ANALGESIC EFFICACY OF A NOVEL TOPICAL FORMULATION FOR TAIL DOCKING IN PIGLETS

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

Master of Science
In
Animal Science

At Massey University, Palmerston North, New Zealand

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2019

Abstract

Tail docking of young piglets is a common management procedure which is carried out on pig farms to prevent tail biting behaviour. In New Zealand, pain relief is not required at the time of the procedure if piglets are less than 7 days old. However, tail docking results in behavioural and physiological pain and stress responses in piglets. A survey of pig husbandry practices was carried out before conducting the experimental procedure. The survey had 15 questions which focused on the occurrence of tail biting, of which only one respondent reported tail biting absence. Most questions were centred on tail biting risk factors coming from housing conformations, use of manipulable substances such as straw and feeding systems. Knowledge of farmers' strategies concerning risk factors can enable stakeholders to advise farmers on the best ways to minimise tail biting. The objective of this research was to evaluate the analgesic efficacy of a novel topical formulation to reduce pain due to tail docking. This was examined in 108 piglets (9 litters of 12 piglets each), which were docked by a gas cautery iron at approximately 2-3 days of age. The study had 3 treatment groups: pain relief provided before docking, ('Before'); pain relief provided after docking ('After'); and the control group which had no pain relief ('Control'). The 9 litters were randomly allocated, such that each treatment group had 3 litters. The behavioural response to docking was recorded during and after docking. The percentages and means for vocalisations and escape attempts before docking were recorded, and these were lowest in the 'before' group ($P<.0001$). Post docking, treatment had a highly significant effect on walking behaviour ($P<0.0002$). Piglets in the control group had the highest likelihood of walking after docking, followed by the after and before groups respectively. Lying in contact, which signifies social coherence, was more apparent in the before group, ($P<0.0001$) followed by the after group and was observed at the lowest frequency in the control group. Conversely, the lying in isolation behaviour was highest in the control group, ($P<0.0002$), followed by the after group and lastly the before group. Sitting and pain related behaviours had the highest occurrences in control piglets, followed by the after group and lastly the before group. Blood sampling was carried out to conduct inflammatory gene expression studies which suggest the level of pain. The results from the study were unbalanced, therefore inconclusive due to sampling challenges which resulted in limited raw data. Blood samples from piglets that had not received pain relief prior to docking were especially difficult to collect. The blood work sought to analyse which cytokines, pro-inflammatory and anti-inflammatory genes would be upregulated following pain due to tail docking. Pre-emptive application of the topical analgesic formulation resulted in fewer pain related behaviour displays but was the most labour-intensive treatment. Further work is required to develop more practical forms of on-farm pain relief during tail docking.

Dedication

For their belief in me and their steadfast love and support; this work is dedicated to my dad, my mum, my brother Tinomutenda, my sisters Rita and Doreen, my brothers in law Anderson and Fanuel, my fiancé Wale and my sister in law Maggie.

Acknowledgements

I would love to express profound gratitude to my main supervisor Professor Patrick Morel for helping me to develop as a scientist. His vast knowledge, enthusiasm and jokes made the research fun. His open-door policy made him accessible and ready to help at any time. I want to express sincere gratitude to Dr. Kirsty Chidgey, one of my co-supervisors. Her knowledge of pig welfare is phenomenal and insightful, so much so that spending a day in her company around pigs will make you feel you can handle all things “pigs”. Her feedback during my literature search made the process enjoyable. I am thankful to my other co-supervisor; Dr. Preet Singh. His handy knowledge in gene expression saved me hours of research. I am grateful to have been part of his team seeking to mitigate pain in piglets, I learnt a lot of veterinary processes which I would not have otherwise grasped.

I wish to acknowledge the Massey University science librarians at the Manawatu campus; Chris Good and Kim Baxter, they are always ready to help with the technical side of thesis writing and this makes the work easier. I wish to acknowledge Jeff, the IT expert in the library, he always seems to go over and above his duties, I am certain I am not the only student grateful for his role.

I extend my acknowledgements to Jamie Hooper, (Logan Tate and Sylvia Hooker who have left) and the rest of the team at the International students’ office. They always had my back and that allowed me to chase my academic dreams harder.

I am grateful for Sam, a fellow student, he would have preferred to handle piglets by conducting cause of death, but he was patient enough to help me without killing them at the end and this became an inside joke. I am thankful for my friend Sandra who put aside her assignments so that she could assist in the practical side of the research.

I acknowledge with love my family for continued support. I am thankful for Professor and Dr. Adeyinka for providing me with love, food and academic assistance. My work was easier because of my good friends Kudzi and Izzy who always reminded me to take a break and have fun with them, every student should have friends like those two. I wish to acknowledge my study buddy and friend, Shakazulu for shared motivation. I am very thankful to my fiancé Wale for being my biggest cheerleader and being there through it all. I wish to thank Vision church in Palmerston North for being a place where I could be part of and have meaningful and healthy relationships outside my school life. They are a heaven-sent group.

I want to acknowledge my classmates and all my Massey friends for their friendship, the way we shared ideas, the misery when suffocating under research and the joy at success that was always at the end. My sincere gratitude extends to the people of New Zealand, their loving nature ensured that I was never homesick, therefore I could concentrate on school work and fun.

My profound gratitude goes to MFAT (New Zealand), from whom this amazing scholarship came from. The generosity of the ministry made my stay in New Zealand very comfortable and happy. It is through this generosity that I got to meet all the wonderful people I have acknowledged above (except my family). I believe MFAT's investment in me will continue to pay off.

Above all, I am thankful to God for His undying love and for bringing me this far!

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CHAPTER 1

Introduction

1 Introduction

The need to evaluate the analgesic efficacy of novel and existing pain relief for piglets during tail docking is brought about by a need to combat tail biting behaviour in pigs. Tail biting is an undesirable behaviour of importance in growing pigs (Stafford, 2010). This behaviour has been the subject of many studies as it results in animal welfare issues, as well as economic losses for pig farmers arising from poor growth rates, deteriorated body condition and carcass condemnations (Kritas and Morrison, 2007, Niemi et al., 2012, Sinisalo et al., 2012). This behaviour is characterised by 'biter' pigs using their mouths to manipulate the tails of littermates, rendering such behaviour as abnormal (van Putten, 1969).

To date, identifying the cause of tail biting has been difficult, but there are multifactorial risk factors which are recorded in literature. The risk factors can be individual pig factors (internal), or environmental factors (external).

Internal risk factors include genetics (Breuer et al., 2005, Schroder-Petersen and Simonsen, 2001), gender (Hunter et al., 1999, Moinard et al., 2003) gastrointestinal discomfort (Amory et al., 2006) health status (Kritas and Morrison, 2007) nutrition (Fraser and Broom, 1990), and age and weight (Wallgren and Lindahl, 1996). External risk factors include lack of manipulable substrates (Van de Weerd et al., 2005), floor type, stocking density, ventilation, feed factors, and climatic conditions (D'Eath et al., 2014, Moinard et al., 2003, Munsterhjelm et al., 2013, Schroder-Petersen and Simonsen, 2001, Scott et al., 2007).

Previous literature provides risk factors and preventative measures for tail biting, and the most widely used prevention measure is tail docking (Moinard et al., 2003, Schroder-Petersen and Simonsen, 2001). Tail docking is routinely carried out on pig farms globally. This is against European Union regulations, which stipulate that other measures must have been exhausted before resorting to tail docking (D'Eath et al., 2016, EFSA, 2007).

Tail docking involves amputation of part of the tail, shortening it to alter the sensitivity around the tail area (Simonsen et al., 1991). Tail docking results in behavioural and physiological responses that denote pain in animals (Noonan et al., 1994, Sutherland et al., 2008, Taylor et al., 2001).

Tail docking is conducted in the absence of anaesthesia and analgesia in some countries. In New Zealand, the Animal Welfare (Care and Procedures) regulations (2018) stipulate that piglets over 7 days of age can only be docked by a veterinarian, and analgesia must be provided at the time of the procedure (MPI, 2018). The absence of pain relief compromises animal welfare with respect to the Five Freedoms whereby pain is experienced at the time of the procedure (Farm Animal Welfare Council, 1993). Table 1.1 lists the five freedoms of animal welfare and some provisions for supporting these freedoms. However, in addition to tail docking, tail biting also compromises the wellbeing of pigs (Sutherland et al., 2009).

Table 1.1: The five freedoms of animal welfare and provisions for their promotion

Freedom	Provision for promotion
Freedom from pain, disease and injury	Preventing pain, practising rapid diagnosis and treatment
Freedom from hunger, thirst and malnutrition	Providing access to fresh water and diets that support full health
Freedom from discomfort and exposure	Provision of adequate shelter and comfortable resting space in an appropriate environment
Freedom from distress and fear	Promoting treatment and conditions which discourage mental suffering
Freedom to express normal behaviour	Providing same species company, enough space and proper facilities

Adapted from(Webster, 1994)

Background

Tail docking is the most widely used preventative measure against tail biting (Moinard et al., 2003, Schroder-Petersen and Simonsen, 2001). However, with the increase in consumer awareness of animal welfare, docking without pain relief nurtures a negative outlook on the pig industry (Amory et al., 2006). In the interest of improved production and welfare of pigs, there is a need to develop pain relief strategies that meet the wellbeing needs of the pigs.

Historically, it was believed that injuries to neonatal pigs and other animals were not recognised as painful (Di Giminiani et al., 2016). Contrary to this, studies have shown that tail docking in piglets is painful (Kells et al., 2017, Sutherland et al., 2008).

Studies which are designed to evaluate pain suffered by pigs due to tail docking mainly focus on behavioural changes (Noonan et al., 1994, Sutherland et al., 2008) and histopathological observations (Simonsen et al., 1991). A dearth of information exists as regarding the use of inflammatory gene expression in combination with behavioural responses, to quantify pain experienced and pain relief during and after tail docking in piglets. The use of inflammatory gene expression as a pain measure has been investigated in castrated calves (Pang et al., 2009), but not in piglets.

Objectives

The first objective of this study was to assess the efficacy of a novel, topical local anaesthetic in reducing pain associated with tail docking in piglets, as quantified by behavioural responses and inflammatory gene expression. Another objective was to assess tail docking in New Zealand pig farms. This was with a view to better understand tail docking and relate it to the New Zealand pork industry perspective.

Research questions

1. Is LA cream (a novel topical local anaesthetic) effective in reducing the pain caused by tail docking in pigs?
2. Are pig husbandry practices linked to tail biting incidences within the New Zealand pig farming community?
3. Can inflammatory gene expression be used as a physiological pain index in pigs?

CHAPTER 2

Literature review

2 Literature review

2.1. Tail Biting

Tail biting is a term which has been used in the description of a wide range of behaviours. This can be described initially as harmless manipulation of the tail. It can escalate to an oral act that grazes the skin, removes part of the tail, or both, with the worst manipulation resulting in a gouged rump (Taylor et al., 2012). When these manipulations result in bleeding, biter pigs intensify the action while other pen mates may be attracted to the blood, causing further damage (van Putten, 1969).

Tail biting had been reported as early as the 1940s on British farms (Moinard et al., 2003). This shows that the phenomenon has been in existence for decades. However, it has been speculated that the promotion of leaner body composition during breeding, together with intensive technical development has led to an increase in the prevalence of tail biting (Edwards, 2006).

Tail biting is deemed an abnormal behaviour in pigs (Moinard et al., 2003, Taylor et al., 2010, Valros and Heinonen, 2015). The behaviour is termed abnormal as it is scarcely reported in semi-natural, extensive or feral conditions (Moinard et al., 2003). Pigs, like other animals, have retained some characteristics that were expressed by their wild ancestors. As pigs were known to forage, it has been hypothesised that tail biting is an abnormal behaviour which stems from the need to exhibit foraging behaviour (Wood-Gush and Vestergaard, 1989). This same reasoning has resulted in other authors describing tail biting as normal behaviour as it is a display of natural exploration and foraging behaviour (van Putten, 1969). However, in nature, pigs would have foraged on substrates rather than pigtails, making biting pigtails abnormal.

2.1.1 Types of tail biting

Tail biting has been categorised into three different types. The basis of the differentiation was that different categories might reveal different aetiologies, giving the potential to solve the issue stage by stage (Valros, 2018).

2.1.1.1. Two-stage biting

This type of tail biting is characterised by two different stages which can occur separately or all together(Schrøder-petersen et al., 2003). The process may start with a harmless tail in mouth (TIM) behaviour which is characterised by gentle manipulation, which is the pre-damaging stage (Fraser and Broom, 1990). This behaviour can escalate to the second stage, which is the damaging stage. This type of biting is not characterised by aggression. The biting has been reported to be a display of foraging or exploratory behaviour which is redirected towards pigtails when the environment is lacking in chewable substrates such as straw (Taylor et al., 2010).

2.1.1.2. Sudden forceful biting

In sudden forceful biting, there is usually no evidence of prior gentle manipulation of the tail. The tail is suddenly and forcefully gripped and is bitten or yanked (van Putten, 1969). This results in the removal of the tail tip at worst or skin and flesh removal in the least (Taylor et al., 2010). This type of biting is not extensively reported in literature (Taylor et al., 2010). This may be because differentiating it from two-stage biting can be challenging. Sudden forceful biting is mostly a result of lack of access to desired resources like feeders (Moinard et al., 2003).

2.1.1.3. Obsessive tail biting

The third distinct type of tail biting is called obsessive tail biting. This type is also known as fanatical tail biting (Van de Weerd et al., 2005). In this type of biting, constant biting is carried out by a single pig or a few pigs that seize and yank tails, resulting in skin removal or amputations as would be expected with the sudden forceful type of biting. What differentiates the two behaviours is that for the obsessive type, the biter is continually moving from tail to tail (Van de Weerd et al., 2005). It is unclear what triggers this type of tail biting. Possibly, some pigs would carry out this type of biting to get access to a resource but discover tail biting to be more rewarding than resource seeking (Taylor et al., 2010).

A summary of the three types of tail biting is shown in table 2.1;

Table 2.1: Summary of tail biting types

Type of biting	Description	Suggested cause	Literature source
Two-stage	Non-injurious TIM behaviour which may escalate to painful biting	Frustration, moderate or chronic stress, lack of chewable substrates	Fraser, 1987, Schrøder-Petersen *et al*., 2003
Sudden forceful	Grabbing and yanking of the tail with force, resulting in abrasions or tail tip removal	Limited access to resources, competition for chewable substrates	van Putten, 1969, Moinard, 2003, Taylor, 2010
Obsessive	Repeated grabbing and yanking at pig tails which cause skin removal and amputations	Unknown, likely problematic protein metabolism causing attraction to blood	Van de Weerd *et al*., 2005, Taylor, 2010, Edwards, 2006

2.1.2 Intrinsic risk factors associated with tail biting

The origins of tail biting are multifactorial (Moinard et al., 2003). Therefore, the risk factors for the behaviour are many. These risk factors may be intrinsic, that is having their point of origin within the animals themselves, or extrinsic, originating from the environment where the animals live (Taylor et al., 2010). It is prudent to note that while the intrinsic and extrinsic risk factors may increase the incidence of tail biting, they do not induce the behaviour. Based on Table 2.1 it is clear that the outstanding cause of tail biting is a failure of pigs to manage the stressors in the environment (Munsterhjelm et al., 2013). Therefore, the failure of a pig to manage stress can be viewed as an intrinsic risk factor. These factors are determined at individual levels. The contribution of the environment is extrinsic, and rather than being determined at individual levels, it is measured at the herd level. The interaction of the intrinsic and extrinsic factors is unclear (Van de Weerd et al., 2005). This unclear interaction causes the determination of the overall risk to be difficult (D'Eath et al., 2014). For better tail biting prevention measures, it is critical to understand the intrinsic and extrinsic risk factors associated with tail biting in pigs.

2.1.2.1 Breed, breeding, genetics and gender

The effect of breed on tail biting has not yet been conclusively corroborated (Valros, 2018). A study showed tail biting to be significantly heritable in Landrace pigs (Breuer et al., 2005). This was supported by authors who reported the Landrace as being the breed with the most incidence of tail biters in comparison to Yorkshire (Sinisalo et al., 2012). It should be noted that the studies were conducted with small samples.

Breeding for leanness may be a contributing factor of tail biting (Edwards, 2006). This is supported by a study that reported tail biting gilts to be phenotypically heavier and faster growing compared to non-tail biting gilts which were characterised by lower back fat potential (Ursinus et al., 2014).

There is a dearth of information regarding genetics and tail biting. An attempt to identify chromosome locations linked to tail biting showed some loci which associated a pig with being either a tail biter or a victim. However, the loci did not have enough basis for concluding the genetic contribution to tail biting (Wilson et al., 2012). While breeding for leanness may exacerbate tail biting, breeding for social cohesion, which is an indirect effect, may result in a reduction of negative social tendencies like tail biting (Camerlink et al., 2015).

Gender is a factor in tail biting risk, but a clear pattern does not exist to highlight which gender is likely to victimise or to be victimised in relation to tail biting (Schrøder-petersen et al., 2003).

Some studies have reported that tail biting damage is widespread in males compared to females (Kritas and Morrison, 2007, Valros et al., 2004). On the other hand, Sinisalo et al. (2012)could not determine gender differences in relation to tail biting damage. Pens with female pigs only have been shown to have more tail biting tendencies when compared to all male or mixed groups. When tail biting was high in male groups, this was linked to competition for food (Wallgren and Lindahl, 1996). Gilts and uncastrated males (barrows) have different dietary needs. If these animals are grouped and fed a single diet, the risk of tail biting may increase as some groups will be lacking in nutrients due to being fed a uniform diet. Ordinarily, the barrows become victims in these mixed groups (Kritas and Morrison, 2007, Valros et al., 2004).

2.1.2.2 Growth, Size and Weight of Pigs

Uniformity of growth within a group of pigs has been noted as an essential parameter in cases of tail biting (Valros et al., 2016). Schroder-Petersen and Simonsen (2001)reported that tail biters were smaller in size compared to other pigs, and this could result from nutrient absorption problems or reduced feed intake culminating in an increased desire to forage and explore. Another school of thought suggests that the smaller pigs often initiate tail biting more than the older pigs because these smaller pigs cannot win fights through normal aggressive behaviour (Fraser and Broom, 1990), therefore, they will launch attacks from behind to stand a chance against bigger sized pen mates (Wallgren and Lindahl, 1996). Suggestions of size being a factor in tail biting were recently supported when Munsterhjelm et al. (2013)showed that pigs that become tail biters or those that carried out TIM after weaning are those which had been born slightly smaller in size. However, Zonderland et al. (2011) did not find any significant size differences between tail biters and neutral pigs despite the biters being smaller.

2.1.2.3 Age of Pigs

The incidences of tail biting do not occur with the same frequency during a pig's life. Generally, tail in mouth behaviour is detected early in life following weaning and seems to decline with age. (EFSA, 2007). Tail biting has been noted to increase around the finishing period. The increase in tail biting during the finishing period occurs when pigs are around 30-60kg(Simonsen, 1995). While tail biting is on the increase during the finishing period, it should be noted that this behaviour has been recorded in other age groups (Schroder-Petersen and Simonsen, 2001). It has been reported that tail biting is observed around pubertal age. This has been attributed to an increased interest in the tail regions of other animals (Valros et al., 2004).

2.1.2.4 Health Status

The risk of tail biting has been linked to the health status of the farm (Edwards, 2006). There is a higher frequency of tail biting in pens that suffer from poor health. For example, there is a high risk of tail biting on farms that have respiratory diseases and rectal prolapse (Kritas and Morrison, 2007, Moinard et al., 2003). Frustration arising from discomfort during illness could contribute to this rise in tail biting. When the immune system is under attack; voluntary feed intake, required amino acid balance and nutrient partitioning all change (Whittemore et al., 2003). Poor health usually leads to a reduced rate of growth, creating size variations within a pen. As mentioned previously, smaller sized pigs have been found to initiate tail biting. However, the association between health status and tail biting has only been measured after the incidents of tail biting, making the cause and effects of the relationship unclear (Niemi et al., 2012).

Gastrointestinal defects or discomforts potentially result in poor nutritional states in pigs; this makes them a risk factor for tail biting (Taylor et al., 2010). Gastric ulcers have the potential to initiate chewing behaviour in pigs, thereby triggering two-stage tail biting. Gastric ulcers in pigs result from infections, stress or feed form (Bolhuis et al., 2006). Pelleted and ground feed may cause gastric ulcers while straw reportedly lowers the risk of these ulcers (Amory et al., 2006).

2.1.3 Extrinsic risk factors associated with tail biting

2.1.3.1 Feeding factors

Feeding factors that pose a risk for tail biting include diet, feed form, feeding method, feeding restrictions and feeder setup.

2.1.3.1.1 Diet

A substandard diet triggers the two-stage type of tail biting (Taylor et al., 2010). Foraging is performed to attain an optimal diet and satiety; therefore, when the diet does not meet the requirements of the pigs, misdirected foraging and exploration occurs, resulting in tail biting. Low salt levels in the diet have been implicated in tail biting (Paul et al., 2007). Salt seeking is a stress response caused by an increase in sodium excretion. Pigs eating a diet low in sodium are at risk of tail biting.

Low protein diets and nutritional imbalances increase foraging behaviour and can subsequently cause tail biting. Pigs on a low tryptophan and low energy diet have been shown to seek and chew bloody tails (McIntyre and Edwards, 2002). However, despite the reported suggestions that diet profile is a significant risk factor for tail biting (D'Eath et al., 2014, McIntyre and Edwards, 2002, Paul et al., 2007, Schroder-Petersen and Simonsen, 2001), some studies are yet to show clearly how feed composition and particular nutrients predispose pigs to tail biting (Valros, 2018).

2.1.3.1.2 Feed Form

Pelleted diets, compared to liquid feeding, was reported to trigger tail biting (Hunter et al., 2001, Watanabe et al., 2015). Contrary to this, there was increased tail biting in pigs exposed to liquid feeding (Moinard et al., 2003, Temple et al., 2012). This inconsistency in reports could result from differences in how different farms mix dry feed with water. Placing the water drinker above the feeding trough could reduce tail biting when using pelleted feed due to reduced dust irritations. When providing a liquid diet, if the water to dry matter ratio is very high, the dry matter intake will be lower, resulting in foraging behaviour. This shows both pellet feeding, and liquid feeding can be manipulated to reduce tail biting. Using accurate feed content has been rated to be critical for the prevention of tail biting (Valros et al., 2016).

2.1.3.1.3 Feeding Methods and restricted feeding

Hand feeding has been reported to reduce the risk of tail biting (Moinard et al., 2003). Additionally, automated feeding has been linked to an increase in the risk of tail biting (Moinard et al., 2003). Farmers have reported that breakdowns of automated machines usually coincide with increased cases of tail biting. Furthermore, any mistake in the programming of the feed mixing and distribution system will result in sudden changes in feed quality or quantity, thereby initiating tail biting (Paul et al., 2007).

The amount of feed or time of feeding restrictions influences tail biting. Feeding multiple times a day as compared to *adlibitum* feeding has been shown to induce a higher risk of tail biting. Increasing the feeding frequency had the same effect of increased tail biting (Temple et al., 2012). This may be due to spreading the same amount of feed into smaller meals, resulting in increased competition for feed if the pigs have been left hungry.

2.1.3.1.4 Feeder set up

The type of feeder and limited space by the feeder can be risk factors. Pigs are social animals and like to feed together (Moinard et al., 2003). Indoor feeding systems offer feed through troughs which maybe multi or single space feeders. This feeding system offers less space per pig compared to grazing or floor feeding. It appears that the optimum number of pigs per feeder is not known. However, a study has determined that when pigs are in excess of five per feeder partition, there is an increased risk of tail biting (Moinard et al., 2003).

2.1.3.1.5 Age and sex appropriate diet

Pigs should be fed according to their stage of growth and sex. If the nutritional requirements are not met within mixed-sex groups, one of the genders will be predisposed to heightened behaviour which can lead to tail biting (Taylor et al., 2010). Feeding in phases in accordance with the dietary protein and energy requirements is not always practised. This results in a deficiency or excess of some nutrients during the growth period. Additionally, unbalanced and unmet nutritional densities increase the risk of tail biting, just as insufficient dietary minerals and proteins promote foraging behaviour which leads to tail biting (Fraser, 1987). Therefore, pigs should be fed based on their age and sex to minimise tail biting problems.

2.1.3.2 Access to manipulable substrates

Using manipulable substrates is one of the main solutions to reducing tail biting (EFSA, 2007). For manipulable substrates to effectively satisfy exploratory behaviour in pigs, the substrates should be complex, destructible, changeable, chewable, containing sparsely distributed eatable parts (Studnitz et al., 2007). This proposed solution has gained more research attention compared to others (D'Eath et al., 2014). The chance to explore and forage is important for pig welfare (Van de Weerd et al., 2005) and it has been reported that pigs that stay in barren pens have increased misdirected foraging behaviour (Simonsen, 1995). Pigs show an extended interest in substrates if they are manipulable (Moinard et al., 2003). Straw is deemed to be very effective in the prevention of tail biting as it can be bitten, chewed, rooted or eaten, thereby assuaging foraging and exploratory tendencies (Van de Weerd et al., 2005). The time devoted to a substrate is relative to the amount of substrate offered. The shape and durability of the substrates determine the interest it gains from the pigs (Van de Weerd et al., 2005). Therefore, substrates should be attractive enough to draw attention away from tails. However, the substrates are usually not as efficient as bedding type material (EFSA, 2014), even though, use of bedding material is not practical in commercial units with partial or fully slatted flooring. For this reason, solid objects (e.g. toys) may be preferred.

2.1.3.3 Housing and climatic conditions

Poor ventilation and floor structure are often implicated as tail biting risk factors (Schroder-Petersen and Simonsen, 2001). Artificial ventilation is reported to lower the risk of biting (Hunter et al., 2001). Draughts and the presence of ammonia in the air, signifying poor air quality, are risk factors for tail biting (Schroder-Petersen and Simonsen, 2001). When tail biting is observed in some pens and not others, it may be that the affected pens are exposed to isolated areas of poor air quality or draughts arising from poorly adjusted ventilation areas (Taylor et al., 2012).

The floor design has been associated with tail biting. Fully or partially slated floors in comparison with solid floors increase the risk of tail biting, more so in grower and finisher pens (Moinard et al., 2003, Schroder-Petersen and Simonsen, 2001). This may be related to the lack of bedding material or substrates which have been shown to reduce tail biting (EFSA, 2014). Slatted floors are associated with higher levels of noxious gases as they allow these gases to move from the manure pit through to the pens (van Putten, 1969). This promotes restlessness within the pigs, thereby inducing tail biting.

The climate environment within the pen contributes to the incidence of tail biting. Changing temperature, gas build up, dust and humidity are stressors which result in chronic stress. Heat and cold stress which results from temperatures outside the thermal comfort zone for pig results in increased tail biting (Schroder-Petersen and Simonsen, 2001).

2.1.3.4 Space and stocking density

High stocking densities induce tail biting (Moinard et al., 2003, Schroder-Petersen and Simonsen, 2001). When pigs have less space to move, there is increased exposure to the bodies and tails of other pigs. This, coupled with the increased competition due to higher densities, results in higher tail biting prevalence (D'Eath et al., 2014). Higher densities restrict movement, and this restricts the avoidance behaviour of the pigs which are victims of tail biting (Moinard et al., 2003).

2.1.3.5 Stressor due to stock person

Studies of tail biting do not usually focus on the stock person even though the human factor has a role to play. The stock person can control tail biting risk factors such as lighting, animal disturbance, ventilation, temperature and unpredictable feeding. These factors affect homeostasis mechanisms and can lead to stress which may ultimately result in tail biting (Jensen et al., 2013). The stock person can have a positive impact through spotting tail biting and intervening. Tail biting has been reported to increase as the number of pens under one stock person increases (Moinard et al., 2003). This highlights the vital role the stock person has in reducing tail biting.

2.1.4 Prevalence of tail biting

The difficulties in preventing tail biting on commercial farms have necessitated the need to dock the pigtails as a prevention measure. This widespread use of docking makes it challenging to assess how the prevalence of tail biting changes over time (Edwards, 2006).

The most common method of assessing tail biting prevalence is tail damage monitoring at abattoirs. This method is advantageous as it is a fast and simple way of monitoring pigs from a number of farms. However, there tends to be an underestimation of the actual prevalence as some tail bitten pigs die or are euthanised at the farms if tail biting is severe or if death due to tail biting is recorded under death due to infections or unspecified injury (EFSA, 2007). Furthermore, assessing the presence or lesions of tails at slaughter does not enable the detection of tail biting lesions that have healed, thus underestimating prevalence.

The incidence of severe tail biting, which includes evidence of fresh tissue damage greatly varies, and docked pigs have a prevalence ranging up to 3% while pigs with intact tails have a higher prevalence, ranging from 2-12% (Valros et al., 2004). In support of these figures, Hunter et al. (1999) reported a tail-biting prevalence of 2.4% in docked pigs compared to 8.5% in pigs that had not undergone tail docking.

2.1.5 Early identification and prediction of tail biting

Mediation in cases of tail biting is more efficient if there is early identification of the behaviour. There are four main behavioural characteristics that have been identified as potential predictors of tail biting outbreaks. These are:

(i) Increased restlessness and activity levels (Larsen et al., 2016). An increase in restlessness and activity levels may be a predictor of tail biting outbreaks.

(ii) Changes in exploratory behaviour towards substrates and pen mates. A higher degree of chewing, coupled with tail and ear biting has been noted before outbreaks of tail biting are officially reported (Zonderland et al., 2011)

(iii) Tail posture; when there is an impending tail biting outbreak, undocked pigs exhibit low tail postures. This may be due to the damage already occurring to the tails (Munsterhjelm et al., 2013), resulting in pigs tucking in the tail between the legs to protect it. This low positioning of the tail is also associated with pigs undergoing negative emotions (Reimert et al., 2013)

(iv) Eating behaviour; at pen level, the daily number of feeding visits at an automated feeder declined as early as ten weeks before an outbreak is visible within a pen (Wallenbeck and Keeling, 2013). A study showed that pigs that later became victims of tail biting had decreased feed intake two to three weeks before the farm staff diagnosing tail biting.

Tail biting behaviour can occur for up to six days without detection of the damage. An intact tail may harbour biting damage; this is an indication that a precise estimation of the start of tail biting may be difficult (Munsterhjelm et al., 2013).

To consolidate early detection and prediction skills, novel ways such as automated recordings of activity levels as detected by animal or pen sensors may be put in place (Larsen et al., 2016). Automatic measurements of manipulable material use can be recorded through the attachment of movement sensors to the materials provided (Bracke and Spoolder, 2008).

2.1.6 Consequences of tail biting

Welfare, pain and health consequences

The welfare of tail bitten pigs is compromised as they are exposed to pain through biting or amputation of their tails (Valros, 2018). Tail biting victims have higher rates of arthritis and abscesses at slaughter, with implications for chronic and high pain levels (Valros et al., 2004).

Tail biting wounds are prone to infection, resulting in secondary infections in organs such as kidneys, lungs and other areas of the body due to pyaemia (Fraser and Broom, 1990).

Cannibalism is a consequence of tail biting. As the tail bleeds, it attracts more bites from the biting pen mates (Fraser, 1987). Ultimately, cannibalism due to tail biting results in the death of the victim (van Putten, 1969), and consequently, a loss of potential income.

Tail biting negatively impacts growth and weight gain (Sinisalo et al., 2012). Pigs that are exposed to biting grow slower than non-bitten pigs. This results in reduced carcass weights at slaughter (Valros et al., 2016), translating to less income from the carcass.

Economic consequences

There are direct and indirect ways in which tail biting impacts the economy of production. Tail biting results in losses due to carcass condemnations, reduced growth, higher costs of medications, increased workload due to increased pig monitoring and increased pig mortality, all which are economic consequences due to tail biting.

Amounts ranging between €5,000 and €10,000 per annum were the estimated costs of a 12% tail biting prevalence on a farm having 1,000 pig places (Niemi et al., 2012). A net cost of €18.96 was suggested per victim and was to be subtracted from net margins (D'Eath et al., 2016). This affects the economic viability of a farm and highlights a negative consequence of tail biting.

2.2 Tail Docking

Tail docking is routinely used as a management tool in the prevention of tail biting on commercial pig farms (Sutherland and Tucker, 2011). Docking is carried out through amputation of a portion of the tail's distal end by use of side cutters or cautery irons (Sutherland and Tucker, 2011). Evidence shows that tail docking decreases tail biting frequency amongst pigs that are farmed in intensive systems (Hunter et al., 2001, Sutherland et al., 2009). The tail length reduction prevents biter pigs from grasping and manipulating the remaining stump. A popular hypothesis suggests that tail docking inflicts neuroanatomical changes to the docked tail tip, resulting in hypersensitivity of the remaining stump. This motivates protective behaviour such that pigs will not stand for the stump manipulation (Simonsen et al., 1991). However, this hypothesis has not been conclusively confirmed(Sutherland and Tucker, 2011)

Tail biting is a welfare issue as it induces stress and pain in pigs (Schroder-Petersen and Simonsen, 2001, Sutherland et al., 2009). By the same token tail, docking is also a welfare problem as its use is undermined by codes of welfare with regards to immediate pain causation and potential long term suffering due to docking without anaesthetics(Sutherland and Tucker, 2011).

2.2.1 Methods of tail docking; physiological and behavioural effects

Tail docking in pigs can be performed using scissors, teeth clippers, scalpel blades, cutting pliers, side cutter clippers or electric or gas cautery irons (Sutherland and Tucker, 2011). Traditionally the common tool has been the side clippers, but of late gas or electric cautery clippers/irons have increased in use as they have the advantage of cauterising the wound upon docking and thereby sealing off the wound (Marchant-Forde et al., 2009).

In some literature, docking with heated cautery irons is referred to as hot docking, while the traditional means is called cold docking or blunt trauma docking (Marchant-Forde et al., 2009, Sutherland et al., 2009). A study has shown that hot docking is responsible for more impaired welfare indicators compared to cold docking (Marchant-Forde et al., 2009). In that study, hot docked piglets emitted the greatest number of vocalisations, with the highest peak and mean frequencies of vocalisations when compared to cold docked and sham docked piglets. Cold docked piglets had intermediate values while sham docked piglets had the least vocalisations and peak and mean frequencies.

Furthermore, hot docking procedure took longer compared to cold docking, thereby prolonging the period of distress. Marchant-Forde et al. (2009)went on to suggest that the greater vocalisation from the hot docking method compared to the cold docking method could be resulting from incidents of superficial burns whereby the cautery iron comes in contact with the skin before docking. Contrary to these findings, hot docking has been found to reduce cortisol release after docking when compared to cold docking (Sutherland et al., 2008). A higher cortisol release after a traumatic or painful experience is associated with a higher degree of pain and welfare impairment (Stafford et al., 2003). To further support hot docking, hot docking has been reported as a better method as it cauterises the wound, and the delay in wound healing was not common after hot docking (Sutherland et al., 2009).

2.2.2 Tail Docking Length

In New Zealand, animal welfare regulations stipulate that when docking a pig, only a third to half of the tail should be removed (Code of Welfare, 2018). The Canadian welfare code concedes that leaving the tail longer is ineffective while cutting too short leads to prolapses or infection risks. The Canadian code recommends cutting the tail to a length that will still cover the anus (Codes Of Practice, 2014).

Tail docking length has been reported to affect pain responses during tail docking; this is shown in Figure 2.1

Figure 2.1: The likelihood of squealing during docking based on tail docking length

Source: Herskin et al. (2016)

The percentages (25, 50 and 75%) represent the lengths of the tail that were left upon docking, while intact signified an undocked tail. Based on these results, the probability of a piglet squealing during docking was highest when leaving 25% of the tail length and this was significantly different to leaving 50% and 75% of the tail intact. Pigs with 50% of the tail remaining had a greater probability of squealing during the procedure compared to those with 75% of the tail left, even though the difference was not significant.

Common pain response during docking is sudden jerking, which is linked to escape attempts. The probability of sudden jerks during docking was found not different amongst the docking lengths (Herskin et al., 2016). The results from this study give strength to the New Zealand tail docking regulations for pigs which require that no more than 50% of the tail should be removed. These results suggest that when 50% or more of the tail is removed, more pain is experienced (Figure 2.1).

In support of the Canadian regulations which deems long lengths to be ineffective in hindering tail biting, it has been demonstrated that leaving medium and long lengths during docking is ineffectual. In a study, medium and long lengths were 5,7 and 7.5 cm respectively. These lengths were not significantly different from undocked tails when it came to the risk of tail biting (Thodberg et al., 2018). Additionally, the pigs amongst the short-docked treatment (2.9cm) showed the least probability of manipulating pen mates' tails. This length is closer to the 2cm length which was concluded to be common and effective in preventing tail biting (Sutherland and Tucker, 2011). Therefore, tail docking should leave a length that only just covers the vulva in females and a corresponding length in males, the length removed should be within a third to half of the tail.

The risk of tail biting is 4.6 fold for undocked pigs compared with those remaining with a quarter of a tail (Valros and Heinonen, 2015), whereas the risk is 3.3 and 3.9 for tails that remain at half and three quarters of the original length of the tail respectively.

2.2.3 The efficiency of tail docking

It has been widely documented that tail docking is efficient in the prevention of tail biting (Lahrmann et al., 2017, Paoli et al., 2016, Sutherland and Tucker, 2011, Thodberg et al., 2018, Valros et al., 2004). Despite the seeming success and widespread use of tail docking the method is not 100% effective as it only lowers the risk rather than eliminating tail biting (Hunter et al., 2001, Sutherland and Tucker, 2011). Tail docking can only effectively reduce biting if at least three quarters of the tail is removed(Thodberg et al., 2018).

In a study comparing docked and undocked pigs, docked pigs had no tail lesions (Lahrmann et al., 2017), this gives credit to literature which claims tail docking reduces the damage due to tail biting (Paoli et al., 2016, Sutherland and Tucker, 2011, Valros et al., 2004). Earlier, Di Martino et al. (2015) demonstrated that there was a higher risk of tail lesions found amongst undocked pigs when compared to docked pigs. Prior to this demonstration, it had been reported that undocked pigs had severe tail lesions in comparison to docked pigs (Sutherland et al., 2009).

When Dutch pig farmers were questioned about tail biting, they stated that the most effective way of reducing the phenomenon was docking(Bracke et al., 2013).

2.2.4 Consequences of tail docking

The consequences of tail docking are a cause of welfare concern; they are discussed as follows;

Pain

Tail docking causes pain. This has been quantified through behavioural and physiological indices of pain (Di Giminiani et al., 2017, Herskin and Di Giminiani, 2018, Sutherland et al., 2008). However, physiological responses to pain are more diverse and seem to be dependent on the tail docking method used (Nannoni et al., 2014, Sutherland and Tucker, 2011).

Behavioural signs denoting pain after docking include tail wagging, scooting, lying prostate and sitting (Herskin et al., 2016, Nannoni et al., 2014, Sutherland and Tucker, 2011).

The presence of neuromas was seen in most of the tails docked using either the cautery iron or clippers (Kells et al., 2017). This suggests long-term pain consequences from tail docking.

Growth

It was reported that tail docking using the hot cautery method resulted in a decreased growth rate up to 14 days after docking (Marchant-Forde et al., 2009). However, this result was not duplicated in another study (Nannoni et al., 2014). These conflicting reports signify that more work is required in the study of pig welfare post docking.

Health

Infections compromise the health of pigs. It has been suggested that tail docking increases infection risks, especially when conducted in non-hygienic conditions (Valros and Heinonen, 2015). This is in line with the report by Valros et al. (2004) after an abattoir survey showed an increase in arthritis and abscesses not only in freshly bitten tails but in those with healed tail damage, with some of these being healings from tail docking wounds.

Social Cohesion

A consequence of tail biting which needs further study is the disruption of social cohesion. It has been proposed that pigtails are important for inter-communication amongst pigs, therefore cutting them off hinders this social activity (Valros, 2018).

2.2.5 Alternatives to tail docking

A warning was issued to the effect that keeping undocked pigs in the European Union without changing management practices and housing systems would result in increases in cases of tail biting (EFSA, 2014). This shows that there is hope of alternatives to tail docking if management practices are altered. Furthermore, a survey on Finnish pig farmers showed that only 21% of the sampled farmers would dock their pigs if this were allowed (Valros et al., 2016). This contradicts Dutch farmers who believe that tail docking is the most effective method of reducing tail biting (Bracke et al., 2013).

The European Union legislation recommends docking only after other risk factors have been removed (EFSA, 2007). The risk factors have been discussed previously. Of all these factors, the ones that have gained attention are the provision of straw as a substrate and manipulation of the stocking density. A combination of straw provision and reduced stocking density is used in Sweden (Larsen et al., 2018).

2.2.5.1 Straw

Straw is the commonly used substrate because it has been shown to attract pigs to a greater extent and for a more extended period when compared to hanging and rubber toys. Pigs have been shown to favour interaction with straw compared to other manipulable substrates (Scott et al., 2007). When 150g of straw was provided daily per pig compared to non-provision, there was a twofold increase in the risk of tail biting where straw was not provided (Larsen et al., 2018). This is in agreement with a study that demonstrated that 100g of straw per pig per day caused pigs to spend less than 5% of their time engaging in redirected behaviour such as TIM.

2.2.5.2 Stocking Density

Lowered stocking density has been suggested as a tail biting preventative measure(Moinard et al., 2003, Schroder-Petersen and Simonsen, 2001). In a study, the stocking density was lowered to provide 1.21m^2 per pig from 0.73m^2 per pig. For this study, reduced stock density did not significantly prevent tail biting damage, but there was a trend of reduced tail biting damage with reduced stocking density (Larsen et al., 2018). This is contrary to a study which demonstrated a lack of relationship between tail biting and reduced stocking density (Kritas and Morrison, 2007). It has been suggested that studies that undertake stocking density trials using densities which are higher than recommended almost always report a corresponding increase in tail biting (Larsen et al., 2018).

2.2.6 Conclusion on alternatives to tail docking

Tail docking is the most effective preventative method against tail biting(Bracke et al., 2013, Paoli et al., 2016). Additionally, in a recent study, tail docking had a higher effect against tail biting compared to provision of straw (Larsen et al., 2018). In the same study, straw provision coupled with lowered stocking densities in undocked pig pens did not result in a higher probability of tail biting damage compared with docked pig pens that had no straw and reduced stocking densities. Therefore, in this study, the use of reduced stocking density coupled with straw provision was as good a method for tail damage prevention as tail docking (Larsen et al., 2018).

2.3 Animal welfare

General animal welfare

Animal welfare has been defined as the state of an animal as regards to the physical and mental state determined by the conditions in which the animal lives and dies,(World Organisation for Animal Health (OIE), 2018). Animals experience good welfare when they are healthy, well-nourished, comfortable, safe, free from pain, distress and fear, all while being capable of expressing behavioural tendencies which are critical for their wellbeing physically and mentally (World Organisation for Animal Health (OIE), 2018).

Animal welfare is an expansive subject which is inclusive of the vast selections of elements which contribute to the quality of life for the animals, the most popular of these elements are the five Freedoms (Mellor, 2016), which have been superseded by the ‘five Domains’. The 5 Domains model was developed in New Zealand to assess welfare compromise in sentient animals. This model distinguishes between four interacting physical/functional domains: nutrition, environment, health and behaviour; and the final domain represented by the mental (Mellor, 2016). The model works by assessing the potential for animal welfare compromise in the first four domains and assigning their consequences to the final mental domain in order to determine an animal’s welfare status.

2.3.1 Animal welfare and tail biting

When the environment is conducive for normal behaviour, pig welfare is promoted. Denying pigs their preferred chewing substrates may be a cause of stress to the animals, resulting in the behaviour of tail biting.

It has been reported that stressful situations in pigs are inclusive of, but not limited to; barren environments, poor ventilation and overcrowding (Schrøder-Petersen et al., 2004). The stressful situations are a breach of welfare and may be accompanied by tail biting behaviour.

The psychological state and behaviour of the victims (tail bitten pigs) has not been extensively covered in the literature. Tail biting or chewing often results in tail irritation, tissue damage, vocalisation and escape behaviour(Schroder-Petersen and Simonsen, 2001). The behaviour of the littermates that are not biters and have not been bitten has not been quantified to conclude if tail biting taking place around them denies them freedom from fear or anxiety.

2.3.2 Animal welfare and tail docking

The process of tail docking results in pain (Prunier et al., 2013, Sutherland et al., 2008). Besides the acute pain that tail docking induces, there is a possibility that docked piglets may be exposed to phantom limb pain and stump pain. Phantom limb pain is a description of pain sensation due to the amputated tail stump. Stump pain, also referred to as residual limp pain is localised in the stump. These phenomena were described by Richardson et al. (2006) in human amputees.

2.3.3 Animal welfare regulations for the New Zealand pig industry

Even after defining animal welfare, individual groups may still have different ideas of what is defined as good or poor animal welfare. This may stem from religious, geographical or personal differences, (Broom, 1988). The participation of countries in signing the global animal welfare guidelines helps in narrowing down the contrasting ideas of animal welfare. 165 countries were signatory to the global guidelines of animal welfare prepared by the World Organisation of Animal Health in 2005 (World Organisation for Animal Health (OIE), 2018). This has encouraged countries to put in place their protective guidelines as some countries were lacking in this before 2005 (Fraser, 2008).

To create animal welfare legislation, the influences of livestock handling protocols and the environment have to be quantified and scientifically validated. The consumers of meat and other animal products have increased concern and awareness of the welfare of animals (Potard, 2015). This has contributed to the ongoing development of animal welfare as a science as well as the continued revisions of animal legislation in many countries.

In New Zealand, there are codes of welfare which are designed to address animal wellbeing concerns relating to specific animal species as well as to specific animal uses (e.g. zoos, circuses). These codes are continuously revised and updated to keep up with good practice, scientific knowledge and technology, and include best practice recommendations. The Codes stipulate minimum standards (good practice) required to provide for welfare under the Animal Welfare Act (1999). Regarding tail docking, the Code of welfare for pigs (2018) stipulates the following:

- When docking the tail of a piglet that is below 7 days old, the operator should ensure a clean cut is created and refrain from tearing the tissue.

- A piglet that is over the age of 7 days can only be docked by a veterinarian or a veterinary student under the supervision of a veterinarian. The piglet must be given pain relief at the time.
- The person docking the tail should have appropriate experience and/or must have received adequate training. Furthermore, the person must be competent and able to identify signs of stress or injury to enable them to take immediate action when necessary.
- The welfare requirements and health of pigs should be met when undertaking docking; therefore, the operator must have suitable equipment and relevant knowledge, training or supervision.

Failure to meet these requirements when conducting tail docking in New Zealand results in regulatory infringements and may lead further to prosecution under the Animal Welfare Act.

2.3.4 Pig welfare regulations in other parts of the world

In Australia, individual states have different governmental responsibilities for farm level animal welfare (Potard, 2015). Australian Animal Welfare Strategy (AAWS) is a collaboration amongst government, the industry and communities. It is mandated to structure a national framework for the improvement of animal welfare. The end goal of the collaboration is to address animal wellbeing issues while promoting scientifically based animal welfare strategies (Gemmell, 2009). Like the code of welfare for pigs in New Zealand, the Australian code of practice for pigs has set up minimum standards for the welfare of pigs. These encompass pain management and the expertise of the stockmen just as is highlighted in the New Zealand code of welfare. Furthermore, Australian Pork Limited has put in place some standards referred to as the Australian Pig Industry Quality Assurance Program (APIQ). The standards are not compulsory; however in some states, export and domestic processors will only accept pigs from APIQ certified farms(Australian Pork Limited, 2010).

In the European Union, a directive was issued in 2003 which stated that tail docking was not to be routinely carried out, rather, it was only to be carried out when there was evidence of injuries due to tail biting or ear biting within the farm population. Furthermore, before tail docking, the farmer should have investigated non-surgical measures of preventing tail biting (D’Eath et al., 2016). This European Union directive notwithstanding, tail docking continues.

Denmark, Germany, Belgium, Ireland, France, Spain and the Netherlands dock 95% or more of piglets while the percentage is over 80 in the United Kingdom (D'Eath et al., 2016). An Irish report documented that 99% of piglets were docked in Ireland(Harley et al., 2012). However, Finland and Lithuania have banned tail docking, while Switzerland and Norway maintain strict regulations. The Swiss regulation prohibits tail docking in pigs (Animal Welfare Ordinance, 2008).

The Zimbabwean pig industry does not appear to have a specific code of welfare for pigs. However, the parliament has a Prevention of Cruelty to Animals' Act which caters for the five freedoms and the necessity of ensuring specialised individuals perform operations (Prevention of cruelty to animals act 19 09, 2018). The pig welfare code for South Africa allows for routine tail docking by trained personnel but does not strictly advise anaesthesia for the procedure (South African Veterinary Council, 2018).

2.4 Pain

Definition of pain

The International Association for the study of pain defines pain as an experience which is unpleasant to the senses as well as emotionally. This is in association with potential or actual tissue damage, (IASP, 1979). This common definition has been discredited as it is said to be heavily reliant on the language and personal report, giving it limitations when discussing nonverbal human beings as well as animals (Anand and Craig, 1996). Following this observed limitation of the earlier definition of pain, Molony and Kent (1997) defined pain as the aversive emotional and sensory experience that represents awareness by the animal to damage or threat of its tissues' integrity, this experience alters behaviour and physiology of the animal in an attempt to reduce the possibility of repetition as well as to promote recovery. In keeping with time, pain has been defined as a somatic experience which is mutually recognisable and reflects apprehension of threat to existential or bodily integrity (Cohen et al., 2018). Anyone of these definitions is sufficient to describe what piglets experience at tail docking.

2.4.1 Aetiology of pain

Pain is not a unitary effect, and its fundamental biology can be fragmented into a number of dimensions. This is seen by the evidence that the processing of discriminative and sensory traits of pain is mostly separate from the cortical level and probably from the early stages.

2.4.1.1.1 Nociceptive pain

Nociceptive pain encompasses the detection of potential or actual damage of tissue cells through stimulation of physiologically and anatomically specialised peripheral sensory neurons called nociceptors (Vinuela-Fernandez et al., 2007). The nociceptors are free nerve endings in unmyelinated C fibres and Aδ fibres. These respond to noxious stimuli, which can be mechanical, chemical or thermal. They then generate action potentials which are transported along efferent axons (Vinuela-Fernandez et al., 2007).

Nociceptive pain safeguards against tissue damage as it gives awareness to the presence of potentially damaging noxious stimuli. For nociceptive pain to carry out its protective functions, the noxious stimulus must be painful enough not to ignore. This type of pain only continues if the noxious stimulus is maintained (Costigan et al., 2009).

2.4.1.2 Inflammatory pain

Inflammatory pain is as a response to injuries to tissue cells and the ensuing inflammatory response. While nociceptive pain can safeguard against tissue damage by provoking protective behavioural responses, inflammatory pain addresses the consequences of tissue damage (Juhl et al., 2008). After tissues undergo trauma, inflammatory mediators are released from surrounding tissues (Costigan and Woolf, 2000). An inflammatory substance is produced and is part of the tissue healing process and also facilitates sensitisation of tissue nociceptors, in that way sensitising higher-order neurons. This provokes hyperalgesia and allodynia which may act as protective measures to avoid further pain and further damage to tissues. This protection is through hyperbolic and sustained noxious stimuli (Juhl et al., 2008). The hyperbolic sensitivity is within the inflamed region and in nearby non-inflamed regions due to the plasticity in central nociceptive pathways and the peripheral nociceptors (Hucho and Levine, 2007). Unless there are underlying chronic factors, inflammatory pain should withdraw after the initial tissue damage has been resolved (Michaud et al., 2007).

2.4.1.3 Neuropathic pain

Neuropathic pain results from nerve injuries and may arise from injuries to sensory transmitting systems in the brain and spinal cord. The combination of paradoxical hypersensitivity and sensory loss is a significant feature of neuropathic pain (Figure 2.2) (Kehlet et al., 2006). As neuropathic pain stems from lesions affecting the somatosensory system through the presence of lesions affecting the peripheral nervous system, in the case of tail docking this may be caused by mechanical trauma and involves several pathophysiological modifications within the central nervous system and the peripheral nervous system (Donato et al., 1999, Dworkin et al., 2003, Mogil, 2009).

Damage to afferent transmission systems results in negative sensory occurrences such as loss of temperature, touch or pressure sensations. This is through a fractional or total loss of input to the nervous system (Kehlet et al., 2006). Contrary to these negative phenomena, the positive occurrence is manifested through dysaesthesia and allodynia whereby pain is induced by a slight pressure, touch and any other innocuous stimuli. This protects against further damage to tissues and nerves. Furthermore, the hypersensitivity within the damaged nerve regions may disguise the sensory loss (Kehlet et al., 2006).

Source; Kehlet et al. (2006)

Figure 2.2: The mechanisms and sites that are responsible for chronic neuropathic pain pot surgery, e.g. post tail docking

The positions numbered 1 to 8 are described as follows; 1- Schwann cells and macrophages, which are located away from the injured nerve sites, work to stimulate pain signalling. 2- Sensory fibres derive excitability from the neuroma at the site of injury. 3- Gene expression changes in dorsal root ganglion transform responsiveness, excitability, survival and transmission of sensory neurons. 4- The dorsal horn is the location of the gene expression changes and activities and produces central sensitisation, microglial activation and loss of inhibitory interneurons, which all intensify the sensory flow. 5- Transmission in the spinal cord is modulated by brain stem descending controls. 6- The hypothalamus and limbic systems give rise to changed behaviour, autonomic reflexes and mood. 7- Based on memories, experiences and expectations, the feeling of pain is generated in the cortex. 8- Chronic pain and reaction to treatment is based on the genomic DNA of the animals

2.4.2 Emotional pain

Pain is contended to encompass sensory and emotional components (Anil et al., 2005). Emotion has a large variety of definitions as psychologists cannot agree on a single one. However, most of the definitions can be used based on the subject matter. It is imperative that the definition chosen should be broad to encompass all the significant traditional aspects of emotion. An example of a working definition of emotion is; a multiplex set of interactions amongst objective and subjective factors which are mediated by hormonal or neural systems, giving to affective experiences which include feelings of pleasure, displeasure or arousal. Furthermore, there can be a generation of cognitive processes like emotionally relevant perceptual effects, labelling processes and appraisals. Lastly, there is activation of extensive physiological adjustments to the arousing factors, leading to actions that are usually goal-directed, expressive and adaptive (Kleinginna and Kleinginna, 1981).

The presence of emotions in nonhumans (animals) sparks controversy. Based on a loose definition, that it is the capacity to relate the past with the present and the future. It has been reported that farm species such as cattle and sheep have the capacity to feel pertaining to anticipated future occasions (Anil et al., 2005). Contrarily, it has been reported that due to the lesser developed neocortex of animals, excluding primates, the less the developed cortex precludes an experience of emotional pain (Bermond, 1997).

From the definitions of emotions, it can be concluded that this sort of pain, like the physical pain, has a function of alerting animals to dangers to their wellbeing, thereby giving the animals some motivation to keep away from the danger (McMillan, 2003).

2.5 Assessment of pain in animals

As of a decade ago in some areas, developing methodologies in pain recognition and awareness of pain in animals was stagnant as some schools of thought rejected the notion that animals were capable of undergoing pain after exposure to diseases, injuries and other noxious events (Molony and Kent, 1997). This has since been disproven as some studies have gone on to demonstrate that even new-born animals, depending on the species, have potential to feel pain as long as certain criteria are met, i.e. the animal should be sentient and conscious to register painful stimuli(Mellor and Diesch, 2006). Animals should have adequately developed neural mechanisms that receive relayed sensory information and transduce the information into sensations. The sensations should be adequately noxious to cause pain. All of this will cause painful sensations to the animal provided the animal is conscious(Mellor and Diesch, 2006). The chronological age and ontigenic period whereby sentience and all neurological features necessary to experience pain develop varies amongst species. This interdicts generalisations with respect to newly born animals. The neonates of species such as cattle, goats, sheep, horses, deer and pigs show behavioural and physiological indications of conscious reactions to sensory stimuli. These species are mature neurologically at birth (Mellor and Gregory, 2003). For species such as dogs, rabbits, cats, rats and mice the neonates are relatively neurologically immature such that consciousness and the capacity to appreciate sensations is delayed (Mellor and Gregory, 2003). The electroencephalographic and the EEC responses to noxious stimuli in rat pups at early postnatal development propose that the pups are unlikely to perceive pain during their first 7 days of life (Diesch et al., 2009)

Concerning pigs in New Zealand, painful procedures such as tail docking are recommended to be carried out within the first 72 hours of life. Furthermore, when pigs exceed 7 days of age, the tail docking must be performed by a veterinarian(NAWAC, 2010).

Relatively limited information is available about the nociceptive responses and sensitivity within the pig species (Herskin et al., 2009). Among other reasons, this is because pain is a personal experience, therefore subjective. This makes it difficult to objectively measure pain in animals (Ison et al., 2016). The unavailability of verbal communication in animals makes the assessment of animal pain a value judgement, which relies on the interpretation of behavioural and physiological indices to present indirect confirmation of the presence of pain(Molony and Kent, 1997).

2.5.1 Behavioural assessment of pain

When in pain, pigs like other animals, display impromptu behaviour. This is more clearly seen when observations are made prior, during and following painful procedures. To assess behaviour related to pain, comparisons are made with control animals or sham animals. To further highlight the behaviour assessment, comparison treatment groups are usually divided between animals on which analgesia or anaesthesia will be used and those on which pain relief is not applied(Hansson et al., 2011). In addition to this, the assessment of pain through behaviour can be analysed through different severities of the nociceptive stimuli (Mohling et al., 2014).

Changes in posture after a pain-inducing procedure indicate the presence of pain and allow for its analysis. The changes in posture can be involuntary or voluntary. Spinal and brain stem reflexes which are involuntary may be prompted by nociceptor activity, some of which can result in hyperreflexia inclusive of increased muscle tone (Molony and Kent, 1997). This involuntary action is exemplified by full extension of the hind limbs as in lambs after they have been castrated using rubber rings (Molony et al., 1993). An example of voluntary posture change signifying pain behaviour is statue standing. This is whereby the animal will adopt an immobile stance after a painful procedure. This is explained as a move to avoid stimulation of hyperalgesic tissues (Sutherland et al., 2008).

Behavioural assessment of pain in animals is also possible through analysing locomotor activity changes. Painful procedures such as tail docking result in increased locomotor activity, inclusive of, tail wagging/ jamming, kicking about, restlessness, easing quarters and stamping (Molony et al., 1993). These changes in locomotor activity and the accompanying behaviour displayed are reported as escape attempts, signifying a need to do away with pain which has been evoked. After the animal changes strategy to adopt passive avoidance attempts, the locomotor changes ease into becoming abnormal postures (Sutherland et al., 2008).

Behavioural indices for assessing acute pain in pigs

As a matter of principle, the pain experienced by animals can never be truly known. Even though several indicators of pain in pigs have been more or less validated, it is imperative to note that there is no gold standard in existence to reliably measure the presence of pain (Ison et al., 2016, Sneddon et al., 2014). This implies that observations of particular measures made on nonverbal animals cannot be used to validate the presence or absence of pain (Rutherford, 2002). However, indices can be used to quantify or assess the pain. Indices or indicators of pain are measurements that provide an indication of the severity and the nature of the pain experienced (Herskin and Di Giminiani, 2018). Some behavioural indices are detailed below.

2.5.1.1 Vocalisation

Several recognised sounds, made in response to different scenarios, are recorded as communication in pigs. Furthermore, the most distinguished aspect of communication in pigs is vocal (Herskin and Di Giminiani, 2018). Animal vocalisations have been known to relay information about the animals' state of being. The rate of calling in birds has been shown to increase with increased exposure to hunger (Weary et al., 1998). With respect to piglets, it has been reported that vocalisations of a high frequency and overall higher vocalisation frequency indicate pain, stress or both (Nannoni et al., 2014, Weary et al., 1998, White et al., 1995). Pigs not only vocalise due to painful stimuli, but they also typically vocalise during handling or upon restraint. However, the vocalisations differ based on the reason behind the vocals. The vocalisations due to a painful procedure are different from those emitted by sham-treated animals (Hansson et al., 2011, Marx et al., 2003). As much as restraint and handling evoke a stress response, when restraint is performed alongside a procedure such as tail docking or castration, there is an alteration in the vocal basal levels shown amongst such piglets and those that have been merely restrained for the same amount of time without undergoing any procedure (Noonan et al., 1994). Piglets have been shown to respond vocally immediately due to painful stimuli such as castration by emitting higher frequency calls such as those exceeding 1000 Hz (Taylor et al., 2001). Piglets also emit vocalisations more frequently, for a longer duration when submitted to a painful stimulus in the absence of local anaesthesia compared to submission to pain under anaesthesia (Hansson et al., 2011, Hay et al., 2003, Leidig et al., 2009). Comparing vocalisations within a treatment is a functional tool when assessing pain in piglets (Marx et al., 2003). It is helpful to have some definitions of the call parameters.

Table 2.2: Definition of vocalisation call parameters

Parameter	Description	SI Unit
Duration of call	Time taken emitting the utterance	Ms
Peak amplitude	Highest amplitude of an isolated time-frequency cell within call	dB
Peak frequency	The frequency of a given peak amplitude	Hz
Occurrence of peak amplitude	Timestamp of peak amplitude given as a percentage of call duration	%
Main amplitude	Highest amplitude in the average spectra of a call	dB
Main frequency	Frequency of highest amplitude for the mean spectra	Hz
Peak level	Level of the time frame with the highest total power in a call	dB
Call level	Level of the average total power of the time frames in a call	dB
Call energy	Discharged call energy standardised for a duration of 1 second	dB(SEL)

Adapted from Marx et al. (2003).

In addition to the description of the technical parameters in Table 2.2, it is interesting to note that the vocalisations can be split by sound in some instances as grunts, squeals, screams and sometimes howls.

Grunts

Grunts typically have a long duration, low fundamental and peak frequencies, low modulation of frequency structures, low amplitude, and lack of harmonies and concentration of spectral energy within narrow ranges of frequency (Marx et al., 2003).

Squeal grunts

These consist of comparatively short duration. Their modulation of frequency structures and peak frequency are relatively higher than those of grunts. Almost no harmonics are shown, and when compared to other vocal types, their amplitudes are intermediate (Marx et al., 2003).

Scream

The main characteristics of screams include long durations of calls with high peak and fundamental frequencies. There are strong modulations of frequency structures for high-frequency range. There are also high amplitudes and harmonics(Marx et al., 2003).

Squeal

These are characterised by short call durations with high fundamental and people frequencies, with comparatively high modulations of frequency structures and high-frequency ranges. These vocalisations have the highest tonality compared with the other vocalisations (Schrader and Todt, 1998).

Marx et al. (2003) focused only on grunts, squeals and screams. In the case of castration without anaesthesia, the screams were most frequent compared to grunts and squeals, thereby indicating pain.

Grunt frequency during tail docking has been recorded as higher compared to other painful husbandry procedures such as ear notching and teeth clipping(Noonan et al., 1994). Furthermore, some authors have reported that within tail docking, the vocalisations may be dependent on the method of docking(Nannoni et al., 2014). Pigs that were tail docked through hot iron cautery squealed more times per second at higher peak and mean frequency(Marchant-Forde et al., 2009), this was attributed to the pain upon hot iron to skin contact with the tail prior to severing the tail. Of note is that the highest energy frequency in kilohertz was recorded as higher in piglets manipulated without anaesthesia, and the kilohertz increased with age (White et al., 1995).

2.5.1.2 Escape reactions

In some literature, escape behaviour is interchanged with avoidance behaviour (Ison et al., 2016). This behaviour has been used to assess and measure pain during and after handling periods when undertaking experiments to assess pain in animals (Leidig et al., 2009). Escape attempts are characterised by jerks, kicks or wriggling by the animals to get out of processing (Kluivers-Poodt et al., 2013), therefore simply put, an escape reaction is a bodily movement carried out to attempt an escape (Marchant-Forde et al., 2009). When piglets are being processed, they have been known to carry out sequential kicks in order to effect an escape, and then pause, only to carry out another bout of kicks (Marchant-Forde et al., 2009). Escape behaviour is scored based on the frequency (Hansson et al., 2011), the duration and intensity of kicks and any other resistance attempt (Leidig et al., 2009). To further assess pain levels, escape behaviour can be analysed using different treatments, such as different methods of docking and presence or absence of anaesthesia at treatments. The intensity and duration of escape behaviour predictably increase when piglets are docked without using local anaesthesia (Hansson et al., 2011, Leidig et al., 2009). The escape behaviour is a vital pain assessment tool when assessing the pain due to acute pain stimuli such tail docking as this helps when evaluating pain reduction approaches such as the use of analgesia and anaesthetics(Ison et al., 2016).

2.5.1.3 Facial grimace

The use of facial expressions to assess pain is a concept that stems in part from considerations that are not a new concept. Facial expressions are indicators of an array of emotions. Furthermore, similar facial expressions are known to be shared amongst different animal species; Akintola et al. (2017), Descovich et al. (2017), Flecknell (2018), Hampshire and Robertson (2015), Leach et al. (2012). The concepts of facial expressions as a pain assessment tool has been developed into a grimace scale in order to objectively assess pain, with the first scale developed in a study with mice (Flecknell, 2018, Langford et al., 2006). This scale achieved success in the detection of changes elicited by painful stimuli to mice. The grimace scale uses a system of scoring which allows the evaluation of facial expressions changes after painful stimuli. This scoring is based on specified morphological characteristics within the face of the study animal (Herskin and Di Giminiani, 2018).

The facial action coding system (FACS) is the most common tool for assessing pain or emotions in the human face in behavioural sciences; this system was developed by Ekman and Friesen in 1978. This system has been manipulated for use in non-humans. The components of the pain face in humans have been extrapolated to suit the pain face in primates and other animals (Vick et al., 2007). FACS recognises any muscular activities in facial orientation (Rahu et al., 2013).

Description of the pain face in pigs

The following table shows some descriptions of some of the common pain faces/ grimaces, in pigs.

Table 2.3: Descriptions of some of the common pain faces/ grimaces, in pigs

Pain face feature	Description
Forehead profile	A slight concave slope of the forehead increases with increased pain
Orbital tightening	The orbital area is continually narrowed until shut with increases in pain
Cheek tension	The cheeks are strained from a rounded appearance to a flatter scrunched appearance
Temporal tension	The ears turn backwards and rather than go upright; they are almost horizontal
Tension over eyes	The eyebrows take on a curved appearance with increasing pain
Lower jaw profile	The skin around the jaw tightens as the jaw takes on a less concaved appearance
Snout plate changes	As the snout plate seems to flatten, its thickness seems to increase
Snout angle	The snout contracts and wrinkles, the angle of the snout relative to the mouth reduces
Upper lip contraction	The upper lip bulges and the canine notch size increases
Nostril dilation	The nostrils appear activated from a resting position to a more evident state

Adapted from Viscardi et al. (2017) and Di Giminiani et al. (2016)

The descriptions in Table 2.2 are shown in Figure 2.3;

Source; Di Giminiani et al. (2016)

Figure 2.3: Examples of pain face in pigs

The pig grimace scale needs further development before it can become an effective tool in pain assessment. The facial grimace is advantageous as facial indicators are concentrated in one area and remain the same regardless of pain origin. This is unlike a full body behaviour pain assessment which requires a number of behaviours to assess, added to that the behaviour may vary based upon the type and the location of the pain (Leach et al., 2012).

The facial action units observed in pig trials may often be subtle, but they appear to have similarities to expressions recorded within other species (Gleerup et al., 2015). The face action units are not always detected concurrently (Di Giminiani et al., 2016, Kunz et al., 2008)

However, there are downsides of using facial grimace as a pain assessment tool. Observers can have difficulties when assigning scores if the captured images are of a suboptimal standard (Di Giminiani et al., 2016), in this case, redoing the whole experiment may become necessary. Furthermore, facial actions which involve muscle contractions or ear positioning such as in temporal tension may be subtle and therefore difficult to objectively detect. In the case of the human pain face assessment, there is the use of an automatic detection system when assessing facial expressions (Lucey et al., 2009). This minimises dependency on individual observations, and such a system should also be employed when assessing pain in pigs using the pain face.

2.5.1.4 Nonspecific behaviour

In addition to the behaviours discussed above, there is also non-specific behaviours which can be observed in experimental piglets when assessing pain. Non-specific behaviour shown by piglets after exposure to painful stimuli is usually a subtle indication of prolonged or acute pain (Leslie et al., 2010) When postnatal animals have been exposed to a painful stressor, they have been observed to display redirected behaviour such as increasing contact with artificial heating sources (McGlone and Hellman, 1988). This mimics the desired temperature of the udder and reduces the stress to the animal (Noonan et al., 1996). More non-specific behaviours are as follows:

Udder massage

Udder massage is when the snout of the piglet makes contact with the sow’s udder and the piglet proceeds to do rhythmic up and down movements of its head (Hay et al., 2003).

Nosing

There is a repetition of the snout massaging another piglet up and down its body, or massaging the sow’s body with the exception of the udder (Noonan et al., 1994).

Suckling.

The piglet employs rhythmic, vigorous movements to suck on the teats (Hay et al., 2003). It has been reported that suckling after a painful procedure may be beneficial to the pained animal as the act of suckling when feeding is said to evoke a release of opioids which can dispense analgesia (Noonan et al., 1996). This was earlier noted by Shide and Blass (1989)when they reported that opioid release stimulated by milk reduced distress vocalisations in infant rats, showing that milk availability resulted in opioids becoming available to the neonates' pain, ingestive and distress systems resulting in an ability cope with distress due to pain. The increased udder activity due to pain as reported by Noonan et al. (1996)is at odds with earlier reports McGlone and Hellman (1988)and later reports Hay et al. (2003)and Van Beirendonck et al. (2011)which found that piglets spent limited time on the udder during their exposure to pain.

Teat seeking

This is when the piglet is trying to find a teat by trying to push away other piglets as they are suckling (Hay et al., 2003). This nonspecific behaviour was not observed for piglets which had undergone pain without analgesia (Van Beirendonck et al., 2011)

Licking and chewing

Licking is the action of rubbing the tongue over littermates and available surfaces. Chewing involves nibbling the ears, tails or feet of littermates (Hay et al., 2003). Licking and chewing are usually put together as they may be difficult to distinguish at times. In an experiment to assess pain, the piglets which went through the painful procedure showed fewer incidences of chewing and licking compared to sham-treated piglets. The subdued oral activities may be a reflection of a prostate state due to pain (Hay et al., 2003).

Social interaction

The proximity of a piglet to its littermates and the sow determines its social cohesion at the time. An active social interaction is shown by proximity (less than 10 cm) away from the sow or littermates, otherwise, the piglet is deemed to be in isolation. Piglets that show isolation behaviour may be signifying a need to protect themselves by avoiding contact with littermates which may lead to the further generation of pain(Mellor et al., 2000). This kind of isolation may also be a result of prostration due to pain (Hay et al., 2003).

2.5.1.5 Quantitative sensory testing

Quantitative sensory testing (QST) procedure offers a non-invasive technique for the assessment of sensory pain. Nociception can be expressed based on the latency needed to evoke an avoidance response. This makes nociception quantifiable (Loeser and Treede, 2008). The latency is evoked by applying mechanical stimulation to an animal with cumulative pressure until the avoidance response occurs. QST only quantifies the sensitivity of animals in the detection of noxious stimulation but not the actual pain sensation. However, it allows for the recognition of anomalous thresholds generated by painful procedures (Jarvis et al., 1997).

In QST study, mechanical thresholds by the tail root in piglets was quantified using von Frey filaments and a plantar stimulator at the foot. Docking the tails did not result in any alterations with these tests (Sandercock et al., 2016) When conducting QST to test the efficacy of analgesia in piglets, the analgesia treated groups have often shown higher threshold levels compared to untreated groups after going through manipulation with handheld algometers (Fosse et al., 2011)

2.5.1.6 Accelerometry

Accelerometery is a non-invasive method of monitoring and assessing the behaviour of animals to determine pain or health status (White et al., 2008). Accelerometers have small silicon beams which deform at acceleration or due to the force of gravity, translating the deformation into a voltage output. Each of the silicon beams measures the acceleration in a single axis. Two-dimensional accelerometers measure acceleration in the vertical x-axis and horizontal parallel to the body y-axis. Three dimensional, also known as tri-axial accelerometers, give measurements like the two-dimensional meter but include measurements of the acceleration from the horizontal perpendicular to the body (z) axis (Theurer et al., 2013). This is shown in Figure 2.4;

Figure 2.4: An illustration of the three-dimensional accelerometer showing the x, y, and z-axes on right leg a standing and sitting calf, A and B respectively

Source; Theurer et al. (2013)

Assessment of animal posture helps in the determination of pain status after painful animal husbandry routines. Accelerometers have not been extensively used in pigs (Escalante et al., 2013) Their use as yet has been limited to activity quantification with a focus on sows (Conte et al., 2014, Oczak et al., 2015)

2.5.2 Summary of main behavioural indices

The primary behavioural indices in the assessment of pain are shown in Table 2.4 as an ethogram that is specific to piglets that have been exposed to tail docking.

Table 2.4: Ethogram for piglets that have undergone tail docking

Behaviour	Description	Literature
Vocalisation	Squealing or grunting, whereby grunting is a more guttural vocalisation form, usually more frequent than a squeal	(Leslie et al., 2010)
Escape attempts	Jerks, kicks or wriggling to get out of processing	(Molony and Kent, 1997)
Trembling	Shivering as though cold while standing or lying down	(Kluivers-Poodt et al., 2013)
Scooting	The caudal part of the body gets dragged across the floor	(Sutherland et al., 2008)
Huddling	Lying almost on top of another pig, with more than half body contact to the other pig	(Leslie et al., 2010)
Lying without contact	Lying in a recumbent position, on one side or on the belly and not in contact with other pigs	(Leslie et al., 2010, Sutherland et al., 2008)
Lying with contact	Lying in a recumbent position, on one side or on the belly and in contact with other pigs	(Leslie et al., 2010, Sutherland et al., 2008)
Walking	Fairly low-speed movement whereby leg action is the propulsion force	(Sutherland et al., 2008)
Sitting	Resting on caudal part of the body	(Herskin et al., 2016)
Standing	The weight of the body is supported on all four legs	(Molony and Kent, 1997)

2.5.3 Conclusion on the behavioural and facial assessment of pain

Behavioural observations are useful tools in assessing pain in animals. However, it should be noted that behavioural assessment of pain has limitations. Often, there is high variation in observed behaviour between subjects while the frequency of observed behaviour is low; this results in floor effects when carrying out data analysis (Mogil, 2009). Also, behavioural assessment of pain depends on indirect measures which may not be predicting the animal's perception (Anil et al., 2005).

Furthermore, there exist dissimilarities in behaviours related to pain between species and sometimes within species. These differences are dependent on gender, age, the environment and the source of nociception (Stafford and Mellor, 2007).

Additionally, pain-related behaviour may not correspond with the level of the pain the animal is subjected to. However, as issues of animal welfare continue to be prioritised. It is hopeful that the assessment of pain through behaviour and facial observations will come to be standardised to achieve fairly accurate pain assessment results.

2.5.4 Physiological Assessment

Changes in the physiological state of animals are useful in the assessment of pain due to noxious circumstances like tail docking in piglets (Hay et al., 2003). Physiological changes due to painful stimuli are primarily as a result of two inter-related mechanisms. Pain is a potent stressor which directly stimulates hormone release from the sympathetic and hypothalamic pituitary adrenal axis in mammals and other species (Prunier et al., 2013). The hypothalamic pituitary adrenal axis is stimulated by physiological and physical stressors for the promotion of recovery through an increase in metabolism and reduction in inflammation (Ison et al., 2016). Secondly, the immune system is activated by tissue damage and releases many inflammatory mediators that may subsequently activate the adrenal axis (Lamont et al., 2000). This is exemplified by interleukin-1, a pro-inflammatory cytokine, which the immune cells release after a tissue lesion. Interleukin-1 stimulates the release of adrenocorticotropic hormone and cortisol and plays a major role in ensuring the brain receives the transmission of nociceptive stimuli (Turnbull and Rivier, 1999). Other physiological indices of pain such as haptoglobin and fibrinogen amongst others that are not participants in pain control but are released upon inflammation may be used as indirect pain indicators because inflammatory conditions are highly likely to generate pains (Sutherland et al., 2008). Accordingly, physiological pain indices encompass the adrenal and sympathetic axis hormones, their metabolites, plasma markers due to inflammatory conditions and the mediators concerned with physiological indicators of pain (Prunier et al., 2013).

Activity from the sympathetic nervous system

The activity from the sympathetic nervous system can be measured through various ways such as changes in the heart rate, peripheral blood flow, skin resistance and pupillary diameter. This system is integrated, and consequently, most of these changes occur in parallel. The easiest way to assess the state of the system is through monitoring the heart rate or through sequential measurements of plasma catecholamines (Molony and Kent, 1997).

Activity from the hypothalamic pituitary adrenal axis

Sequential measurements must be taken before and after treatments to cater for the release and elimination characteristics of the hormones and also to ascertain the changes produced (Molony and Kent, 1997).

Physiological indices for assessment of pain

The primary physiological indices that are used in pain assessment are listed in Table 2.5;

Table 2.5: Physiological indices that show the presence of pain in animals

System affected	Response to be measured
Adrenal axis hormones in saliva, blood or urine	Adrenocorticotropic hormone Cortisol
Sympathetic axis hormones in saliva, blood or urine	Adrenaline Noradrenalin
Blood metabolites (energetic)	Glucose Lactate Free fatty acids
Haematological concentrations of inflammatory markers	White blood cells Haptoglobin Fibrinogen Interleukin 1
Autonomous nervous system activity	Heart rate Respiratory rate Arterial blood pressure Cutaneous, internal and eye temperature Sweating
Brain activity	Electroencephalography

Adapted from (Hay et al., 2003, Mellor and Gregory, 2003, Prunier et al., 2005)

2.5.4.1 Cortisol

When animals experience pain and distress at the hand of painful procedures such as tail docking and castration, cortisol levels are altered in response. Pain can be assessed by measuring the releaseof cortisol into the saliva, blood stream or urine (Hay et al., 2003). Plasma cortisol may be used objectively as a stress and pain indicator (Hansson et al., 2011). However, if an animal undergoes an experience resulting in a consequential elevation in plasma cortisol concentration, it may not always be suitable to use the plasma cortisol as an indicator of pain but it can be used as a pointer in the assessment of comparative intensity of the experience (Stafford et al., 2003). An acute activation of the sympathetic nervous system and hypothalamic pituitary adrenal axis is caused by castration, with the subsequent increase in cortisol levels (Hansson et al., 2011, Hay et al., 2003, Marchant-Forde et al., 2009).

Figure 2.5: Plasma concentrations of cortisol 1day old piglets after (CAST; castration, SHAM; sham castration and NOHA; non handling and the cortisol profile after DOCK; docking the tail, SHAMD; sham tail docking; NOHA; non handling; n = 5 or 6 in each group) the

Sourced from Prunier et al. (2005)

Figure 2.5 shows the cortisol profiles of day-old piglets after painful husbandry procedures castration and docking. Plasma cortisol increase had a slight delay after castration. The peak values occurred between 30 and 60 minutes post-surgery with significant increases in cortisol levels for the castrated pigs. Within 3 hours, cortisol levels had resumed to pre-surgery levels. With tail docking, there was no effect of the procedure on plasma cortisol levels when comparing docked and sham docked piglets (Prunier et al., 2005). However, the experimental data were too scarce for a definitive conclusion as to the reason for the unchanging plasma cortisol levels due to tail docking. The findings above concur with a later report by Prunier et al. (2013) where there was an analysis of a number of studies involving animals that had been exposed to pain. The painful procedures resulted in high plasma cortisol increases except for the tail docking in very young piglets. However, the term ‘very young’ piglets leave room for further thought. Some animals have been known to show limited response to stimuli at early neonatal stages. Other authors have reported high cortisol levels upon tail docking. An example is (Sutherland et al., 2008)who reported an acute cortisol response in piglets which had been docked via blunt trauma method. Tail docking using a cautery iron resulted in a lesser cortisol response compared with those docked by blunt trauma. Sutherland et al. (2008) went on to report that since tail docking had elicited an acute cortisol response when blunt trauma was used, it may be that the high heat levels from the cautery iron may result in third degree burns, resulting in the destruction of the nociceptors in the immediate area, resulting in the reduction of the pain perception experienced by that piglets. If this presupposition is true, then it is not known if these pigs would experience any distress when the numbed nociceptors regenerate.

While Prunier et al. (2005) reported no effect of treatment on cortisol levels, they had studied 1-day old piglets. Conversely,(Sutherland et al., 2008) studied this effect in approximately 6 day old piglets. This may have been why they had different results. It has been postulated that the lack of effect of tail docking on cortisol may be explained by the fact that the pituitary-adrenocortical axis may be unresponsive to stress in the day old piglets, if responsive, then the unchanging cortisol levels previously reported may be due to variations related to the additional procedures undertaken in the piglets such as blood sampling, thereby masking the experimental procedure effects (Prunier et al., 2005). Additionally, it has been hypothesized that the activity in the pituitary adrenocortical axis is considerably stimulated around the time of birth and may not be responsive to supplementary stimulation, or maybe nociceptive stimuli resulting from tail docking are insufficient to invoke a physiological stress response (Prunier et al., 2005).

Nevertheless, this last point would need further investigation as there are authors that have reported a physiological response due to tail docking.

2.5.4.2 Adrenocorticotropic hormone

The activity of the hypothalamic pituitary adrenal axis can be used to assess pain (Prunier et al., 2013). Psychological and physical stressors activate the hypothalamic pituitary adrenal axis to stimulate recovery through an increase in metabolism and a reduction in inflammation (Mellor et al., 2000). The hypothalamus responds to psychological and physical stress by releasing corticotropin hormone to stimulate secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary which produces cortisol trough acting on the cortisol gland (Ison et al., 2016). In some studies, plasma ACTH variations were more distinct when compared to plasma cortisol variations following husbandry procedures that induce pain (Merlot et al., 2011, Prunier et al., 2005)

ACTH concentrations are typically markedly higher in piglets that have undergone castration compared to those that have undergone tail docking. This difference has been explained by the theory that the heat which is associated with cautery iron docking may result in third-degree burns resulting in the destruction of nociceptors within the surrounding vicinity, thereby causing a reduction in the pain perception experienced by piglets (Sutherland et al., 2008).

2.5.4.3 Adrenaline and noradrenaline

Adrenaline and noradrenaline are catecholamines. They were earlier characterized as the adrenal medulla emergency function, or commonly, flight or fight response (Cannon, 1914). The excretion of catecholamines is increased in stressful periods (Huskisson, 1974). The fight or flight response is regulated by the sympathetic nervous system. This system responds to an animal’s emergency, such as exposure to pain. Pain drives a response from the amygdala in the brain’s medial temporal lobe. This causes the hypothalamus to produce corticotrophin releasing hormone (CRH), which when transmitted to the anterior pituitary gland triggers the sympathetic nervous system (SNS) (Swift, 2018). The clinical effects of adrenaline and noradrenaline are shown in the following table.

Table 2.6: Physiological consequences of adrenaline and noradrenaline

System	Effect	Clinical consequence
Brain	Activation of amygdala	Heightened awareness
	Balance alteration between the frontal cortex and limbic control of micturition	Fear
		Incontinence and urges to urinate
Heart	Elevated heart rate	Increased blood pressure and heart rate
Blood vessels	Constricted flow of blood to the skin	Cold, pale skin
	Blood vessels to muscles become dilated	Increased capacity for exercise
Skeletal muscle	Rhythmic contraction	Shivering or shaking
		Chattering teeth

Adapted from Swift (2018)

2.5.4.4 Blood metabolites as pain indicators

Lactate and glucose

Painful procedures carried out on pigs resulted in an elevation of plasma lactate (Prunier et al., 2013). This may be a consequence of muscular glycogen catabolism. This catabolism may be due, in part, to the adrenaline surge which is as a result of a painful stimulus (Merlot et al., 2011). Plasma lactate has been shown to increase after some routine husbandry practices such as ear notching in pigs (Leslie et al., 2010). Castrated piglets have been shown to present higher blood lactate concentrations when compared to sham castrated piglets (Prunier et al., 2005). Contrary to the findings for ear notching and castrations, the blood lactate levels of piglets that had undergone tail docking and teeth resection were not shown to be different compared to sham treated piglets. This may imply that lactate is only produced during severe stress or pain (Leslie et al., 2010). Catecholamines, as previously discussed are produced in response to pain as it is a stressful event. This causes increased glycogenolysis and glycogen mobilization primarily from muscle tissue. This, in turn, increases glucose and lactate circulation and production (Landa, 2012).

Insulin and glycogen

Insulin resistance has been seen to accompany nociceptive or stressful conditions such as trauma, surgery, sepsis or burns (Thorell et al., 1994) These conditions are denoted by tissue injuries and elevated afferent input going to the central nervous system. This is inclusive of increased activities within the nociceptive pathways (Greisen et al., 2001). Surgical operations typically increase glucagon secretion by the pancreas and decrease insulin secretion, resulting in hepatic glycogenolysis (Muir, 2015). Insulin and glucagon have not been validated as pain indicators in piglet tail docking.

Thyroid hormones

There is suggestive evidence linking stressful procedures to a decrease in the central drive of the hypothalamic pituitary thyroid axis. However, the mechanisms of this stress-induced regulation are unknown (Helmreich and Tylee, 2011). Thyroid hormones thyroxine and triiodothyronine increasingly sensitise beta-adrenergic receptors in the heart, sensitising it to respond to the circulating catecholamines (Muir, 2015).

2.5.4.5 Haematology

White blood cells

White blood cells also referred to as leukocytes, are part of the immune system defence mechanism. Cell types included are lymphocytes, macrophages, basophils, neutrophils, monocytes and eosinophils. White blood cells biology is complex since their functions can be adaptive based on the environment such that they can have roles in both tissue healing and inflammation (King et al., 2018) When the animal is subjected to inflammation, neutrophils are the prevalent white blood cells. Macrophages and lymphocytes become more prevalent after 24 to 48 hours of the inflammation induction (Lingen, 2001). These changes in white blood cells numbers can be used as a pain assessment tool as the severity of stress and inflammation can be monitored. When tail docking was undertaken in piglets using different docking methods, white blood cells counts reduced when piglets were docked using blunt trauma or cautery iron as compared to the controls which were sham docked. This is shown as follows;

Figure 2.6: White blood cell profiles after tail docking

Source: Sutherland et al. (2008)

Figure 2.6 shows total white blood cell counts for piglets which were tail docked using cautery docking, blunt trauma docking or sham docked at 0, 30, 60 and 90 min post handling. White blood cell counts declined for cautery and blunt trauma docked piglets 30 minutes after docking(Sutherland et al., 2008). It has been suggested that this may be due to white blood cells trafficking owing to stress. It has been hypothesised that stressful periods induce white blood cells to move out from the peripheral blood into the skin, bone marrow and lymph nodes to prepare the animal for a potential assault (Dhabhar and McEwen, 1997). Further work in mice revealed that lymphocyte numbers reduced immediately following a 2-hour restraint test, and again after 30 seconds of handling. This suggests that white blood cells trafficking can take place immediately after acute stress.

Acute phase proteins

Acute phase proteins (APP) is a generic term for a group of about 30 biochemically and functionally active proteins (Bishara, 2012). The synthesis and subsequent circulation of these plasma proteins are regulated as a response to tissue injury, inflammation or infection. The generic name came about because C-reactive protein, which was the first such recorded, was initially discovered within the serum of patients who were suffering from the acute phase of pneumococcal pneumonia (Pepys, 1998).

The response of APP is termed the acute phase reaction. The reaction may be positive or negative, whereby the levels of the protein secretion in the serum is increased or reduced respectively. This occurs about 90 minutes after exposure to injury, inflammation, infection or stress (Bishara, 2012). The prime positive acute phase proteins which have been identified in pigs are C-reactive protein, haptoglobin, serum amyloid A and pig-major acute protein (Pig-MAP) (Candiani et al., 2008, Pineiro et al., 2007). As previously noted, different APPs have separate functions. C-reactive protein specializes in restricting the inflammatory response. Haptoglobin primarily functions in preventing iron loss by forming complexes which are stable with free haemoglobin. Serum amyloid A's role in host defence is undetermined in animals, whereas in human beings it has been noted to have important roles in processes such as inflammation, thrombosis, atherosclerosis, rheumatoid arthritis and amyloid-A amyloidosis (Heinonen et al., 2010).

C-reactive protein and haptoglobin have been shown to be good indicators of inflammatory stress in pigs (Eckersall et al., 1996). In an experiment to evaluate acute phase response in 6-day old piglets, the piglets were treated as docked or control (not docked). At 3 weeks post docking, there was no difference in the blood samples collected for the different treatments. However, at 7 weeks, there were higher concentrations of c-reactive proteins amongst the control group when compared to the docked group (Sutherland et al., 2009). This was supported by Heinonen et al. (2010) who demonstrated increased serum concentration of C-reactive protein, serum amyloid-Aand haptoglobin working as inflammation markers in tail bitten pigs. This suggests that C-reactive proteins could be validated as a useful pain assessment tool as it reported the presence of inflammation in tail bitten control piglets. In addition to the assessment of pain due to inflammatory stress, APP response has been reported in non-inflammatory events such as road transportation. C-reactive protein, haptoglobin, pig- MAP and serum amyloid-A increased while apolipoprotein A-1 concentration levels decreased after pigs were transported via the road within 24-48 hours (Pineiro et al., 2007)

Tear Staining

Tear staining is also referred to as chromodacryorrhoea, literally meaning a profuse production of coloured tears. Tear staining is characterised by reddish-brown secretions overflowing from the Harderian gland (Clement, 1994).

Tear staining is a validated, qualitative and non-invasive tool for the assessment of inflammation and stress levels in rats. This non-invasive method has been used for the identification of social stress in rats (Mason et al., 2004) and for identifying pain and inflammation in the same species (Harper et al., 2001) However, tear staining has not yet been validated as a pain and stress assessment tool in pigs. This is not to say the method has no potential for use as a pain marker. Pigs exhibit tear stains, but such stains have been historically attributed to environments that expose pigs to high dust or ammonia levels (Straw et al., 2006). Therefore, most tear staining studies relating to pigs have been focusing on tear straining as a measure of poor environment and not as a broader welfare assessment tool (Telkanranta et al., 2016).

With a view of broadening the use of tear staining, studies have been undertaken on pigs. As a result of these further studies, a weak correlation between tear staining and evidence of tail and ear bites has been reported (Telkanranta et al., 2016). To graduate tear staining into becoming a validated tool for pain and stress assessment in pigs, the tear staining method requires further investigation and testing on a wider range of stressors common to pigs.

2.5.4.6 Tachykinins

Tachykinins belong to a neuropeptide family. The members are made up of structurally similar peptides which are derivatives of alternate processing of three tachykinin genes (Steinhoff et al., 2014). Tachykinins are expressed in the immune system and throughout the nervous system, where they participate in physiological processes such as inflammation and nociception(Euler and Gaddum, 1931).

Tachykinin 1 (TAC 1)

TAC 1 is conserved in mammals and is involved in inflammation and pain transmission among other physiological roles (Borbely et al., 2013). While expression of the TAC 1 gene can be modulated by different stimuli, this gene encodes substance P, a neuropeptide which is also implicated in physiological processes concerning pain (Shanley et al., 2011).

Substance P

Substance P is a neuropeptide that is produced by the immune cells and other neuronal and non-neuronal cells (Suvas, 2017). Substance P is considered to have a role in neurotransmission and neuromodulation in the peripheral and central nervous systems, while also undertaking biological roles such as the regulation of inflammatory and immune responses (Krause et al., 1992, Suvas, 2017). The effects of substance P extend to the involvement in pain and stress integration (DeVane, 2001).

In piglets undergoing castration, there was no evidence of significant differences of Substance P levels when comparing castrated and sham castrated piglets (Sutherland et al., 2012) However, earlier work in castrated calves had shown increased levels substance P in the castrated calves when compared to non-castrated calves (Coetzee et al., 2012). More work is required to assess the role of substance P as a pain biomarker in swine.

2.5.4.7 Inflammatory gene expression and cytokines

Cytokines are small, hydro-soluble regulatory peptides or glycoproteins (de Oliveira et al., 2011) This is a general term and specific cytokine names include lymphokines, chemokines, monokines and interleukin. Lymphokines, monokines and interleukins are made by lymphocytes, monocytes, and leukocytes respectively while chemokines exhibit chemotactic activities (Zhang and An, 2007). Cytokines are released by a variety of cells for the modulation of cell to cell interaction and immune responses such as inflammation (Shubayev et al., 2010).

Inflammation is an immediate reaction to infection or injury. The characteristics of inflammation are pain, heat, swelling and redness (Ison et al., 2016). In case of inflammation or immune response, cytokines display pleiotropy, such that the same cytokine can be secreted by different cell types, or the same cytokine can exert action on a variety of cell types (Zhang and An, 2007). This is shown in Figure 2.7:

Figure 2.7: The cytokine network and a display of pleiotropy in cytokines

Source; Zhang and An (2007)

Figure 2.7 illustrates how a wide variety of cell types correlate their efforts to support the immune system. This includes macrophages, neutrophils, basophils, eosinophils, mast cells, T cells and B cells. These cell types have specific roles within the immune system and communicate with the other immune cells via the secreted cytokines.

Cytokines can be pro-inflammatory or anti-inflammatory. Pro-inflammatory cytokines are primarily produced by activated macrophages.

Pro-inflammatory cytokines

As implied by their name, pro-inflammatory cytokines have a role in upregulating inflammatory reactions. Examples of pro-inflammatory cytokines are interleukin 1, interleukin 6 (IL6) and tumour necrosis factor alpha (TNF-α) (Zhang and An, 2007).

Interleukin 1 alpha (IL-1α) and Interleukin 1 beta(IL-1β)

IL-1α and IL-1β are the 2 known types of IL-1. IL-1α is primarily associated with cell membranes, exerting its functions through cell contact, on the other hand, IL-1β is expressed in nociceptive neurones and produces systemic inflammation(Wolf et al., 2008) The expression of IL-β is enhanced following peripheral nerve injury and increases substance P production, amongst other roles (Yan et al., 1992) IL-β is predominantly released by macrophages and monocytes. Fibroblasts and endothelial cells, which are non-immune cells also release IL-β during injury or inflammation (Copray et al., 2001).

The biogenesis of IL-β has received wider coverage in literature compared to that of IL-1α, whose peculiar responsibility in the process of inflammation remains deficiently defined(Di Paolo and Shayakhmetov, 2016).

IL-6

IL-6 plays a major role in neuronal responses to nerve damage and in the regulation of the expression of neuronal peptides. IL-6 is a contributing factor in neuropathic pain behaviour development after injuries at the peripheral nerves (Ramer et al., 1998) Furthermore, it has been implicated in the induction of thermal hyperalgesia and tactile allodynia in nerve injured and intact rats respectively (Zhang and An, 2007).

TNF-α

TNF-α has a central role in pain models. Inflammation can be activated by nuclear factor kappa light chain enhancer of activated beta cells (NF-kβ), this process is regulated by TNF-α, giving TNF-α a huge role in inflammatory responses (Boka et al., 1994).

CXCL8 also known as IL-8

CXCL8, a chemokine referred to in older literature as IL8is a major mediator in inflammation (Turner et al., 2014). Chemokines are a family of chemotactic cytokines and are known promoters of differential leukocyte trafficking (Gangur et al., 2002). The structure of the chemokine family comprises of 4 amino acid residues which are highly conserved, 2 cysteines (C) residues and is a non-cysteine amino acid which structured between the cysteines. This brings about the CXC nomenclature (Joseph et al., 2015). CXCL8 has been implicated in inflammatory responses (Gangur et al., 2002), making it a pro inflammatory chemokine.

Anti-inflammatory cytokines

The immune system has control elements for immune regulation and these act together to direct the immune response. Anti-inflammatory cytokines are part of that system as they function to control the response of pro-inflammatory cytokines(Sultani et al., 2012, Zhang and An, 2007).

A number of anti-inflammatory cytokines have been reported, including but not limited to; interleukin 4 (IL-4), interleukin 10, (IL-10) and transforming growth factor beta (TGFβ).

IL-4 is produced by T cells, basophils and eosinophils and acts on and T and B lymphocytes. IL-4 has a number of important immunological roles (de Oliveira et al., 2011). With regards to its anti-inflammatory status, IL-4 is a suppressant in the synthesis of the inflammation inducing IL-1β. Additionally, IL-4 promotes the expression of interleukin 1 receptor antagonist (IL-1RA) which prohibits pro inflammatory cytokines; IL-1α and IL-1β from bonding to their receptors (Vannier et al., 1992) this effectively downregulate the expression of pro inflammatory cytokines.

Neuro endocrine tissues, neural tissues and immune cells synthesise IL-10 (de Oliveira et al., 2011). IL-10 controls the process of inflammation through the suppression of TNF-α, IL-1 and IL-6 among other cytokines produced by the macrophages and monocytes. Furthermore, IL-10 impairs the expression of the TNF-α receptor and stimulates its subsequent loss into the systemic circulation (Clarke et al., 1998) In essence, IL-10 works to lessen the potency of the inflammation reaction through downregulating pro inflammatory cytokines.

TGFβ obstructs the production of TNF-α, IL-1, IL-2 and IL-6. Aside from this, it triggers the production of IL-1RA which further antagonises the action of IL-1 (de Oliveira et al., 2011)

Summary: inflammatory gene expression

Exposure to nociceptive stimulation of a surgical nature results in an inflammatory response (Molony et al., 1995). Inflammatory reactions are an important tool in pain assessment, however, it should be noted that the extent of the inflammatory response is not necessarily proportional to the pain experienced (Wall, 1979). The inflammatory response is cytokine-dependent, both for the initiation and sustenance (Zhang and An, 2007).

2.5.4.8 Electroencephalography

Electroencephalography (EEG) is a neurological examination which is electrophysiological in nature as it makes use of an electronic device that measures and monitors electrical activities in the brain. EEG is customarily non-invasive as electrodes are arranged on the scalp. However invasive electrodes can be used when performing an EEG (Sheehy, 1984).

Traditionally it was accepted that the cerebral cortex had no role in processing pain, however, physiological and anatomical experiments in animals, coupled with neurophysiologic and functional neuro imaging in humans have revealed that the cerebral cortex is active in pain processing (Schnitzler and Ploner, 2000). The EEG has been found to be a reliable and safe method of pain assessment. The frequency of EEG is reportedly proportional to metabolic activities in the cerebral cortex, these activities can be altered in response to blood flow, changes in the availability of oxygen, anaesthetics and other pharmacological agents. This allows the electroencephalogram to represent cortical activity as it is recorded by changes in the frequency spectrums that are a result of the electrical activities of cortical neurons (Boveroux et al., 2008). Following this, a number of studies have reported that pain effects significant changes in EEG signalling within multiple regions of the brain(An et al., 2017, Jensen et al., 2013, Nir et al., 2010, Pinheiro et al., 2016).

EEG in relation to tail docking in piglets has been useful in pain determination. Piglets which were docked 2 days after birth showed modicum EEG response to tail docking when compared to piglets that had been docked at 20 days of age. Those docked at 20 days of age showed significant EEG response (Kells et al., 2017).

2.5.4.9 Infrared thermography

Infrared thermography is a non-invasive method of measuring the activity of the autonomic nervous system. This method precisely measures infrared radiation that is emitted by an object. This allows the determination of surface temperatures based on relatively simple laws of physics (Mccafferty, 2007). The information from this technique is called a thermogram and is presented in the form of a picture. Individual pixels of the thermogram represent the surface temperatures which have been measured. The thermogram may be in grey tones or may come out in colour. White or red depict the warmest areas, while blue or black highlight the coolest areas (Eddy et al., 2001).

Infrared thermography was used to successfully detect septic arthritis in the metatarsophalangeal joint in a Friesian heifer. When compared to the healthy limbs, the diagnosed one presented higher temperatures within the metatarsophalangeal joint (Dopfer et al., 2012). Infrared thermography has been applied in cattle to analyse limb and foot disorders. The results revealed higher temperatures in the lame limbs when compared with the sound limbs (Nikkhah et al., 2005). The same temperature pattern was recorded in a study with piglets undergoing castration, with and without (control) analgesia. Infrared thermography measured the ear temperature of piglets 24 hours after castration. The temperature in control piglets were found to be significantly higher than that of piglets which had been given a combination of lidocaine and meloxicam or just lidocaine (Hansson et al., 2011).

Infrared thermography is an advantageous tool as it is noninvasive and highly sensitive, making it useful when used with biomarkers in the detection of pain. However, it should be noted that Infrared thermography needs to be used where there is no exposure to wind drafts and direct sunlight. Furthermore, the coat of the study animal should not have moisture, dirt or foreign materials. Moisture promotes heat loss to the environment while dirt changes conductivity and emissivity of the coat, giving false measurements (Palmer, 1981)

2.6 Anaesthesia and analgesia for painful husbandry processes

Pain detection, measurement and analysis have been discussed in the preceding sections. Major concerns in the management of pain in farm animals are the challenges of recognizing, quantifying and evaluating the pain experience of individual farm animals without disturbing the whole group (Giorgi et al., 2016). For the management of pain to be effective, there is a need for sufficient anaesthesia and analgesia medication protocols(Anil et al., 2005). This highlights the importance of objectively assessing pain so that the correct dosages of anaesthesia and analgesia can be used.

Anaesthetics and analgesics are pharmacological agents that are used for pain reduction. Anaesthesia suggests numbness to sensation while analgesia suggests removal or a marked decrease in the pain sensation. Therefore, conclusively, anaesthesia supports analgesia(Schulz et al., 2007) The analgesic pharmacological agents which are accessible for use in pigs are inclusive of local anaesthesia (LA), Nonsteroidal Anti-inflammatory drugs (NSAIDs), glucocorticoids, opioids and alpha-2 agonists(Bradbury et al., 2016, Sutherland et al., 2008). A highly functional approach to addressing pain in pigs is the use of LA and NSAIDs in combination. This is a highly effective form of analgesia as it seeks to manage both inflammatory and acute pain pathways (Mellor and Stafford, 1999). Notably, anaesthesia requires a veterinarian and is therefore not practical for routine (on-farm) painful husbandry procedures.

2.6.1 General anaesthesia

General anaesthesia is a medically induced state characterised by loss of consciousness added to sensational and protective reflex losses. This state is induced to allow the undertaking of a painful procedure in a way that minimizes painful sensations. The drug in use may be injectable or inhaled and acts upon the central nervous system (Carroll and Hartsfield, 1996).

2.6.2 Injectable anaesthesia

Injectable anaesthesia is usually administered intravenously. In a study by McGlone and Hellman (1988), 18 piglets aged 14 days old were given general anaesthesia by ketamine hydrochloride, xylazine and glyceryl guaiacolate. Five died within 24 hours of general anaesthesia administration. This may have been because the piglets were not yet physiologically mature to completely catabolise or metabolise the anaesthetic. On the other hand, the deaths may have been caused by malpractice during the injections. The same experiment was repeated with 6-7week old piglets. The piglets which were injected with general anaesthesia had behavioural anomalies such as decreased nursing time and increased lying down time. The behavioural anomalies lasted for 6-8 hours. Conversely, a similar trial was conducted in 8 week old piglets and concluded that the intravenously administered general anaesthesia did not result in any behavioral anomalies in regards to nursing and lying times (McGlone et al., 1993) This difference in results may be attributed to the differences in age, therefore possible differences in physiological maturity to handle the effects of the general anaesthesia, or perhaps the dosages and drugs used were not uniform and resulted in different reactions.

2.6.3 Inhalation anaesthesia

Inhalation anaesthesia has some advantages compared to injectable anaesthesia. It requires a shorter induction period and less animal contact as it does not require intravenous injections. Inhalation anaesthesia also has short term effects and offers reversibility (Rault and Lay, 2011). The disadvantages of inhalation anaesthesia include the fact that most of these drugs are controlled and require special instruments, making it impractical for continued farm use. Furthermore, the inhalation anaesthesia may result in increased operational costs, and intensity of the handling period and behavioural stress during painful husbandry practices (Rault and Lay, 2011).

A number of inhalation anaesthesia agents have been used in a bid to reduce pain in piglets undergoing painful management practices. When isoflurane was used during piglet surgical procedures, it was reported that there was a reduced pain reaction in the anaesthetised piglets, however, there were no differences in pain biomarkers such as endorphin and ACTH (Walker et al., 2004). Contrary to these findings, isoflurane was deemed ineffectual as a pain management drug in piglets, especially at castration (Schulz et al., 2007).

Carbon dioxide has also been used as an inhalant. Sutherland et al. (2012) concluded that carbon dioxide as an inhalant did not significantly reduce the pain experienced by piglets after surgical procedures. Carbon dioxide as an inhalant was applauded for the speed at which it can be administered, the ease of piglets regaining consciousness and the unrestricted nature of the gas (Sutherland et al., 2012). However, the same gas was dismissed as an ineffective method that increases stress-related behaviour in piglets (Zimmermann et al., 2011).

2.6.4 Local anaesthesia

Analgesics that are both local and topical are commonly used during painful animal husbandry practices. Local anaesthesia works by obstructing the depolarization and conduction of nerves through sodium channel impediment (Anderson and Muir, 2005). One of the most commonly used local anaesthetics is lidocaine. Lidocaine is known to stabilize membranes while abolishing the capability of an electrical stimulus from reaching the central nervous system. The contributing factors to the popularity of lidocaine are that it is known to have limited toxicity and it is not expensive (Edmondson, 2014). Lidocaine is reported to reduce pain responses when administered to piglets prior to castration. Piglets treated with lidocaine had fewer escape attempts and lower vocalization frequencies in comparison to control piglets which had not been exposed to lidocaine (Kluivers-Poodt et al., 2013). Furthermore, pigs that were given lidocaine prior to castration were reported to nurse for a similar duration with the sham treated piglets, which had not undergone castration, while those piglets that were castrated without lidocaine were reported to nurse 8.5% less for a day after castration. In addition, lidocaine was found to reduce pain behaviour indices such as huddling and lying (McGlone and Hellman, 1988).

Topical anaesthesia can potentially decrease double piglet handling through reduced needle use. Other examples of local and topical anaesthesia are tetracine hydrochloride, cetacaine, butamben and benzocaine which are all short acting, as well as the long acting ones; bupivacaine, adrenaline, cetrimide and ligocaine. Of note, bupivacaine takes longer for the onset of action and retains a longer activity duration(Sutherland et al., 2010)

Long and short acting topical anaesthesia applied after castration were reported to be ineffective in the reduction of pain-induced stress in piglets. However, the piglets that did not receive any anaesthesia presented more pain behaviour such as lying in isolation when compared to anaesthetised piglets (Sutherland et al., 2010). Providing anaesthesia before castrating piglets may be more effective in reducing pain associated with castration.

2.6.5 Nonsteroidal Anti-inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs), are a group of drugs with anti-inflammatory, antipyretic and analgesic properties. NSAIDs are able to reduce inflammation through the inhibition of cyclooxygenase (COX) which is an enzyme (Hudson et al., 2008). COX is a glycol and haemo protein which is membrane-bound and primarily found in prostanoids forming cells, specifically in the endoplasmic reticulum. When NSAIDs are administered, the formation of prostaglandins is inhibited, thereby reducing pain stimuli to the brain (Vane and Botting, 1998). NSAIDs are primarily COX inhibitors, however, they exhibit secondary anti-inflammatory, analgesic mechanisms (Cunningham and Lees, 1994).

NSAIDs like aspirin activate lipoxin synthesis. Lipoxin formation triggers leukocyte downregulation through prevention of neutrophil adhesion, thereby impeding the release of cytokines (Gewirtz et al., 1998).

NSAIDs commonly used in the pig industry include; ketoprofen, flunixin meglumine, meloxicam and tolfenamic acid. Early research with NSAIDs saw the use of paracetamol and orally administered aspirin. The aspirin did not show significant effects in reduction of pain, while paracetamol showed positive results through reduced physiological pain biomarkers such as blood pressure, heart rate and temperature. However, a disadvantage of paracetamol is its short half-life of 62 minutes compared to the 480 minutes of meloxicam half-life (McGlone et al., 1993, Reyes et al., 2002).

When ketoprofen was administered before painful husbandry procedures, plasma cortisol was reduced compared to those that were not provided ketoprofen before a procedure (Cassar et al., 2014)

Flunixin meglumine has been reported to have antipyretic effects in piglets. Furthermore, it has been noted to reduce plasma cortisol levels and piglet vocalisations when administered before a painful procedure versus non-administration. However, in the case of pain that results in wounds or lesions, administration of flunixin meglumine has been shown to delay wound healing (Reiner et al., 2012).

In a survey, 92.3% of veterinarians prescribed meloxicam while 41% of farmers used this drug for pain management in pigs (Ison and Rutherford, 2014). Meloxicam has been shown to be an effective pain reliever in pigs(Friton et al., 2003). After treatment with meloxicam, lame pigs had improved lameness scores and feed intake, with reduced signs of depression when compared to the control group that was not provided meloxicam. However, in terms of physiological biomarkers of pain, meloxicam showed no difference in haptoglobin levels when compared to a control (Friton et al., 2006, Keita et al., 2010)

2.6.6 Glucocorticoids

Glucocorticoids are hormones which are secreted into the blood after synthesis in the adrenal cortex (van der Velden, 1998). These corticosteroids have the ability to inhibit a wide variety of humoral and immunologic cellular reactions. Examples are hydrocortisone, dexamethasone and cortisol, all of which strongly inhibit 1L-1β, TNF-α, IL-1 through to IL-6, IL-8, IL9, IL-12, IFN-γ and the synthesis of granulocyte macrophage colony stimulating factor (Bednarek et al., 1999). Inhibition of pro-inflammatory cytokines promotes the reduction of inflammation. Another way with which the inflammatory response is affected by glucocorticoids is through the inhibition of phospholipase A_2 activity which is required for arachidonic acid release. Without arachidonic acid, prostaglandins are inhibited (Vane and Botting, 1998). This results in lesser chances of pain and inflammation as prostaglandins work to magnify pain responses like inflammation.

2.6.7 α_2 agonists

α_2 agonists activate the α_2 adrenoreceptors in the peripheral and central autonomic nervous system. The agonists evoke a negative effect on noradrenalin release and sympathetic activity, culminating in sedation and analgesia (Hudson et al., 2008). The most common α_2 agonist is xylazine. The use of these drugs for analgesia in animals provokes side effects such as reduced respiratory rate, reduced cardiac output, depressed gastrointestinal motility and relaxation (Coetzee, 2013).

2.6.8 Opioids

Opioids offer analgesic effects through binding to the spinal and supraspinal mu (μ), sigma (σ) and kappa (k) receptors. This binding is responsible for the activation of receptor linked potassium channels, while obstructing voltage gated calcium channels, leading to a decrease in the generation of nociceptive signals (Coetzee, 2011). One of the most common opioids in the pig industry is fentanyl, which is synthetic in nature and between 75-100% more potent when compared to morphine but has short half-lives (Harvey-Clark et al., 2000). Short half-lives are a disadvantage as there arises a need to repeatedly restrain the pigs and administer the opioid, which may lead to exacerbated stress behaviour.

2.6.9 Challenges in the use of anaesthetics and analgesics during painful procedures

There are several constraints associated with the provision of anaesthesia in livestock. Farmers will be more open to using a drug that takes less time to take effect while having a long half-life (Mellor and Stafford, 1999). When a drug takes a long time to take effect during a routine procedure such as tail docking, animal processing is slowed down, resulting in longer working hours and subsequently higher labour costs. This presents a disincentive to the farmers, causing them to avoid pre-emptive analgesia routines (Coetzee, 2011). A drug with a short half-life offers a constraint as it requires repeated administration for maximum effectiveness. This becomes strenuous to the drug administrator, increases labour costs increases animal distress and increases the costs through drug use as it has to be used frequently. Furthermore, there is the risk that dosages are missed, and therefore animal welfare will be compromised.

Analgesics that require the presence of a veterinarian are a challenge and a barrier to on-farm use. The presence of these specialists for procedures that occur frequently on a farm is not economically viable.

The pig industry has a number of licenced products for use during painful procedures. Some of them are NSAIDs which contain active ingredients such as meloxicam, flunixin meglumine, ketoprofen, tolfenamic acid, the glucocorticoid dexamethasone and mild paracetamol (Coleman et al., 1998). These products are under the classification of restricted veterinary medicine (RVM) meaning they should be prescribed by a veterinarian after assessing the animals (Ison and Rutherford, 2014). This factor is a challenge as it requires paying for the services of a veterinarian each time an animal may require analgesia.

Another challenge is the extent of knowledge of toxicity and pharmacokinetics of the analgesics in use. If farmers do not have full knowledge of these matters, there is bound to be a reluctance to take up the use of analgesics. Lidocaine is a common livestock local anaesthetic but is known to cause toxicity in goats at certain plasma concentrations (Venkatachalam et al., 2018). To counteract this challenge, there should be more research towards toxicity and pharmacokinetics of the commonly used drugs to ensure farmer confidence and uptake of analgesic routines.

The general public are increasingly concerned about animal welfare and are particularly interested in food producing animals and pain management during husbandry procedures (De Vito, 2015). Sensitive consumers and animal welfare professionals have encouraged pain alleviation drugs to be provided to animals undergoing painful husbandry procedures. A further limitation to this is concern about drug residues in meat, and currently, there is limited information on the withholding periods (WHP) of anaesthetics which are characterised by amnesia, analgesia and catalepsy. The WHP for some opioids are also relatively unknown (Cornish et al., 2016).

This study seeks to explore the potential for alternative pain relievers, which are not only safe for consumers but provide analgesia that meets the requirements of animal welfare stipulations without the need for a veterinarian and the accompanying costs.

2.6.10 Concluding remarks

After going through literature, it can be concluded that tail docking causes pain and this was evidenced through behavioural and physiological indices of pain. However, the physiological indices of pain in pigs have been limited to hormones, blood metabolites, infrared thermography and electroencephalography. Currently, there exists a dearth in literature as there is no research done on pigs to quantify pain through inflammatory gene expression, which is a physiological pain index that has been researched in cattle. Furthermore, through the pain behavioural studies and pig husbandry survey, this research seeks to add to the existing literature that links pig husbandry procedures to the incidence of tail biting.

CHAPTER 3

Materials and methods

3 Materials and methods

3.1 Pig husbandry survey

A survey was undertaken to review the incidence of tail biting against the pig husbandry protocols in use on commercial pig farms. The survey was conducted through the Qualtrics online survey platform. The aim of the survey was explained, and participation was voluntary.

A link to the survey was sent via an email list to commercial New Zealand pig producers. The link was sent to farmers on three successive weeks. A total of 97 pig farmers are on the list. The survey was closed one week after the last email was sent.

The survey consisted of 15 questions which had a combination of multiple choice and open-ended questions. The survey was laid out as follows;

- Tail docking- whether it is carried out, at which age it is carried out, the tools used for docking and the length of tail that is removed
- Tail biting- occurrence of the incident
- Housing conformation- the housing system for sows, whether there is gender mixing in production pens, production pen sizes and flooring systems
- Use of manipulable substances like straw
- Feeding- type of feed and the feeding system used

The complete survey questionnaire is presented in Appendix 1 in the appendices section.

3.2 Pilot study

The Massey University Animal Ethics Committee reviewed and approved the animal procedures for this project (MUAEC 17/106 and 18/43). The project was conducted on a commercial pig farm in Whanganui, located on the West coast of New Zealand’s North Island (39.9° S, 175.1° E). Whanganui experiences a temperate climate and approximately 900mm of rainfall annually and an average temperature of 15^{o}C with humidity of 75%.

The objective of the experiment was to determine the efficacy of a topical anaesthetic on the behavioural responses of piglets to tail docking. A pilot study was carried out to determine an appropriate method of topically applying a local anaesthetic to piglet tails before docking and to determine the length of time taken for loss of sensation in the area upon which the formulation was applied. A litter of 12 piglets was used for these determinations. The piglets were removed from the sow and shut in the creep area.

The formulation used was Lignocaine and Articaine (LA) cream. The LA cream was a novel formulation which is confidential at this stage. The active ingredients were at a 4% concentration. Approximately 100mg of the LA cream was applied to the tail of each piglet, approximately equating this to 4mg per piglet. One person restrained a piglet, while another person applied the LA cream to the first two thirds of the tail. The LA cream has been previously shown to permeate goat skin, of which that data is withheld as it is beyond the scope of the current project. To prevent evaporation of the drug, it is necessary to occlude the tail, therefore a cotton swab and a layer of VetWrap was used.

In the absence of an algometer, a needle was used to poke the pigtails every 10 minutes. As the pain response provoked by the needle could not be measured technically, the trained pain behaviour observer determined the point in time at which the needle could no longer elicit a notable noxious reaction. This time point was 40 minutes after the application of the cream. During this test to determine at which time the cream would have taken effect, one person restrained the piglet under one arm. Two fingers from one hand gently held either side of the tail, while the tail was poked with a blunt ended needle using the other hand.

3.3 Animals and docking procedure

The study was conducted on a 480-sow farrow to finish commercial pig farm in New Zealand. The farm routinely carried out tail docking and iron injection administration 2-3 days after birth. The type of tail docking done on the farm was hot docking, using gas heated cautery clippers. The docking procedure removed one third to half of the tail. The experimental piglets were the progeny of Large White and Landrace cross sows. The temperature in the farrowing room was maintained at 24^0C while the creep area was maintained at 33^0C using under floor heating and infrared lamps.

A total of 9 litters with 12 piglets per litter were randomly allocated to 3 treatment groups (3 litters per group):

(i) Application of LA cream before docking; referred to as the before group.

(ii) Application of LA cream after docking; referred to as the after group.

(iii) Docking with no cream; referred to as the control group.

To carry out the docking, individual litters were processed at different times. Piglets from the same litter were removed from the sow at the same time and confined to the creep area. For the docking procedure, one person restrained the piglet under one arm. Two fingers from one hand gently held either side of the tail, while the other hand docked the tail, approximately cutting off one third to one half of the tail with a gas heated cautery iron cutter. For the before group, the bandaging around the tail was removed before docking as per the described method.

For the after group, immediately following docking, the tail stump was completely covered with the same amount of cream applied to the before group. After application of the LA cream in the after group, there was no bandaging as with the before group. Since the cream was applied on the wound it would penetrate faster through the compromised skin (broken skin), minimizing chances of drug evaporation, thereby cancelling the need to cover the tail with a bandage.

The control group was also docked as described. After docking, piglets in each litter were marked with a number between 1 and 12 for identification purposes during behavioural observations.

3.4 Behavioural study

The behaviour of piglets during docking was scored by one trained person who recorded whether the piglets vocalised during the procedure (Rand et al., 2002) and how many vocalisations were emitted. The incidence of escape attempts (Marchant-Forde et al., 2009) and number of escape attempts were also recorded. The docking procedure and the resulting behaviours during and after docking were video tapped using a Sony handycam digital video camera recorder. The video cameras were used to record the footage after docking to allow 30 minutes after the docking of the last piglet. Scoring at each time point was based on whether or not piglets performed a particular behaviour at each time period.

Piglets were returned to the creep after docking in all treatments. Upon completion of the video recording, piglets were returned to the sow. The behaviour of the piglets was not measured or recorded prior to tail docking as the objective was to compare behaviour between treatments, not before and after docking.

A trained person who was not blind to the treatments reviewed the video footage and recorded post docking behaviours via scan sampling at set time points across all 9 litters at 0 (immediately after docking) 4, 7, 20 and 30 minutes after docking. These time points were selected randomly. At each time point, the behaviour of each piglet within a litter was recorded. An ethogram of the behaviours recorded after docking is shown in Table 3.1, which includes four types of behaviours which are associated with behavioural displays that indicate an animal is in pain. These behaviours are; sitting, scooting, lying prostrate and tail wagging. These behaviours were pooled in the data and classified as pain behaviour to avoid repetitions in the descriptions of behavioural types.

Table 3.1: Ethogram for piglet behaviour after tail docking

Behaviour	Description	Behavioural type	Literature
Vocalisation	Screams and grunts, showing deviation from normal communication vocals	Pain	(Herskin and Di Giminiani, 2018)
Escape attempts	Bodily movement such as jerks, kicks and wriggling carried out to attempt an escape	Pain	(Kluivers-Poodt et al., 2013)
Scooting	The caudal part of the body gets dragged across the floor	Pain	(Sutherland et al., 2008)
Lying without contact	Lying in a recumbent position, on one side or on the belly and not in contact with other pigs	Social anomaly	(Leslie et al., 2010, Sutherland et al., 2008)
Lying with contact	Lying in a recumbent position, on one side or on the belly and in contact with other pigs	Normal	(Leslie et al., 2010, Sutherland et al., 2008)
Lying prostrate	The body is stretched out while the head is in a position below the shoulders	Pain	(Hay et al., 2003)
Walking	Fairly low speed movement whereby leg action is the propulsion force	Restlessness	(Sutherland et al., 2008)
Sitting	Resting on caudal part of the body	Pain	(Herskin et al., 2016)
Tail wagging	The tail is moved about 1cm to either side in quick motions	Pain	(Herskin et al., 2016)

3.5 Physiological study; blood sampling for gene expression study

The LA cream application and the docking protocol for the behaviour study were repeated for the gene expression study. A 2 ml blood sample was collected from either the brachial or saphenous vein using a 24-gauge needle and stored in heparinised vacutainers on ice. Samples were collected from one litter of 12 piglets at the following time points: immediately before docking and at 2, 4 and 24 hours post docking. These 12 piglets were randomly allocated into 4 groups of 3 to represent before, after and control treatments.

The gene expression methodology following blood collection from the piglets is as follows;

RNA extraction and purification

One ml heparinised blood was aliquot into a 50 ml conical tube containing10 ml pre-warmed tris-buffered ammonium chloride (TAC) buffer (46 mMTris-Cl, pH 8.1, and 1 mM CaCl2), incubated at 37°C for 10 min and centrifuged at 350xg for 7 min at room temperature. The supernatant was discarded and the leucocyte pellet re-suspended in 0.5 ml RNeasy Lysis Buffer (RLT buffer, catalogue # 79216,Qiagen GmbH, Germany),Total RNA in the lysates purified using QIAamp RNA Blood Mini Kit (catalogue # 52304, Qiagen GmbH, Germany) and quantity and quality assessed using Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). RNA concentrations in the samples were adjusted to $\geq$50 ng/µl and stored at -80°C.

Enumeration of gene-specific RNA

Detection and enumeration of RNA specific to 24 genes (21 of interest and 3 references) were performed using a multiplexed digital gene expression assay (nCounterPlexSet, NanoString, Seattle, USA). Fifteen RNA samples (from blood collected at different time-points) pertaining to the 8 animals were analysed using a custom-designed probe panel for 24 genes. Details of the genes, their Gene bank accession numbers and position of target sequences are shown in Table 3.2. PlexSet technology enables simultaneous amplification-free gene expression assay for up to 96 RNA targets in a given sample. Further, it enables processing up to eight RNA samples in a single lane on the nCounter cartridge. The assay was performed as per the manufacturer’s protocol outlined in ‘PlexSet Reagents for Gene Expression User Manual’, MAN-10040-04 February 2018 (NanoString, Seattle, WA, USA). In brief, optimal RNA amount for the actual expression assay was standardised by an initial titration assay. A 200 ng RNA per sample was found to be ideal. Subsequently, probe-RNA hybridization reactions (22 hours at 65°C) were set up and samples pooled (a set of eight into one tube). Excess probes in the samples were removed using a 2-step magnetic bead-based purification and samples were transferred on to a cartridge. This step was performed in a robotic nCounter Prep Station (NanoString), using the high-sensitivity protocol. Subsequently, the RNA-probe hybrids immobilised on the surface of the cartridge were counted by scanning the cartridge on the nCounter Digital Analyzer (NanoString) with maximal sensitivity (555 fields of view, FOV).

Table 3.2: Details of gene analysis

Gene	Gene category	Genebank accession	Probe target sequence position
CXCL8	Pro-inflammatory cytokine	NM_213867.1	421-520
IL1B	Pro-inflammatory cytokine	NM_214055.1	544-643
IL1A	Pro-inflammatory cytokine	NM_214029.1	1086-1185
IL6	Pro-inflammatory cytokine	NM_214399.1	332-431
TNFa	Pro-inflammatory cytokine	NM_214022.1	1119-1218
IFNG	Pro-inflammatory cytokine	NM_213948.1	427-526
IL17A	Pro-inflammatory cytokine	NM_001005729.1	583-682
TAC1	Neuroactive-ligand-receptor	XM_003130164.6	312-411
NGF	Neuroactive-ligand-receptor	XM_021089997.1	23-122
CCK	Neuroactive-ligand-receptor	NM_214237.2	338-437
PTGES	Neuroactive-ligand-receptor	NM_001038631.1	744-843
PTGS2	Neuroactive-ligand-receptor	NM_214321.1	1342-1441
CGRP	Neuroactive-ligand-receptor	XM_021086359.1	379-478
NOS1	Neuroactive-ligand-receptor	XM_021072698.1	2743-2842
AGTR2	Neuroactive-ligand-receptor	XM_005673847.3	1323-1422
CRH	Neuroactive-ligand-receptor	NM_001113062.1	615-714
PLA2G3	Secreted phospholipase A2	XM_021072944.1	999-1098
IL24	IL10 family of cytokines	XM_021063319.1	2016-2115
IL4R	Interleukin-4 receptor activity	NM_214340.1	972-1071
OPRM1	Mu opioid receptor	NM_001001538.1	854-953
NPY	Peptide-ligand-binding	NM_001256367.1	421-520
YWHAZ	Reference	NM_001315726.1	430-529
B2M	Reference	NM_213978.1	250-349
GAPDH	Reference	XM_021091114.1	426-525

Processing mRNA expression data

Tabulated data in comma separated value (CSV) format, obtained from the nCounter Digital Analyzer as reporter code count (RCC) file, was input into nSolver Analysis Software, version 4.0 (https://www.nanostring.com/products/analysis-software/nsolver) and analysed as per "All About PlezSet Technology Data Analysis in nSolver Software" manual, MAN-10044-02 2017 (NanoString, Seattle, USA). The reporter library file containing the CodeSet information specific for the genes in this study was used to undertake the quality control routine. Default quality control settings were used: an Imaging QC (a measure of the percentage of requested fields of view successfully scanned in each cartridge lane) of 75 and a Binding Density (a measure of reporter probe density in each cartridge lane) range of 0.1 to 2.25 were used. All samples passed the quality control. Positive control normalisation of RNA counts was then performed using the geometric mean of the top 3 positive controls included in the PlexSet assay. Finally, biological normalisation of gene-specific RNA counts was undertaken based on the RNA counts of the chosen three mRNA reference genes (Table 3.2) and the final counts were exported into an Excel workbook.

3.6 Statistical analysis

The data were tested for normality using the univariate procedure of SAS version 9.4. For the survey, some descriptive statistics were done. For the behaviour during docking, the following variables from piglet responses to docking were calculated:

(i) Response to docking by vocalisation (yes or no) and the number of vocalisations per piglet

(ii) Response to docking by making escape attempts (yes or no) and the number of escape attempts made

The number of vocalisations and the number of escape attempts were not normally distributed and were analysed using non-parametric tests; Kruskal-Wallis, PROC NPAR1WAY, (SAS 9.4). The behaviour variables were: vocalisation, attempt to escape during docking, lying in contact, lying in isolation, lying prostrate, tail wagging, sitting, scooting and walking after docking and were recorded as binomial variables. These are as presented in Table 3.1. A linear model with treatment and time and their interaction was fitted to these categorical data (PROC GENMOD, SAS 9.4). The resulting logit Least Square means were back transformed into percentages of various behavioural outcomes. This was done using the formula (exp (x) / (1 +

exp (x)). Male and female pigs were used in the study, the piglet was the experimental unit and only the treatment effect was included in the model.

CHAPTER 4

Results

4 Results

4.1 Survey outcome

Responses to the survey came from Canterbury, Manawatu/Wanganui, Wellington, Auckland and Waikato regions. The survey outcome was not statistically analysed due to low response. The results are shown in Table 4.1.

Table 4.1: Survey outcome

	Responses 1	2	3	4	5	6	7
Do you carry out tail docking	Yes	Yes	Yes	No	Yes	Yes	Yes
At which age/ days after birth	1,2,3,4	3	1	N/A	2	2	2
Method for docking	Clippers	Clippers	Cautery	N/A	Clippers	Clippers	Cautery
What length is removed	More than 1/3 to 1/2	less than 1/3 to 1/2	No response	N/A	More than 1/3 to 1/2	1/3 to 1/2	less than 1/3 to ½
Have you observed signs of tail biting	Yes	No	Yes	Yes	Yes	Yes	Yes
Tail biting nature	Intermittent	N/A	Consistent	Intermittent	Intermittent	Intermittent	Intermittent
Housing for farrowing and lactating sows	Outdoor hut	Outdoor hut	Pen	Outdoor hut	Crates	Crates	Crates
Predominant flooring system post weaning	Solid floor	Outdoors	Partially slatted	Outdoors	Fully slatted	Partially slatted	Fully slatted
Do you separate males and females post weaning	Separate	N/A	Separate	Mixed	Separate	Separate	Separate
If single sex, which gender has more biting prevalence	Cannot recall	N/A	Cannot recall	N/A	Cannot recall	Cannot recall	Males
If mixed sex, which gender has more biting prevalence	N/A	N/A	N/A	No response	N/A	N/A	N/A
Do you provide straw or other manipulable objects	Straw	N/A	Molasses block	Straw	Salt blocks	Straw	Straw
Do you provide deep litter bedding	Straw	Straw	No	Straw	No	Straw	No
Form of feed post weaning	Dry feeding	Dry feeding	Dry feeding	Mozzarella cheese	Dry feeding	Dry feeding	Wet feeding
Feeding method used	Adlib automated	Adlib hand	Adlib automated	Adlib hand	Adlib automated	Adlib automated	Adlib automated

One out of seven respondents indicated that they do not carry out tail docking on their farm. The age at which docking is mostly carried out was 2 days old as is seen in Table 4.1.

Four out of six respondents who practice tail docking made use of clippers, while two out of six made use of cautery irons. The length of the tail removed varied, with an equal number of respondents removing more than one third to half of the tail, and less than one third to half of the tail. Of the seven respondents, only one reported a lack of tail biting incidents on the farm, furthermore, of the six who have experienced tail biting, five farmers reported intermittent biting while one farmer reported consistent tail biting within the farm.

One farmer used pens for housing farrowing sows while an equal proportion used either outdoor housing or farrowing crates. One farmer reported solid floor housing for post weaning production, two farmers reported using fully slatted housing, two farmers reported partially slatted housing, while two reported that pigs were kept outdoors post-weaning.

Separation of weaners based on gender was not applicable to one farmer who transferred the weaners to a different producer. Only one out of the six applicable farmers reported keeping mixed batches.

One respondent could say for certain that the gender which had the most prevalence of tail biting was the males, the other respondents could not recall.

All the six applicable respondents provided manipulable materials. Four farmers provided straw and the remaining two provided either molasses or salt blocks. Four out of seven respondents provided deep litter bedding in the form of straw, while the remaining three farmers did not provide deep litter bedding. The form of feed post weaning was mostly dry feeding. This is shown in Table 4.1.

Five out of seven respondents provided dry feeding, while one farmer provided Mozzarella cheese and the other provided wet feeding. The most common feeding method used was adlib automated feeding.

4.2 Behavioural responses during tail docking

The treatment levels comprised of LA cream before docking (before), LA cream after docking (after) and docking without LA cream (control). The treatment levels had highly significant effects (P<.0001) on the dependent variables recorded during docking. These variables were vocalisations and escape attempts. The use of LA cream before docking led to a reduced percentage of vocalisation and escape attempts during docking. The control group and after group did not significantly differ in terms of vocalisations and escape attempts during docking as there was no differentiating factor between treatment groups at this stage.

Piglets in the before group had the least percentage of vocalising during docking treatment, with a percentage of 44% (Table 4.2). The piglets in the control group and the after group were the most likely to vocalise during docking at 92% and 97% respectively. The before group was significantly different from the other two levels (P<0.0001). Control and after groups were not different in terms of piglet vocalisation responses to docking.

Table 4.2: Percentage for vocalisation during tail docking in piglets

Treatment	Mean	SE	Percentage[1]	Chisq	P
Control	2.3979 ^{a}	0.603	92	35.16	<.0001***
Before	-0.2231 ^{b}	0.3354	44		
After	3.5553 ^{a}	1.0142	97		

^{a,b} Least square means across a row with different superscripts are significantly different between treatments, P<0.05

[1] Percentage value was derived through logit back transformation

*** p<0.0001, the sources have highly significant effects on the dependent variables

The percentage of piglets performing escape attempts during tail docking was 31% in the before group, and this was significantly lower compared to the control group (83%) and after group (72%) (P<0.0001) (Table 4.3). There was no significant difference in the percentages of performing escape attempts between the control and after groups.

Table 4.3: Percentage for escape attempts during tail docking in piglets

Treatment	Mean	SE	Percentage[1]	Chisq	P
Control	1.6094 ^{a}	0.4472	83	24.1	<.0001***
Before	-0.821 ^{b}	0.3618	31		
After	0.9555 ^{a}	0.3721	72		

^{a,b} Least square means across a row with different superscripts are significantly different between treatments, P<0.05

[1]Percentage value derived through logit back transformation

*** p<0.0001, the sources have highly significant effects on the dependent variables

The mean number of vocalisations and the number of escape attempts during tail docking illustrates that piglets in the control and after treatments vocalised more and performed more escape attempts during tail docking compared to piglets in the before treatment (P<0.0001). This is seen in Table 4.4.

Table 4.4: Mean number of vocalisations and escape attempts by piglets during tail docking

Variable	Treatment	Mean score	Df	Chisq	P
Vocalisation	Control	0.682	2	40.34	<0.0001***
	Before	0.159			
	After	0.659			
Escape attempts	Control	0.691	2	24.41	<0.0001***
	Before	0.241			
	After	0.568			

*** p<0.0001, the sources have highly significant effects on the dependent variables

4.3 Post docking behavioural responses

The post docking behaviours observed recorded were walking, lying in contact, sitting, lying in isolation, and pain-related behaviours. Pain related behaviour was a pooled group made up of behaviours known to be due to pain, these were; sitting, scooting, lying prostrate and tail wagging. These behaviours were pooled in the data and classified as pain behaviour to avoid repetitions in the descriptions of behavioural types

The effects of treatment, time, and their interaction all had highly significant effects on walking behaviour post docking (Table 4.5 and Figure 4.1). At time 0, the after group had the highest percentage of walking after docking, while the before group had the lowest percentage. These percentages were significantly different from each other. The percentage of walking in the after group was numerically higher than that of the control group; however the difference was not significant. The percentage of walking post docking was also numerically higher in the control group compared to the before group, however, the difference was not statistically significant. At 4 minutes post docking, the before group had the least percentage of walking. This was significantly different from the other treatment groups.

At 7 minutes post docking, there was no significant difference between the control and after groups regarding the percentage of walking. However, the percentage of walking in the before group was significantly lower than the after group but not the control group.

At 20 minutes, the percentage of walking in the after group was significantly lower than the control group. The before group was not significantly different to the control. At 30 minutes post docking, there was no difference between treatments with regard to walking behaviour.

Table 4.5: Effect of treatment and time on walking behaviour of docked pigs

Treatment		Time (minutes)					Significance P value		
		0	4	7	20	30	Treat	Time	Treat* time
After	Mean	1.14^{a}	0.45^{a}	-0.22^{a}	-3.56^{a}	-2.40^{a}	0.0002	<.0001	0.0005
	SE	0.406	0.342	0.335	1.014	0.603			
	%	76	61	44	3	8			
Before	Mean	0.06^{b}	-2.08^{b}	-2.08^{b}	-1.42^{ab}	-3.56^{a}			
	SE	0.338	0.530	0.530	0.421	1.014			
	%	51	11	11	19	3			
Control	Mean	0.75^{ab}	-0.34^{a}	-1.10^{a}	-0.69^{b}	-2.40^{a}			
	SE	0.429	0.338	0.385	0.354	0.603			
	%	68	42	25	33	8			

^{a,b,} Least square means within a column with different superscripts are significantly different between treatments, P<0.05

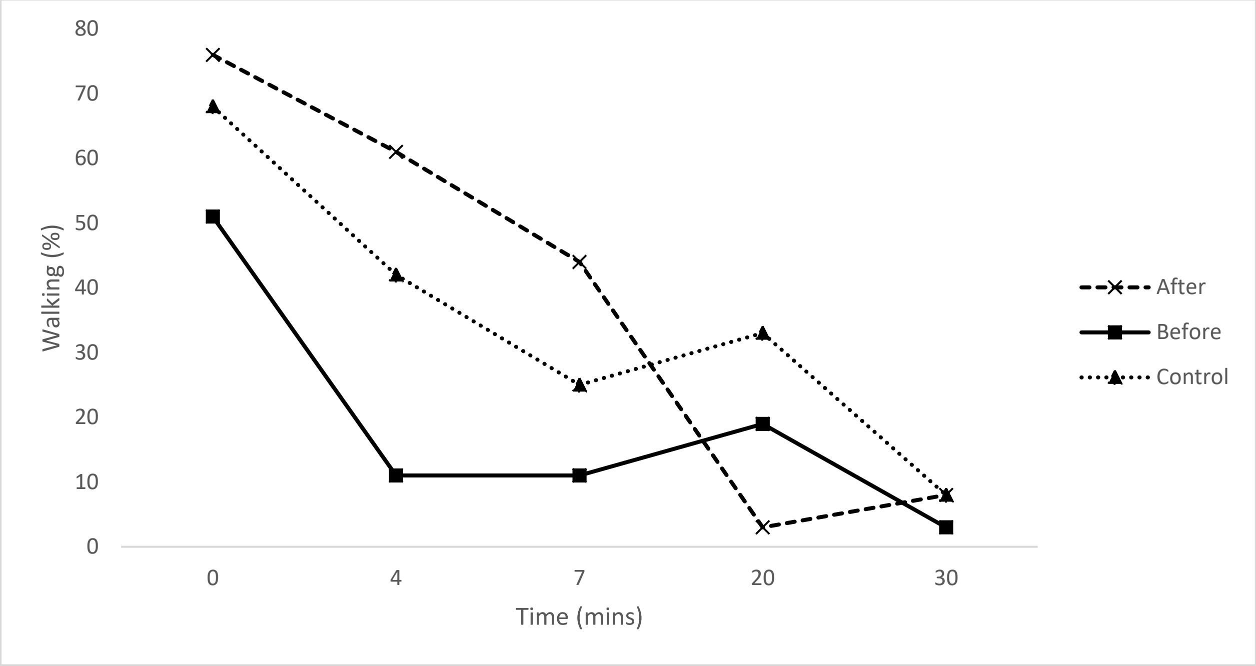

Figure 4.1: Effect of treatment and time on walking behaviour

Treatment, time and treatment by time interaction all had highly significant effects on the lying in contact behaviour of docked pigs as shown in Table 4.6. At time 0, 4 and 7 minutes, there was no difference between the control and the after treatments in regard to lying in contact behaviour (Table 4.6, Figure 4.2). At 20 minutes, the piglets in the after treatment group were lying in contact with their littermates significantly more than those in the control group, but at 30 minutes post docking, there was once again no difference between these treatments. Piglets in the before treatment group were lying in contact with littermates significantly more than the control and the after treatment throughout the observation period, with the exception of the 20-minute time point, when there was no difference between the before and after treatments.

Piglets in the before group had a consistently high percentage of lying in contact with other piglets after docking (Figure 4.2). Observations of piglets lying in contact in the control group gradually increased over time after docking.

Table 4.6: Effect of treatment and time on lying in contact behaviour of docked pigs

		Time					Significance (P Value)		
		0	4	7	20	30	Treat	Time	Treat*Time
After	Mean	-25.37^a	-1.82^a	-1.10^a	1.82^a	-0.22^a	<0.0001	<0.0001	<0.0001
	Se	-	0.482	0.385	0.482	0.335			
	%	0	14	25	86	44			
Before	Mean	-0.82^b	1.42^b	2.08^b	0.96^a	2.08^b			
	Se	0.362	0.421	0.530	0.372	0.530			
	%	31	81	89	72	89			
Control	Mean	-3.56^a	-1.42^a	-0.96^a	-0.57^b	-0.82^a			
	Se	1.014	0.421	0.372	0.347	0.362			
	%	3	19	28	36	31			

[a,b,] Least square means within a column with different superscripts are significantly different between treatments, P<0.05

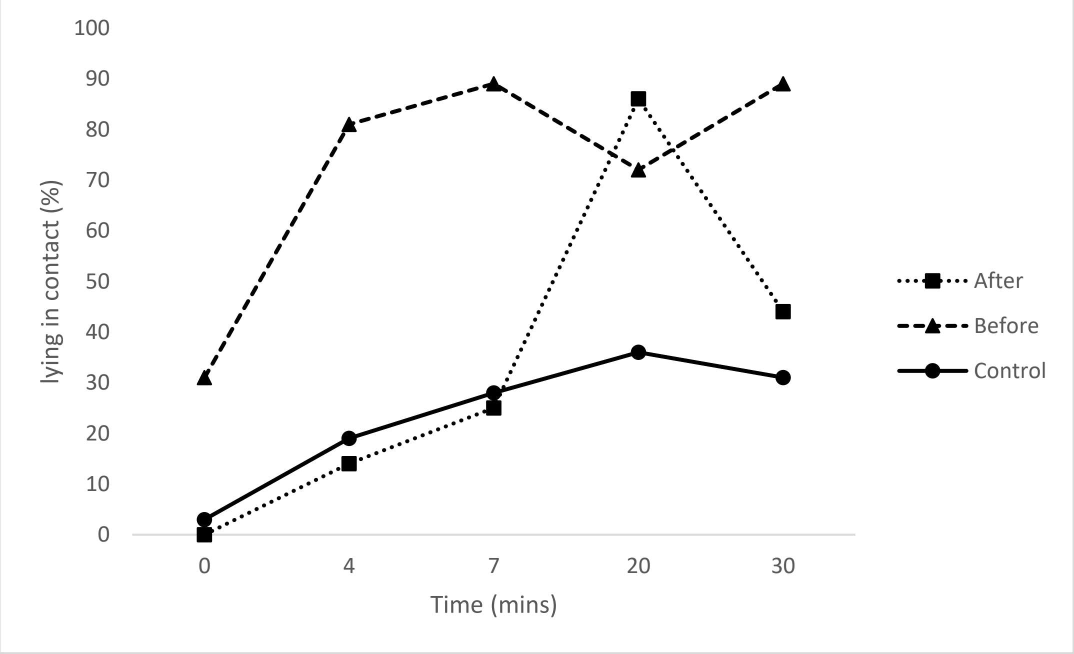

Figure 4.2: Effect of treatment and time on lying in contact behaviour

There was a clear effect of treatment on sitting behaviour post docking, whereby piglets that did not receive the LA cream either before or after docking were significantly more likely to be observed sitting (Table 4.7; Figure 4.3). At time 0, the percentage of sitting after docking was zero in the before and after groups; whereas the control group recorded 6% sitting after docking. The percentage of sitting continued to increase in the control group over time after docking. Sitting behaviour increased in the after group from 20 minutes after docking and was not significantly different between the after and control treatments at 30 minutes (Table 4.7; Figure 4.3).

Table 4.7: Effect of treatment and time on sitting behaviour of docked pigs

Treat		Time					Significance (P Value		
		0	4	7	20	30	Treat	Time	Treat*Time
After	Mean	-28.37^{a}	-3.56^{a}	-28.37^{a}	-28.37^{a}	-1.82^{ab}	0.0001	0.001	0.03258
	Se	-	1	-	-	0.482			
	%	0	3	0	0	14			
Before	Mean	-28.37^{a}	-28.37^{a}	-28.37^{a}	-28.37^{a}	-3.56^{a}			
	Se	-	-	-	-	1.014			
	%	0	0	0	0	3			
Control	Mean	-2.83^{b}	-1.42^{b}	-1.10^{b}	-2.08^{b}	-0.96^{b}			
	Se	0.728	0.421	0.385	0.530	0.372			
	%	6	19	25	11	28			

^{a,b,} Least square means within a column with different superscripts are significantly different between treatments, $P<0.05$

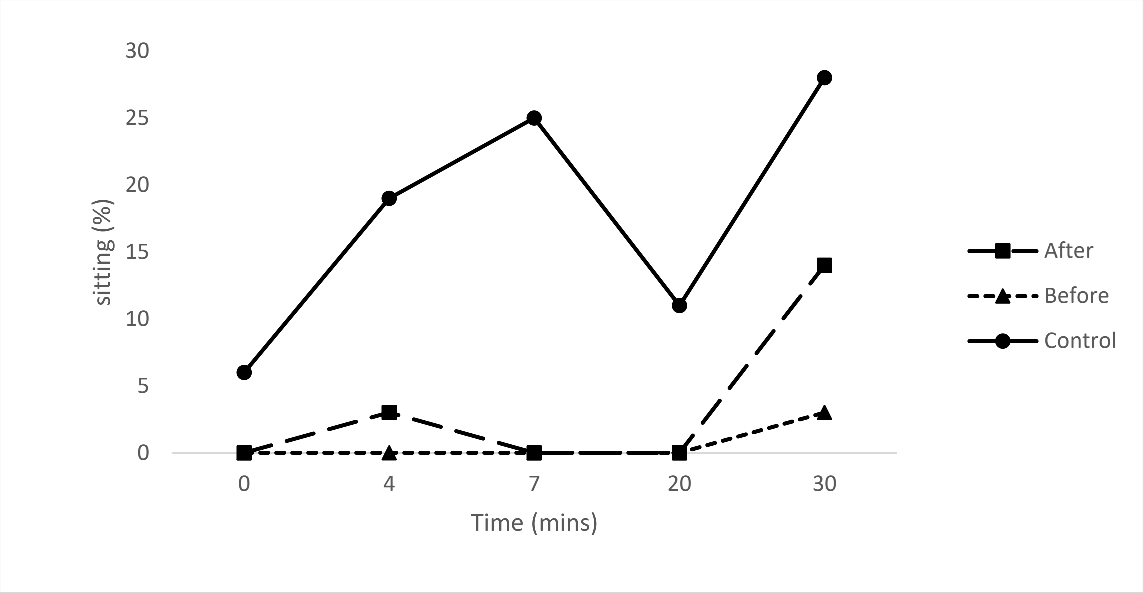

Figure 4.3: Effect of treatment and time on sitting behaviour

There were no differences between the after and control treatments for lying in isolation behaviour after docking (Table 4.8; Figure 4.4). The main effects of treatment and time were significant; however, the interaction of treatment and time was not significant (Table 4.8). Over time, piglets in the before group recorded reduced percentages of lying in isolation. This percentage increased over time in the control and after treatments.

Table 4.8: Effect of treatment and time on lying in isolation behaviour of docked pigs

Teat	Time						Significance (P Value)		
		0	4	7	20	30	Treat	Time	Treat*Time
After	Mean	-27.37^{a}	-2.83^{a}	-1.82^{b}	-2.08^{b}	-1.82^{b}	0.002	0.009	0.1948
	Se	-	0.728	0.482	0.530	0.482			
	%	0	6	14	11	14			
Before	Mean	-3.56^{b}	-2.83^{a}	-27.37^{a}	-27.37^{a}	-3.56^{a}			
	Se	1.014	0.728	-	-	1.014			
	%	3	6	0	0	3			
Control	Mean	-27.37^{a}	-2.40^{a}	-1.61^{b}	-2.08^{b}	-1.61^{b}			
	Se	-	0.603	0.447	0.530	0.447			
	%	0	8	17	11	17			

^{a,b,} Least square means within a column with different superscripts are significantly different between treatments, P<0.05

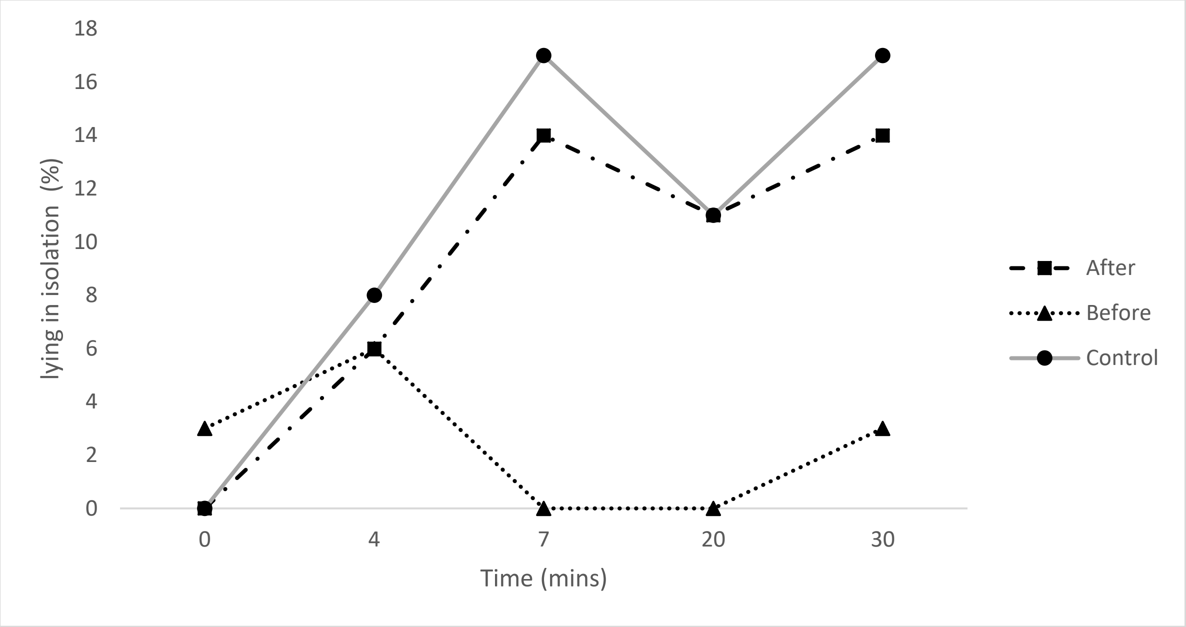

Figure 4.4: Effect of treatment and time on lying in isolation behaviour.

The main effects of treatment and time had highly significant effects on pain-related behaviours after tail docking (Table 4.9; Figure 4.5). There was no evidence of an interaction between treatment and time. At time 0, the percentage of pain related behaviours being expressed was highest in the control group, followed by the after and before groups respectively. At 4 minutes, pain related behaviour was not observed in the before group, and at 7 and 20 minutes, pain related behaviour in the before and after groups were not observed. At 30 minutes, pain related behaviour was observed in all treatment groups and the differences between each treatment were significantly different (P<0.05). At this time point, pain related behaviour was lowest in the before, highest in the control with the after treatment intermediate.

Figure 4.5 illustrates that piglets in the before treatment exhibited very few pain related behaviours except for the immediate post docking period and 30 minutes following docking. The after group followed a similar trend with no pain related behaviours being observed between 7 and 20 minutes after the procedure. The control group had a different pattern whereby pain related behaviour was significantly higher that before and after treatments at each time point (P<0.05, Figure 4.5).

Table 4.9: Effect of treatment and time on pain behaviours[1] of docked pigs

Treat		Time					Significance (P Value		
		0	4	7	20	30	Treat	Time	Treat*Time
After	Mean	-2.08	-3.56^{b}	-27.37^{a}	-27.37^{a}	-1.82^{b}	0.0001	0.000	0.3418
	Se	0.53	1.101	-	-	0.482			
	%	11	3	0	0	14			
Before	Mean	-3.56	-27.37^{a}	-27.37^{a}	-27.37^{a}	-3.56^{a}			
	Se	1.014	-	-	-	1.014			
	%	3	0	0	0	3			
Control	Mean	0.00	-1.42^{c}	-1.10^{b}	-1.83^{b}	-0.96^{c}			
	Se	0.333	0.421	0.385	0.482	0.372			
	%	50	19	25	14	28			

a,b,c Least square means within a column with different superscripts are significantly different between treatments, $P<0.05$

[1] Pain behaviour was the combination of scooting, lying prostrate and tail wagging

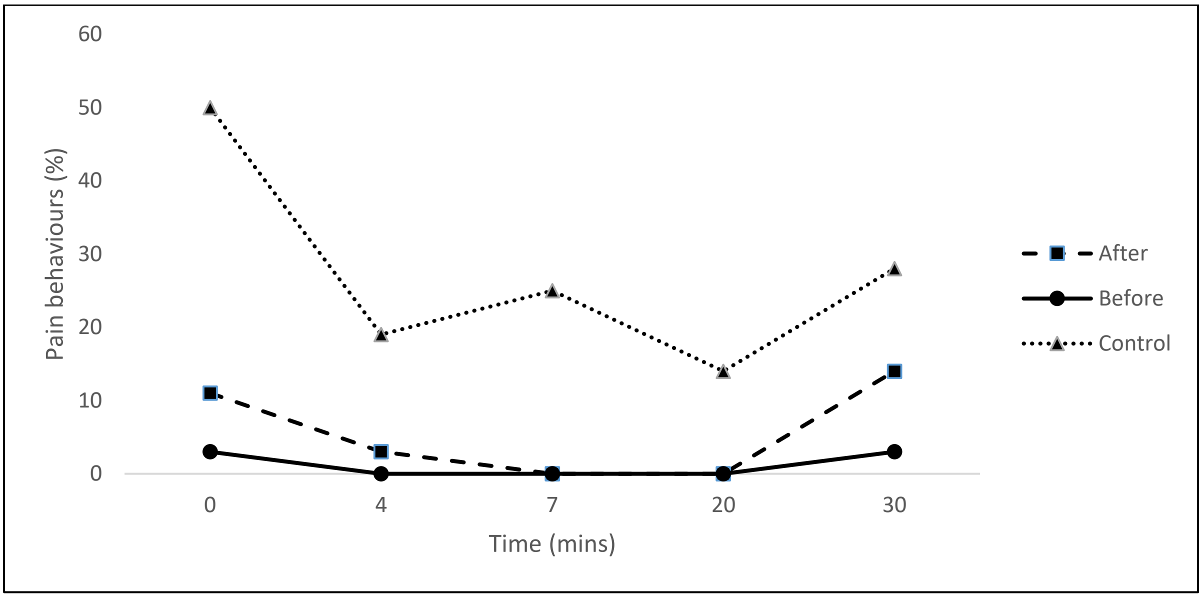

Figure 4.5: Effect of treatment and time on pain behaviours

4.4 Physiological study (Blood samples)

The physiological assessment required blood samples from each of 12 piglets. The piglets were randomly assigned to each of the three treatment groups. Blood was to be collected intravenously from all piglets before docking, then 2, 4 and 24 hours after docking. There were some difficulties in humanely collecting the blood samples from all the piglets at all the time points, resulting in the experiment dropping the 4 hours' time point. Even then, the samples were highly unbalanced as blood was collected from very few piglets (Table 4.10). The scarcity in raw data for the physiological study renders this part of the results in-conclusive. Only the results for the genes which showed responsiveness are shown in this section (Figure 4.6), the rest of the results are shown as Appendix 2 in the appendices section.

Table 4.10: Number of piglets per treatment giving blood samples per collection time point

	Number of piglets per treatment		
Time/hrs	Before	After	Control
0	4	3	1
2	2	1	0
24	2	2	0

Table 3.2 shows a list of 21 genes which were analysed from the samples collected from the information shown above. When the results were plotted on to scatter plots, only three genes indicated a tendency for up or downregulation after 24 hours.

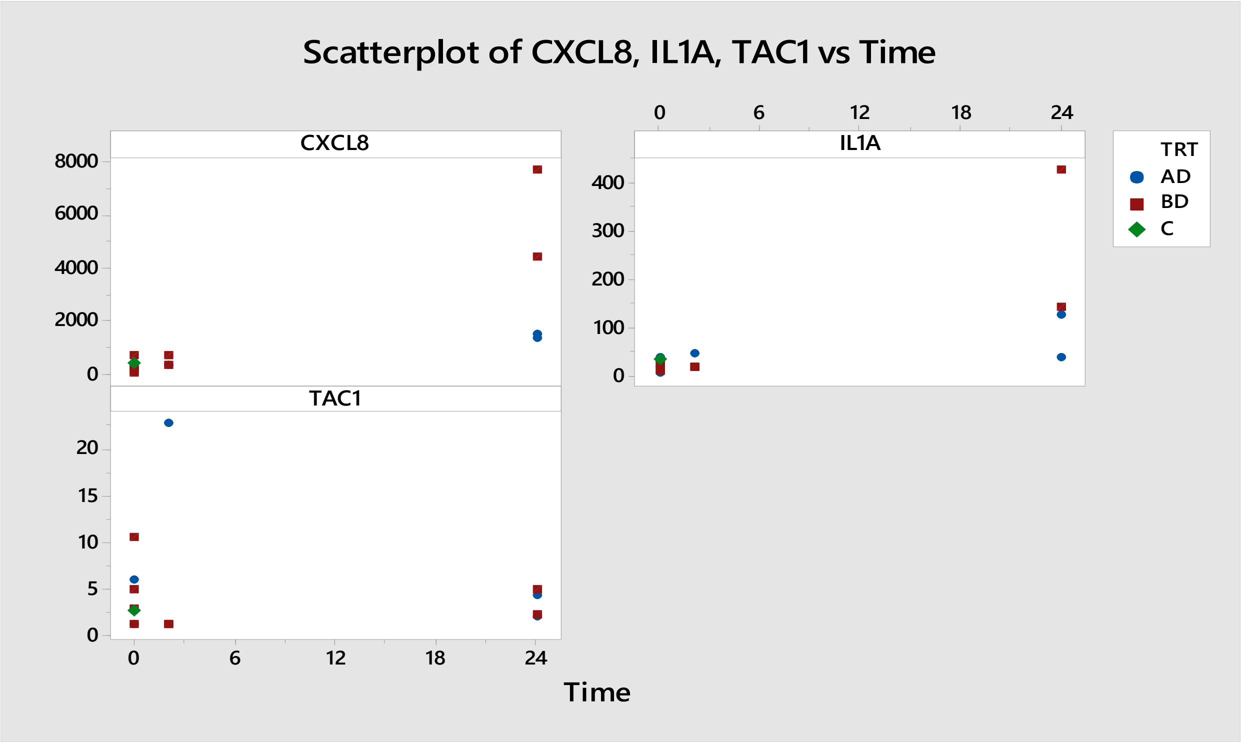

Figure 4.6: Inflammatory gene expression for 0, 2 and 24 hours after tail docking

CXCL8, IL-1α and TAC 1 showed changes in regulation. CXCL8 and IL-1α were upregulated at 24 hours, while there was downregulation of TAC 1 at 24 hours.

CHAPTER 5

Discussion

5 Discussion

Worldwide, pig farms routinely conduct tail docking as a measure to prevent tail biting (Schroder-Petersen and Simonsen, 2001, Sutherland et al., 2009). However, while tail biting is a welfare issue for the victimized pigs; tail docking, despite being a preventative measure, is also a welfare challenge (Sutherland et al., 2010), especially as tail docking takes place without anaesthesia.

This study was designed to determine the efficacy of a novel topical anaesthetic as a means of mitigating pain caused at tail docking. The study evaluated the behaviour of pigs during and post docking. The presence of pain behaviour corroborates earlier reports which illustrated that neonatal piglets can experience pain during tail docking (Marchant-Forde et al., 2009, Noonan et al., 1994, Sutherland et al., 2008).

5.1 Survey analysis

From the survey, it was concluded that tail biting is a cause of concern. Tail docking is also widely carried out. However, it is prudent to present that the survey response was low. The survey had been targeted at potentially 97 pig farmers who would see the survey link through a weekly newsletter. The minimum sample size would be 30 responses. The online platform for the survey received 25 responses but out of those, only 7 had responses which were answered in full and readable. This low response affects one of the research questions which sought to link husbandry procedures to the incidence of tail biting. While the low response to the survey prevents the summation of a concrete conclusion to this research question, the survey is still useful as it provides the baseline for future surveys which can be conducted given a wider space of time.

The most common docking age from the survey is 2 days old. This is in line with the Best Practice Recommendations for tail docking piglets under the New Zealand Code of welfare for pigs (2018), which encourages piglets to be docked before they exceed the age of 7 days, after which a veterinarian would be required to carry out the docking process, with provision of pain medication (Code of Welfare, 2018).

Clippers, which are a form of blunt trauma, were the tool of choice for tail docking. Notably, during a lamb tail docking survey in New Zealand, use of blunt trauma was the least favoured method of tail docking, with only 10 of the responding farmers reporting to use this tool, while 721 farmers reportedly used cauterising irons. During tail docking, a cauterising iron result in more pain vocalisations, which could be due to superficial burns (Marchant-Forde et al., 2009). However, the cautery method is favoured as it cauterises the wound, thereby reducing the chances of excessive bleeding and infections (Sutherland et al., 2009).

From the survey data, the lengths of the tail removed fell within the stipulated length in New Zealand laws. Only a third to half of the tail should be removed (Code of Welfare, 2018). The length should be maintained as such because leaving out a long tail is ineffective, while leaving out a very short tail may lead to infection and prolapse risks (Codes Of Practice, 2014).

All the possible flooring systems for post weaning pig production were reported in the survey. These were solid floors, fully and partially slated floors and outdoor production. This highlighted the fact that there is no standard of flooring systems in the New Zealand production system. Interestingly, the only farmer who reported not to have tail biting problems engaged in outdoor post weaning production. The other farmer practising outdoor production did not carry out tail docking on the farm. This may point to the idea that outdoor systems may result in less tail biting incidents. Slatted floors have been known to support tail biting due to the restlessness in pigs following the promotion of noxious gases as they permit movement of the from the manure pit through to the pens (van Putten, 1969). Solid floors are to be favoured over partially or fully slated floors because the lack of bedding materials in partial or fully slated floors supports the incidence of tail biting behaviour (Moinard et al., 2003).

The survey did not provide answers as to which gender is more prone to tail biting or whether production units should be of mixed gender. Only one farmer was certain that males were more prone to tail biting and only one farmer practised mixed sex production pens. Literature is contradictory when it comes to which sex displays more tail biting behaviour. The best solution is keeping the pigs separately based on gender to allow for adequate feeding in accordance with nutrient requirements as determined by the gender. Adequate nourishment lessens tail biting behaviour.

The survey respondents who kept their pigs post weaning all provided manipulable materials. This was mostly in the form of straw, but also as salt blocks and pork CRC molasses. It has been shown that when manipulable substances such as straw are not provided, there is a 2 fold increase in the risk of tail biting (Larsen et al., 2018).

Three out of the 7 respondents did not provide deep litter bedding post weaning. Deep litter bedding is not practical for commercial production sites which use slated floors. However, deep litter bedding, especially straw, takes the attention away from biting tails and may be a factor in reducing tail biting (EFSA, 2014).

Most respondents reported the use of dry feeding as a feed form. Literature is contradictory as to which feed form results in a lower tail biting risk. From the survey, farmers who practised wet or dry feeding still encountered tail biting. It would be beneficial to have other factors constant, except for feed form, to determine the lower risk feeding factor.

In terms of feeding method, 5 respondents reported the use of *adlib* automated feeding, while 2 respondents reported *adlib* hand feeding. Hand feeding has been reported to reduce tail biting risk, furthermore, automated feeding has been known to increase the incidence of tail biting (Moinard et al., 2003). However, the increase due to automated feeding has been seen to coincide with periods of auto breakdowns or poor programming of feed mixes (Paul et al., 2007). This shows that the problems lie not in automated feeding, but in the feed restrictions that come with machine failure. *Adlib*hand feeding would seem to reduce tail biting behaviour, especially in this survey as one of the respondents who has not encountered tail biting on their farm practices *adlib* hand feeding.

More research is needed to individually examine the tail biting risk factors which were covered in the survey.

5.2 Behavioural responses during docking

Vocalisation

Treatment level had a highly significant effect on vocalisation. Vocalisation can be an indicator of pain and distress (Noonan et al., 1994). The percentages of vocalisation during docking was 97% in the after group and 92% in the control group. The results of these two groups were not significantly different from one another. This is because, during docking, the after group did not have LA cream application, making these groups identical during the process of docking. The before group had the least percentage of vocalising during docking (44%). This value was significantly different from the other groups. These results agree with a previous study which concluded that pigs which had been exposed to anaesthesia produced stress vocalisations at a similar level to piglets in the present study, before and during painful procedures. This indicated that the anaesthesia provided pain relief during tail docking (Sutherland et al., 2010). The study by Sutherland et al. (2010) reported that pigs docked without pain relief vocalized less before the actual docking. This vocalisation may be due to the stress of handling before actual pain due to docking. In a separate study, sham docked piglets were shown to have a vocalisation likelihood of 0.1 (Herskin et al., 2016). This puts into perspective the difference that anaesthetics could make, if, as reported, there would be similarity between vocalisations due to handling without pain and the actual pain procedure during docking with anaesthesia.

In this study, the mean number of vocalisations within the three treatment levels followed the same pattern as the percentage of vocalizing.

Escape attempts

An escape attempt is a behaviour that shows the presence of pain or distress and a need to take evasive action to avoid it. This behaviour is described as sequential kicking carried out in bouts and intercepted by a pause before the kicking resumes (Marchant-Forde et al., 2009). Treatment level had a highly significant effect on escape attempts. Piglets docked with pain relief presented fewer escape attempts. These results are in agreement with earlier studies which show that more escape attempts are recorded in docked rather than sham treated piglets (Marchant-Forde et al., 2009). Earlier work reported that piglets provided with a local anaesthetic prior to docking had significantly lower escape attempts compared to control piglets (Hansson et al., 2011, Leidig et al., 2009). Escape attempt behaviour has been used to assess pain before, during and after animal handling procedures to compare pain levels between husbandry routines and sham procedures as well as comparing treatments where pain relief was or was not provided (Walker et al., 2004).

Behavioural responses post docking

Walking

While walking is a normal behaviour in pigs, increased walking, especially in pigs subjected to painful stimuli is a sign of restlessness, which points to stress or pain (Molony et al., 1993). In this study, treatment, time and treatment by time interaction had highly significant effects on the walking behaviour.

The before group was the least likely to walk post docking, while the after group started showing a reduced tendency to walk compared to the control at 20 to 30 minutes post docking. The increased restlessness constantly exhibited by the control group indicates that higher levels of pain were experienced, leading to restless behaviour. Increased restlessness is a defence strategy carried out by an animal experiencing pain, and in piglets, this may prevent further pain caused by littermates which may be the case if the victim stays in one place which is accessible to littermates (Mellor et al., 2000).

Pain behaviour

Lying prostrate, scooting and tail wagging are all noted as being pain behaviours and pigs, therefore they were pooled together to avoid repetitions. There have been various definitions of pain behaviour across studies, but the constants within these definitions are the involvement of the tail region and hind limbs (Herskin et al., 2016, Keita et al., 2010). Analysis of pooled behaviour is more effective when the behavioural elements have the same underlying motivations (Herskin et al., 2016). The motivator, in this case, was pain due to tail docking.

The control group showed the highest levels of pain behaviour following tail docking. The after group had significantly lower levels of exhibiting pain related behaviour compared to the control group. When compared to the before group, in some instances, the percentage of pain behaviour displays in these two groups was not significantly different, but it was consistently lower in the before group. These results suggested that the LA cream was effective in reducing pain caused by tail docking relative to the other treatment groups. This is in agreement with previous work illustrating that anaesthesia reduced pain during docking.

Sitting

The sitting behaviour was lowest in the before group, closely followed by the after group, while the control group had a significantly higher percentages of sitting after tail docking. Treatment and time had highly significant effects on the sitting behaviour. These results support previous work which reported that tail docked piglets show more pain compared to sham treated piglets (Sutherland et al., 2008). In the case of this study, all piglets were docked but the two groups which received pain relief at a point showed the least tendency to sit, once again demonstrating the efficacy of the LA cream in mitigating pain associated with tail docking. It has been suggested that sitting stems from a need to relieve the painful sensation (Nannoni et al., 2014). In this study, all the piglets had the sitting behaviour peaking at the observation times 20 to 30 minutes, with the control maintaining the highest percentage of sitting behaviour. The peak in the tendency to sit in the groups treated with pain relief may be due to the effects of the anaesthetic wearing off at this time. Local anaesthesia works by blocking the initial pain sensations caused by tissue damage at docking. Once the local anaesthetic begins to wear off, the painful stimuli begin to affect the animal, causing it to register pain (Sutherland et al., 2009).

Lying in contact and lying in isolation behaviours

Lying in contact and lying in isolation are opposite behaviours. Pigs that lie in contact with each other or with the sow display a normal social cohesion. When piglets experience pain, they display a reduction in social cohesion and lie in isolation (Hay et al., 2003). This is in agreement with the findings of the present study, where piglets in the control group were lying in isolation more compared to the after or the before group. The groups that were provided with pain relief had more social cohesion after tail docking, therefore more lying in contact and less lying in isolation.

Pigs undergoing the most pain become desynchronized and isolated as a protective measure to avoid stimulation of further pain due to contact with litter mates (Mellor et al., 2000). In this study, the control group had a gradual rise in the percentage of piglets lying in contact, possibly signifying a gradual return to normal social cohesion after the painful stimuli. The after group had a gradual rise in the percentage of piglets lying in contact, however, up until the observation time at 7 minutes, this rise was below that of the control. After 7 minutes, the incidence of lying in contact for the after group surpasses that of the control group, possibly signifying the working effect of the LA cream applied to the tails after docking. The before group had a higher percentage of lying in contact during each time point, likely due to the pain relief effects of the LA cream applied before docking.

In this study, applying the LA cream before docking produced the most favourable results with regards to reducing pain during and after tail docking.

5.3 Physiological responses to tail docking

While the results of the blood samples are inconclusive due to limited sample numbers, there were some gene expressions which behaved as would be expected if a study of this nature had been undertaken in pigs before.

Detection of circulating IL-1α is rare even though it is steady in healthy tissues(Garlanda et al., 2013). This is illustrated in Figure 4.6, where IL-1α is detected at time 0 but more so at 24 hours after a noxious event. The expression of IL-1α is increased due to pro-inflammatory or stress associated stimuli (Di Paolo and Shayakhmetov, 2016). The upregulation of IL-1α in Figure 4.6 is consistent with the experimentally confirmed theory which states that following tissue injury or cell death, IL-1α passively leaks out, thereby activating inflammation (Eigenbrod et al., 2008). This active role of IL-1α has led to it being referred to as a key ‘alarmin’ whose role is alerting the body system to stress or injury (Garlanda et al., 2013, Rider et al., 2013).

It has been reported that CXCL8 expression is upregulated due to wounding and infectious stimuli (de Oliveira et al., 2013). Furthermore, CXCL8 is practically undetectable in cells that have not undergone stimulation (Ha et al., 2017). CXCL8 becomes detectable following stimulation via cytokines (IL1, IL6 and TNF), hypoxia and environmental stressors such as injuries (de Oliveira et al., 2013, Wald et al., 2013). This is consistent with Figure 4.6 where there was upregulation of CXCL8 following tail docking which is known to be painful.

Instead of upregulation of TAC1, there was downregulation. Available literature advises that there are diverse regulation mechanisms which control TAC 1 promoter activity, and these are unclear (Shanley et al., 2011). There is a need to clearly understand these mechanisms to understand the expression. It would be interesting to discover whether or not downregulation of TAC 1 is a deviation from the standard procedure as a result of pain.

5.4 Limitations of the study

A research question sought to analyse if there was a link between the pig husbandry practices in New Zealand and the incidence of tail biting. This was to be analysed through a survey. Insufficient responses were generated by the survey thereby posing a limitation to that aspect of the study. Responses which had enough data to work with were only 7 out of 25 responses that came in. There were technical issues with 18 of the other responses which came in, these included a change of language into one that was not recognized and duplicated answers. This resulted in the survey outcome being limited by a low response and a survey platform malfunction. This could be corrected in future by taking a more direct approach whereby the survey enumerator could be physically present to give out the questions and put down the responses based on the response of the farmer and the evidence on the ground. This would be time-consuming but it would generate important information.

Blood sampling was carried out so as to answer the research question which had to do with testing if inflammatory gene expression could be used as a physiological pain index in pigs. This outcome of this aspect of the study was limited by insufficient blood work from the pigs due to blood collection difficulties. The sample pigs were 2-3 days old therefore the veins were quite small. There was need for specialized equipment for the collection, such as powerful lighting and restriction tables and a wider range of needle sizes. A lack of these resources resulted in a low number of blood collections across all the treatments, causing the final result to be inconclusive. However, this type of pain indexing has not been done before, therefore it is a promising venture if it is to be redone under improved circumstances.

CHAPTER 6

Conclusion and recommendations

6 Conclusion and recommendations

This study evaluated the efficacy of a novel topical local anaesthetic for providing pain relief during and after tail docking. Pain relief has become an important issue in the swine industry due to increased consumer concern with regards to animal welfare (Anil et al., 2005). While assessing pain in animals is difficult, behavioural and physiological indices can be used to quantify pain severity (Ison et al., 2016, Sutherland et al., 2008). In this study, the novel LA cream appeared to have a pain-relieving effect during and post tail docking.

Piglets that received the LA cream before docking were the least likely to express pain related behaviours, followed by those that received the cream post docking. The control group showed the highest likelihood of pain related behaviour. After docking, the piglets that were provided LA cream had higher social cohesion compared to the control piglets which expressed more social desynchronization.

Applying the LA cream before docking showed the best results in terms of supporting animal welfare. However, this treatment was the most labour intensive of the three treatments as it included handling the piglets twice and waiting 40 minutes for the LA cream to take effect. The cream application was also time consuming as it required bandaging to keep the cream in place. This labour intensity does not provide an effective option for farmers to adopt this technique as it translates to a higher labour requirement, and therefore an increased cost. It would be beneficial to have the same formulation but with a more practical on farm application technique such as a consistency that would allow spraying rather than requiring bandaging.

For the research questions, it can be concluded that LA cream is effective in reducing the pain caused by tail docking in piglets. There is need for more survey data before accurately linking pig husbandry practices to tail biting incidences in the New Zealand farms. While inflammatory gene expression is a promising physiological tool in measuring pain in pigs, the study was inconclusive due to limited raw data.

It is a recommendation of this study that in future, electroencephalography should be used in conjunction with an inflammatory gene expression study. Electroencephalography (EEG) ensures that piglets are unconscious. This would circumvent the unfortunate event of blood sampling struggles with conscious piglets, which leads to limited raw data and inconclusive results. When taken together, EEG could validate the gene expression study; this added to the behavioural study would make a strong case for pain alleviation in piglets undergoing tail docking.

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8 Appendices

Appendix 1; Pig husbandry questionnaire

Pig husbandry survey

My name is Fadzai Roselyn Magadzire and I am a Master’s student studying Animal Science at Massey University in Palmerston North. I am currently undertaking a project to evaluate a novel form of pain relief for piglets undergoing tail docking. As part of this project, I would like to invite New Zealand pig farmers to participate in a short survey on tail docking and tail biting, to better understand this and relate it to the New Zealand pork industry perspective. The survey is mostly multiple choice for your convenience and will only take approximately 5 minutes to complete. The data obtained from the survey will be treated with confidentiality as the survey is completely anonymous. The use of the data will not extend beyond this project.

Your participation is very much appreciated.

This project has been evaluated by peer review and judged to be low risk. Consequently, it has not been reviewed by one of the University's Human Ethics Committees. The researcher(s) named in this document are responsible for the ethical conduct of this research. If you have any concerns about the conduct of this research that you want to raise with someone other than the researcher(s), please contact Professor Craig Johnson, Director (Research Ethics), email; humanethics@massey.ac.nz

Q1. Do you carry out tail docking of piglets on your farm?

☐ Yes, on all piglets

☐ Yes, when there is an incidence of tail biting

☐ No

Skip To: Q5 If Do you carry out tail docking of piglets on your farm? = No

Q2. At which age do you carry out tail docking? (Please tick all that apply)

☐ 1 day old

☐ 2 days old

☐ 3 days old

☐ 4 days old

☐ 5-7 days old

Q3. Which method do you use for tail docking piglets? (Please select all that apply)

☐ Clippers

☐ Cautery iron

☐ Other (specify) ____

Q4. What length of the tail is removed?

☐ Less than one third to half of the tail

☐ One third to half of the tail

☐ More than one third to half of the tail

☐ Other (Specify) ____

Q5. Have you observed signs of tail biting on your farm before?

☐ Yes

☐ No

Skip To: Q7 If Have you observed signs of tail biting on your farm before? = No

Q6. What is the nature of the tail biting you have observed?

☐ Consistent e.g. there are usually pigs present on farm with tail biting injuries

☐ Intermittent e.g. at times there are no pigs on farm with tail biting injuries

Q7. What kind of housing is available for farrowing and lactating sows? (Please tick all that apply)

☐ Crates

☐ Pen

☐ Outdoor hut

☐ Other (Specify) ____

☐ Not applicable (no farrowing/lactating sows)

Q8. What is the predominant flooring system available post weaning?

☐ Solid floor

☐ Fully slatted floor

☐ Partially slatted floor

☐ Outdoors

Q9. Do you separate males and females, or keep mixed sex pens post weaning?

☐ Separate

☐ Mixed

☐ Some pens are separate some are mixed

Q10. If you maintain **single sex** pens, was tail biting was more prevalent in a particular gender?

☐ Yes (Please specify which gender) ____

☐ Cannot recall

☐ Not applicable

Q11. If you maintain **mixed sex** pens, was tail biting was more prevalent in a particular gender?

○ Male

○ Female

○ Not applicable

Q12. Do you provide straw or other manipulable items to pigs post weaning e.g. toys or objects?

☐ Yes (Please describe) ____

☐ No

Q13. Do you provide deep litter bedding in the pens post weaning?

☐ Yes (Please describe e.g. straw, sawdust) __________________________________

☐ No

Q14. Which form of feed do you use post weaning?

☐ Dry feeding

☐ Wet feeding

☐ Other (Please specify) ____

Q15. Which feeding method do you use?

☐ Restricted hand feeding

☐ Restricted automated feeding

☐ Adlib hand feeding

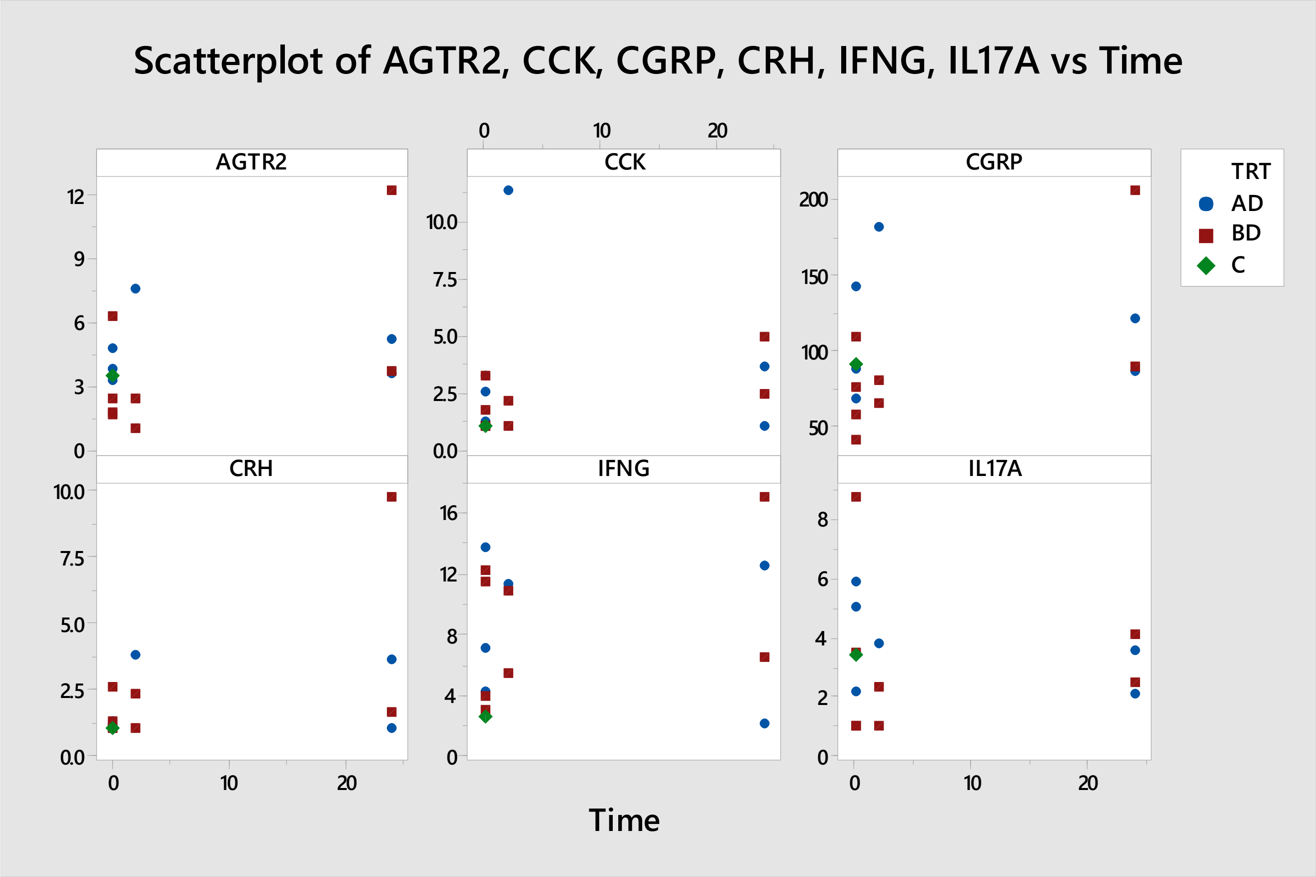
☐ Adlib automated feeding, other (Specify) (5) ____

This is the end of the survey. Your responses have been recorded, thank you for your participation.

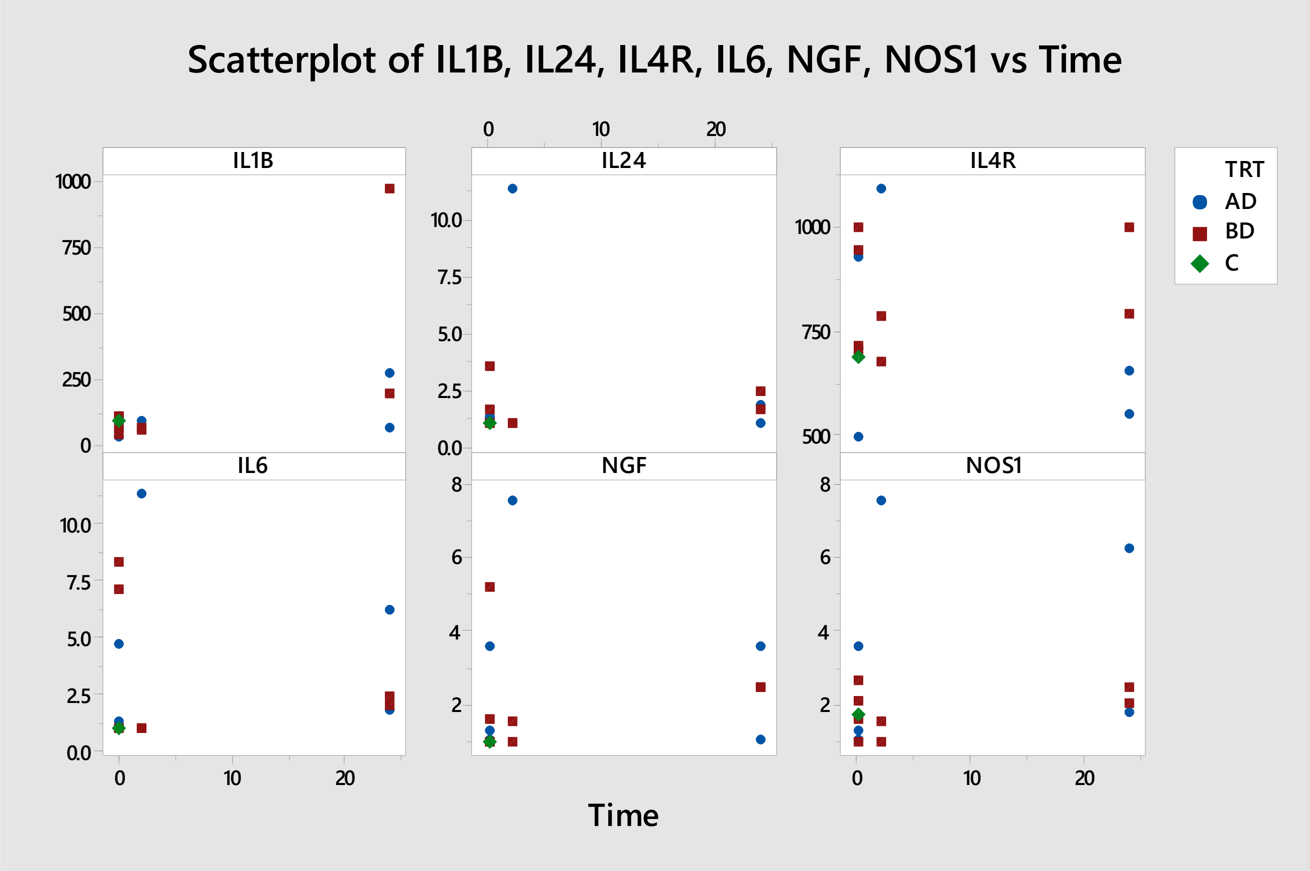
Appendix 2; Physiological study scatter plotss

The scatter plot results for three of the twenty-one genes that were analysed were shown in section 4 and discussed in section 5. There has not been emphasis on these results as they are unbalanced having come from limited raw data. The scatter plots for the remaining genes as shown below do not show any activity in terms of gene expression regulation;

Scatterplot of AGTR2, CCK, CGRP, CRH, IFNG, IL17A vs Time



Scatterplot of IL1B, IL24, IL4R, IL6, NGF, NOS1 vs Time



Scatterplot of NPY, OPRM1, PLA2G3, PTGES, PTGS2, TNFa vs Time