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Commercially-viable strategies to reduce the acute pain of tail docking in piglets

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2.0 Executive Summary

There is increasing pressure from animal welfare groups to provide pain relief for elective husbandry procedures such as tail docking of piglets. The RSPCA's position is "that any procedure that may cause pain to the animals should be undertaken at the earliest possible age and only by competent and accredited operators. Appropriate pain-relieving products and treatments, and/or anaesthetics, must be used" (RSPCA, 2016).

Our previous Australian Pork Limited (APL) project (2010/1018.348) investigated the physiological, behavioural and neurophysiological responses of pigs to tail docking and showed that tail docking two-day old piglets using the clipper and cauterisation methods caused an acute, short-term stress response. Cauterisation appeared to be less aversive than clipper method.

This APL project examined commercially-viable strategies to reduce the acute pain of tail docking in piglets. Part 1 examined the long-term welfare implications of cauterisation, such as the formation of neuromas (swellings or thickenings caused by abnormal regeneration of nerve fibres secondary to nerve transection), which have been linked to increased pain sensitivity, spontaneous pain generation and pain perception in response to benign stimuli. In the current project, piglets that had their tails left intact did not have any formation of neuromas on their tails at slaughter, despite some gross evidence of tail tip trauma resulting from tail biting. Tail docking by either clipper or cauterisation method resulted in a higher proportion of tails with neuromas. There was a trend for less severity of neuroma formation in tails of pigs that were docked using cauterisation compared to the clipper treatment. Further research is required to identify if these neuromas are in fact painful for the pig.

In this project, Part 2 examined the stress physiology and pain-related behavioural responses of piglets after tail docking with clipper or cauterisation provided with injectable meloxicam treatment compared to sham (handling alone) and no handling treatments. There were physiological and behavioural responses of piglets to tail docking by clipper and cauterisation methods. There was a cortisol response at 15 minutes post-treatment in both tail docking treatments, however this stress response diminished by 30 minutes post-treatment which provides further evidence that tail docking causes an acute, short-term stress response. The cauterisation method appeared to be less aversive than clipper treatment and appeared to mitigate this acute pain response. Injectable meloxicam administered 60 minutes prior to tail docking also appeared to alleviate this acute stress response. During the tail docking treatment pigs in all tail docking treatments exhibited more vocalisations and escape attempts. Pigs in the clipper treatment exhibited more pain-related behaviour 60 minutes post-treatments compared to those in the cauterisation and meloxicam treatments.

The need for pain relief to be provided for a husbandry procedure that causes an acute short-term response remains controversial. The administration of meloxicam pain relief 60 minutes prior to tail docking appears to mitigate the stress response, however increases cost of production through additional labour, piglet handling and medication costs. Methods of providing pain relief through piglet

injection/topical application increase labour requirements because each piglet must be treated individually. For most medications, the time delay between drug administration and effectiveness requires that animals to be handled twice. The concept of providing analgesia through the sow's milk (translactational anaesthesia) is a less laborious method of pain medication that could provide producers with a practical method of improving animal welfare. There may also be additional benefit to the sow post-farrowing. International research is currently investigating this as an option, however to date there are not any commercial recommendations for this procedure.

The current project showed that tail docking two-day old piglets using the clipper and cauterisation method caused an acute, short-term stress response. Cauterisation appeared to be less aversive than clipper method based on effects on stress physiology, pain-related behaviour post-treatment and trend for lower severity of neuroma formation. However, caution should be exercised when considering cauterisation as an alternative to the clipper treatment. This project was conducted under experimental conditions using new equipment and trained operators. The cauterisation method involves equipment that requires a high level of maintenance and an extremely high standard of operator competence to ensure that the procedure is conducted efficiently and humanely. The impact on piglet growth health and survival requires further investigation on a larger sample size to fully understand the commercial-viability of the cauterisation method.

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3.0 Background to Research

Tail biting is both an economic and welfare problem of pigs that involves destructive chewing of pen-mates' tails, which become attractive to other pigs in the group once the tail bleeds. Tail biting occurs in two stages, a pre-injury and an injury stage, and it is the second stage that results in a wound and bleeding and more severe consequences such as infection, spinal abscess, paralysis, and in extreme cases, death (Schroder-Petersen and Simonsen, 2001). As a result, the pork producer can incur severe economic losses when the pigs are marketed, and there are serious welfare consequences for the pig. Tail biting behaviour is likely to cause both acute and chronic pain in the short-term due to the actual tail biting and longer-term as a result of weight loss and infection (Sutherland and Tucker, 2011). The etiology of tail biting remains poorly understood and potential factors pre-disposing tail biting behaviour are numerous and include crowding, health, poor ventilation, breakdown in the food or water supply, poor quality diets and breed type (reviewed by Taylor *et al*., 2010). Despite years of research focusing on this area the underlying behavioural mechanisms for tail biting are still not well understood.

While management and housing factors should be carefully examined in cases of tail biting, tail docking is a common method for prevention and is routinely conducted on pig farms world-wide (EFSA, 2007), and there is substantial evidence that the tail docking procedure reduces the likelihood of this detrimental behaviour (Sutherland and Tucker, 2011). Tail docking is usually performed by removing at least half of the tail using either side-cutter pliers (clippers) or a cauterising tail-docking iron (cauterisation). The docking should occur between 1.5 and 2.5 cm from the base of the tail and care should be taken to dock in between vertebra (Simonsen *et al*., 1991). It is common practice to leave at least 2 cm of tail from the base to cover the vulva in females and equivalent length in males (Sutherland and Tucker, 2011).

There is increasing pressure from animal welfare groups to provide pain relief for elective husbandry procedures such as tail docking (RSPCA, 2016). However, there is limited information in the scientific literature on methods of tail docking, and whether or not the procedure of tail docking causes significant pain, the duration of the pain caused by the procedure, and in fact whether it is necessary to provide pain relief for this procedure.

Pain is difficult to study because it is an inherently subjective experience. While humans can report pain, only indirect indices of pain are available for use in animals. Pain and welfare can be assessed in animals using a range of physiological and behavioural measures. The physiological response is measured by assessing total cortisol concentrations after a stressor is imposed to determine activation of the hypothalamic-pituitary (HPA) axis (see review by Barnett and Hemsworth, 2009). Corticosteroids are generally accepted as a measure of stress (Barnett, 2003), however it should also be recognised that non-painful components of a surgical husbandry procedure such as restraint, isolation, presence of humans etc. may also increase cortisol concentrations. Furthermore, corticosteroids also have anti-inflammatory and immunosuppressive properties in response to tissue injury (Yeager *et al*., 2004).

The behavioural response is assessed by behavioural indicators of pain such as vocalisation, escape attempts and standing with head lowered (Hemsworth *et al*., 2009; Hay *et al*., 2003). More recently, neurophysiological responses (activity of the cerebral cortex) of the animal, recorded by electroencephalographic (EEG) responses recorded using a minimal anaesthesia model have been successfully used to assess nociception in a range of domesticated mammals (Johnson, 2007; Johnson

et al., 2005a; Johnson *et al*., 2005b) and are now used in combination with behavioural and physiological responses of the animals to measure pain.

Neurophysiological, physiological and behavioural changes have been used to assess the pain of tail docking in piglets. In our recent Australian Pork Limited (APL) project (2010/1018.348), we used a range of neurophysiological, physiological, behavioural responses of the animal to assess acute and chronic pain caused by the procedure of tail docking. The results from these experiments and evidence in the scientific literature indicate that tail docking causes an acute, short-term pain. Kells *et al*. (2013) examined the EEG responses of two-day old piglets to tail docking using either the clipper or cauterisation method and concluded that cauterisation is less aversive than clipper in the short-term. Morrison *et al*. (2013a) investigated the physiological response of two-day old piglets to tail docking and showed that docking tails using either clippers or cauteriser elicited a significantly greater stress response at 15 and 30 minutes post-treatment compared to the control treatment (handling alone). The cortisol concentrations at 30 minutes were lower in the cauterisation treatment indicating cauterisation may be less aversive. In a further experiment, Morrison *et al.* (2013b) repeated this effect, however by 30 minutes there was no difference in cortisol concentrations between tail docked and control pigs. These results are similar to Sutherland *et al*. (2008) who found that cortisol concentrations were greater in the clipper treatment at 60 minutes post-docking compared to the cauterisation treatment and that cortisol concentrations were similar 90 minutes post treatment.

The behaviour of pigs that are tail docked is also affected during and post-docking. Pigs that are tail docked produce more squeals with higher peak vocal frequencies during the treatment (Morrison *et al*., 2013 a,b; Marchant-Forde *et al*., 2009; Noonan *et al*., 1994), perform more escape attempts (Morrison *et al*., 2013a,b) and perform more tail jamming (clamping of tail stump between hind limbs) and tail wagging (Noonan *et al*., 1994), standing with head lowered (Morrison *et al*., 2013) and posterior scooting (Sutherland *et al*., 2008) post-treatment compared to control pigs. These data, using a range of neurophysiological, physiological and behavioural measures to assess pain indicate that tail docking causes an acute, short-term response and cauterisation may be a less aversive method.

Whilst there is evidence in the scientific literature that tail docking causes an acute, short-term response, there is no evidence that tail docking causes chronic, long-term pain in pigs. The formation of neuromas as a consequence of tail docking, have been identified in pigs (Simonsen, *et al*., 1991 and Herskin *et al*., 2014). Neuromas are swellings or thickenings caused by abnormal regeneration of nerve fibres secondary to nerve transection (Lewin-Kowalik *et al*., 2006). The development of neuromas has been linked to increased pain sensitivity, spontaneous pain generation and pain perception in response to benign stimuli (Bennet and Xie, 1988; Jensen and Nikolajsen, 1999). The potential for heightened pain perception or spontaneous pain as a result of tail docking should be considered from a welfare point of view. Eicher *et al*. (2006) reported increased sensitivity of the ventral docked tail in dairy heifers relative to controls. On the other hand, Sandercock *et al*. (2011) reported no difference in response to mechanical or noxious stimuli between pigs that had been docked with clippers compared to intact tails, however in that experiment the stimuli was applied to the tail root not the tail tip where the neuroma formation occurs. The long-term welfare implications, in particular the formation of neuromas requires further investigation before cauterisation is recommended as an alternative to the clipper method.

There is limited information in the scientific literature comparing the different methods of tail docking (i.e. clipper vs. cauterisation method) and practical medication strategies to reduce the acute pain of

tail docking. Non-steroidal anti-inflammatory drugs (NSAIDs) are becoming licensed for use in food-producing animals and beginning to be investigated as an opportunity to address pain associated with husbandry procedures. Opiate-based analgesics are potentially addictive to humans and thus their widespread use in commercial production systems would provide challenges, and therefore there is limited research being conducted using these medications routinely in commercial production systems (Tenbergen *et al*., 2014). The relatively long-acting meloxicam is a non-steroidal anti-inflammatory drug that becomes effective approximately 30 to 60 minutes after administration. Meloxicam is recommended for use in the EU for surgical castration of piglets (European Declaration, 2010). Our previous APL project (Morrison *et al*., 2013b) showed that meloxicam (Metacam® 5mg/ml administered via piglet intramuscular injection 60 minutes prior to tail docking) was effective at reducing the stress response 15 and 30 minutes post-docking. A topical lignocaine cream (EMLA cream-2.5% Lignocaine; 2.5% Prilocaine) applied 60 minutes prior to tail docking treatment was also effective at reducing the piglet neurophysiological response to tail docking. However, this topical anaesthetic or other medications that are not registered for use in pigs in Australia were not able to be investigated in this experiment. Furthermore, the practicality of applying a topical anaesthetic cream to the base of each tail 60 minutes prior to docking and becoming an attractant to other piglets requires consideration. The comparison of potential analgesic strategy tail docking method and/or the assessment of anti-inflammatory medication requires further investigation.

The aim of this project was to assess the long-term welfare implications of the cauterisation method of tail docking and to identify practical docking methods or medications to reduce the acute pain of tail docking in piglets.

4.0 Objectives of the Research Project

Part 1: Establish if cauterisation has long-term welfare implications for the animal (i.e. formation of neuromas on the tail).

Part 2: Identify a practical medical strategy and/or tail docking technique to reduce the acute pain of tail docking.

5.0 Part 1: Assess the long-term welfare implications of cauterisation

5.1 Introduction

Morrison *et al*. (2013 a, b) examined the behavioural, physiological and neurophysiological response of piglets to tail docking and concluded that tail docking causes a short-term, acute pain response. Furthermore, cauterisation appeared to be less aversive than clippers. These results support those of Sutherland *et al.* (2008). However, the long-term welfare implications of cauterisation such as formation of sensitive neuromas on the tail stump, growth and survival are unknown and further investigation is required before this technique can be recommended as an alternative to clipper treatment for tail docking.

Cauterisation causes intense heat at the site of tail docking which may cause third degree burns, destroying nociceptors in the area, thereby reducing perception of pain in these areas, resulting in a lower neurophysiological and physiological response. However, when the nerve fibres regenerate, neuromas may form (swellings or thickenings caused by abnormal regeneration of nerve fibres secondary to nerve transection) (Lewin-Kowalik *et al*., 2006), causing the tail stump to become highly sensitive later in life when the nociceptors regenerate. The development of neuromas has been linked to increased pain sensitivity, spontaneous pain generation and pain perception in response to benign stimuli (Bennet and Xie, 1988; Jensen and Nikolajsen, 1999).

Therefore, cauterisation should not be recommended in the immediate future as a practical alternative to clippers for tail docking, until the long-term welfare implications of cauterisation are investigated**.** The aim of this experiment (Part 1) was to assess the long-term welfare implications of cauterisation. The neuroanatomy in healed tail tips from slaughter-age pigs docked using clippers or cauterisation docking technique was assessed.

5.2 Research Methodology

This experiment was approved by the Rivalea Australia Animal Ethics Committee (13B068C). The experiment was conducted at the Rivalea Australia, Research and Innovation Unit, Corowa NSW, Australia. The experiment was conducted between March and September 2014. Fourty sows (Large White x Landrace) and their litters were selected. The sows farrowed in individual farrowing crates. Three healthy, viable entire male piglets were selected per litter when they were two days post-birth. The pigs were randomly allocated to treatment and a treatment letter (i.e. A-C) was written on their back with a black stock marker pen. Data were collected from 120 piglets.

The following treatments were imposed:

Treatment A: No Tail docking
Treatment B: Tail docking using clippers
Treatment C: Tail docking using cauteriser (Stericut® Tail Docker)

The piglets were handled in the same manner and for approximately the same time in all treatments. Piglets were quietly picked up from their home pen and were held, supported under the arm of the technician with their hind area exposed. The piglets in treatment A were held the same way for approximately 30 s and were put back into their pen. The pigs in treatment B had their tail docked with clean, disinfected side-cutters (clippers). The pigs in treatment C had their tail docked with a clean disinfected gas operated Stericut® cauteriser. In both treatments the tail was cut approximately 2cm from the base in between the first and second vertebrae and a disinfectant was applied immediately post-docking. At the time of tail docking 20 tails were collected from piglets in the clipper treatment and placed in a solution of formalin (10% neutral buffered) and stored in a refrigerator at 4°C. The pigs remained in a conventional housing (semi-slatted system) system until market. The pigs were individually weighted prior to tail docking treatment at weaning and at market at approximately 150 days (approximately 21 weeks) of age. The individual pig was the experimental unit in the experiment.

5.3 Laboratory methodology

5.3.1 Collection and preparation of tail tissue

A random sample of 20 tail tips removed at the time of tail docking (2 days of age) was fixed and stored in 10% neutral buffered formalin until the time of slaughter. Following slaughter, 20 tails from each treatment (all treatment groups) were cut off at the base and individually fixed in 10% neutral buffered formalin.

5.3.2 Histology and immunohistochemistry

Histological analysis was conducted by Massey University, Institute of Veterinary, Animal and Biomedical Sciences, New Zealand. Prior to sectioning, all tail samples were examined for gross evidence of trauma (indicative of tail biting), and the tail length was measured and recorded. All tails were cross-sectioned 5 mm from the distal tip. For control (undocked) tails, an additional cross section was prepared 92 mm from the base of the tail in the control samples. This represented the average length of all docked tails, allowing visualisation of nerve morphology in this region in the absence of tail docking. Tail tips collected at the time of docking (2 days-old) were cross-sectioned at the docking site, in order to examine nerve morphology in this region at 2 days-of-age.

All tail sections were decalcified and dekeratinised in potassium hydroxide and Veet©, and then processed through graded alcohols and xylene before embedding in paraffin. The formalin-fixed, paraffin-embedded tissues were sectioned at 4–5 µm and stained with haematoxylin and eosin. S-100 immunohistochemical stain was applied to selected samples to optimise nerve visualisation. Tissue sections were deparaffinised then blocked for endogenous peroxidase and treated with primary antibodies towards S-100 (polyclonal rabbit anti-S-100, (Dako)) as described by Nielsen *et al.* (2011). Selected samples were also stained with Masson's trichrome and S-100 to further optimise nerve visualisation". Due to problems with tissue preparation, sections from 15/20 intact tails, 19/20 clipper-docked tails and 18/20 cautery-docked tails were stained and scored.

Tail sections were examined using light microscopy at 1.5, 20 and 40x magnification. Two pathologists, both blinded to treatment, examined sections independently and assigned scores of 1, 2, or 3 as follows:

1 = discrete well organised nerve bundles;
2 = moderate proliferation and disorganisation within fibrous connective tissue, affecting less than half the circumference of the tail;
3 = marked proliferation to form almost continuous disorganised bundles OR non-continuous enlarged bundles compressing the surrounding, densely fibrous, connective tissue.

The presence of disorderly, proliferative nerve bundles embedded in fibrous connective tissue (i.e. scores of 2 or 3), indicated neuroma formation (Simonsen *et al.*, 1991, Devor and Seltzer, 1999, Dahl-Pedersen *et al.*, 2013).

5.4 Statistical analysis

Neuroanatomical scores for control, clip-docked and cautery-docked tails were compared using the Kruskal-Wallis test in SAS version 9.3.1 (SAS Institute Inc., Cary NC, USA, 2012). Post-hoc pairwise comparisons were conducted using the Wilcoxon rank-sum test, with Bonferroni adjustment for multiple comparisons. Fisher's exact test was used to further analyse the neuroanatomical scores. Inter-rater reliability of scores (based on initial independent scores) was determined using the weighted Cohens Kappa coefficient.

Growth performance data were analysed using SPSS version 22 (SPSS, USA, 2015) General Linear Model using sow as a random factor. Chi-squared analysis was used to analyse treatments effects on number of piglets that died or were removed between treatment and weaning, evidence of tail damage and further analysis of neuroanatomical scores.

5.5 Results

Table 1. Number of piglet deaths and removals between treatment and slaughter

Cause of death/removal	Tail intact	Tail docked using clippers	Tail docked using cauteriser
Overlain by sow	5	1	4
Scours/HE	1	1	3
Unthrifty	1	5	1
Tail bite (Euthanasia)	2	0	0
Other	1	2	1
Total	10/40 (25%)	9/40 (23%)	9/40 (23%)

As shown in Table 1, there was no significant difference (X^2=0.09; P=0.95) between the number of piglet deaths and piglet removals due to illness and injury between treatments. These results should be interpreted with caution due to low sample size.

Table 2. Number of pigs with evidence of tail damage at market (i.e. slight scab on end of tail)

	Tail intact	Tail docked using clippers	Tail docked using cauteriser
Number of pigs with tail damage prior to slaughter	20/25=80%	6/25=24%	10/25=40%

As shown in Table 2, the pigs in the intact tail treatments had a significantly higher (X^2=16.67; P=0.000) incidence of tail damage recorded prior to slaughter (scab on the end of their tail indicative of tail biting damage) compared to the combined docked treatments.

Table 3. Effect of treatment on growth performance of piglets

	Tail intact	Tail docked using clippers	Tail docked using cauteriser	SEM	P value
Live weight prior to treatment (kg)	1.85	1.84	1.71	0.03	0.12
Weaning weight (kg) *	7.0	6.2	6.9	0.16	0.06
Rate of gain (g/day) from treatment to weaning*	0.217[a]	0.184[b]	0.213[a]	0.006	0.04
Market weight (kg) *	104.3	101.7	104.4	1.63	0.70

[abc] Within rows values with different superscripts are significantly different (P<0.05).
*Individual live weight prior to treatment used as a covariate in analysis.

As shown in Table 3, there was no significant difference (P>0.05) in the live weight of piglets prior to treatment. There was a strong trend (P=0.06) for higher weaning weight and significantly higher (P<0.05) rate of gain from treatment to weaning in the intact tail and cauterisation treatment. There was no significant difference (P>0.05) in market weight of pigs. These results should be interpreted with caution due to low sample size.

5.5.1 Descriptive neuroanatomy

Descriptive neuroanatomy was used to define and describe the formation of nerve bundles on the tails.

Tail tips from 2-day-old pigs: Discrete, well-organised nerve bundles were observed in all tail tips, indicating innervation at the site of amputation.

Intact tails: No abnormal nerve proliferation was observed in tail sections from control, undocked, pigs (Table 4). Gross evidence of marked tail tip trauma was observed in 5/15 tails, with corresponding evidence of inflammation and/or scarring on histological analysis. Because tails had been formalin fixed it wasn't easy to discern scarring/minor trauma, therefore only severe trauma was noted. Neural anatomy at the site approximating the point of tail docking (section x) was unremarkable, with discrete nerve fibres present and no signs of proliferation observed.

Docked tails: The average length of docked tails was 92 (range 75–140) mm. Evidence of neural proliferation consistent with neuroma formation was observed in tails from pigs docked using both clippers and cautery iron (Table 4).

Neuroanatomical Score
The tails were scored as follows:
1 = discrete well organised nerve bundles;
2 = moderate proliferation and disorganisation within fibrous connective tissue, affecting less than half the circumference of the tail;
3 = marked proliferation to form almost continuous disorganised bundles OR non-continuous enlarged bundles compressing the surrounding, densely fibrous, connective tissue.

Image 1. Microscopy images of neuroanatomical scores.

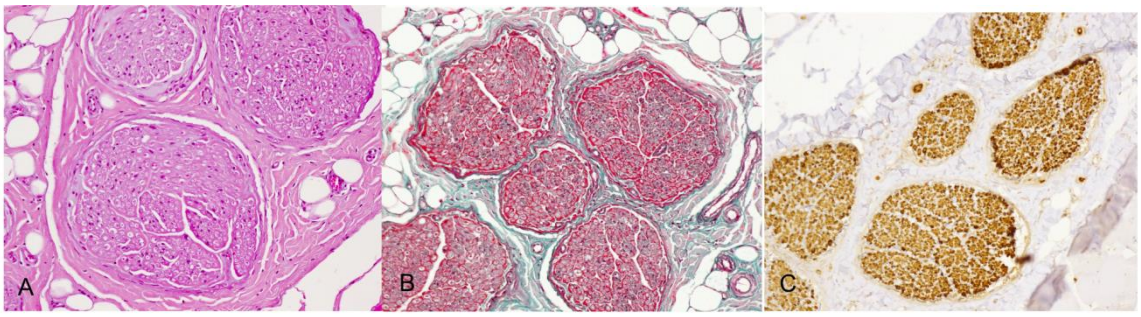

Fig 1. Control pig, nerve histomorphology at the base of the tail (equivalent level to docking site) (magnification 20X). A: H&E; B: Masson's trichrome: C: S100.

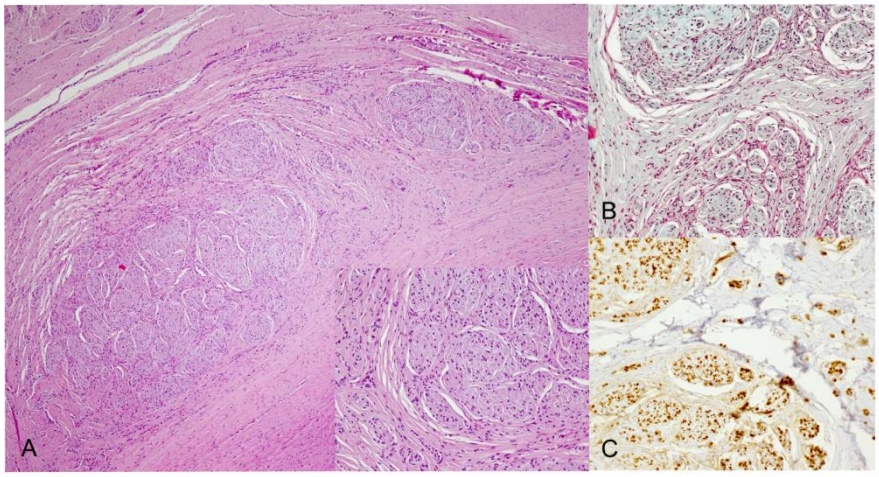

Fig 2. Docked pig, nerve histomorphology scored as 2. A: Moderately disorganised proliferative nerve bundles of varying sizes, surrounded by thin, frequently loosely arranged layers of fibrous connective tissue. H&E (magnification 4X). Inset: 20X magnification at same site. B: Masson's trichrome (magnification 20X); C: Small to medium sized nerve fibres stain brown with S100 (magnification 20X).

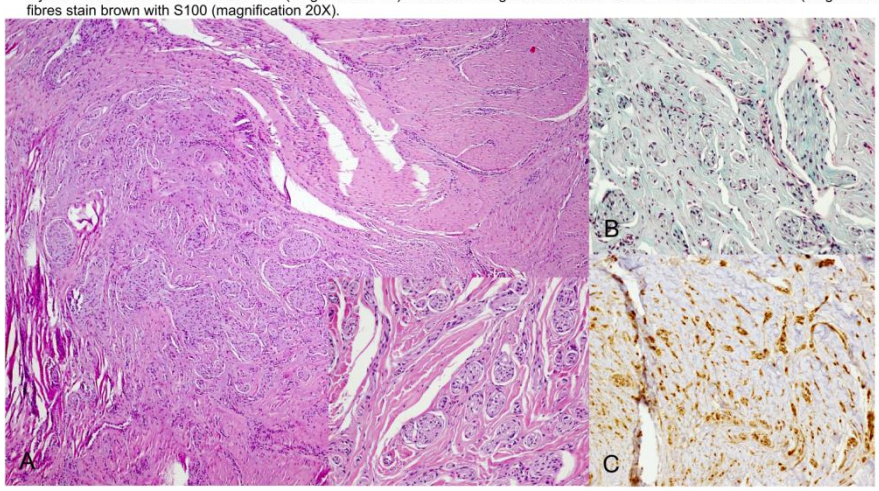

Fig 2. Docked pig, nerve histomorphology scored as 3. A. Poorly organised highly proliferative nerve bundles, often within dense fibrous connective tissue. H&E (magnification 4x). Inset: 20X magnification at same site. B. Note numerous small proliferating nerves within connective tissue. Masson's trichrome (magnification 20X). C: Large numers of small disorganised immunopositive nerve fibres. S100 (magnification 20X).

Table 4. Distribution of neuroanatomical scores in cross sections of the distal tail.

Score	Tail intact	Tail docked using clippers	Tail docked using cauteriser
1	15	2	1
2	0	9	14
3	0	8	3
n	15	19	18

Table 4 shows the distribution of neuroanatomical scores of the distal region in pigs. There was a significant effect of treatment on neuroanatomical scores (χ^2= 31.25; P < 0.0001). The mean scores in both the clipper and cauterisation treatments were significantly higher than the intact tail treatment (Wilcoxon rank means = 35.0, 31.7 and 9.5 respectively for clipper, cauterisation and intact tail treatments) (P< 0.001). Comparison of clipper and cauterisation means revealed no difference in mean scores (p = 0.23), although there was a trend for the proportion of tails scored as 3 (marked neural proliferation) to be higher in tails docked using clippers than those docked using cauterisation (42 vs. 17%, respectively; P=0.15).

5.6 Discussion

The aim of the present study was to assess the long-term welfare implications of cauterisation. The neuroanatomy in healed tail tips from slaughter-age pigs docked using clippers or cauterisation docking method was compared.

Due to the haphazard degree of neural proliferation observed in the tips of docked tails, the number and/or size of individual neuromas could not be determined, as it was not clear whether large regions of proliferation resulted from the regeneration of single or multiple severed nerves. Instead, a descriptive scale was used to rate the degree of neural proliferation. Evidence of abnormal neural proliferation, consistent with neuroma development, was observed at the site of amputation in tails docked using both clippers and cauterisation. These results are similar to that found by Herskin *et al.* (2015). In contrast, no evidence of neuroma formation was found in tail tips of control, undocked tails, despite some gross evidence of tail tip trauma (assumed to be the result of minor tail biting from other pigs).

Given the reported associations between neuroma formation and the occurrence of neuropathic pain (Zimmermann, 2001, Lewin-Kowalik *et al.*, 2006), the presence of neuromas in the tail stumps of pigs docked using clippers and cauterisation suggests that tail docking by either method has the potential to induce long-term alterations in pain perception in pigs. This does however need to be confirmed before any robust conclusions about pig welfare can be drawn. This might be achieved by assessing thermal and mechanical nociceptive thresholds in the tail tip over the lifetime in commercial pigs with intact tails and those docked using clippers or cauterisation.

The piglets in the intact tail and cauterisation treatment had a higher rate of gain from treatment to weaning than those in the clipper treatment. There was no difference in piglet survival between the treatments. The difference in growth rate may be attributed to the fact that cauterisation involves the burning of the tail tissue and searing the wound which may reduce the risk for bacteria to gain entry via the tail wound (Hungerford, 1990) post-tail docking, compared to the clipper method which leaves an open wound. It is well known that activation of the hypothalamic-pituitary-adrenal axis (HPA axis) can lead to suppression of growth hormone and corticosteroids can induce resistance to growth factors in target tissues (Kaltas and Chrousos, 2007). Corticosteroids and adrenocorticotrophic hormones can also have a catabolic effect on the body (Elsasser *et al.*, 2000).
Morrison *et al.* (2013) showed increased cortisol response at 15 minute post-treatment in pigs tail docked with the clippers than those cauterised. Furthermore, an electroencephalographic assessment of nociceptive responses to tail docking revealed that cauterisation was less aversive than clipper method (Kells *et al.*, 2013). It is unclear whether the stress response was implicated in the reduction in growth performance in the clipper treatment in the current experiment. This requires further investigation involving larger numbers of animals, as a possible long-term welfare benefit in terms of growth and survival of pigs docked using the cauterisation method.

In conclusion, tail docking by either clipper or cauterisation method resulted in a higher proportion of neuromas in the healed tail tip. There was a trend for less severity of neuroma formation following tail docking by cauterisation than clippers. Further research is required to identify if these neuromas are in fact painful for the pig. There was no incidence of neuroma formation on intact tails, despite some gross evidence of tail tip trauma resulting from tail biting.

6.0 Part 2: Development of practical medication strategies to reduce the acute pain of tail docking.

6.1 Introduction

Our previous Australian Pork Limited (APL) project (2010/1018.348) investigated the physiological, behavioural and neurophysiological responses of pigs to tail docking showed that tail docking two-day old piglets using the clipper and cauterisation method caused an acute, short-term stress response, and indicated that cauterisation is less aversive than clipper method.

The need for pain relief to be provided for a husbandry procedure that causes an acute short-term response remains controversial. There is limited information in the scientific literature assessing practical medication strategies to reduce the acute pain of tail docking. Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) that becomes effective approximately 30 to 60 minutes after administration. Meloxicam works by blocking the action of a substance called cyclo-oxygenase, which is involved in the production of prostaglandins. Prostaglandins are produced by the body in response to injury and certain diseases and conditions, and cause pain, swelling and inflammation. Meloxicam blocks the production of these prostaglandins and is therefore effective at reducing inflammation and pain (Cashman 1996, Isiordia-Espinoza *et al*., 2012). Meloxicam is recommended for use in the European Union for surgical castration of piglets (EU Directive, 2010). Our previous APL project (Morrison *et al*., 2013b) showed that meloxicam (Metacam® 5mg/ml administered via piglet intramuscular injection 60 minutes prior to tail docking) was effective at reducing the stress response, based on cortisol concentrations 15 and 30 minutes post-docking. Interestingly, the piglets treated with meloxicam were more active post-tail docking which could not be explained.

Morrison *et al*. (2013b) also showed a topical lignocaine cream (EMLA cream-2.5% Lignocaine; 2.5% Prilocaine) applied 60 minutes prior to tail docking treatment was also effective at reducing the neurophysiological (EEG) response to tail docking. In the same experiment the use of cauterisation also appeared to mitigate the acute nociceptive response, although to a lesser extent than the topical anaesthetic. The topical anaesthetic cream contained the anaesthetic agents lignocaine and prilocaine, which penetrate the skin and block signals generated by the activation of nociceptors in the dermal and sub dermal regions, preventing any generated nociceptive signals from reaching the brain (Thurmon *et al*., 1996). However, this topical anaesthetic or other medications that are not registered for use in pigs were not able to be investigated in this experiment. Furthermore, the practicality of applying a topical anaesthetic cream to the base of each tail 60 minutes prior to docking and becoming an attractant to other piglets requires consideration.

The implications of an anti-inflammatory medication on piglets tail docked using cauterisation or clippers requires further investigation. Therefore, the aim of this experiment (Part 2) was to assess the efficacy of cauterisation and meloxicam in mitigating acute stress responses to tail docking.

6.2 Research Methodology

This experiment was approved by the Rivalea Australia Animal Ethics Committee (14B052C). The experiment was conducted at Rivalea Australia, Corowa NSW, Australia. The experiment was conducted between October and December 2014. Seventy two sows (Large White x Landrace) and their litters were selected. The sows farrowed in individual farrowing crates. Fostering was conducted within the first 24 hours post-birth to standardise litters and ensure there were 6 viable males in each experimental litter. Six entire, healthy pigs were selected per litter when they were approximately two days post-birth. The pigs were randomly allocated to treatment and a treatment letter (i.e. A-F) was written on their back with a black stock marker pen. Data were collected from 432 piglets.

The following treatments were imposed:
Treatment A: No handling
Treatment B: Sham treatment (handling alone)
Treatment C: Tail docking using clippers
Treatment D: Tail docking using cauteriser (Stericut® Tail Docker)
Treatment E: Meloxicam-Metacam®-5 mg/ml (0.1ml/1.25kg pig) injected intramuscular 1 hr prior to tail docking using clippers.
Treatment F: Meloxicam-Metacam®-5 mg/ml (0.1ml/1.25kg pig) injected intramuscular 1 hr prior to tail docking using cauteriser.

Piglets in treatment E and F were picked up quietly and injected with Metacam® 60 minutes prior to tail docking. During tail docking treatments, the piglets were handled in the same manner and for approximately the same time in all treatments (except for treatment A). Piglets were quietly picked up from their home pen and were held, supported under the arm of the technician with their hind area exposed. The piglets in treatment B (sham) were held the same way at tail docking for approximately 30 s and were put back into their pen. In all treatments the tail was cut approximately 2cm from the base of the tail in between the second vertebrae. The pigs in treatments C and E had their tail docked with clean, disinfected side-cutters (clippers). The pigs in treatments D and F had their tail docked with a clean disinfected gas operated Stericut® cauteriser.

Piglets in treatment A and B had their tails removed after blood samples and behavioural observations were completed as the piglets were not able to remain in the commercial herd with their tails intact as the risk of these piglets being tail bitten was too high. Therefore, the data for growth performance of piglets in treatment A and B is not included in analysis. An iron injection was given to all piglets and an individual ear tag placed into the ear of each piglet approximately 90 minutes after treatment (once behavioural observations were completed).

6.2.1 Stress physiology

Blood samples were collected by jugular venipuncture. The blood samples were taken at 15 minutes and 30 minutes post-tail docking. The blood sampling was conducted by trained personnel who were able to obtain a blood sample within 20 s of the piglet being picked up. The blood was collected into 2 ml Vacutainer tubes (BD, Franklin Lakes, NJ, USA) treated with Lithium Heparin and stored on ice. The individual samples were centrifuged at 7000 rpm and the plasma was poured off and stored frozen at -20°C until analysed. The samples were assayed for total cortisol at University of Western Australia. Plasma concentrations of cortisol were measured in duplicate by radioimmunoassay using Immuchem™ Coated Tube Cortisol ^{125}I RIA kits (MP Biomedicals, Belgium). The limit of detection

was 0.2 µg/dL. Quality control samples (7.1 and 25.2 µg/dL) were used to estimate inter- (6.4 % and 3.8 %) and intra-assay (7.4 % and 4.8 %) coefficients of variation.

6.2.2 Behaviour

During the treatment an escape attempt was defined as a body movement carried out to effect an escape (i.e. rapid leg thrust while being held by the technician) as described by Marchant-Forde *et al*., 2009). The duration of vocalisations was recorded during treatment, from the time piglets were picked up to when they were placed back in the pen after treatment. The behaviour of the six treatment pigs in each litter was videotaped by using mounted cameras (HD Sports cameras) that enabled view of the whole farrowing crate. The behaviour of the piglets for the first 60 minutes post-treatment was measured by continuously observing each piglet for 60 sec every 5 minutes. (i.e. a total of 12 minutes in the first 60 minutes post-treatment).

The following ethogram was used to describe behaviours:

Table 5. Ethogram of behaviour of the piglets (modified from Hay et al., 2003, Hurnik et al., 1995).

Posture:	
Standing (normal)	Upright position with bodyweight supported by all four legs.
Standing (head lowered)	Upright position with bodyweight supported by all four legs. Head lower than shoulders.
Sitting	Body weight supported by the hind-quarters and front legs.
Lying (with sow contact)	Maintaining a recumbent position in contact with a part of the sow.
Lying (without sow contact)	Maintaining a recumbent position not in contact with a part of the sow.
States:	
Idle	Not performing any behaviour
Walking /Running	Slowly moving forward one leg at a time/ Trot or gallop
Massaging udder/ Nursing	Nose in contact with the udder and/or teat in mouth-assumed to be suckling.
Asleep	Lying down assumed to be sleeping.
Playing/frolicking	Head shaking, springing (sudden jump or leap), running with horizontal and vertical bounces.

The total active behaviours were calculated as the combination of walking, running, playing and frolicking. Total resting behaviours were calculated as the total of time lying with and without sow contact. The term "out of view" was used when the piglet could not been seen within the field of view of the camera.

6.2.3 Growth performance

The piglets were weighed individually immediately prior to the treatment and then at 7 days post-treatment and at weaning (average of 26 days of age).

6.2.4 Statistical analysis

Statistical analysis were performed using SPSS (Version 21 -SPSS Inc., Chicago, Illinois, USA). All data were analysed for normality and transformed (square root) where appropriate. Analysis was conducted using univariate General Linear Model, using each piglet as the experimental unit and the

sow as the random factor. *Post hoc* tests were conducted to identify differences between individual viewing treatments and period using Least Significant Difference tests. Chi-squared analysis was used to analyse treatments effects on number of piglets that died or were removed between treatment and weaning.

6.3 Results

Table 6. Number of piglet deaths and removals between treatment and weaning.

Cause of death/removal	No handling*	Sham*	Tail docked using clippers	Tail docked using cauteriser	Meloxicam + clipper	Meloxicam + cauterisation
Overlain by sow	3	3	3	1	7	2
Scours/HE	1	1	2			3
Unthrifty		2	3	1		1
Other	1	1	1	1	3	1
Total	5/72	7/72	9/72	3/72	10/72	7/72

*Note that piglets in the no handling and sham treatment had their tails docked with clippers after behavioural observations were completed.

As shown in Table 6, there was no significant difference (X^2=3.68; P=0.505) between the number of piglet deaths and piglet removals due to illness and injury between treatments. Combining data for the tail docking method (i.e. data were pooled for each clipper method) revealed a trend for more deaths when clippers were used compared to cauterisation (13.2% and 6.9% mortality post-treatment, in clipper and cauterisation method, respectively. X^2=3.11; P=0.078).

Table 7. Effect of treatment on mean total cortisol concentrations (ng/ml).

Cortisol (ng/ml)	No handling	Sham	Tail docked using clippers	Tail docked using cauteriser	Meloxicam + clipper	Meloxicam + cauterisation	SEM	P value
15 minutes post-taildocking	88.6^{a}	138.4^{b}	186.7^{c}	169.5^{cd}	163.2^{bcd}	144.3^{bd}	3.97	0.000
30 minutes post-taildocking	212.6^{a}	276.2^{bc}	317.7^{b}	267.5^{c}	261.8^{c}	238.7^{ac}	6.73	0.001

^{abc} Within rows values with different superscripts are significantly different (P<0.05).

As shown in Table 7, there was a significant treatment effect (P=0.000) on cortisol concentrations at 15 minutes post-tail docking. In comparison to the Sham treatment, cortisol concentrations at 15 minutes post-treatment were higher (P<0.05) in the clipper and cauterisation treatment. The two tail docking treatments with meloxicam were similar to the sham. Cortisol concentrations in the no handling treatment were lower than in the other four treatments.

There was a significant (P=0.001) treatment effect on cortisol concentrations at 30 minutes post-tail docking. All tail docking and meloxicam treatments were similar (P>0.05) to the sham treatment. All treatments apart from the meloxicam and cauterisation were higher than the No handling treatment. The cauterisation and meloxicam treatments were lower (P<0.05) than the clipper treatment.

Table 8. Effect of treatment on behaviour of piglets during and 60 minutes after treatment. Mean total time (sec) spent in each posture or state during observation period.*

	No handling	Sham	Tail docked using clippers	Tail docked using cauteriser	Meloxicam + clipper	Meloxicam + cauterise	SEM	P value
Duration of vocalisations during treatment (sec)	-	1.2[a] (1.4)	2.1[b] (4.4)	1.9[b] (3.6)	2.0[b] (4.0)	1.9[b] (3.6)	0.04	0.000
Number of escape attempts during treatment	-	0.9[a] (0.8)	1.8[b] (3.2)	1.8[b] (3.2)	1.9[b] (3.6)	1.7[b] (2.9)	0.04	0.000
Posture (sec):								
Standing (normal)	10.2 (104.0)	11.8 (139.2)	11.8 (139.2)	10.4 (108.2)	14.8 (219.0)	15.5 (240.3)	0.19	0.149
Standing (head lowered)	4.2[a] (17.6)	4.1[a] (16.8)	10.7[b] (115.5)	5.2[a] (27.0)	4.2[a] (17.6)	5.8[a] (33.6)	0.15	0.000
Sitting	6.0 (36)	6.2 (38.4)	3.7 (13.7)	6.8 (46.2)	6.1 (37.2)	6.5 (42.3)	0.33	0.686
Lying (with sow contact)	11.1 (123.2)	13.5 (182.3)	6.1 (37.2)	13.0 (169)	10.3 (106.1)	14.0 (196.0)	0.35	0.056
Lying (without sow contact)	20.1 (404.0)	20.8 (432.6)	17.9 (320.4)	21.4 (457.9)	18.0 (324.0)	18.1 (327.6)	0.30	0.875
Out of view	6.6 (43.6)	4.9 (24.0)	6.4 (41.0)	6.1 (37.2)	5.9 (34.8)	5.5 (30.3)	0.19	0.983
States (sec):								
Idle	7.3 (53.3)	6.9 (47.6)	8.5 (72.3)	7.2 (51.8)	7.6 (57.8)	8.1 (65.6)	0.15	0.965
Active (play, run, walk, frolick)	6.8 (46.2)	6.3 (39.7)	11.5 (132.3)	7.6 (57.8)	9.2 (84.6)	8.8 (77.4)	0.16	0.134
Massaging udder/Nursing	11.5 (132.3)	10.6 (112.4)	8.7 (75.7)	7.6 (57.8)	13.6 (185.0)	13.6 (185.0)	0.27	0.347
Asleep	20.3 (412.1)	21.3 (453.7)	18.4 (338.6)	21.5 (462.3)	18.4 (338.6)	18.3 (334.9)	0.21	0.667
Out of view	6.5 (42.3)	4.9 (24.0)	6.5 (42.3)	6.1 (37.2)	5.8 (33.6)	5.5 (30.3)	0.19	0.985

[abc] Within rows values with different superscripts are significantly different (P<0.05).

* Data transformed prior to statistical analysis. Transformed means are presented and back transformed means presented in parentheses.

As shown in Table 8, there were significantly more ($P<0.05$) vocalisations and escape attempts in all of the tail docking treatments compared to the sham treatment. Piglets in the clipper treatment spent more time ($P<0.05$) standing with their head lowered compared to all other treatments. There was no significant difference ($P>0.05$) between treatments in other postures and states observed in the 60 minutes period post docking.

Table 9. Effect of treatment on growth performance of piglets.

	No handling*	Sham*	Tail docked using clippers	Tail docked using cauteriser	Meloxicam + clipper	Meloxicam + cauterise	SEM	P value
Live weight prior to treatment (kg)	-	-	1.90	1.96	1.95	1.97	0.025	0.797
Weaning weight(kg) *	-	-	6.34	6.33	6.33	6.31	0.120	1.00
Rate of gain (g/day)	-	-	0.209	0.207	0.206	0.207	0.005	0.997
Treatment -weaning *								

*data not included for no handling and sham treatment as these piglets had their tails docked immediately after behaviour and physiology samples were collected. Individual weight prior to treatment was used as a covariate in analysis.

As shown in table 9, there was no significant difference (P>0.05) in weaning weight or rate of gain between the clipper, cauterised and meloxicam treatments.

Table 10. Cost of tools and medications.*

	Sham	Tail docked using clippers	Tail docked using cauteriser	Meloxicam + clipper
Cost/piglet treatment ($)	-	One off purchase for clippers $60	One off purchase for cauteriser $150 + $8 gas refill	One off purchase for clippers $60+ $0.53 + labour

*These prices are estimates only. These costs were calculated based on commercial costs to purchase the medication/equipment at the time of the experiment. This price may vary subject to costs of medications. The additional labour required is not included but is estimated to be 1 minute/pig to catch and inject piglet prior to tail docking (approx. $0.80/piglet if labour charged at $50/hr).

6.4 Discussion

The aim of this experiment was to assess the efficacy of cauterisation and meloxicam in mitigating acute stress responses to tail docking.

There was no significant difference in the number of medical treatments, piglet mortality and growth between treatments indicating that administration of meloxicam prior to tail docking did not provide any long-term benefits to the piglets in terms of health, weight gain and survival. It is well known that activation of the hypothalamic-pituitary-adrenal axis (HPA axis) can lead to suppression of growth hormone and corticosteroids can induce resistance to growth factors in target tissues (Kaltas and Chrousos, 2007). Corticosteroids and adrenocorticotrophic hormones can also have a catabolic effect on the body (Elsasser *et al*., 2000). Although in the current experiment piglets in the tail docked treatments had activation of the HPA axis 15 minutes after treatment, the response was not significant to cause a reduction in growth performance. Furthermore, there were no differences in duration spent nursing post-treatment indicating that nursing behaviour was not disrupted post-treatment. These data provide further evidence that tail docking causes a short-term stress response which does not impact on biological fitness of the animal and the results are in agreement with other reports which found no relationships between pain-control and weight gain of piglets (Tenbergen *et al*., 2014; Hansson *et al*., 2011; Keita *et al*., 2010). It was not possible to compare the growth performance of tail docked pigs to those with intact tails in the current experiment and tails were unable to be left intact due to increased risk of tail biting.

However, when the data for tail docking methods were pooled (i.e. both clipper vs cauterisation treatments), there was strong trend for less deaths in the cauterisation treatment. Cauterisation involves the burning of the tail tissue and searing the wound which may reduce the risk for bacteria to gain entry via the tail wound (Hungerford, 1990) post-tail docking, compared to the clipper method which leaves an open wound. This requires further investigation involving larger numbers of animals, as a possible long-term welfare benefit in terms of growth and survival of pigs docked using the cauterisation method.

Total cortisol concentrations were measured at 15 and 30 minutes after the tail docking treatment. Tail docking using the clippers and cauteriser elicited a significant stress response at 15 minutes post-treatment compared to the sham treatment. At 30 minutes after docking all tail docking treatments were similar to the sham treatment, indicating that tail docking causes a short-trem acute stress response. At 30 minutes after tail docking cauterisation treatment elicited a lower stress response compared to the clipper treatment. These results are similar to that of Sutherland *et al.* (2008) who showed that cauterisation was less aversive than clipper treatment. Care must be taken when comparing these experiments as the pigs in Sutherland *et al*. (2008) were considerably older (6 days of age), nevertheless there appear to be similarities between experiments. Prunier *et al*. (2005) also showed that cortisol concentrations did not differ between cauterised and handling alone treatments for up to 180 minutes post-tail docking, providing further evidence that cauterisation is less aversive than the clipper treatment. Marchant-Forde *et al.* (2009) showed no difference in cortisol concentration 45 minutes post tail docking using clipper or cauteriser. It is speculated that cortisol has already peaked and returned to baseline levels in that experiment prior to the 45 minutes blood sampling measure.

In comparison to the Sham treatment, cortisol concentration at 15 minutes post-treatment was higher in the clipper and cauterisation treatment, but not the two tail docking treatments with meloxicam.

Thus the stress response at 15 minutes appeared to be mitigated by the cauterisation alone or either tail docking methods and meloxicam. At 30 minutes post-treatment, none of the tail docking methods with and without meloxicam differed from the Sham treatment in terms of cortisol concentrations. The cortisol concentrations at 30 minutes post treatment were similar in the sham, clipper and meloxicam treatments. Therefore, the administration of injectable meloxicam 60 minutes prior to tail docking with either method reduced the stress response at 15 minutes after tail docking. Meloxicam, like other non-steroidal anti-inflammatory drugs, is believed to exert anti- nociceptive effects mainly through inhibition of peripheral inflammatory responses and there is some evidence that it may also have central and pre-emptive analgesic effects (Cashman 1996, Isiordia-Espinoza *et al*., 2012).
Tail docking using clippers or cauterisation caused an increase in piglet vocalisations and escape attempts during the tail docking treatment and these behaviours were not mitigated by the use of meloxicam. These results agree with those of Tenbergen *et al*. (2014). Meloxicam has been shown by other authors to mitigate acute pain associated immediately post-tail docking (Tenbergen *et al*., 2014), however it does not appear to be effective at blocking the pain associated with the surgery itself. This is supported by the assessment of pain based on neurophysiological analysis by Morrison *et al.* (2013b) in which the administration of meloxicam 60 minutes prior to tail docking did not affect the nociceptive response during the actual tail docking surgery. Marchant-Forde *et al*. (2009) showed that piglets that had their tail docked by cauterisation emitted more squeals per second with higher mean and peak frequencies compared to the clipper treatment. The authors state that the cauterisation treatment took 20% longer than the clipper method, thereby exposing the piglet to handling of longer duration. In the current experiment piglets were all held for the same amount of time to ensure that there were no confounding factors involved.

In the current experiment piglets that had their tail docked by clipper spent more time standing with their head lowered in the 60 minutes post-treatment. Head lowered has been previously suggested to be an indicator of pain (Hay *et al*., 2013). Meloxicam with either tail docking method appeared to mitigate this behavioural response post-treatment in the present experiment. Morrison *et al*. (2013) also found a similar effect with tail docking with the clipper and, unlike the present experiment with cauterisation.

In conclusion, the physiological and behavioural evidence indicates that tail docking of piglets by either clipper or cauterisation caused an acute pain response both during treatment and in the short term after treatment. Cauterisation appeared to be less aversive. The administration of meloxicam did not mitigate the behavioural response during and after tail docking, however appeared to mitigate the stress response after treatment.

7.0 Implications & Recommendations

In conclusion, based on physiological and behavioural responses, tail docking with either clippers or cauterisation causes a short-term acute pain response. The use of cauterisation method appears to be less aversive than clipper treatment. Injectable meloxicam administered 60 minutes prior to tail docking with either method also alleviated this acute pain response. The need for pain relief to be provided for a procedure that causes an acute short-term response remains controversial. The administration of meloxicam pain relief 60 minutes prior to tail docking increases cost of pig production through additional labour, piglet handling and medication costs. The use of the cauterisation method is a more commercially-viable method (no additional labour and medication costs) that appears to provide similar welfare benefits to the piglet in the short-term.

Tail docking by either clipper or cauterisation method resulted in a higher proportion of tails with neuromas. There was a trend for less severity of neuroma formation in cauterisation compared to the clipper treatment. There was no neuroma formation on intact tails even though there was evidence of gross tissue damage. Furthermore, there was a trend less piglet deaths in the pooled cauterisation alone and cauterisation plus meloxicam which may be due to cauterisation searing the tail wound reducing the risk of bacteria entering the body. The impacts on piglet growth and health require further investigation on a larger sample size.

Cauterisation appeared to be less aversive than clipper method based on effects on stress physiology, pain-related behaviour post-treatment and trend for lower severity of neuroma formation. However, caution should be exercised when considering cauterisation as an alternative to the clipper treatment. This project was conducted under experimental conditions using new equipment and trained operators. The cauterisation method involves equipment that requires a high level of maintenance and an extremely high standard of operator competence to ensure that the procedure is conducted efficiently and humanely. The impact on piglet growth health and survival requires further investigation to fully understand the commercial-viability of the cauterisation method.

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