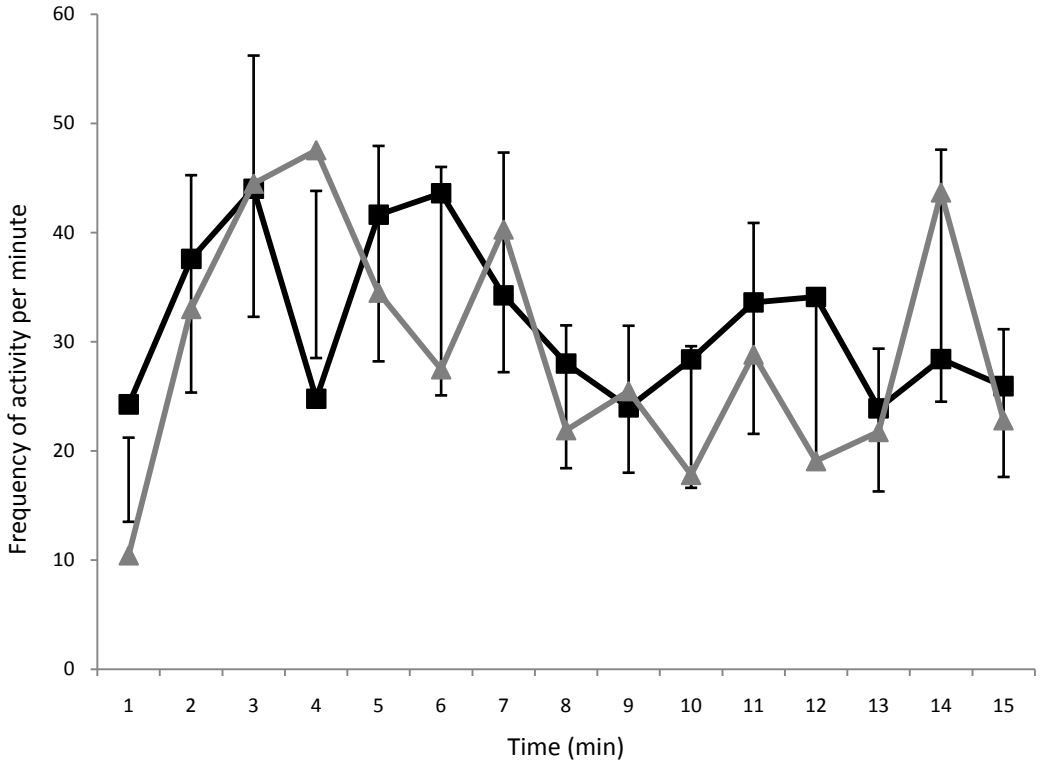

Figure 2.12. Average total activity for negative (■, n=20) and positive (▲, n=19) treatment groups over the 15 minute restraint period. Mean ± standard error of the mean.

EAR TAGGING PROCEDURE

HEART RATE

There were no significant differences in heart rate before and after the ear tagging procedure between treatment groups ($F_{1,32}$=0.245, p=0.624) with the average

heart rate for the 20 minute period being 92.5±4.5 and 88.0±4.4 (±s.e.d) beats/min for positive and negative treatment groups respectively. There was a significant change in heart rate within treatment groups in response to the ear tagging procedure with an increase in heart rate of 8.7±3.1 and 10.3±3.0 beats/ min for the positive and negative treatment groups respectively ($p<0.01$), when comparing five minutes before and after ear tagging (Fig 2.13).

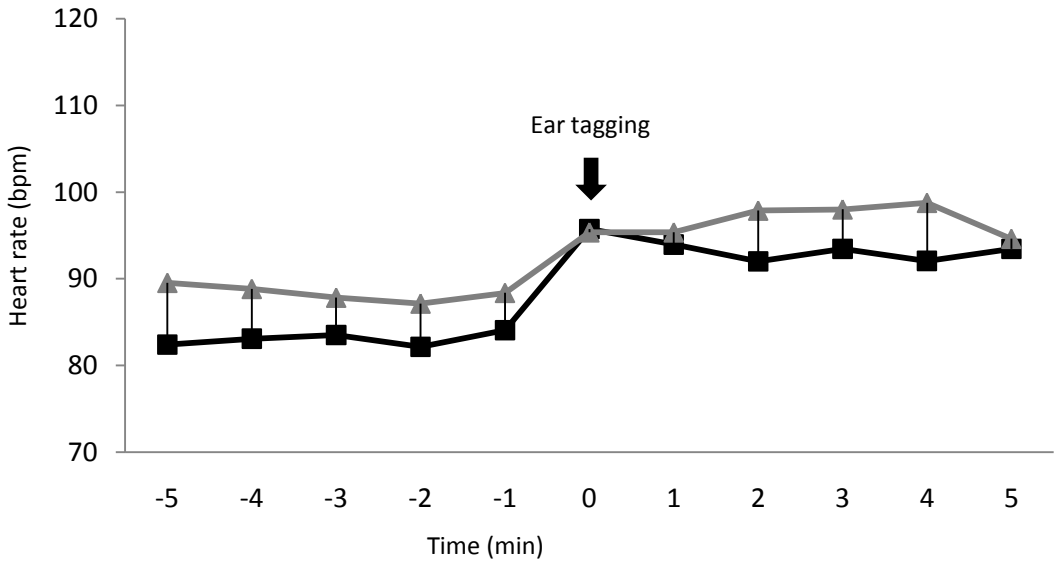

Figure 2.13. Average heart rate of negative (■, n=20) and positive (▲, n=19) treatment groups for five minutes pre- and post-ear tagging procedure. Mean ± standard error of the difference.

HEART RATE VARIABILITY

There were no significant differences in any heart rate variability parameter at any stage of the ear tagging procedure between treatment groups. Table 2.4 shows the 512 R-R beat intervals immediately after compared to immediately before ear tagging.

Table 2.4. Average change in heart rate variability (HRV) parameters (±s.e.m) in time domain, the root mean square of successive R-R interval differences (RMSSD), and frequency domain, high frequency (HF) and low frequency (LF) power and the LF/HF ratio immediately before and after ear tagging (n=39).

HRV parameter	Positive	Negative	ANOVA	F Value
HF (n.u.)	1.54±0.31	1.64±0.29	p= 0.947 ns	$F_{1,28}$=0.004
LF (n.u.)	0.99±0.04	0.99±0.04	p= 0.960 ns	$F_{1,28}$=0.003
HF/LF ratio	1.00±0.34	1.32±0.32	p= 0.970 ns	$F_{1,28}$=0.001
RMSSD (ms)	4.34±8.78	13.20±8.20	p= 0.466 ns	$F_{1,28}$=0.547

ns – non significant n.u. = Hz in normalised units

RESPIRATION RATES

There was no significant difference between treatment groups for the twenty minute ear tagging procedure (p>0.244). Although not significantly, respiration rates did increase within treatments post ear tagging compared to baseline levels by 3.6±2.4 and 6.1±2.3 (±s.e.d) breaths/min for the positive and negative treatment groups respectively ($F_{1,37}$=0.588, p=0.448).

EYE TEMPERATURE

There was no significant change in eye temperature before and after ear tagging between or within positive and negative treatment groups ($F_{1, 37}$=0.122, p=0.729; Fig 2.14.), with the average change in eye temperature (°C) from pre to post ear tagging increasing 0.08±0.06 and 0.05±0.06 (±s.e.d) for the positive and negative treatments respectively (p>0.729).

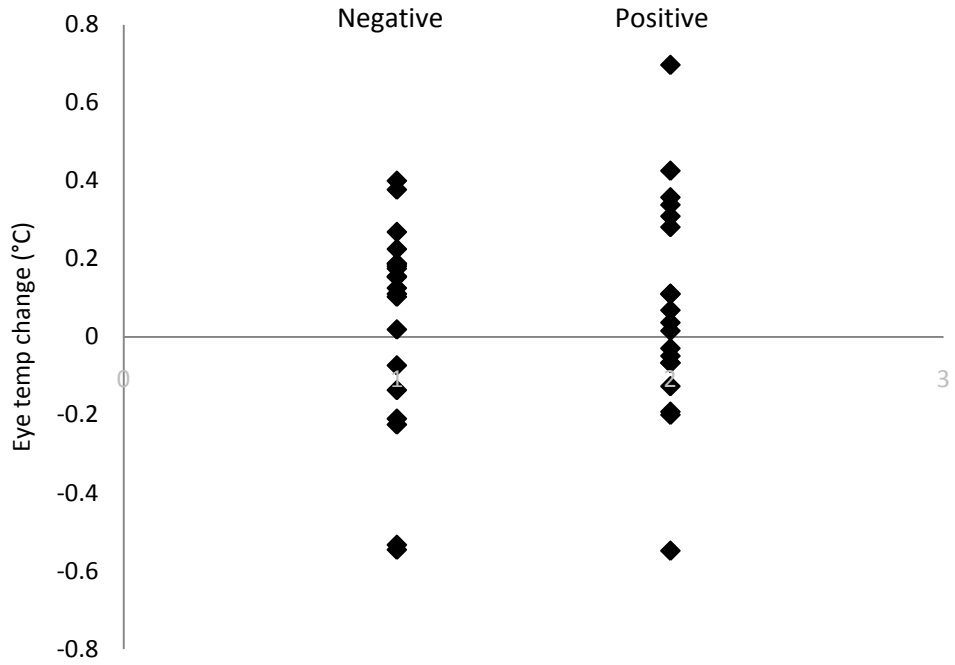

Figure 2.14. Change in eye temperature (°C) for negative (n=20) and positive (n=19) treatment groups five minutes before and after ear tagging procedure.

BEHAVIOUR

There were no significant treatment differences in behaviour throughout the ear tagging procedure ($F_{1, 37}$=0.320, p=0.575). When comparing the rate of tail flicking five minutes before and five minutes after the ear tagging procedure there was an increase, although not significant, of 9.6 and 5.6 (±5.5 s.e.d) tail flicks for the positive and negative treatments respectively ($F_{1, 34}$=0.53, p=0.472). When comparing the change in total movement five minutes before and after tagging there was a tendency for movement to increase with an average of 2.74 and 1.08 (±1.44 s.e.d) movements/min for the positive and negative treatment groups respectively ($F_{1,37}$=1.32, p=0.257).

DISBUDDING PROCEDURE

HEART RATE

There were no significant differences in baseline heart rate ($F_{1, 32}$=0.336, p=0.556), pre-disbudding ($F_{1, 32}$=0.015, p=0.903) and post-disbudding ($F_{1, 32}$=0.227, p=0.637) heart rates between positive and negative treatment groups. However heart rate did increase for both treatments in response to the disbudding procedure with positive and negative groups increasing 14.7±4.5 and 18.6±4.3 (±s.e.d) beats/min respectively from base heart rate levels (p<0.001; Fig 2.15.).

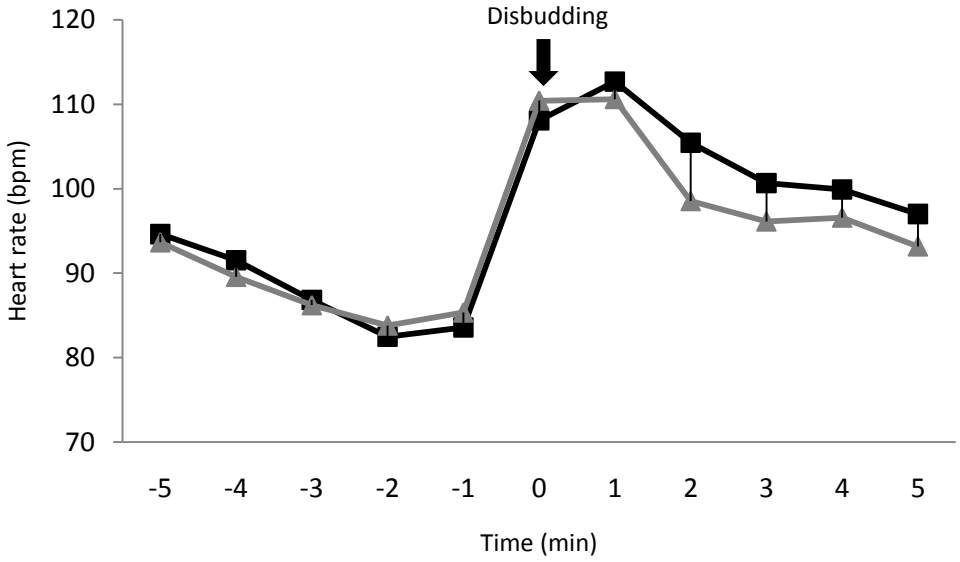

Figure 2.15. Heart rates for negative (■, n=19) and positive (▲, n=18) treatment groups over the 10 minute procedure including disbudding. Mean ± standard error of the difference.

HEART RATE VARIABILITY

There were no significant differences in any heart rate variability parameter at any stage of the disbudding procedure between treatment groups when comparing changes in parameters immediately before and after disbudding (Table 2.5).

Table 2.5. Average change in heart rate variability (HRV) parameters (±s.e.m) in time domain, the root mean square of successive R-R interval differences (RMSSD), and frequency domain, high frequency (HF) and low frequency (LF) power and the LF/HF ratio immediately before and after disbudding (n=39).

HRV parameter	Positive	Negative	ANOVA	F Value
HF (n.u.)	1.47±0.25	1.24±0.25	p=0.864 ns	$F_{1,28}$=0.030
LF (n.u.)	0.96±0.11	1.21±0.11	p=0.108 ns	$F_{1,28}$=2.760
HF/LF ratio	1.79±0.60	1.87±0.60	p=0.564 ns	$F_{1,28}$=0.342
RMSSD (ms)	8.10±10.75	-4.01±1.75	p=0.432 ns	$F_{1,28}$=0.635

ns= non significant n.u. = Hz in normalised units

RESPIRATION RATES

There were no significant differences in respiration rates between treatments in response to disbudding ($p>0.27$). Respiration rates did increase for both treatments by 8.2±3.4 and 9.3±3.3 breaths/min, positive and negative respectively ($p<0.05$).

BEHAVIOUR

There were no significant treatment differences in behaviour throughout the disbudding procedure ($F_{1, 33}$=0.29, p=0.596). However, there were significant within treatment differences, with an increase in struggling behaviour (Fig 2.16.) and tail flicking (Fig 2.17.) five minutes after compared to five minutes before the disbudding procedure for both the positive and negative treatment groups ($p<0.001$).

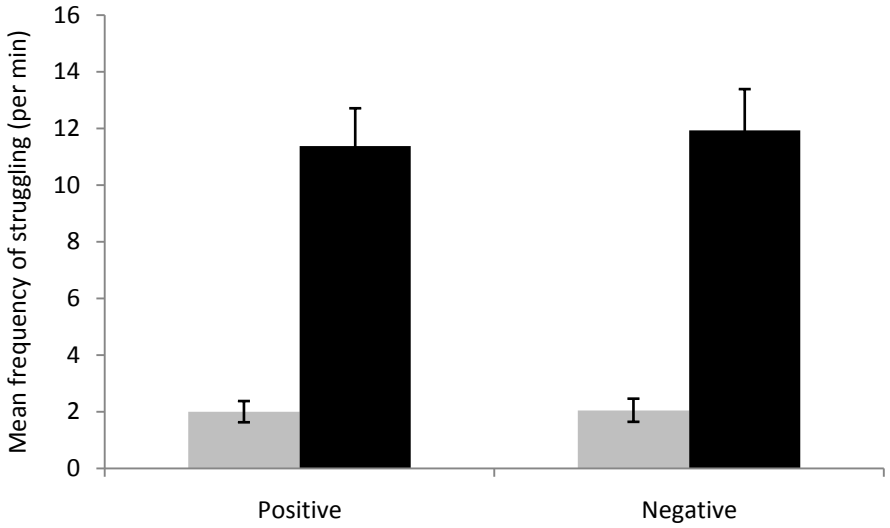

Figure 2.16. Comparison of struggling behaviour for positive (n=18) and negative (n=19) treatment groups five minutes before (■) and after (■) the disbudding procedure. Mean ± standard error of the difference.

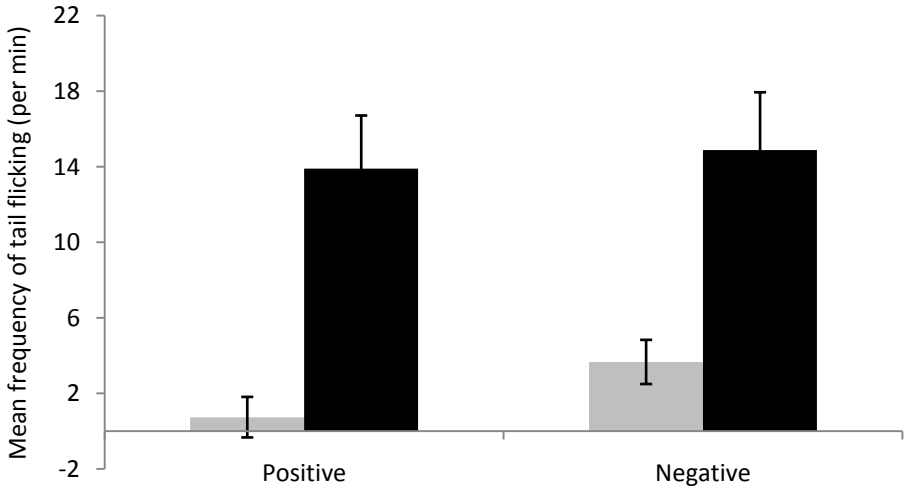

Figure 2.17. Comparison of mean tail flicking behaviour for positive (n=18) and negative (n=19) treatment groups five minutes before (■) and after (■) the disbudding procedure. Mean ± standard error of the difference.

CORTISOL

There was no significant difference between increases in the mean cortisol concentrations (ng/ml) for the positive and negative treatment groups for either the -20 minute to 40 minute periods, 7.1 ±1.2 ng/ml and 8.8 ±1.2 ng/ml respectively ($F_{1, 35}$= 1.06, p=0.310) or the initial -20 to 20 minute change after disbudding, 10.3 ±1.1 ng/ml and 12.3 ±1.1 ng/ml respectively ($F_{1, 35}$=1.64, p=0.209) (Fig. 2.18). There were however significant increases in mean cortisol concentrations within treatment groups in response to the disbudding procedure for positive ($F_{1, 35}$=83.5, p<0.001) and negative ($F_{1, 35}$=125.9, p<0.001) groups.

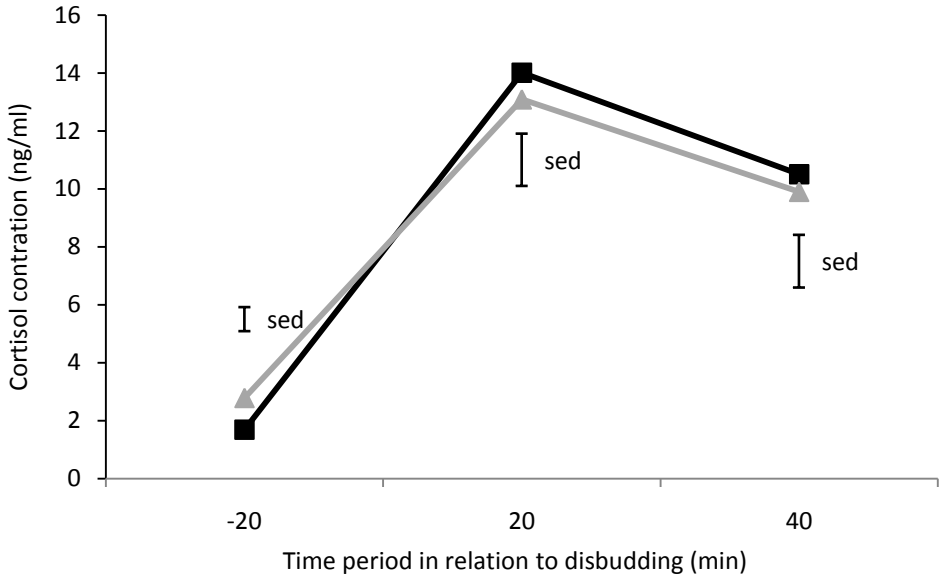

Figure 2.18. Average cortisol concentrations (ng/ml) for negative (■, n=19) and positive (▲, n=18) treatment groups -20 min, 20 min and 40 mins relative to disbudding time. Mean ± standard error of the difference.

DISCUSSION

The results from this study do not support the hypothesis that calves handled positively, early in life, would show overall lower levels of behavioural and physiological responses in comparison to negatively handled calves during routine husbandry procedures. Findings that heart rate responses were not significantly different between treatment groups during routine husbandry procedures are not consistent with those of Waiblinger et al. (2004) who found that positively handled cows had significantly lower heart rates during a rectal palpation/insemination procedure. This difference may be due to the age difference in cattle used for the two trials; Waiblinger et al. (2004) used cattle 3-11 years in age which could have caused significant differences in perception of human interactions and also previous experiences with humans could have had an influence on responses. There were however changes in calves' heart rates within treatment groups in response to each procedure. In particular the increase in heart rate to ear tagging and disbudding procedures indicates that the animals did experience a certain level of pain and/or stress. During restraint, the significant decrease in heart rate during the final ten minutes compared to the first five for both treatment groups suggests that the calves may have habituated to the crush and the restraint situation. The rise in heart rate for both treatment groups during the five minutes post ear tagging and disbudding procedures is consistent with previous findings of disbudding procedures (Grøndahl-Nielsen et al., 1999; Stafford & Mellor, 2005; Stewart et al., 2008), which suggests that the animals experienced pain during these procedures. An increase in heart rate has been shown to be an indicator of pain as it reflects changes in the sympathetic

branch of the ANS, which is activated when an animal is stressed (Hagen et al., 2005). However, as heart rate is a limited physiological measure, parameters of HRV were also assessed (von Borell et al., 2007). Changes in HRV were not significantly different at any stage of the restraint, ear tagging and disbudding procedures between positive and negative treatment groups. These findings are consistent with findings of Stewart et al., (2008) who found that disbudding, using a local anaesthetic, did not cause any changes in HRV, whereas disbudding without using a local anaesthetic caused an increase in LF/HF ratio, which indicates an acute sympathetic response to pain. Respiration rates between treatment groups were not significantly different at any stage of the three procedures, but animals did show an increase in respiration rate following ear tagging and disbudding procedures. This was expected as both respiration rate and heart rate responses are related, with an increase in respiration rate typically resulting in a corresponding increase in heart rate (Mellor & Stafford, 1999). This change again indicates a pain response, due to activation of the sympathetic branch of the autonomic nervous system, to the ear tagging and disbudding procedures (Dantzer & Mormede, 1983; Maule & VanderKooi, 1999). Findings that eye temperature between treatment groups were not significantly different at any stage of the restraint and ear tagging procedures indicate that although these procedures may have been stressful (indicated by increase of HR and RR), they may not have been painful/noxious enough to elicit a change in eye temperature. Stewart et al. (2008) found that calves disbudded without local anaesthetic, deemed to be a very painful procedure, had a rapid drop in eye temperature during the five minutes following disbudding; eye temperature then increased and remained higher than baseline temperatures over the rest of the sampling period. If calves in this trial had experienced a high level of pain it would be expected that they would have shown this

rapid drop in eye temperature as seen in Stewart et al. (2008). Stewart et al. (2008) found that calves disbudded with a local anaesthetic had only a small non-significant decrease in eye temperature. Struggling behaviour between positive and negative treatment groups were not significantly different at any stage of the restraint, ear tagging and disbudding procedures. During these procedures many recorded behaviours, back leg lunges, rump squats, eliminations, rears, falls and lateral body movement were not performed frequently enough to be analysed statistically. The increase in tail flicking and change in total movement within treatment groups five minutes after the ear tagging procedure indicates an acute stress response to the tagging procedure. There was also an increase in struggling behaviour and tail flicking during the five minutes after the disbudding event which is similar to the behavioural responses of lambs during tail docking (Molony et al., 1993). Tail docking in lambs produced increases in locomotor activity including kicking, jumping and tail wagging (Molony et al., 1993; Molony & Kent, 1997). Tail flicking behaviour has the potential to be a reliable and easily detected indicator of discomfort and agitation during routine farm procedures. Tail flicking was observed in this research as a distinctive, forceful behaviour which increased in occurrence in response to both ear tagging and disbudding procedures. Researchers investigating the behavioural responses of beef cattle to different types of branding used tail flicking as an indicator of acute pain (Schwartzkopf-Genswein et al., 1998). Their results showed that tail flicking increased during the more painful procedures along with incidences of kicking and falling behaviour and also increased vocalisations. Plasma cortisol concentrations between positive and negative treatment groups were not significantly different at any stage of the disbudding procedure but cortisol levels did increase within treatments to disbudding. These findings are consistent with other disbudding

research, which found that following the disbudding procedure, there is generally an increase in plasma cortisol levels, which can remain elevated hours after the initial procedure (Petrie et al., 1996; Stafford & Mellor, 2005).

The lack of significant differences between positive and negative treatment groups may have been due to a number of factors. It is possible that the quality and quantity of the handling techniques used in this research were not intensive or long enough to cause significant differences in the perception of humans between the positive and negative handling treatments. Previous research in pigs has shown that these causes are unlikely, with handling treatments as short as 2.5 minutes a day for five days a week, and 2 minutes for 3 days a week over a ten week period causing a significant difference in behavioural and physiological responses (Paterson & Pearce, 1989; Hemsworth & Barnett, 1991); however a species difference could be possible as pigs may simply be a more sensitive and intelligent animal than calves. Handling imposed on non-lactating dairy heifers twice daily for two to five minutes per session over five weeks also resulted in significant treatment effects (Breuer et al., 2003). It is also possible that the negative treatment animals became habituated to treatments or handlers during the five week period. It has been well established that the aversiveness of a stimulus can be substantially reduced if it is predictable to the animal (Boissy, 1995; Boissy & Bouissou, 1995). The negative handling treatments were organised to avoid this occurring by changing the tool used by the handler each week, and also rotating the order of the handlers each session. A more aversive approach during the negative handling, such as electric shocks that are often used with pigs (Gonyou et al., 1986; Hemsworth & Barnett, 1991) may have had more of an effect on the calves. Handling techniques could have also been more unpredictable and inconsistent for the calves, such as research by Hemsworth et al., (1987) who

used a combination 1:5 ratio of unpleasant and pleasant handling to avoid habituation to treatments. The pre-treatment feeding of calves before handling may have had an overriding effect on the handling treatments by pairing humans with a positive influence (feeding) to the calves; however it was attempted to avoid this by ensuring that if one handler fed a positive group then they would handle the negative group for that session. Research has shown that feeding may influence young calves' responses to humans more than handling itself so it is quite possible that the calves were too young and dependent on humans to respond to treatment differences (Jago et al., 1999; Krohn et al., 2001). Satiation may have also played a role in reducing treatment effects of handling. It is possible that the animals were satisfied and content enough due to feeding that handling did not have as significant effect as if the animals were stressed from hunger. It is also possible that the routine procedures, restraint, ear tagging and disbudding, themselves were not severe and fear provoking enough to cause treatment differences between the groups. This is possible, but there was within treatment differences caused by the procedures, such as increased heart rate and behavioural responses so it is unlikely that the procedures themselves were not severe enough, especially in the case of disbudding. It is therefore also possible that the procedures were too severe to allow a treatment difference. The procedures themselves may have overridden the expression of treatment effects. The behavioural and physiological measures used to record responses to the procedures may not have been appropriate to pick up treatment differences; however there were again within treatment differences and a large range of commonly used and proven techniques were used. Another limitation to the research was the lack of a minimally handled control group, the inclusion of a control group would have allowed for a comparison

of both handling techniques with a minimal handling approach which may have highlighted treatment differences.

In conclusion, this research did not find any evidence to indicate that early positive and negative handling techniques cause a difference in the behavioural and physiological responses of calves to common on-farm husbandry procedures. There was however evidence that these procedures cause acute distress and discomfort to the animals and it is possible that our measures were not sufficient to pick up any treatment differences. However, it is more likely that the animals were either too young and dependent on humans and feeding to be influenced by these handling techniques or that the quality and quantity of the handling treatments were not intensive or long enough to cause a significant difference. It is also possible that the animals became habituated to handling and, particularly in the case of the negative treatment; handling no longer influenced their perception of humans and the routine procedures they were subjected to. The effects of early human-animal interactions in dairy calves warrants further investigation to distinguish whether early handling does affect the fear of humans to stock and therefore their ease of management later in life. Thorough research is crucial to New Zealand's export industry as any improvements in this area will improve animal welfare, reduce management time for farmers and therefore improve on-farm efficiency and profit, whilst improving New Zealand's 'animal friendly' image.

CHAPTER 3

The effects of positive and negative handling on dairy calves physiological and behavioural responses at three months of age to routine farm procedures

INTRODUCTION

Humans and animals interact daily in a general farming situation. This makes the quality and quantity of these interactions important to the welfare and productivity of these animals (Lewis & Hurnik, 1998; Breuer et al., 2000). While fear thresholds have been reduced by hundreds of years of domestication, fear responses to humans have not been totally eliminated in farm animals (Hemsworth & Coleman, 1998; Hemsworth, 2009). Fear in animals can be caused by aversive handling and being exposed to painful routine procedures; this fear of humans can decrease the welfare and productivity of animals and also affect ease of handling and decrease farm manageability (Boissy & Bouissou, 1995; Lewis & Hurnik, 1998; Hemsworth, 2003; Waiblinger et al., 2004). An animal that is frightened is said to enter a state of 'fight or flight' during which the animal shows increased heart and respiration rates and often performs unpredictable movement and behaviour (Dantzer & Mormede, 1983). To assess an animal's fear of humans, ease of movement and human approach tests are often used (Lewis & Hurnik, 1998; Bertenshaw & Rowlinson, 2008). Lewis & Hurnik (1998) assessed the ease of handling of 80 dairy cows six months after their

exposure to a routine aversive procedure, either hoof trimming or injections for mastitis treatment, while restrained in a crush. Their results showed that cows which received a negative experience during a routine procedure were harder to move into the crush and that overall, leading and movement into a crush caused the greatest problems during testing. Breuer et al. (2003) investigated the effects of positive and negative handling on the behavioural and physiological responses of young heifers. They showed that negatively handled cows were slower to approach a human and had a greater flight distance than positively handled animals; negatively handled cows also took less time to complete the force test, received less positive interactions and were significantly more agitated when held in the crush. Research on an animal's exit speed (often referred to as flight speed) from a confinement situation is also used to assess temperament and ease of handling. Animals with a fast exit speed are thought to have poor temperament and are thought to be typically more fearful of humans and harder to handle (Fisher et al., 2000; Petherick et al., 2002; Müller & von Keyserlingk, 2006; Petherick et al., 2009a, 2009b). Fisher et al. (2000) suggests that animals with quicker flight speeds are more fearful of humans in a confined area and this speed reflects motivation to rejoin group-mates. Petherick et al. (2009a, 2009b) investigated the quality of handling (good, poor and minimal handling) on innate and acquired temperaments of beef cattle. Results showed that animals in poor and minimal handling groups found the crush a more aversive situation than good handling groups. Previous good handling reduced fearfulness of humans with flight speed decreasing more over repeated trials in the good handling group. This experiment shows that flight speed can be affected by previous handling treatments and also by previous experiences in the crush. Bertenshaw & Rowlinson (2008) used flight distance to assess ease of handling of positively handled dairy heifers. Results

showed that cows within the positive treatment group had lower flight distance scores than control animals which received minimal human handling.

An animal's behavioural response to a situation provides an insight into the underlying stress levels of that animal; however, care is needed during interpretation, because behavioural responses to a stressor may vary between animals due to individual characteristics, previous experiences, breed and sex of the animal (Boissy & Bouissou, 1995; Stewart et al., 2005; Van Reenen et al., 2005). A range of physiological measures are also commonly used to assess stress responses and the following two measures were used in this research: (a) Heart rate was used and is a reliable tool for assessing stress responses, it is a common measure of physiological stress in animals having been used to assess welfare in cattle (Rushen et al., 1999a; Waiblinger et al., 2004; Van Reenen et al., 2005). Heart rate variability was not assessed to complement heart rate during these tests as the two minute recording period was too short to obtain required lengths of data needed for analysis. (b) Respiration rate was measured as it generally increases in relation with increasing heart rate and indicates an automatic response; to increase the level of oxygen provided to the body during increased activity or a fight or flight reaction (Mellor & Stafford, 1999).

In the present study, calves were assessed for their ease of movement by measuring the time taken and force needed to move a single calf down a raceway and into a crush. Struggling behaviour, heart rate and respiration rates were recorded while calves were restrained in the crush and the exit speed once released from the crush was recorded. The aim of this trial was to reassess the effects of early handling on calves' fear responses and behaviour towards humans at three months of age and also to compare their responses to minimally handled control calves. This assessment

is important because treatment differences and responses to humans may have become apparent in groups at an older age, without the influence of feeding and regular handling. A minimally handled control groups was included in this trial to allow for a comparison of calves reared under normal on-farm practise, which was unable to be assessed in the initial trial in this research. It was hypothesised that negatively handled animals would be more fearful and therefore may be harder to move and handle than positive and control animals. It was predicted that a fearful animal would have increased levels of stress in situations of solitude and move quickly to join other paddock mates, resulting in a quick force test time. It was expected that fearful and agitated animals would perform more struggling movement in the crush and have increased heart and respiration rates, and also have faster exit speeds from the crush.

MATERIALS AND METHODS

The experiment described below was approved by the University of Waikato Animal Ethics Committee (Protocol No. 742) and the Ruakura Animal Ethics Committee (Protocol No. 11576).

ANIMALS, HOUSING AND HUSBANDRY

Sixty Holstein-Friesian heifers, approximately three months of age, were used in this study. The forty animals used in the previous study which received positive and negative handling until the age of five weeks were used; these animals

received no further treatment handling after the initial five week period. The additional 20 animals were obtained from the Tokanui farm to act as control animals. These animals were of the same breed and similar age and weight (Average: 85 kg Range: 62-115.5 kg) to the original forty animals and had been reared in the same facility under normal farm conditions with minimal handling. Normal farm conditions consist of handling only for the purpose of feeding and cleaning; no additional attention is given. The group of sixty animals were kept as one group at pasture from two months of age. All animals were still supplemented Ancalf ^{TM} calf milk replacer up until three months of age. The follow up trial was performed at Tokanui in the upper stockyards (19.7m length x 18.0m width; Fig. 3.1.) on the farm. At three months of age, the 60 animals were split into two groups of 30 animals each consisting of ten positive, ten negative and ten neutrally handled calves (appendix I) to balance the ages of the animals. Calves were tested on consecutive days in December 2008. On testing days each group of 30 calves was further split into treatment groups consisting of five animals (two groups of five animals for each treatment; negative and positive animals were put into their original treatment groups). Each group was kept in a separate pen for the duration of the testing day.

Figure 3.1. Race and crush in stockyards at Tokanui farm.

TESTING PROCEDURES

RACE, FORCE AND CRUSH TEST

The animals were held in their groups of five in a holding pen (4.5m length x 3.8m width) within the yards. Heart rate monitors were attached (as described in Chapter 2) and the animals were left for five minutes to settle. Baseline respiration rates were taken after the settling period, before testing started. Each calf was then individually walked down the stock race (15.7m length x 0.76m width) and restrained in a cattle crush (2.67m length x 0.35m width; Maxi Power Master, Racewell Ltd, Te Kuiti, New Zealand) for two minutes (Fig 3.3.). Heart rate, time and force required to move the animal down the race was recorded. Force was assessed on a scale of assistance (Table 3.1.) needed by the handler to move the

animal forward. Heart rate, respiration rate and movement (Table 3.2.) was also recorded for the two minutes the animal was held in the crush.

Table 3.1. Scoring requirements of the force test.

Score	Activity of handler
0	Handler follows at 1m per 0.5sec using voice only
1	Handler follows at same speed using movement by waving and clapping hands and using voice
2	Handler has to push calf with hands only using voice
3	Handler has to push calf with hands and also use legs to create more pressure to move calf forwards, also using voice
4	Handler has to partially lift weight of calf to move it forwards, also using voice

Table 3.2 Scoring requirements of movement behaviour.

Score	Activity of handler
0	No movement whilst in crush
1	Less than 15 seconds of movement whilst in crush
2	Moderate movement whilst in crush, lasting no longer that 1 ½ minutes of the restraint period
3	Constant and severe movement whilst in crush involving fast movement and banging into the sides of the crush. Behaviour lasts longer than 1 ½ minutes of the restraint period

EXIT SPEED TEST

After two minutes in the crush, the front gate of the crush was removed allowing the calf to exit. The calf was scored on whether or not it left the crush voluntarily, without assistance (score 0), or had to be pushed out with physical contact being made with the calf (score 1). The exit speed (m/s) from the crush was also measured when the calf was released. This was measured by recording the time taken for the animal to firstly exit the crush and then to move through a given distance (1.95m) by tripping two sets of laser beams set up at mid calf height on posts, one at the crush exit and the other 1.95m from the exit (Fig 3.4.).

Figure 3.2. Crush set up with front gate in place.

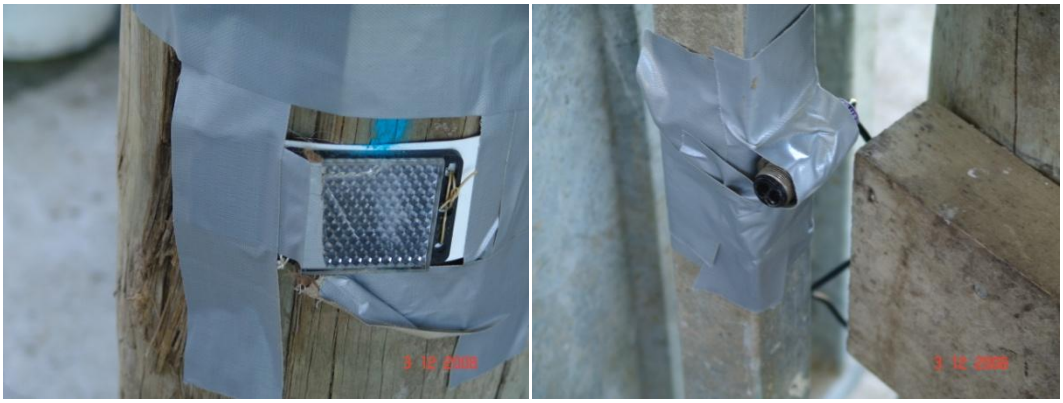

Figure 3.3. Laser beam and sensor set up on posts at the crush exit.

STATISTICAL ANALYSIS

Data was interpreted with the assistance of Neil Cox, a Ruakura statistician. Heart rate data was initially analysed using Polar Precision Performance software, described in chapter 2. All obvious outlier readings were identified on scattergraphs as single data points remote from the bulk of the data and then checked and removed if false, for example, a single HR reading of 200 when all reading surrounding are 60 beats/min. These outliers were removed from data and treatment effects were analysed using an ANOVA. Initially test data was assessed for any influence that the different test dates may have had on results using an ANOVA. There was no effect of day, therefore, it was not included in analysis. All test data obtained from the trial was analysed using Genstat (version 10.2) statistics program. Data obtained from the force test was analysed using a general analysis of variance. Entry time was analysed the same way, with one outlier points being removed. Assisted exit was analysed with a chi squared test and then a regression analysis under a generalised linear model. Exit speed data was log transformed and then analysed using an ANOVA. Log data was back transformed and presented in results. Data are presented as the mean ± the standard error of the mean (s.e.m) or the standard error of the difference (s.e.d).

RESULTS

ENTRY TIME

The control group had significantly faster entry times into the crush with an average of 36.1±1.4 (± s.e.d) seconds compared to the positive and negative treatment groups with an average of 39.3±1.4 and 40.9 ±1.4 seconds respectively (p=0.047; Fig 3.5).

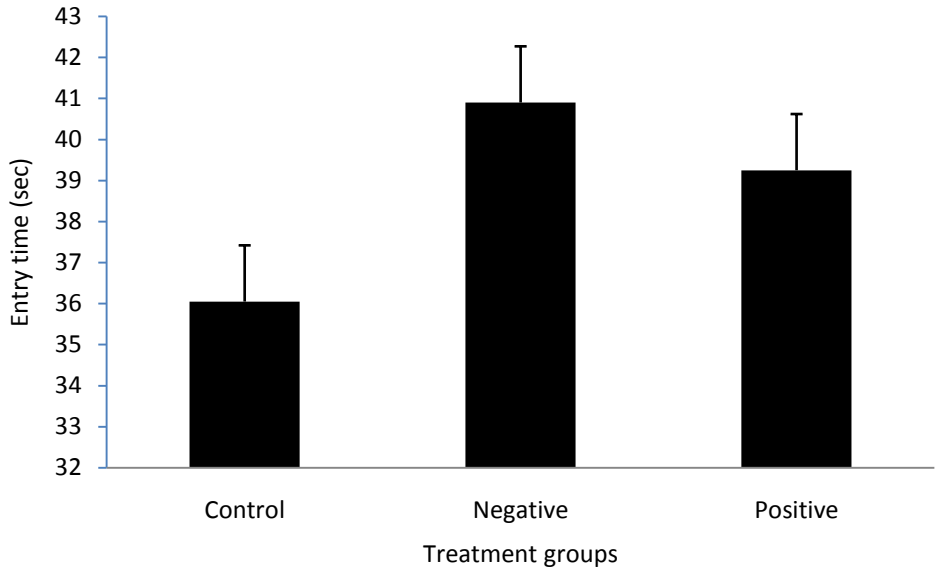

Figure 3.4. Average entry times for control, negative and positive treatment groups during the race test. Mean ± standard error of the difference (n=60).

FORCE TEST

There was no significant difference in scores for the force test between treatment groups (p=0.592). The average score was 1.2±0.3, 1.3±0.3 and 0.9±0.3 (± s.e.d) for the positive, negative and control treatment groups respectively (Fig 3.6).

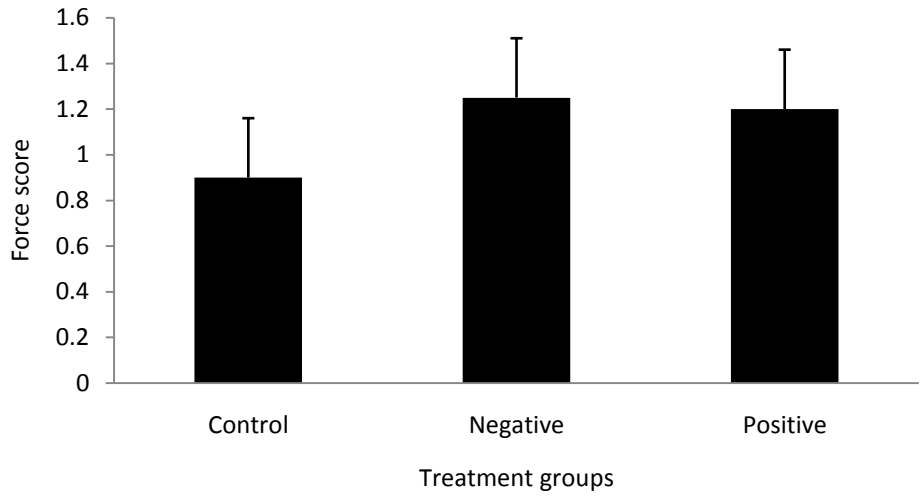

Figure 3.5. Average force scores for control, negative and positive treatment groups during the force test. Mean ± standard error of the difference (n=60).

HEART RATE

There were no significant differences between treatment groups for heart rate during the two minute crush test ($F_{2, 42}$= 0.393). Average heart rates for this time were 82.5 ±4.1, 78.0 ±3.6 and 80.8 ±3.7 (± s.e.d) for positive, negative and control groups respectively (p=0.677).

MOVEMENT IN CRUSH

There were no significant differences in scores for movement in crush test between treatment groups ($F_{2, 57}$=0.364). The average movement score was 1.5 ±0.2, 1.4 ±0.2 and 1.3 ±0.2 (± s.e.d) for the positive, negative and control treatment groups respectively (p=0.695; Fig 3.7).

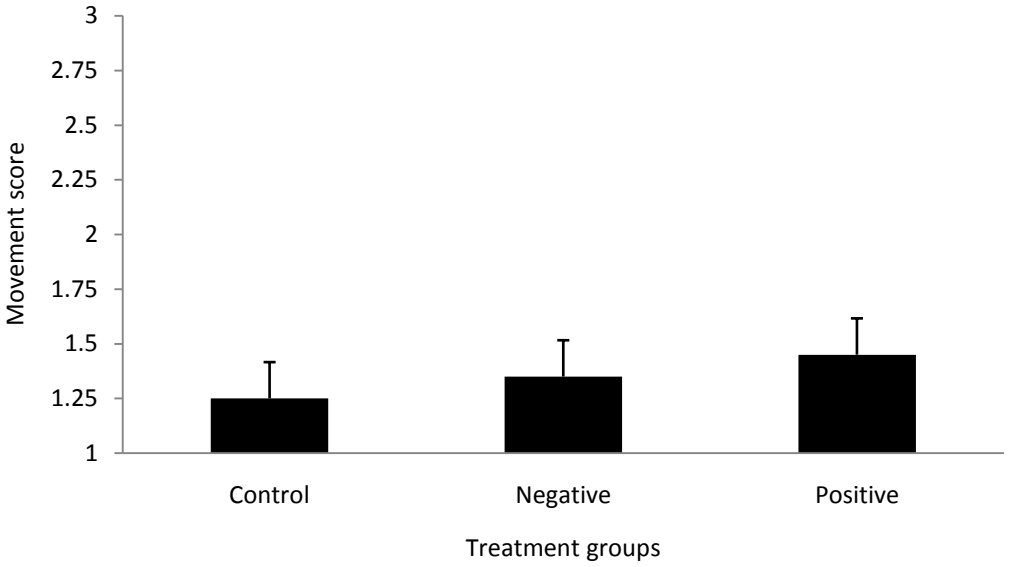

Figure 3.6. Average movement scores for control, negative and positive treatment groups during the two minute restraint period. Mean ± standard error of the difference (n=60).

ASSISTED EXIT

The positive treatment group had significantly more assisted exits from the crush (p=0.040), with an average of 95% assisted exits, when compared to negative and control treatment groups, 65% and 70% respectively (Fig 3.8).

EXIT SPEED

There were no significant differences between treatment groups (p=0.322) with an average time of 0.55 ±0.32 m/s, 0.77 ±0.32 m/s and 0.51 ±0.32 m/s (± s.e.d) for the positive, negative and control groups respectively (Fig 3.9).

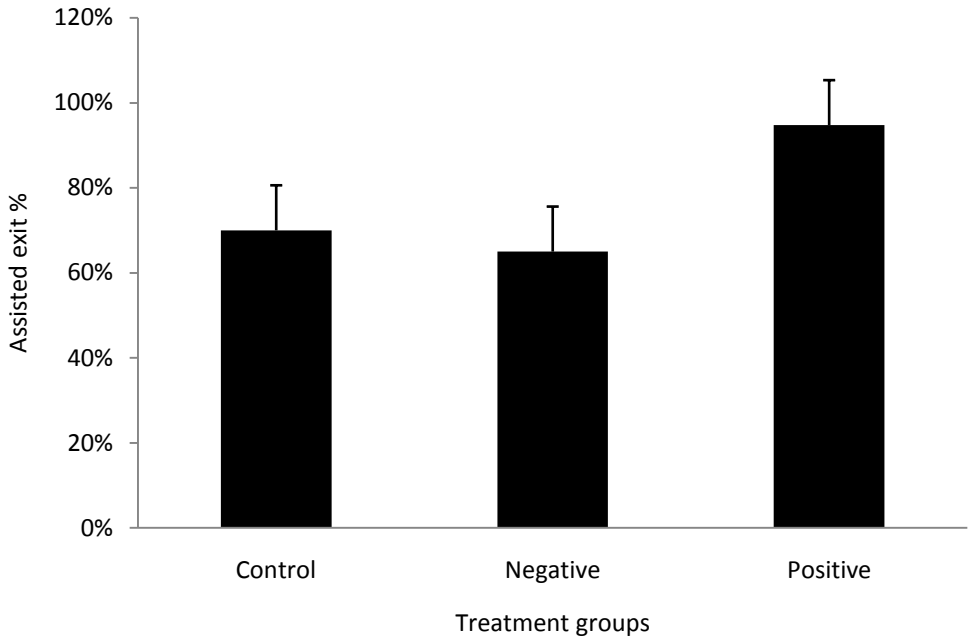

Figure 3.7. Percentage of animals with assisted exit from the crush for control, negative and positive treatment groups. Mean ± standard error of the difference (n=60).

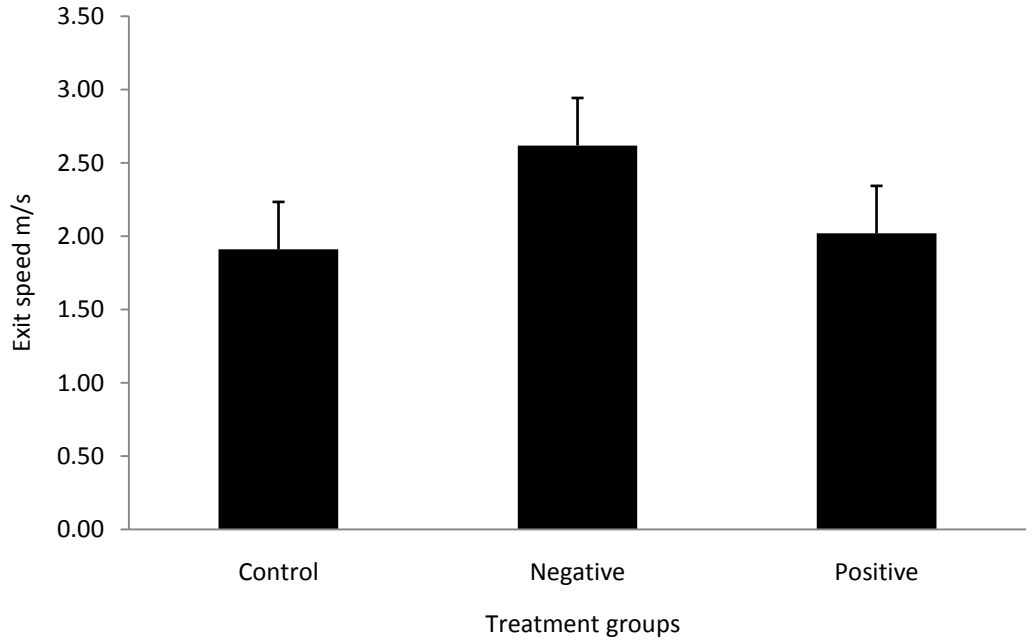

Figure 3.8. Average exit speed for control, negative and positive treatment groups after the two minute restraint period. Mean ± standard error of the difference (n=60).

DISCUSSION

The results of this study do not support the hypothesis that calves handled negatively, early in life, would be harder to control and show more fear responses in situations of solitude in comparison to positive and minimally handled calves. Results instead suggest that minimal handling of calves may result in a higher fear of humans than regularly handled animals. However, to fully understand the effects of quality versus quantity of handling, further research would be required. Interestingly, control calves had faster entry times when put through the race test into the crush, and also required less force to be moved into the crush. These results may reflect a fear of the human behind them in the race and also a possible fear of isolation with a need to quickly find and rejoin other paddock mates. This is consistent with the research of Fisher et al. (2000) who found that more fearful stock will show a greater tendency to move faster and rejoin paddock mates after times of solitude. Petherick et al. (2009b) also found that fear from lack of contact and then the novelty of being handled may be stressful for minimally handled animals. Results are inconsistent with Breuer et al. (2003) who found that negatively handled heifers took less time to move to the crush than positive heifers, however Breuer et al. (2003) did not include minimally handled control animals in their research. There were no significant differences in respiration rate, heart rate or behaviour while the calves were held in the crush; this is in contradiction with Breuer et al. (2003) who found negatively handled calves to be significantly more agitated than positively handled calves while in the crush. This result may, however, reflect a calming effect of regaining visual contact with other stock which were held within view of the crush; this may have overridden treatment

effects. It would therefore have been interesting to investigate the influence of visual contact with paddock mates on the behavioural and physiological components of calves whilst in the crush. Positively handled calves had significantly more assisted exits from the crush than negative and control animals; this may reflect a level of ease of the positive treatment within the crush. The lack of difference between exit speeds from the crush may also reflect a calming effect of regaining visual contact with group mates; however control calves did have quicker exit speeds than the positive treatment group. Interestingly results from human approach tests performed on these animals, done in correspondence with tests and results already presented and discussed, indicate that these control animals were more fearful of humans, with the average flight distance in metres being 3.3, 3.7 and 4.9 (± 0.4 s.e.d) ($p<0.001$) for the positive, negative and control groups respectively. Results from a calf approach test showed that the average contact score, out of a maximum score of 4, was 1.5, 1.0 and 0.3 for the positive, negative and control groups respectively ($p<0.001$) (± 0.2 s.e.d; see Appendix IV), which also indicates that control animals had a greater fear of humans.

There are a possible number of reasons that there were not more significant differences between positive, negative and control treatment groups. It is possible that the time period between positive and negative handling treatments, routine farm procedures and these follow up tests was too long for treatment effects to still be seen. It is possible that the initial handling treatments were not intensive or long enough to cause long lasting differences in behaviour of these animals. Research on the influence of early handling of dairy heifers found that only prolonged handling over nine months would substantially influence human-animal relationships (Boissy & Bouissou, 1988). However, Waiblinger et al. (2004) gained significant treatment

effects after four weeks of handling. Individual variation within animals may have also made it difficult to distinguish fearfulness and responses to the test procedures (Koolhaas et al., 1999; Van Reenen et al., 2005; Kilgour et al., 2006). Koolhaas et al. (1999) states that individuals in the same situation may react similarly for different reasons due to different coping styles; for example a frightened animal may freeze and remain still in a crush while an animal which is calm may remain still because it is comfortable with the confinement situation. It is possible that the testing procedures may not have been specific enough to pick up treatment differences; however this is unlikely because all tests are well established and frequently used methods to assess fear responses in livestock (Boivin et al., 1992; Boissy & Bouissou, 1995; Burrow & Dillon, 1997; Breuer et al., 2003; Bertenshaw & Rowlinson, 2008).

In conclusion, results from this research suggest that initial early positive and negative handling does not cause long lasting effects on calves' behavioural and physiological responses to human-animal interactions and standard farm management procedures. It is however possible that individual variation within animals may have made it difficult to distinguish fearfulness and responses to the test procedures or that too much time had passed between testing and initial handling procedures. There was evidence however from the control group that indicates minimal contact with humans in early life may lead to a level of fear and distress in the presence of humans later in life. These findings are consistent with results obtained from the previous trial in this thesis and findings indicate that treatment differences have not appeared with elapsed time since the initial handling treatments. The implications of this research are important to the general welfare of dairy calves and further research into the effects of quality and quantity of human handling during rearing could have an influence on standard farm management practices and ultimately the welfare of cattle.

CHAPTER 4

Conclusions and Recommendations

There is little scientific information regarding the effects of early handling on the behavioural and physiological responses of calves to various routine husbandry procedures or the effects of this early handling on responses of these animals later on in life. Therefore, this research aimed to investigate the effects of early positive and negative handling on calves heart rate, respiration rate, eye temperature, heart rate variability, plasma cortisol levels (assessed only during disbudding) and behaviour to three routine husbandry procedures: restraint, ear tagging and disbudding. The main hypothesis for this research was that positively handled animals would show lower levels of behavioural and physiological responses during these procedures when compared to negatively handled animals. The results did not support this hypothesis; the main finding (Chapter 2) was that there were no significant differences between positive and negative treatment groups for any behavioural or physiological measure during any of the three routine husbandry procedures. However, the response to the procedures in both treatment groups were consistent with previous studies and confirmed that such procedures are a stressful and/or painful experience for calves, especially ear tagging and disbudding. The second study (Chapter 3) aimed to investigate the effects of this initial handling on the same calves at three months of age, and compare calves to a control group of minimally handled animals. This study

investigated the calves' responses to a force test into a crush, heart rate and behaviour during this time and then to an exit speed test. The results did not support the hypothesis that negatively handled animals would be more fearful and therefore may be harder to move and handle during testing than positive and control animals. Theresults suggested that there were no lasting effects of previous early handling, and that minimally handled calves appeared to be most fearful of humans.

Findings from both research trials were unexpected and may be explained by one or a combination of the following reasons. Possible reasons for the lack of effect of handling at 5 weeks of age (Chapter 2):

- The effects of early handling were overridden by the severity of the stress and/or pain caused by the routine husbandry procedures.

- The handling techniques used in this research were not intensive or long enough to cause significant differences in the responses to the husbandry procedures.

- Animals exposed to the negative treatment became habituated to treatments or handlers and treatments were no longer perceived as negative; and that the quantity of the handling was of higher importance. Additional treatments would have been required to tease apart the effects of the quality vs. the quantity of handling and would warrant further investigation in future studies. In the present study, one improvement may have been to include a control (minimal handling group) in the first study.

- Pre-treatment feeding of calves may have had an overriding effect on the handling treatments by pairing humans with a positive influence; satiation may have also played a role in reducing treatment effects.

- Behavioural and physiological measures used to record responses to the procedures may not have been appropriate to detect treatment differences.

Possible reasons for the lack of effect of handling at 3 months of age (Chapter 3):

- It is possible that the time period between positive and negative handling treatments, routine farm procedures and these follow up tests was too long for treatment effects to be detected.

- It is possible that the initial handling treatments were not intensive or long enough to cause long lasting differences on the behavioural responses of these calves.

- It is possible that individual variation within animals made it difficult to distinguish fearfulness and responses to the test procedures, or that the testing procedures were not sensitive enough to detect these differences.

FUTURE RESEARCH

Handling of stock is a standard requirement of modern farming practices. It is therefore important that animals handled well to ensure good welfare, high levels of productivity and high levels of farm management. The following recommendations for future research are suggested as follows:

- Investigate the effects of different ages of calves/cows and their perceptions of humans and different handling treatments.

- Investigate the effects of more intensive handling techniques, using longer durations of handling and more aversive negative handling treatments such as shocks or hitting, as used in pig research (Hemsworth & Barnett, 1991; Hemsworth, Barnett et al., 1981), and include a control group.

- Examine the effects of the quality versus the quantity of handling on responses of cattle.

- Assess the use of behavioural responses such as tail flicking as stress indicators to minimise stress and fear of humans caused during husbandry procedures.

IMPLICATIONS FOR ANIMAL WELFARE AND FINAL CONCLUSIONS

Overall, the dairy industry and more broadly the livestock industries are crucial to New Zealand's export market. Public awareness of farm animal welfare has become increasingly important to today's society. Worldwide, consumers are starting to demand 'animal friendly' products, better living conditions for farm animals and reduced use of painful husbandry procedures, which is putting increasing pressure on our livestock industries to improve welfare standards. Therefore, research into improving stockmanship, rearing conditions and handling techniques is extremely important for our livestock industries and to maintain our export markets.

Although the present studies did not find evidence that early positive handling had an effect on responses of calves, potentially, if we could demonstrate that good handling and stockmanship during calf rearing does have positive effects on reducing pain/stress during routine husbandry procedures, this would have major implications for animal welfare and livestock industries. This could provide information for handling/rearing guidelines that could be issued in recommendations and codes of practice. It could also make on-farm management easier and less labour intensive for farmers, which in turn could improve on-farm productivity and profitability and most importantly improve overall animal welfare, as well as improving New Zealand's 'welfare friendly' image. Currently the effect of early quality and quantity of handling in dairy calves is largely unexplored, and therefore warrants further investigation.

Appendix I

All raw data, statistical analysis and recording sheets are provided on the enclosed cd-rom. Codes are given at the top of each spreadsheet to explain the terms used in analysis. Colour codes are also given as below:

Code	**Colour highlighted**
P values	Yellow
F values	Orange
Means	Green
SEM of means	Blue
Instructions on how to look at analysis	Red

Appendix II

CALF HEALTH TREATMENTS AND ANY ABNORMALITIES DURING TRIALS

MAIN TRIAL

- Calf 3 (positive) was removed from the trial due to ill health – data obtained from this animal was excluded from all analysis.

- Ear tagging procedure - calves 23 (positive), 25 (positive), 26 (negative) and 30 (negative) were tagged above the top cartilage ridge due to prior tagging in the correct location. All response data was included in analysis as normal.

- Disbudding – Calves 35 (positive) & 40 (negative) were not disbudded as calves had no horn buds (polled animals). Calf 3 (positive) was disbudded to ensure normal farm practise but data was still excluded from the trial.

FOLLOW UP TRIAL

- All 60 calves were included in analysis.

Appendix III

SCHEDULE OF TESTING FOR ALL ROUTINE PROCEDURES

Group 1	5-Oct	7-Oct	9-Oct
Trt	**Restraint**	**Ear Tag**	**Disbud**
Positive	Red	Yellow	
Negative	Red	Yellow	Green
Positive	Pink	Red	Green
Negative	Pink	Red	Yellow
Positive	Blue	Pink	Yellow
Negative	Blue	Pink	Red
Positive	Green	Blue	Red
Negative	Green	Blue	Pink
Positive	Yellow	Green	Pink
Negative	Yellow	Green	Blue
Positive			Blue

Group 2	11-Oct	13-Oct	15-Oct
Trt	**Restraint**	**Ear Tag**	**Disbud**
Positive		Pink	Yellow
Negative	Blue	Pink	Yellow
Positive	Blue	Blue	Red
Negative	Green	Blue	Red
Positive	Green	Green	Pink
Negative	Yellow	Green	Pink
Positive	Yellow	Yellow	Blue
Negative	Red	Yellow	Blue
Positive	Red	Red	Green
Negative	Pink	Red	Green
Positive	Pink		

Group 3	14-Oct	17-Oct	18-Oct
Trt	**Restraint**	**Ear Tag**	**Disbud**
Positive	Green	Red	
Negative	Green	Red	Pink
Positive	Yellow	Pink	Pink
Negative	Yellow	Pink	Blue
Positive	Red	Blue	Blue
Negative	Red	Blue	Green
Positive	Pink	Green	Green
Negative	Pink	Green	Yellow
Positive	Blue	Yellow	Yellow
Negative	Blue	Yellow	Red
Positive			Red

Group 4	20-Oct	22-Oct	24-Oct
Trt	**Restraint**	**Ear Tag**	**Disbud**
Positive			Red
Negative	Yellow	Blue	Red
Positive	Yellow	Blue	Pink
Negative	Red	Green	Pink
Positive	Red	Green	Blue
Negative	Pink	Yellow	Blue
Positive	Pink	Yellow	Green
Negative	Blue	Red	Green
Positive	Blue	Red	Yellow
Negative	Green	Pink	Yellow
Positive	Green	Pink	

Appendix IV

HUMAN APPROACH TESTS

These tests were performed on the same animals and test days as other measures, I took part in the testing procedures, but these results were not intended to be part of this thesis research. However, results discussed complement results from my research and were mentions to provide more information on the follow up trial, giving a more complete assessment of the fearfulness of these animals in the presence of humans.

Methodology for ease of handling tests

Flight distance

Animals were held in their group of five next to the flight distance arena. Each animal was individually moved into the 20 meter test arena, which was marked in individual meters, where a human stood at the far end. The other four calves were held in that pen in visual contact of the individual calf. Once the calf had settled and stopped moving, the human approached at approx1m/second towards the animal. The human stopped approaching when the calf took a step away from the human using any leg. The test was performed twice on each animal and the average distance at which the human could approach before the calf moved was recorded as that animal's flight distance. A score of zero was awarded if the human reached and touched the calf.

Calf approach test

Animals are held in their groups of five. The human enters the pen and stands approximately two meters in front of a calf and attempts to make eye contact with each calf individually. Once eye contact is made the human then attempts to move closer to the calf. Animals' are scored on the amount of visual and physical contact made with a human whilst in this group situation. This test was performed twice on each animal and an average was given as the calves approach test score.

Scoring requirements of calf approach test.

Score	Activity of calf towards human
0	Awarded if no eye contact is made with the human
1	Awarded if eye contact is made with human
2	Awarded if human can take one step towards the animal without the animal moving
3	Awarded if human can take two steps towards the animal without the animal moving
4	Awarded if the human touches the animal.

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