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Assessment of Pain Induced by Tail Docking in Piglets and Strategies to Reduce this Pain

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Executive Summary

There is increasing pressure from animal welfare groups to provide pain relief for elective husbandry procedures such as tail docking of piglets. 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 and whether it is necessary to provide pain relief. The limited number of experiments that have been conducted to assess the painfulness of tail docking have only used stress physiology and/or changes in pain-related behaviour to measure pain. More recently, neurophysiological tools (i.e. the Electroencephlogram-EEG which reflects changes in the central nervous system function) have been developed, and in combination with stress physiology and behaviour are a useful tool to assess the pain caused by tail docking.

The first aim of this project (Part 1) was to assess the pain caused by tail docking using either clippers or cauterisation by measuring the neurophysiological, physiological (stress) and behavioural responses of the animal. There were two experiments conducted. The neurophysiological component (Experiment 1) was conducted by Massey University, New Zealand and the physiological and behavioural component (Experiment 2) was conducted by Rivalea Australia. Whilst the design of the experiments was similar and the methodology of treatment application were the same, care must be taken when interpreting the results as the experiment were conducted at different locations (i.e. different environment, genetics of pigs etc). Nevertheless, there were some consistencies between the results of the two experiments.

Experiment 1 examined the neurophysiological response (EEG) to docking with clippers versus cauterisation. Tail docking caused a significant EEG response and a larger and sustained EEG response was caused by clippers, suggesting that tail docking causes an acute pain response and that clipping is more aversive than cauterisation.

Experiment 2 examined the stress physiology and pain-related behavioural responses of piglets to clippers and cauterisation treatment compared to surgical castration and a sham treatment (handling alone). Tail docking piglets using the clipper or cauterisation method caused a significant cortisol response at 15 and 30 min post-treatment, caused an increase in vocalisations and escape attempts during treatment and increased in pain-related behaviour in the 60 min period post-treatment. This impact on stress physiology and pain-related behaviour had diminished by 24 hours post-treatment. There were no significant differences in growth performance between treatments which indicates that the tail docking procedure did not impact on biological fitness of the piglet.

Cauterisation appeared to be less aversive than clippers, in terms of the neurophysiological (Experiment 1) and physiological response (Experiment 2). However the long term welfare implications of cauterisation are not known (i.e. formation of sensitive neuromas on the tail stump) and this technique requires further investigation before it is recommended as an alternative to clipper treatment.

The second aim of the project (Part 2) was to investigate practical strategies that could be used to reduce or eliminate the acute pain caused by tail docking procedure. There is pressure from animal welfare groups to provide pain relief for management husbandry procedures, regardless of the duration of the pain. 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, 2013). Therefore based on this premise, the decision was made to investigate commercially-available medications that may alleviate the acute pain of tail docking.

There were two experiments conducted in Part 2. The neurophysiological component (Experiment 3) was conducted by Massey University, New Zealand and the physiological and behavioural component (Experiment 4) was conducted by Rivalea Australia. The three following commercially-available medications were investigated. Cauterisation was included as it is a cheaper alternative to the medications.

i) Topical anaesthetic cream- applied to the base of the tail 60 min prior to tail docking with clippers (product contained 2.5% Lignocaine, 2.5% Prilocaine).
ii) Topical anaesthetic spray- applied to the docked wound immediately after tail docking with clippers (product contained 40.6g/L Lignocaine, 4.2g/L Bupivacaine, 24.8 mg/L Adrenaline, 5.0 g/L Cetrimide).
iii) Anti-inflammatory-Meloxicam given either orally or by injection 60 min prior to tail docking with clippers.
iv) Cauterisation.

Experiment 3 investigated the neurophysiological response after the application of a topical anaesthetic cream, oral meloxicam and cauterisation compared to clipper. Application of a topical anaesthetic cream abolished the EEG responses observed with clippers alone. Cauterisation also appeared to mitigate the EEG response, although to a lesser extent than the topical anaesthetic. Oral meloxicam had little effect on EEG responses to tail docking. Further research is required to assess the physiological and behavioural responses of piglets to tail docking after application of a topical anaesthetic cream.

Experiment 4 investigated the physiological and behavioural response of piglets after clipper, cauterisation, a topical anaesthetic spray or injectable meloxicam treatment compared to clipper and sham (handling alone) treatments. There were physiological and behavioural responses of piglets to tail docking. There was a cortisol response at 15 min post-treatment in the clipper treated pigs, however these stress responses diminished by 30 min post-treatment which provides further evidence that tail docking causes an acute stress response. Piglets in the meloxicam treatment had a lower cortisol response at 15 min post-treatment compared to the clipper treatment.

Piglets in the cauterised, topical anaesthetic spray and meloxicam treatment all exhibited the same amount of vocalisations and escape attempts during treatment as the clipper treatment, therefore it was concluded that these medications/techniques were not effective at reducing pain-related vocalisations and behaviour at the time of the tail docking procedure. Furthermore the cauterisation, topical anaesthetic and meloxicam did not influence pain-related behaviours in the 60 min period post-treatment. Interestingly, in Experiment 4 there were no significant differences in pain-related behaviours between the sham treatment and tail docked treatments, which is contrary to Experiment 2. It was speculated that the environmental conditions under which Experiment 4 was conducted were hotter than those of Experiment 2 which may have influenced piglet behaviour in the period post-treatment. Meloxicam treated pigs were more active and were more aroused compared to the sham and clipper treatment. These results cannot be explained and further research is required to assess the use of meloxicam as a possible pain relief for tail docking.

In conclusion, based on neurophysiological, physiological and behavioural responses, tail docking caused an acute pain response. This pain response had diminished by 24 hours. The use of

cauterisation appears to be less aversive than clippers, however further research is required to assess long-term welfare implications of cauterisation. Topical anaesthetic cream and injectable meloxicam administered prior to tail docking appear to mitigate this acute pain, however further research is required to investigate physiological and behavioural responses to a topical anaesthetic cream and behavioural changes observed with the use of meloxicam. The commercial-viability and practicality of using these pain relief medications requires further investigation.

Table of Contents

Background to Research/Introduction

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 wounding 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 at when the pigs are marketed, and in the mean time there are serious welfare consequences for the pig. The etiology of tail biting remains poorly understood and potential factors pre-disposing tail biting are numerous, e.g. crowding, poor ventilation, breakdown in the food or water supply, poor quality diets and breed type. Despite years of research focusing on this area the underlying behavioural mechanisms for tail biting are 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 there is substantial evidence that the procedure reduces the numbers of tail-bitten pigs. Removing at least half of the tail has been recommended, 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). Docking tails too short may lead to infections or prolapses (Smith, 1999), or left too long, may reduce the tail dock's effectiveness (Hunter et al., 2001).

There is increasing pressure from animal welfare groups to provide pain relief for elective husbandry procedures such as tail docking. 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. Furthermore, the limited number of experiments that have been conducted to assess the painfulness of tail docking (Marchant-Forde et al., 2009; Sutherland et al., 2008; Prunier et al., 2005) have not used the range of neurophysiological, physiological, behavioural responses of the animal to assess acute and chronic pain caused by the procedure of tail docking.

It should be recognised that 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. Furthermore, many of the traditional behavioural and physiological indices that have been used to study pain are also measures of non-painful stressors. For example, measures such as heart rate, hormone response and behaviour are not specific to pain. 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).

Neurophysiological tools are now widely used in humans to assess pain in both research and clinical settings. Studies in human volunteers and in patients experiencing pain have demonstrated that in contrast to physiological measures, electroencephalographic (EEG) variables correlate well with subjective evaluations of pain, indicating the value of quantitative EEG analysis as an indicator of the degree of pain perceived by humans. These neurophysiological tools have also been applied and demonstrated in animal studies in search of a monitor of adequacy of anaesthesia and to assess the efficacy of analgesic agents. Recently, neurophysiological responses of the animal, recorded by EEG

have been shown to provide valuable insights into the perception of pain by animals (Johnson, 2007; Murrell and Johnson, 2006), and are now used in combination with behavioural and physiological responses of the animals to measure pain.

It is essential to conduct experiments using neurophysiological, behavioural and physiological responses to evaluate whether tail docking causes significant pain in piglets, and if required, strategies and medications to reduce the pain associated with tail docking should be investigated.

Objectives of the Research

Part 1: Assess the pain induced by tail docking using two common methods for tail docking.

(i) Neurophysiological responses of tail docking-acute pain response.
(ii) Physiological and behavioural responses of tail docking-acute and chronic pain response.

Part 2: Depending on the outcomes of part 1, short and/or long term strategies (i.e. medications) to reduce or eliminate the pain caused by tail docking procedure will be investigated.

Part 1: Assess the Pain Induced by Tail Docking Using Two Common Methods for Tail Docking

Experiment 1: Neurophysiological Responses of Tail Docking - Acute Pain Response

The aim of this experiment was to compare the neurophysiological responses of piglets when they were tail docked using clippers or cauterisation at 2 and 20-days of age.

The electroencephalogram (EEG) reflects changes in the central nervous system function and it gives an indication of the activity of the cerebral cortex (Silva, 2004). Particular structures in the brain such as the anterior cingulated gyrus play an important role in the perception of pain, and recent studies in humans have shown that EEG variables correlate well with subjective evaluation of pain (Johnson, 2007). Power spectral analysis gives an indication of the frequencies that form the EEG and a number of descriptive variables have been derived from the power spectrum, which include the median frequency, 95% spectral edge frequency and total EEG power. Power spectral analysis of the EEG has been used in many species as an indication of noxious stimulation. In general, the response of the EEG to a noxious stimulus manifests as a decrease in low frequency activity and an increase in high frequency activity. This results in increases in 95% spectral edge and median frequency and a concurrent decrease in total EEG power (Johnson, 1997).

Murrel and Johnson (2006) developed a 'minimal anaesthesia' technique which enables the animal to be anaesthetised so they cannot experience the pain, however their cerebral cortex responds to the noxious stimulus in the same way as when the animal in conscious. This technique enables a negative control treatment to be imposed with detrimental effects of animal welfare and due to the sensitivity of the EEG response, and fewer animals can be used in experiments. Assessment of the neurophysiological response through spectral analysis of the EEG response is becoming a common approach in pain research.

Research Methodology

This experiment was approved by the Massey University Animal Ethics Committee. The experiment was conducted at the neurophysiology laboratory, Institute of Veterinary, Animal and Biomedical Sciences (IVABS), Massey University, Palmerston North, NZ. The experiment was conducted between March and April 2011. Fifty (male) piglets (Large White x Landrace) piglets were handled in the same manner in all treatments. They were selected from a commercial piggery and were transported in a piglet carrier to the surgery. The piglets were given time to settle down in a warm quiet pen in a temperature controlled (29°C) ventilated room with straw bedding. Piglets had *ad libitum* access to fresh water prior to testing and were hand fed with lamb milk replacement formula upon return to the recovery pen at the conclusion of the experiment.

The following treatments were imposed:

The five treatments were:
A: Docking by clippers at 2 days of age (2clip)
B: Docking by cauterisation at 2 days of age (2caut)
C: Docking by clippers at 20 days of age (20clip)
D: Docking by cauterisation at 20 days of age (20caut)
E: Castration at 20 days of age (cast).

The piglets were handled in the same manner in all treatments. Piglets were quietly picked up from their enclosure and transported individually to the neurophysiology lab for testing. Anaesthesia was induced using halothane (46%) vaporised in oxygen (4 L/min) delivered through a face mask. When adequate depth of anaesthesia was reached (recumbency, loss of muscle tone and no response to toe pinch), stainless steel 20 gauge needle electrodes were positioned subcutaneously to monitor EEG and ECG activity. The animal was placed on a circulating warm-water heating blanket for thermal support during anaesthesia. Following instrumentation halothane was adjusted to an end-tidal concentration of 1.2%. Body temperature, end-tidal CO_2, halothane, respiration rate and ECG activity were monitored continuously.

Subcutaneous 27-gauge stainless steel needle electrodes (Viasys Healthcare, Surrey, England) were positioned to record electroencephalograph (EEG) and electrocardiograph (ECG) activity. A five-electrode montage was used to record EEG from both the left and right cerebral hemispheres, with inverting electrodes placed parallel to the midline over the left and right frontal bone zygomatic processes, non-inverting electrodes over the left and right mastoid processes and a ground electrode placed caudal to the occipital process (see Murrell & Johnson, 2006). ECG was recorded using a base-apex configuration.

Both EEG and ECG signals were fed via breakout boxes to separate amplifiers (Iso-Dam isolated biological amplifier, World Precision Instruments Inc.). The signals were amplified with a gain of 1000 and a band-pass of 1.0 –500Hz and digitised at a rate of 1kHz (Powerlab 4/20, ADInstruments Ltd, Colorado Springs, Co). The digitised signals were recorded on an Apple Macintosh personal computer for analysis off-line at the conclusion of the experiment.

Baseline EEG was recorded for 5 minutes prior to the specified treatment (A, B, C, D or E) being carried out and for a further 5 minutes following treatment. Digitised EEG data was analysed off line at the conclusion of the experiment.

The pigs in treatments A and C had their tail removed with clean, disinfected side-cutters (clippers). The tail was cut approximately 2cm from the base of the tail in between the second vertebrae. The pigs in treatment B and D had their tail docked with a clean disinfected gas operated Stericutter cauteriser. Their tails were docked at the same location as clipper treatment. The pigs in negative control were surgically castrated. The piglet's anogenital region was exposed and a scalpel was used to make a 10mm long incision on each side of the scrotum to expose each testical. The testicals were removed by cutting the testicular cord. A disinfectant was applied to the wounds in all treatments. The piglets were returned to a recovery pen after the procedure.

Results

Statistical Analysis of Piglet EEG Data

Raw data from the EEG were inspected manually and any artefacts excluded from further analysis. The total power (Ptot), median frequency (F50) and spectral edge frequency (F95) were calculated for consecutive 1-second epochs, using purpose-written software (Spectral Analyser, CB Johnson, Massey University). A single mean value for F50, F95 and Ptot was calculated for the 5 minutes of baseline prior to castration or docking, and for consecutive 15 second blocks following castration or docking, using Microsoft Excel 2008 for Mac (Microsoft Corporation, Redwood, USA). Data from individual animals were standardised to a percentage of baseline and combined for statistical analysis.

Mean values for each 15-second block after stimulus application, up until 180 seconds, were compared to baseline using repeated measures analysis of variance in SAS® 9.1 (SAS Institute Inc., Cary, NC, USA), with p values manually adjusted to incorporate multiple comparisons. The linear mixed model for repeated measures included the fixed effects of treatment and time, and the random effect of animal. The five treatments were: docking by clippers at 2 days of age (2clip), docking by cauterisation at 2 days of age (2caut), docking by clippers at 20 days of age (20clip), docking by cauterisation at 20 days of age (20caut), and castration at 20 days of age (cast).

In the present study, only the median frequency (F50) of the piglet EEG varied significantly with treatment (F=5.57, p=0.0011) (Table 1). However, there were significant effects of time, along with a significant treatment x time effect, on the change in F50, F95 and Ptot of the piglet EEG (Table 1).

Table 1: Results of repeated measures analysis of variance, showing the overall effects of treatment and time on the change in F50, F95 and Ptot of the piglet EEG.

	Treatment		Time		Treatment*Time	
	F value	p value	F value	p value	F value	p value
F50	5.57	0.0011	11.95	<0.0001	1.76	0.0018
F95	2.28	0.0767	5.99	<0.0001	2.01	0.0001
Ptot	2.33	0.0716	11.21	<0.0001	1.99	0.0002

Surgical castration or tail docking of 20 day-old piglets by either clippers or cauterisation induced a rise in the F50 of the EEG relative to baseline (Figure 1). I n contrast, tail docking of 2 day-old piglets by either clippers or cauterisation resulted in a transient reduction in F50.

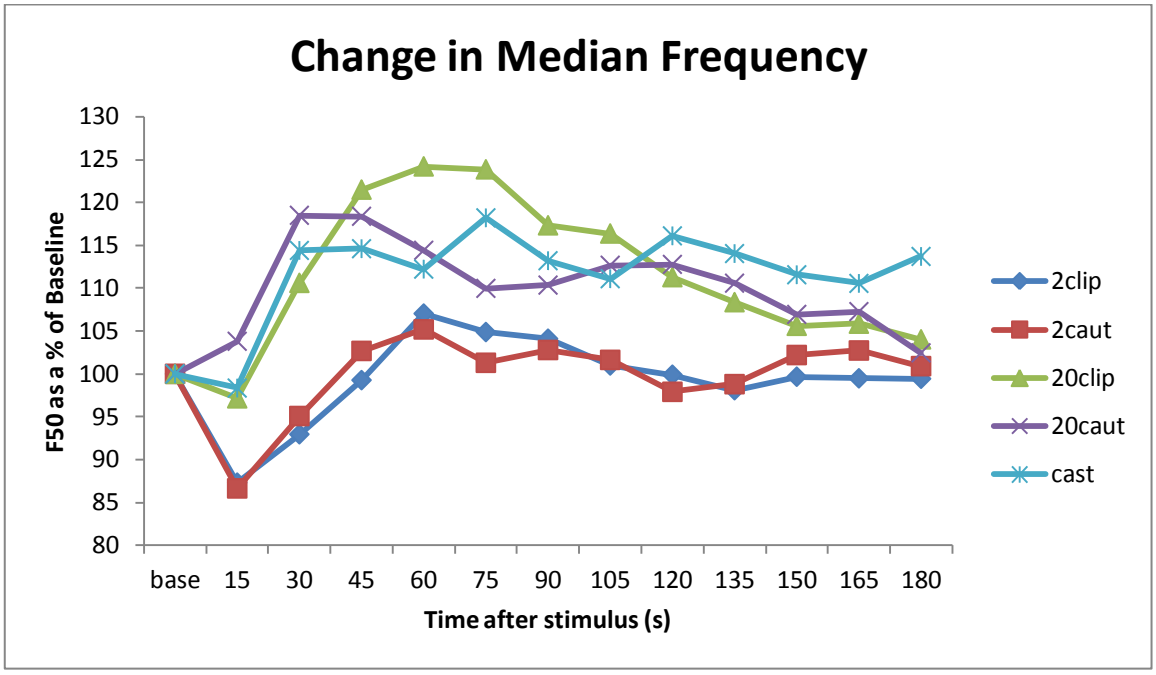

Figure 1: Percentage change in the mean median frequency (F50) of the piglet EEG relative to baseline, for consecutive 15-second blocks following castration or tail docking by clippers or cauterisation at 2 or 20 days of age

In 20 day-old piglets docked using clippers, F50 rose significantly above baseline 45 seconds after docking, and remained significantly elevated until 105 seconds after docking (Table 2). A peak increase of 24% (relative to baseline) was recorded 60 seconds after docking.

In 20 day-old piglets docked by cauterisation, a significant rise in F50 was observed 30 seconds after docking, returning to baseline levels at 75 seconds, then increasing again from 105 to 120 seconds (Table 2). A peak increase of 19% was recorded 30 seconds after docking.

In 2 day-old piglets docked by either clippers or cauterisation, a reduction in F50 occurred in the 15 seconds following docking, returning to pre-treatment values by 30 seconds after docking (Table 2). This transient reduction in F50 was significant in the group docked by cauterisation ($p=0.03$), and showed a trend toward significance in the group docked by clippers ($p=0.07$). In both groups the peak reduction in F50 was around 13%, relative to baseline (Table 2).

Surgical castration of 20 day-old piglets induced a significant rise in F50 30 seconds after castration (Table 2). Unlike the tail-docked pigs, the F50 of castrated pigs remained elevated for the entire 180s period of comparison, although the increase was not significant for all time points analysed (Table 2). A peak increase of 18% was recorded 75 seconds after castration was begun.

Table 2: Mean (SEM) percentage change in median frequency (F50) of the piglet EEG, relative to baseline, over 15-second intervals following tail docking or castration. An asterisk indicates that the mean value differs significantly to the baseline F50 ($p<0.05$).

Treatment	Base	15	30	45	60	75	90	105	120	135
2day clip	100	87.3	93.0	99.3	107.0	104.9	104.1	101.0	99.9	98.1
		(3.7)	(4.4)	(2.7)	(2.5)	(2.1)	(2.2)	(3.1)	(1.8)	(3.4)
2day caut	100									
		86.6*	95.1	102.7	105.2	101.3	102.8	101.6	97.9	98.8
		(3.7)	(3.6)	(2.8)	(2.2)	(3.2)	(4.0)	(2.2)	(3.8)	(3.2)
20day clip	100									
		97.4	110.6	**121.5***	**124.2***	**123.9***	**117.3***	**116.4***	111.3	108.4
		(6.5)	(5.2)	(6.2)	(4.9)	(6.1)	(4.7)	(4.1)	(3.3)	(3.6)
20day caut	100									
		103.8	**118.5***	**118.4***	**114.4***	110.0	110.4	**112.7**	**112.8**	110.6
		(5.1)	(5.3)	(4.9)	(3.5)	(4.4)	(4.1)	(3.3)	(4.1)	(4.4)
castrate	100									
		98.3	**114.4***	**114.6***	112.2	**118.2***	113.2	111.1	**116.1***	**114.0***
		(4.3)	(4.9)	(3.9)	(3.4)	(4.1)	(5.1)	(4.3)	(9.4)	(6.5)

There was little change in the F95 of 2 day-old piglets docked with either clippers or iron, or in 20 day-olds docked by cauterisation (Figure 2). In castrated piglets and in 20 day-old piglets docked using clippers, F95 increased following treatment application, peaking 45 seconds after start of treatment (Figure 2).

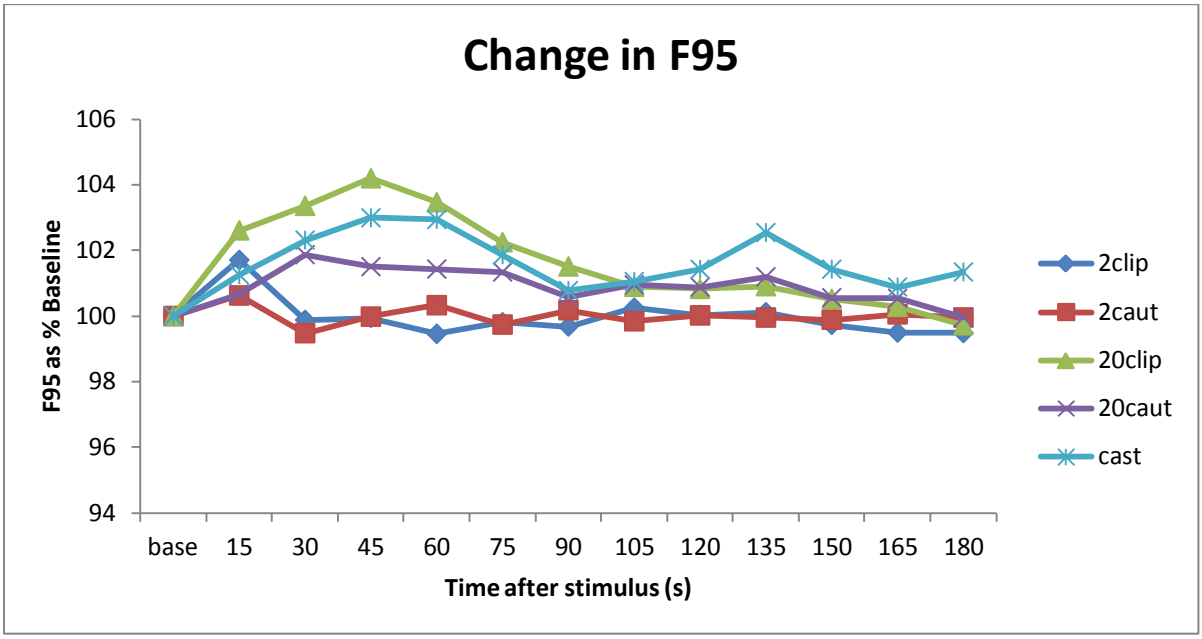

Figure 2: Percentage change in the mean 95% spectral edge frequency (F95) of the piglet EEG relative to baseline, for consecutive 15-second blocks following tail docking by clippers or cauterisation at 2 or 20 days of age.

There was no significant change in F95 relative to baseline in 2 day-old pigs tail docked by either cauterisation or clippers, or in 20 day-old pigs docked by cauterisation (Table 3). In 20-day old pigs docked using clippers, F95 increased significantly 15 seconds after docking and remained elevated until 75 seconds after docking (Table 3). A peak increase of 4.2% was recorded 45 seconds after docking. In the castrated pigs, F95 increased significantly 30 seconds after the start of castration, remaining significantly elevated until 75 seconds (Table 3). A second rise in F95 was observed 135 seconds after start of castration (Figure 2 and Table 3).

Table 3: Mean (SEM) percentage change in 95% spectral edge frequency (F95) of the piglet EEG, relative to baseline, over 15 second intervals following tail docking or castration. An asterisk indicates the mean value differs significantly to baseline ($p<0.05$).

Treatment	Baseline	15	30	45	60	75	90	105	120	135
2day clip	100	101.7	99.88	99.95	99.46	99.81	99.68	100.3	100	100.11
		(0.7)	(0.7)	(0.6)	(0.6)	(0.5)	(0.5)	(0.3)	(0.5)	(0.4)
2day caut	100	100.6	99.48	99.98	100.3	99.74	100.2	99.84	100	99.95
		(0.5)	(0.4)	(0.3)	(0.3)	(0.3)	(0.4)	(0.3)	(0.4)	(0.4)
20day clip	100	**102.6***	**103.4***	**104.2***	**103.5***	**102.3***	101.5	100.9	100.8	100.9
		(0.9)	(1.1)	(0.9)	(0.9)	(0.9)	(0.7)	(0.9)	(0.8)	(0.6)
20day caut	100	100.7	101.9	101.5	101.4	101.3	100.6	101	100.9	101.2
		(0.9)	(0.7)	(0.7)	(0.9)	(0.9)	(0.7)	(0.7)	(0.8)	(1.1)
castrate	100	101.3	**102.6***	**102.8***	**102.9***	**102.3***	101.2	101.5	101.9	**102.5***
		(1.3)	(1.1)	(1.2)	(1.5)	(1.1)	(1.2)	(1.2)	(1.2)	(1.4)

There was a reduction in total power of the EEG immediately following castration or tail docking by either method in both 2 and 20 day-old pigs (Figure 3). In the 2 day-old piglets the reduction was briefer, with Ptot returning to baseline values by 30 seconds after docking (Figure 3). In the other groups, the reduction in Ptot was more prolonged. The magnitude of the reduction in total power appeared similar in the 2 day-old piglets docked by either method, the 20 day-old piglets docked by cauterisation, and the castrated pigs, whereas the 20 day-old piglets docked using clippers exhibited a greater reduction in total power (Figure 3).

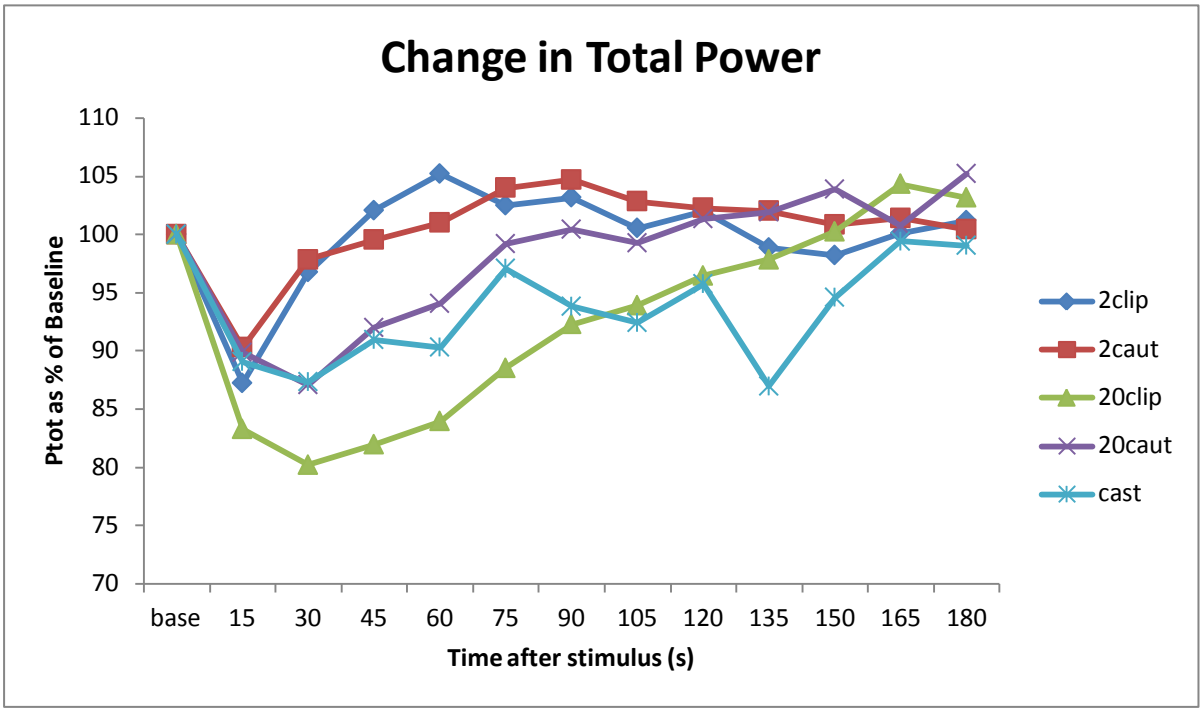

Figure 3: Percentage change in the mean total power (Ptot) of the piglet EEG relative to baseline, for consecutive 15-second blocks following tail docking by clippers or cauterisation at 2 or 20 days of age.

In the 2 day-old piglets docked using side clippers, the reduction in Ptot was significant 15 seconds after docking, dropping 12.8% below baseline (Table 4).

In 2 day-old pigs tail docked using cauterisation, Ptot was reduced by 9.7% at 15 seconds after docking, but did not differ significantly from baseline (p=0.17) (Table 4).

In the 20 day-old pigs docked using clippers, Ptot fell significantly below baseline in the 15 seconds immediately following docking, remaining depressed until 90 seconds post docking. A peak reduction of 19.8% was recorded 30 seconds after docking (Table 3). In contrast, 20 day-old pigs docked using cauterisation exhibited a briefer reduction in Ptot, with mean Ptot dropping significantly below baseline in the 15-30 seconds following docking only (Table 3). In addition, the magnitude of the reduction in Ptot was less for 20 day-olds docked using cauterisation (12.8% compared with 19.8% for clippers).

In the castrated 20 day-old pigs, Ptot was significantly lower than baseline at 30 and 135 seconds after start of castration (Table 4). A peak reduction of 14.3% was recorded 30 seconds after start of castration.

Table 4: Mean (SEM) percentage change in total power (Ptot) of the piglet EEG, relative to baseline, over 15 second intervals following tail docking or castration. An asterisk indicates the mean value differs significantly to baseline ($p<0.05$).

Treatment	Baseline	15	30	45	60	75	90	105	120	135
2 clip	100	**87.2***	968	102.1	105.2	102.5	103.2	100.5	102	98.9
		(3.6)	(3.0)	(1.8)	(3.6)	(1.8)	(2.0)	(1.5)	(1.8)	(2.4)
2 caut	100	90.3	97.8	99.6	101	104	104.7	102.8	102.3	102.0
		(4.0)	(3.0)	(3.0)	(2.5)	(2.5)	(2.8)	(2.5)	(2.5)	(2.8)
20 clip	100	**82.9***	**80.2***	**82***	**84***	**88.5***	92.3	93.9	96.5	97.8
		(4.7)	(3.3)	(3.5)	(3.8)	(4.4)	(3.7)	(3.7)	(3.1)	(2.9)
20 caut	100	89.8	**87.1***	92	94.1	99.2	100.4	99.2	101.3	101.9
		(3.8)	(4.0)	(5.1)	(4.7)	(3.8)	(4.0)	(3.3)	(2.7)	(3.7)
castrate	100	89.1	**85.7***	91.4	90.3	95.0	91.7	90.3	93.6	**86.9***
		(4.5)	(4.6)	(5.0)	(6.1)	(3.6)	(4.4)	(4.4)	(3.9)	(5.9)

Discussion

The typical response to noxious stimulation in the mammalian EEG is an increase in median frequency (F50) along with a reduction in total power (Ptot), often accompanied by an increase in 95% spectral edge frequency (F95).

The EEG responses of piglets to docking by clippers and cauterisation varied both with age and with docking method.

In 2 day-old piglets, a reduction in total power of the EEG, a typical response to noxious stimulation, was observed in the period immediately following docking, but was only significant in the group docked using clippers. A transient reduction in median frequency of the EEG also occurred in 2 day-old pigs docked by either method. Although the typical mammalian EEG response to noxious stimulation is an increase in median frequency, reductions in F50 have been reported in anaesthetised wallaby pups in response to toe clamping (Diesch, Mellor et al. 2009), in anaesthetised rat pups in response to tail clamping (Diesch, Mellor et al. 2009), and in anaesthetised infants and children in response to skin incision (Oshima, Shingu et al. 1981). The observed reduction in F50 in 2 day-old pigs following tail docking may thus be considered evidence of a nociceptive response in this age group. The reduction in F50 was significant in the group docked by cauterisation and nearing significance in the group docked by clippers. There was no significant change in spectral edge frequency in 2 day-old pigs docked by either method. Although the duration and magnitude of EEG responses were similar in clipper and cauterisation docked pigs in this age group, the greater reduction in Ptot observed in the clipper docked pigs indicate that docking with clippers induces a greater nociceptive response than docking with cauterisation in 2 day-old pigs.

In 20 day-old pigs docked using either clippers or cauterisation, an increase in median frequency and decrease in total power of the EEG was observed following docking, indicating a nociceptive response. In comparison to the 2 day-old piglets, the observed reduction in total power of the EEG was more sustained in the 20 day-olds, suggesting a more sustained nociceptive response in this age group. Although the direction of the shift in median frequency differed between 2 and 20 day-olds, the duration of the shift was greater in the 20 day-olds, again suggesting a more sustained

nociceptive response to tail docking in this age group. A comparison between docking methods in the 20 day-old pigs revealed a greater and more prolonged increase in median frequency and decrease in total power in those pigs docked using clippers. In addition, docking with clippers elicited a significant increase in F95 that was not observed in the pigs docked using cauterisation. These data indicate that docking by clippers produces a more intense and sustained nociceptive response than docking by cauterisation in 20 day-old pigs.

Surgical castration of piglets is a known noxious stimulus. In comparing the EEG responses of tail docked 20 day-old pigs to those of castrated pigs, the responses of 20-day old pigs docked using cauterisation were similar to those of castrated pigs in terms of the magnitude of changes in F50 and Ptot. In contrast, docking of 20 day-olds by clippers induced larger magnitude increases in F50 and F95 and decrease in Ptot than cauterisation, providing further evidence that clipping is more noxious than cauterisation at 20 days of age. The EEG responses to castration were more persistent than the responses to docking by either method. This is likely due to the differences in time taken to perform the procedures, with castration taking 1-2 minutes to perform, compared with 1-2 seconds for tail docking.

Conclusion

In conclusion, it is likely that the differences in the median frequency of the EEG after tail docking in 2 day-old versus 20 day-old pigs reflect differences in the maturation of peripheral and central nociceptive pathways as a function of age (Mellor et al., 2009). These maturational effects may also account for the shorter duration of EEG responses observed in the 2 day-old pigs compared with 20 day-olds. It appears that tail docking induces a larger nociceptive response in 20 day-old pigs than in 2 day-old pigs, suggesting that it is in animals' best interests that docking be performed within the first few days after birth.

In terms of the nociceptive response to docking with clippers versus cauterisation, the larger and more sustained EEG responses observed following docking by clippers in the 20 day-old pigs suggest that clipping is more aversive than cauterisation. Although the EEG responses of 2 day-old piglets were similar after docking with either clippers or cauterisation, the magnitude of the observed reduction in Ptot after docking with clippers suggests this may induce a larger nociceptive response. Based on the observed differences in EEG responses to tail docking by clippers or cauterisation in 20 day-old pigs, it may be concluded that, in welfare terms, the use of a cauterisation is preferable to the use of clippers for tail docking of piglets.

Experiment 2: Physiological and Behavioural Responses of Tail Docking - Acute and Chronic (24hr) Pain Response

The aim of this experiment was to compare the physiological and behavioural responses of piglets when they were tail docked using two common methods (clippers and cauterisation) at two days of age.

A broad examination of the physiological, health and fitness responses of piglets was used in this experiment to examine piglet welfare (Barnett and Hemsworth, 2009) in response to tail docking treatment. The stress response commences once the central nervous system firstly perceives a potential challenge (stressor) to homeostasis and one of the key general biological defence responses is that of the neuroendocrine system with the activation of the HPA axis and the release of corticosteroids (Barnett and Hemsworth, 2009). The physiological response was measured by assessing total cortisol concentrations after a stressor is imposed to determine activation of the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA axis can also lead to suppression of growth hormone and corticosteroids can also induce resistance to growth factors in target tissues (Kaltas and Chrousos, 2007). Corticosteroids and adrenocorticotrophic hormones can have a catabolic effect on the body (Elsasser et al., 2000) therefore assessment of changes in live weight can also be used to assess biological response to a stressor.

There is evidence in the scientific literature that surgical castration causes acute pain in piglets. Piglets that are surgically castrated exhibit a stronger vocal response compared to piglets that are sham castrated or castrated under local anaesthesia (Taylor and Weary, 2000). Surgical castration also induces activation of the hypothalamic pituitary adrenal axis and the sympathetic nervous system (White et al., 1995; Prunier et al., 2001) and also leads to abnormal pain-related behaviours (Taylor et al., 2001). Therefore surgical castration was used in this experiment as a negative control and the positive control was a sham handling treatment.

Research Methodology

This experiment was approved by the Rivalea Animal Ethics Committee. The experiment was conducted at the Rivalea Australia Research and Innovation unit, Corowa NSW, Australia. The experiment was conducted between January and March 2011. Seventy two sows (Large White x Landrace) and their litters were selected over six weeks. The sows farrowed in individual farrowing crates. Four entire male piglets greater than 1.2kg in live weight were selected per litter when they were approximately 2 days post-birth. The pigs were randomly allocated to treatment and a number was written on their back with a black stock marker. Data were collected from 288 piglets.

The following treatments were imposed:
Treatment A: Sham treatment
Treatment B: Surgical castration
Treatment C: Tail docking using side-cutters (clipper)
Treatment D: Tail docking using a Stericut® Tail cauteriser (cauterisation).

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 approximately 30 s and were put back into their pen. The pigs in Treatment B (negative control) were surgically castrated. The piglet's anogenital region was exposed and a scalpel was used to make a 10mm long incision on each side of the scrotum to expose each testicle. The testicles were removed by cutting the testicular cord. A disinfectant was applied to the wound and the piglet was

returned to the pen. The pigs in treatment C had their tail docked with clean, disinfected side-cutters (clippers). The tail was cut approximately 2cm from the base of the tail in between the second vertebrae. The pigs in treatment D had their tail docked with a clean disinfected gas operated Stericut tail docker. Their tails were docked at the same location as treatment C. A disinfectant was applied to all castration and docking wounds.

An iron injection was given to all piglets and an individual ear tag placed into the ear of each piglet after the 24 h blood sample was taken.

Stress Physiology

Blood samples were collected by jugular venipuncture. The blood samples were taken at 15 min, 30 min and 24h post-treatment. 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 Monash University (Clayton, Vic). Plasma concentrations of total cortisol were determined in duplicate 100-μL aliquots using an extracted radioimmunoassay according to the protocol developed by Bocking and Harding (1986) and validated for pig plasma using hydrocortisone H-4001 (Sigma Chemical Co., St Louis, MO) as standard.

Behaviour

During the treatment an escape attempt was defined as a body movement carried out to effect an escape (as described by Marchant-Forde *et al., 2009*). Vocalisations were recorded during treatment. A bout criterion interval of 1 second was used, i.e. if a piglet squealed for approximately 10 sec a score of 10 was given.

The behaviour of the four treatment pigs in each litter was videotaped by using mounted cameras (Signet Model QV-3063) that enabled view of the whole farrowing crate. The behaviour of the piglets for the first 60 min post-treatment was measured by continuously observing each piglet for 60 sec every 5 min. (i.e. a total of 12 min in the first 60 min post-treatment). The behaviour of the piglets 23 hrs post-treatment was measured by continuously observing each piglet for 60 sec every 5 min. (i.e. a total of 12 min between 23-24 hrs post-treatment). The total active behaviours were calculated as all behaviours combined with the exception of lying behaviour. Total resting behaviours were calculated as the total of time lying with and without sow contact and when the piglet was idle. The term other was used when the piglet could not been seen within the field of view of the camera.

The following ethogram was used to describe behaviours:

Table 1: 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
Walk/Run	Slowly moving forward one leg at a time/ Trot or gallop
Massaging udder/ Nursing	Nose in contact with the udder/ Teat in mouth. Vigorous and rhythmic suckling movements.
Asleep	Eyes closed while lying down.
Playing/frolicking	Head shaking, springing (sudden jump or leap), running with horizontal and vertical bounces.
Events:	
Scooting	Causal part of body being dragged across ground.
Scratching	Scratching the rump against the floor or walls of the pen.
Shivering	Shivering as with cold.

Tail Lesion Scoring

The tail lesion score was measured as described by Marchant-Forde et al. (2009). The lesion score was carried out on those piglets that had their tail docked (treatments C and D). The lesions were scored from 0 to 5 as follows; 0=intact skin with no swelling or reddening, complete healing with no scab; 1=swelling, but intact skin or healing lesion with a scab; 2= severe swelling, but skin intact or a narrow, red, ulcerated wound around the perimeter of the injury site with little or no exudate. A healing lesion showing a large scab with underlying granulation;3=wider band of red, ulcerated skin surrounding injury side, but with no excessive exudate present;4=red, ulcerate lesion covered by exudate, swelling of the surrounding tissues and 5=large red, ulcerated lesion with much pus and exudate and a strong smell of necrosis, severe swelling.

Growth Performance

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

Statistical Analysis

Statistical analysis was performed using SPSS (Version 21 -SPSS Inc., Chicago, Illinois, USA). All data were analysed for normality and data transformation (square root) was performed when required. Analysis was conducted using Univariate General Linear Model, using each piglet as the experimental unit and the sow as the random factor. Chi-squared analysis was used to analyse treatments effects on number of piglets that died or were removed between treatment and weaning.

Results

Table 1: Number of piglet deaths and removals between treatment and weaning.

Cause of death/removal	Sham	Surgical castration	Tail docked using clippers	Tail docked using cauteriser
Overlain by sow	5	3	6	6
Scours		1		
Unthrifty		1		2
Other		1	1	
Total	5/73 piglets	6/73 piglets	7/73 piglets	8/73 piglets

There was no significant difference (X^2=0.70; P=0.951) between the number of piglet deaths and piglet removals due to illness and injury between treatments.

Table 2: Effect of treatment on mean total cortisol concentrations (ng/ml).

	Sham	Surgical castration	Tail docked using clippers	Tail docked using cauteriser	SEM	P value
Cortisol (ng/ml)						
15 min post-treatment	91.24[a]	128.93[c]	110.73[b]	106.43 [b]	2.204	0.000
30 min post-treatment	115.27 [a]	145.80 [c]	125.91 [b]	121.77 [ab]	1.862	0.000
24 hr post-treatment	48.98	45.33	41.92	43.95	1.920	0.536

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

Table 2 shows cortisol concentrations at 15min, 30min and 24 hr post treatment. Cortisol concentrations 15 min and 30 min post-treatment were significantly (P<0.001) higher in both tail docking treatments and the surgical castration treatment compared to the sham treatment. The cortisol concentration of the surgical castration treatment was significantly higher than the tail docked and sham treatments. Cortisol concentrations at 30 min post-treatment were significantly higher in the tail docked and surgically castrated treatments compared to the sham treatment. Cortisol concentrations were significantly (P<0.001) lower in the cauterisation treatment compared to the clipper treatment 30 min after treatment. There was no significant difference (P>0.05) in cortisol concentrations between treatments 24 hours after treatments were imposed.

Table 3: Effect of treatment on frequency of vocalisations and escape attempts and behaviour of piglets 60 min after treatment. Mean total time (sec) spent in each posture or state during observation period are presented. Transformed means are presented and back transformed means presented in parentheses.

	Sham	Surgical castration	Tail docked using clippers	Tail docked using cauteriser	SEM	P value
Duration of vocalisations during treatment (sec)	1.6[a] (2.6)	3.9[c] (15.2)	2.0[b] (4.0)	2.0[b] (4.0)	0.07	0.000
Number of escape attempts during treatment	1.7[a] (2.9)	4.1[c] 16.8)	2.0[b] (4.0)	2.1[b] (4.4)	0.07	0.000
Posture (sec):						
Standing (normal)	17.3 (299.3)	17.0 (289.0)	16.4 (269.0)	16.5 (272.3)	0.22	0.604
Standing (head lowered)	2.3[a] (5.3)	4.8[b] (23.0)	4.3[b] (18.5)	4.1[b] (16.8)	0.26	0.007
Sitting	1.5 (2.3)	2.1 (4.4)	1.5 (2.3)	1.9 (3.6)	0.18	0.565
Lying (with sow contact)	9.2 (84.6)	7.1 (50.4)	9.1 (82.8)	8.3 (68.9)	0.41	0.055
Lying (without sow contact)	14.1 (198.8)	15.1 (228.0)	13.5 (182.3)	14.7 (216.1)	0.38	0.436
Out of view	2.9 (8.4)	2.3 (5.3)	2.6 (6.8)	3.4 (11.6)	0.22	0.639
States (sec):						
Idle	10.7 (114.5)	12.8 (163.8)	11.4 (130.0)	11.0 (121.0)	0.24	0.160
Walk/Run/Frolicking	9.6 (92.2)	8.7 (75.7)	8.8 (77.4)	9.3 (86.5)	0.17	0.199
Massaging udder/Nursing	12.3 (151.3)	11.3 (127.7)	12.1 (146.4)	12.4 153.8)	0.33	0.749
Asleep	16.8 (282.2)	16.6 (275.6)	16.4 (269.0)	16.2 (262.4)	0.24	0.902
Total active	16.0 (256)	14.8 (219.0)	15.7 (246.5)	16.2 (262.4)	0.27	0.605
Total inactive	20.4 (416.2)	21.4 (458.0)	20.5 (420.3)	20.0 (400.0)	0.21	0.538
Out of view	3.0 (9.0)	2.3 (5.3)	2.6 (6.8)	3.4 (11.6)	0.22	0.625

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

* Data square root transformed prior to statistical analysis.

Table 3 shows the vocalisations and behaviour of piglets during treatment and the 60 min post-treatment. Scooting and shivering were not observed during the observation period. Frolicking observations were rare and were only observed on five occasions in short bouts. These data were combined with the walking and running data. Piglets in the surgical castration treatment performed significantly (P<0.001) more bouts of vocalisations and performed more escape attempts than the

sham and tail docked treatments. Piglets in the tail docked treatments exhibited significantly (P<0.001) more vocalisations and performed more escape attempts during treatment than the sham treatment. Piglets in both tail docked treatment and surgical castration treatment spent more time standing with their head lowered compared to the sham treatment. There was a trend (P=0.055) for the piglets in the surgical castration treatment to spend less time lying in contact with the sow compared to the other treatments.

Table 4: Effect of treatment on behaviour of piglets 24 hrs after treatment. Mean total time (sec) spent in each posture or state during observation period are presented. Transformed means are presented and back transformed means presented in parentheses.

	Sham	Surgical castration	Tail docked using clippers	Tail docked using cauteriser	SEM	P value
Posture (sec):						
Standing (normal)	10.3 (106.1)	10.3 (106.1)	10.3 (106.1)	10.4 (108.2)	0.29	0.996
Standing (head lowered)	0.7 (0.5)	0.9 (0.8)	0.8 (0.64)	1.3 (1.7)	0.14	0.448
Sitting	1.4 (2.0)	1.2 (1.4)	1.2 (1.4)	0.5 (0.25)	0.16	0.397
Lying (with sow contact)	10.3 (107.0)	8.2 (67.2)	9.4 (88.4)	8.9 (79.2)	0.56	0.622
Lying (without sow contact)	17.3 (299.3)	19.4 (376.4)	18.8 (353.4)	19.0 (361.0)	0.48	0.393
Out of view	1.6 (2.6)	0.5 (0.25)	0.9 (0.8)	1.0 (1.0)	0.18	0.323
States (sec):						
Idle	5.1 (26.0)	5.6 (31.3)	6.0 (36.0)	4.9 (24.0)	0.27	0.410
Walking/Running/Frolicking	3.9 (15.2)	4.3 (18.5)	4.1 (16.8)	4.2 (17.6)	0.23	0.962
Massaging udder/Nursing	10.7 (114.5)	10.1 (102.0)	10.8 (116.6)	10.4 (108.2)	0.30	0.787
Asleep	22.0 (484.0)	22.1 (488.4)	22.1 (488.4)	22.4 (501.8)	0.19	0.868
Total active	12.1 (146.4)	11.8 (139.2)	12.1 (146.4)	11.8 (139.2)	0.28	0.959
Total inactive	23.13	23.25	23.34	23.35	0.16	0.947
Out of view	1.58	0.48	0.93	1.1	0.18	0.353

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

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

Running, scooting, scratching and shivering were not observed during the observation period. Frolicking observations were rare and were only observed on five occasions in short bouts. These data were combined with walking and running data. There was no significant difference (P>0.05) in any postures, behavioural states or behavioural events 24 hours after the treatments were performed.

There was no significant difference (P>0.05) of the tail lesion score of piglets at 7 seven days post-treatment (1.3 and 1.3 in the clipper and cauterisation treatments, respectively) and at weaning the tail lesion scores were 0.03 and 0.0, in the clipper and cauterisation treatments, respectively).

Table 5: Effect of treatment on growth performance of piglets.

	Sham	Surgical castration	Tail docked using clippers	Tail docked using cauteriser	SEM	P value
Live weight prior to treatment (kg)	1.9	2.0	1.9	1.9	0.02	0.281
Weight 24 hrs post-treatment (kg)	2.1	2.1	2.0	2.0	0.02	0.310
Weight 7 days post-treatment (kg)	3.5	3.5	3.2	3.4	0.05	0.193
Weaning weight(kg)	8.0	7.7	7.3	7.7	0.13	0.273
Rate of gain (g/day) 0-24 hrs post-treatment	151.1	128.9	135.4	127.4	6.94	0.611
Rate of gain (g/day) 0-7 days post-treatment	228.9	216.1	200.7	215.1	4.97	0.259
Rate of gain (g/day) Treatment-weaning	223.0	207.6	199.7	207.9	4.09	0.235

There was no significant difference (P>0.05) between live weight of piglets prior to treatment, at 24 hours post-treatment, 7 days post-treatment and at weaning. There was no significant difference (P>0.05) in rate of gain of piglets 24 hours post-treatment or 7 days post-treatment and between treatment and weaning (Table 5).

Discussion

This experiment assessed the acute and chronic (24 hours post-treatment) physiological and behavioural responses of piglets to tail docking using clippers or cauterisation compared to a negative control (surgical castration) and a positive control (sham treatment-handling alone).

The results clearly show that surgical castration of two day old piglets causes substantial behavioural and physiological responses in the 30 to 60 minute period after treatment. Piglets in the surgically castrated treatment had more vocalisations and escape attempts during treatment and had significantly higher cortisol concentrations 15 and 30 min after treatment. Surgically castrated pigs spent more time in the 60 min after treatment with their heads lowered and idle and there was a trend for them to spend a higher proportion of time lying without sow contact in inactive states compared to the sham treatment. These results are consistent to those found in other studies (McGlone and Hellman, 1988; Hay et al., 2003; Prunier et al., 2005; Carroll et al., 2006; Llamas Moya et al., 2008). Interestingly, in the present study these behavioural and physiological differences diminished between 30 minutes post-treatment and 24 hours post-treatment, as there were no differences between treatments in physiology of behaviour 24 hrs after the treatment. Other studies have shown that castrated piglets can experience pain for up to four days after surgical castration (McGlone and Hellman, 1988; Hay et al., 2003).

Total cortisol concentrations were measured at 15 min, 30 minutes and 24 hours after the treatment to assess the stress response of the tail docking procedure. Tail docking using either the clippers or cauteriser elicited a significant stress response at 15 and 30 minutes post-treatment compared to the sham treatment. The cortisol concentrations at 30 minutes post-treatment were

lower in the cauterisation treatment compared to the clipper treatment which indicates that cauterisation may be less aversive than clipper treatment. These results are similar to that of Sutherland et al. (2008) who found that cortisol concentrations of clipped piglets were greater than the cauterised and sham treatments 60 min post-treatment and cortisol concentrations were similar between all treatments 90 min after treatment, indicating tail docking causes an acute stress response. Care must be taken in comparing studies as the pigs in the experiment by Sutherland were older (6 days of age) and there was considerable handling involved and repeated blood sampling within a short period of time. Nevertheless there appears to be some similarities between the two experiments. Prunier et al (2005) also showed that cortisol concentrations did not differ between cauterisation and handling for up to 180 minutes after treatment of one day old piglets, which were younger than piglets used in Sutherland et al (2008).

In the current experiment, cortisol concentrations were similar between treatments at 24 hours post- treatment, which indicates that the stress response diminished between 30 min and 24 hours post-treatment.

Piglets in both tail docking treatments exhibited behavioural responses during and 60 minutes after treatment that are indicative of pain. Piglets in the tail docked treatments performed more vocalisations and escape responses during tail docking compared to the sham treatment. These behavioural responses were not as high as the responses of the piglets in the surgically castrated treatment. Piglets in the tail docked treatment spent more time with their head lowered in the 60 min period post- treatment compared to the sham treatment which is an indication of pain (Hay et al., 2003). Although cauterisation induced less of a physiological response 30 min after treatment compared to clipping, the behavioural responses post-treatment were similar to the clipper treatment.

There were no significant difference between the behaviours of pigs 24 hours after treatment, which indicates that in the current experiment the pain response had diminished between 60 min and 24 hours post-treatment.

There were no impacts of treatment on growth performance in the present experiment. 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 piglets in the surgically castrated and tail docked treatments had activation of the HPA axis 15 min and 30 min after treatment, the response was not significant enough to have a biological impact on the piglet and cause a reduction in growth performance. These data provide further evidence that tail docking causes a short-term pain response which does not impact on biological fitness of the animal.

There was no difference in tail lesion scores between the clipper and cauterisation treatment at day 7 post-treatment and at weaning. The average lesion score at day seven indicated some swelling, but intact skin and healing lesions with a scab and by weaning there was intact skin with no swelling or reddening and a complete healing with no scab. These results indicate that there was no benefit of a particular tail docking treatment in terms of wound healing after docking. However, the long-term detrimental welfare implication of cauterisation, such as the formation of neuromas when the nociceptors regenerate (as found by Simonsen et al., 1991) are not known. Sutherland et al (2005) stated that the intense heat associated with cauterisation may destroy nociceptors in the immediate area and may reduce the perception of pain in these areas, resulting in a lower physiological

response. It is not possible to detect neuromas visually, therefore the tail lesion score conduced in the current experiment would not have identified neuromas. Eicher et al., (2006) showed that the tail stumps of heifers that had been docked with a cauteriser were more sensitive to heat and cold. Further research is required to determine the long-terms implications of cauterisation for tail docking of piglets.

Conclusion

In conclusion, tail docking 2 day old piglets using clipper or cauterisation method caused a cortisol response at 15 and 30 min post-treatment. Tail docking caused an increase in vocalisations and escape attempts during treatment and increase in pain-related behaviour in the 60 min period post-treatment. The impact on stress physiology and pain-related behaviour had diminished by 24 hours post-treatment. Cauterisation appeared to be less aversive, in terms of the stress response, however the long term welfare implications of cauterisation are not known (i.e. formation of sensitive neuromas on the tail stump) and this technique requires further investigation before it is recommended as an alternative to clipper treatment.

Part 2: Strategies to Reduce or Eliminate the Pain Caused by Tail Docking Procedure

Introduction

Part 1 of this project was conducted to assess the pain induced by tail docking using either clippers or cauterisation by measuring the neurophysiological, physiological and behavioural responses of the animal. The results showed that tail docking using clippers or cauterisation caused an acute pain response and that cauterisation is less aversive than clipper treatment.

The second aim of the project (Part 2) is to investigate practical strategies that could be used to reduce or eliminate the acute pain caused by tail docking procedure. There will be considerable discussion in the future between the pork industry, animal welfare groups, animal welfare scientists and customers in regard to whether it is deemed necessary to provide pain relief for the tail docking procedure, that has been shown in this part 1and by others (Sutherland et al., 2008; Prunier et al., 2005) to cause an acute, short term pain response. Nevertheless, there is pressure from animal welfare groups to provide pain relief for management husbandry procedures, regardless of the duration of the pain. 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" (RSPCAwebsite-http://kb.rspca.org.au/Why-are-painful-procedures-performed-without-anaesthetic_83.html). Therefore based on this premise, the decision was made to continue with part 2 of the project and investigate commercially-available medications that may alleviate the pain of tail docking.

There are a plethora of commercially available medications that may provide pain relief for piglets during tail docking. The authors discussed possible options with other animal scientists, veterinarians and pork production managers, and decided on four commercially available products. Opiate-based analgesics that sedate the animal were not investigated in the experiments as they are potentially addictive to humans and they need to be administered by a Veterinarian, thus their widespread use in commercial pig production would not be feasible at this stage. Furthermore, medications that sedate the piglet may increase the risk of piglets being overlain by the sow once they return to the home pen (piglets drowsy). Piglets would need to be removed from the sow and managed in a hospital pen until they had fully recovered from the medication. Therefore, in this experiment commercially available, practical medications that can be used by stockpeople under veterinary supervision were investigated. The medications investigated required piglets to be quietly removed from their home pen, treated and placed back immediately with the sow. Some of the some medications were not able to be assessed via EEG technology as they are applied after the tail docking procedure is conducted and the EEG is not responsive at that stage.

The three following commercially-available medications were investigated:

i) Topical anaesthetic- cream applied to the base of the tail 60 -90 min prior to tail docking (product contained 2.5% Lignocaine, 2.5% Prilocaine). The cream contains 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.

ii) Topical anaesthetic- spray applied to the docked wound immediately after tail docking (product contained 40.6g/L Lignocaine, 4.2g/L Bupivacaine, 24.8 mg/L Adrenaline, 5.0 g/L Cetrimide). This commercially available topical anaesthetic and antiseptic solution has been developed to provide pain relief following mulesing in lambs and to reduce blood loss and infection to improve wound healing.

iii) Anti-inflammatory-Meloxicam. Meloxicam works by blocking the action of a substance in the body called cyclo-oxygenase (COX). Cyclo-oxygenase is involved in the production of various chemicals in the body, some of which are known as 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. Meloxicam is widely used in Europe to reduce the pain associated with surgical castration.

iv) Cauterisation was investigated as a cheaper alternative to medications.

Experiment 3: Neurophysiological Responses

Aim

The aim of this experiment was to use changes in EEG variables of minimally-anaesthetised pigs to assess the efficacy of cauterisation, oral meloxicam and a topical anaesthetic in mitigating acute nociceptive responses to tail docking.

Research Methodology

This study was undertaken with the approval of the Massey University Animal Ethics Committee Forty white line (Large white x Landrace) pigs (26 male, 14 female) aged 18–23 days (mean= 20.9 days), weighing 4.2–8.3 kg (mean=6.07 kg) were obtained from a commercial piggery. Pigs were transported to Massey University on the day of testing and held in groups in a 30°C temperature-controlled, ventilated room on deep straw litter with ad libitum access to water until the time of testing. Testing was carried out in the Neuroscience laboratory at Massey University. At the completion of data collection, pigs were returned to a separate pen in the same holding room and offered creep feed and lamb milk replacement (AnLamb, NZAgBiz, Hamilton, NZ) in addition to fresh water. All piglets were relocated to a local private farm at the end of the same day.

Pigs were randomly assigned to receive one of four treatments:

i) Clipper: Tail docked using sanitised side clippers

ii) Cauterisation: Tail docked using cauterising iron (Stericut® Tail Docker, Cotran Corp., Portsmouth, RI, USA)

iii) Meloxicam: Tail docked using sanitised side cutters at least 60 minutes (mean=92; min=74, max=103 minutes) following oral administration of 0.4 mg/kg meloxicam (Metacam® 1.5 mg/mL oral suspension, Boehringer Ingelheim NZ Ltd., Manukau, NZ)

iv) EMLA: Tail docked using sanitised side cutters at least 60 minutes (mean=99; min=86, max=119 minutes) after application of 1g of a topical anaesthetic cream (EMLA cream, 2.5% lignocaine 2.5% prilocaine, AstraZeneca, NSW, Australia) to the base of the tail.

Oral meloxicam was administered in preference to subcutaneous injection as to not interfere with EEG analysis.

Each pig was transported individually to the laboratory for testing. Pigs were gently restrained whilst anaesthesia was induced with 4% halothane (Halothane BP, Nicholas Piramal India Ltd., Ennore, Chennai, India) vaporised in oxygen (4 L/min) delivered through a facemask. When adequate depth of anaesthesia was reached (recumbency, loss of muscle tone, absence of palpebral reflex, no response to toe pinch), stainless steel 27-gauge needle electrodes (Ambu, Ballerup, Denmark) were positioned subcutaneously to record EEG from the left and right cerebral cortices. A five electrode montage was used (Murrell & Johnson 2006), with inverting electrodes positioned parallel to the midline over the zygomatic processes of the left and right frontal bones, non-inverting electrodes positioned over the left and right mastoid processes and a ground electrode positioned caudal to the occipital process. EEG signals were amplified (Iso-Dam isolated biological amplifier, World Precision Instruments Inc.) with a gain of 1000 and a band-pass of 1.0 –500Hz and digitised at a rate of 1kHz (Powerlab 4/20, ADInstruments Ltd, Colorado Springs, Co, USA).

Following electrode placement, halothane delivery was adjusted to achieve an end-tidal concentration of 1.10 ± 0.1%. Body temperature was maintained with the aid of a circulating 37°C warm-water heating blanket (Gaymar, New York, NY, USA). Once end-tidal halothane was stable in the desired range, baseline EEG was recorded for 10 minutes. The tail was then docked approximately 2 cm from the base of the tail, taking care to cut between vertebrae, according to the treatment protocol, and EEG recording was continued for a further 10 minutes.

Heart rate, respiration rate, body temperature, O_2 saturation, end-tidal CO_2 and end-tidal halothane were monitored throughout anaesthesia. At the conclusion of the recording period, the tail stump was sprayed with a disinfectant (0.5% chlorhexidine in methylated spirits) and pigs were given 2 mg/kg carprofen (Rimadyl, Pfizer NZ, Auckland, New Zealand) via subcutaneous injection, then allowed to recover from anaesthesia. EEG data were analysed off line at the conclusion of the experiment.

Data Analysis

Raw EEG recordings were inspected manually and any artefact (out of range data) was excluded from subsequent analyses. The total power (P_{TOT}), median frequency (F50) and 95% spectral edge frequency (F95) were calculated for consecutive 1-second epochs using purpose-written software (Spectral Analyser, CB Johnson, Massey University). For each EEG variable, the following was completed.

For each individual animal, data were standardised to a percentage of baseline using a mean baseline value calculated over the 60 seconds immediately prior to docking.

For statistical analysis, averages were taken over consecutive 30 second blocks of data, from 30 seconds before until 180 seconds after docking (seven data points per pig). These data were analysed using a mixed model (SAS version 9.1, SAS Institute Inc., Cary, NC, USA) with time as the repeated measure and treatment, day of testing and gender as fixed effects. Where significant effects were found, *post hoc* tests, corrected for multiple comparisons, were used to identify differences among times within each treatment and between treatments at each time point.

Results

There was no effect of day of testing or gender on the EEG variables after tail docking (p >0.5 for both).

There were significant treatment x time effects on F50 and P_{TOT}, along with significant overall treatment and time effects on F95 (Table 1).

Table 5 Results of repeated measures analysis of variance, showing the overall effects of treatment and time on F50, F95 and P_{TOT} of the EEG of anaesthetised piglets following tail docking with or without prior analgesia

	Treatment		Time		Treatment*Time	
EEG variable	**F value**	**p value**	**F value**	**p value**	**F value**	**p value**
F50	5.81	0.0086	19.30	<0.0001	2.21	0.0041
F95	3.99	0.0303	9.26	<0.0001	1.28	0.2025
P_{TOT}	1.91	0.1738	30.98	<0.0001	2.82	0.0002

F50 (Median Frequency)

F50 increased significantly above baseline after tail docking in the control and meloxicam treatments, but not in the EMLA or cautery treatments (Figure 1 and Table 2). Thirty seconds after docking F50 in the control group was significantly higher than that of the EMLA group. Control F50 was significantly higher than that of the EMLA and cautery treatments at 60 and 90 seconds after docking. By 120 seconds after docking, F50 did not differ between treatment groups. F50 did not differ between the control and meloxicam treatments at any time point (Table 2).

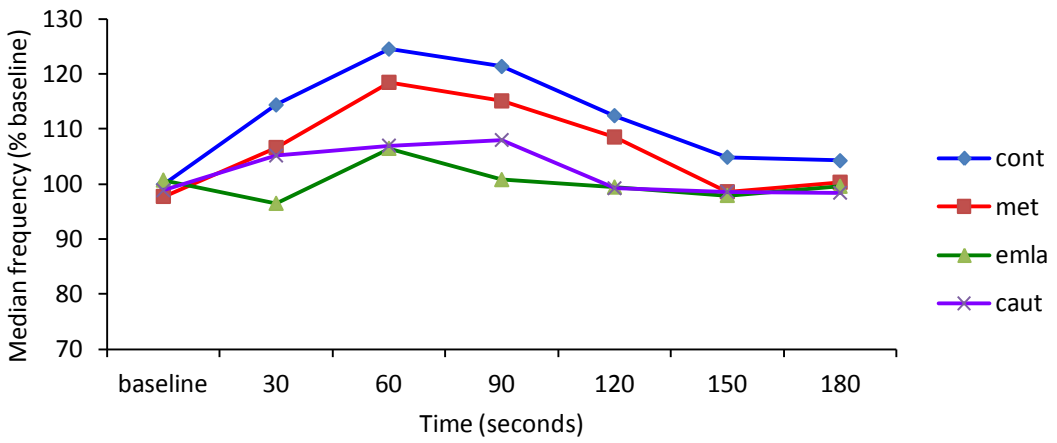

Figure 1: Mean standardised (% baseline) median frequency (F50) of the piglet EEG over consecutive 30 second blocks beginning 30 seconds prior to tail docking and ending 180 seconds after tail docking using either: clippers with no prior analgesia (cont); cautery iron with no prior analgesia (caut); clippers with prior administration of Meloxicam (met); or clippers with prior application of topical anaesthetic cream (EMLA)

Table 2: Mean (SEM) standardised (% baseline) median frequency (F50) of the piglet EEG over consecutive 30-second intervals following tail docking with or without analgesia

		Elapsed time (seconds)					
Treatment	**Baseline**	**30**	**60**	**90**	**120**	**150**	**180**
Clipper	99.9	**114.4**^{*y}	**124.5**^{*y}	**121.4**^{*y}	112.4	104.9	104.2
	(1.51)	(5.31)	(2.37)	(1.66)	(3.18)	(2.95)	(1.94)
Meloxicam	97.8	106.6	**118.5***	**115.2***	108.6	98.6	100.3
	(1.16)	(7.21)	(5.75)	(5.86)	(3.50)	(3.77)	(2.16)
EMLA	100.7	96.5^{z}	106.5^{z}	100.8^{z}	99.5	97.9	99.6
	(1.05)	(1.08)	(3.24)	(2.31)	(3.92)	(2.90)	(2.60)
Cautery	98.9	105.2	107.0^{z}	108.0^{z}	98.2	97.2	98.4
	(1.07)	(2.33)	(3.55)	(3.17)	(2.28)	(2.38)	(2.65)

* indicates value differed significantly to baseline within the same row

^{yz} values in the same column with different superscripts differed significantly (P <0.05)

P_{TOT} (Total Power)

P_{TOT} decreased significantly below baseline after tail docking in the control, Meloxicam and cautery treatment groups (Figure 2 and Table 3). At 30 seconds after docking P_{TOT} in the control treatment was significantly lower than that of the EMLA treatment. At 60 seconds control P_{TOT} was lower than that of both the EMLA and cautery treatments. At 90 seconds post docking P_{TOT} did not differ between treatments. P_{TOT} did not differ between the control and Meloxicam treatments at any time point (Table 3).

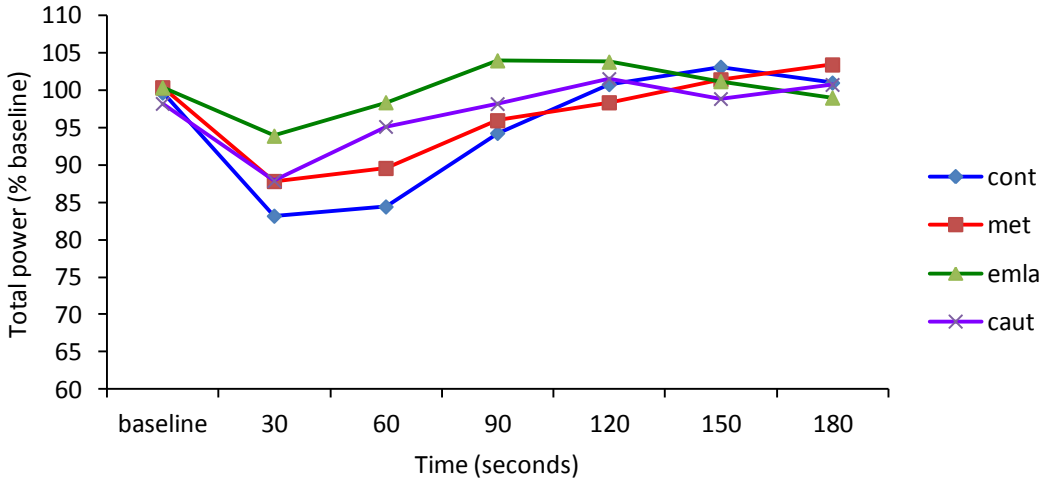

Figure 2: Mean standardised (% baseline) total power (P_{TOT}) of the piglet EEG over consecutive 30 second blocks beginning 30 seconds prior to tail docking and ending 180 seconds after tail docking using either: clippers with no prior analgesia (cont); cautery iron with no prior analgesia (caut); clippers with prior administration of Meloxicam (met); or clippers with prior application of topical anaesthetic (EMLA)

Table 3: Mean (SEM) standardised (% baseline) total power (P_{TOT}) of the piglet EEG over consecutive 30-second intervals following tail docking with or without analgesia

		Elapsed time (seconds)					
Treatment	**Baseline**	**30**	**60**	**90**	**120**	**150**	**180**
Control	99.7	**83.2**^{*y}	**84.4**^{*y}	94.3	100.8	103.1	101.0
	(1.08)	(2.61)	(2.28)	(3.14)	(2.89)	(2.15)	(1.01)
Meloxicam	100.3	**87.8***	**89.5***	95.9	98.3	101.4	103.4
	(0.65)	(2.78)	(2.48)	(3.19)	(1.84)	(1.75)	(1.33)
EMLA	100.3	93.9^{z}	98.4^{z}	103.9	103.8	101.2	99.0
	(0.89)	(2.01)	(3.15)	(1.52)	(1.27)	(1.38)	(1.10)
Cautery	98.2	**87.9***	95.1^{z}	98.2	101.5	98.8	100.7
	(0.80)	(3.06)	(3.28)	(3.04)	(2.70)	(1.50)	(1.73)

* indicates value differed significantly to baseline within treatment

^{yz} values in the same column with different superscripts differed significantly ($p < 0.05$)

F95 (Spectral Edge Frequency)

F95 did not differ significantly from baseline in any treatment group, nor were there any differences between treatment groups at any time point. There was an overall time effect, with F95 being higher than baseline at 30, 60 and 90 seconds after docking (Figure 3, Table 4). Control F50 was significantly higher than EMLA overall (p=0.0037).

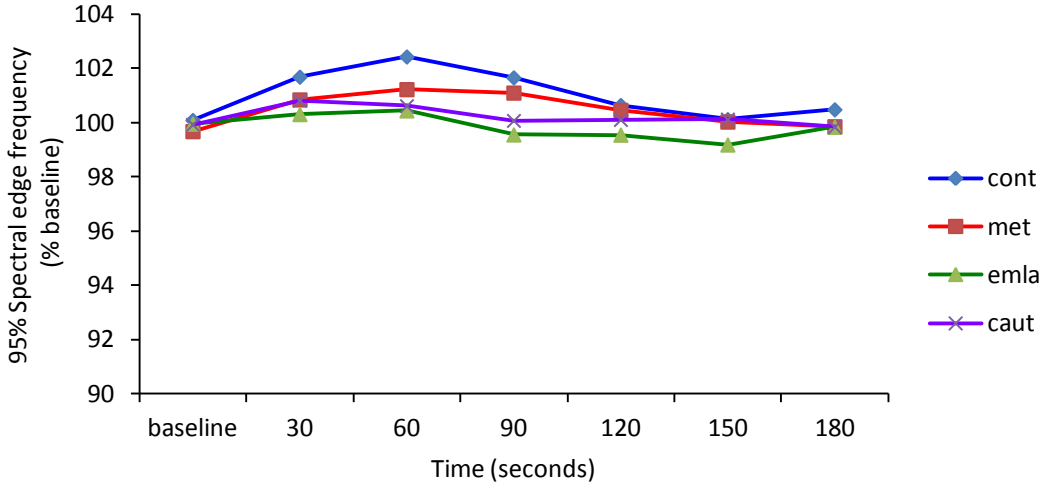

Figure 3: Mean standardised (% baseline) spectral edge frequency (F95) of the piglet EEG over consecutive 30 second blocks beginning 30 seconds prior to tail docking and ending 180 seconds after tail docking using either: clippers with no prior analgesia (cont); cautery iron with no prior analgesia (caut); clippers with prior administration of Meloxicam (met);or clippers with prior application of topical anaesthetic (EMLA)

Table 4 Results of mixed model analysis showing the effects of treatment and time on F95 of the EEG of anaesthetised piglets following tail docking with or without prior analgesia

Variable	F95 (mean)	SE
Treatment		
Control	101.09	0.256
EMLA	99.61[y]	0.261
Meloxicam	100.54	0.248
Cautery	100.11	0.251
Time (seconds)		
0	99.87	0.202
30	100.88[z]	0.202
60	101.14[z]	0.202
90	100.56[z]	0.202
120	100.15	0.202
150	99.83	0.202
180	99.95	0.204

[y] differed to control F95 (p=0.0270)
[z] differed significantly to F95 at time 0 (p <0.05)

Discussion

The typical mammalian EEG response to noxious stimulation is desynchronisation, or a shift to high frequency low voltage activity, with a corresponding increase in F50 and decrease in P_{TOT} (Murrell & Johnson 2006). A number of studies have identified an increase in F50 and decrease in P_{TOT} of the EEG of anaesthetised animals subjected to painful stimuli using the minimal anaesthesia model (Murrell et al 2003, Johnson et al 2005, Gibson et al 2007, Murrell et al 2007).

Consistent with this nociceptive response, control animals tail docked using clippers without prior analgesia demonstrated an increase in F50 and decrease in P_{TOT} of the EEG following docking.
Application of a topical anaesthetic (EMLA cream) to the base of the tail 60–90 minutes prior to docking with clippers abolished the EEG responses observed with clippers alone. EMLA cream contains 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).

In contrast, administration of oral meloxicam 60–90 minutes prior to docking with clippers had little effect on the change in F50 after docking, and no effect on the change in P_{TOT}. Median frequency was elevated at 60 and 90 seconds after docking, compared with 30–90 seconds in the control group. The magnitude of the increase in F50 did not differ between the two groups; however, whilst F50 in the control group was significantly higher than that of the EMLA and cautery groups after docking, F50 of the meloxicam group was not different. These results suggest that meloxicam may have had a weak anti-nociceptive effect on the acute response to tail docking. Although meloxicam, like other non-steroidal anti-inflammatory drugs, is believed to exert anti nociceptive effects mainly through inhibition of peripheral inflammatory responses, there is some evidence that it may also have central and pre-emptive analgesic effects (Cashman 1996, Isiordia-Espinoza et al 2012).

Pigs in the cautery treatment exhibited no such change in F50 in response to docking. A transient reduction in P_{TOT} was, however, observed immediately following docking by cautery iron suggesting some nociceptive processing still occurred. The significantly smaller changes in F50 and P_{TOT} observed compared to the control treatment at 60 and 90 seconds post docking indicate a reduction in nociceptive processing following docking by cautery compared with clippers. In a previous study comparing the EEG responses of pigs to tail docking using clippers or cauterisation, it was found that docking by cauterisation reduced nociceptive responses relative to docking with clippers (Kells et al 2013). This was the basis for inclusion of cauterisation as a potential analgesic strategy in the present study.

Despite an overall increase in F95 in the 90 seconds following docking, F95 did not differ significantly to baseline after docking in any treatment. An increase in F95 following noxious stimulation has been reported in some studies using the minimal anaesthesia model (Johnson et al 2005, Gibson, et al. 2007), whereas other studies report no change in F95 in response to noxious stimulation (Murrell, et al. 2003, Murrell, et al. 2007, Kongara et al 2010). Changes in F95 are thought to be associated more with adequacy of anaesthesia (Johnson et al 1994) than nociception, therefore the absence of an increase in F95 in the present study should not be equated with the absence of a nociceptive response.

Although tail docking and other routine husbandry procedures are normally carried out within the first days of birth, this study used 20 day-old pigs, as the methodology employed has previously been validated in this age group (Kells, et al. 2013).

Conclusion

In conclusion, based on the analysis of EEG variables, it appears that prior application of topical anaesthetic (EMLA) is effective in mitigating acute nociceptive responses to tail docking in pigs. The use of a cauterising iron to dock the tail also appeared to mitigate the acute nociceptive response, although to a lesser extent than EMLA. Prior administration of meloxicam had little effect on acute nociceptive responses to tail docking.

Experiment 4: Acute Physiological and Behavioural Responses

Aim

The aim of this experiment is to use the physiological and behavioural responses of piglets to assess the efficacy of cauterisation, meloxicam and a topical anaesthetic in mitigating acute responses to tail docking.

Research Methodology

This experiment was approved by the Rivalea Animal Ethics Committee. The experiment was conducted at the Rivalea Australia, Research and Innovation Unit, Corowa NSW, Australia. The experiment was conducted between November 2012 and March 2013. Seventy two sows (Large White x Landrace) and their litters were selected. The sows farrowed in individual farrowing crates. Five entire male piglets greater than 1.2kg in live weight 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-E) was written on their back with a black stock marker pen. Data were collected from 360 piglets.

The following treatments were imposed:
Treatment A: Sham treatment (Handling alone).
Treatment B: Tail docking using clippers.
Treatment C: Tail docking using cauteriser (Stericut® Tail Docker)
Treatment D: Topical anaesthetic/antiseptic- Tail docked using clippers and 4ml (2 sprays of 2ml applicator) applied directly after tail docking. The medication contained 40.6g/L Lignocaine, 4.2g/L Bupivacaine, 24.8 mg/L Adrenaline, 5.0 g/L Cetrimide.
Treatment E: Meloxicam-Metacam®-5 mg/ml (0.1ml/1.25kg pig) injected 1 hr prior to tail docking. Tail was docked using clippers.

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 approximately 30 s and were put back into their pen. The pigs in treatments B, D and E had their tail docked with clean, disinfected side-cutters (clippers). The tail was cut approximately 2cm from the base of the tail in between the second vertebrae. The pigs in treatment C had their tail docked with a clean disinfected gas operated Stericut® cauteriser. Their tails were docked at the same location as other treatments.

Piglets in treatment A had their tails removed after blood samples and behavioural observations were completed. 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 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).

Stress Physiology
Blood samples were collected by jugular venipuncture. The blood samples were taken at 15 min and 30 min post-procedure. 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 total cortisol were determined in duplicate 100-µL aliquots using an extracted radioimmunoassay according to the protocol developed by Bocking and Harding (1986) and validated for pig plasma using hydrocortisone H-4001 (Sigma Chemical Co., St Louis, MO) as standard.

Behaviour
During the treatment an escape attempt was defined as a body movement carried out to effect an escape (as described by Marchant-Forde et al., 2009). Vocalisations were recorded during treatment. A bout criterion interval of 1 second was used. i.e. if a piglet squealed for approximately 10 sec a score of 10 was given.

The behaviour of the five treatment pigs in each litter was videotaped by using mounted cameras (Signet Model QV-3063) that enabled view of the whole farrowing crate. The behaviour of the piglets for the first 60 min post-treatment was measured by continuously observing each piglet for 60 sec every 5 min. (i.e. a total of 12 min in the first 60 min post-treatment). The total active behaviours were calculated as all behaviours combined with the exception of lying behaviour. Total

resting behaviours were calculated as the total of time lying with and without sow contact and when the piglet was idle. The term "out of view" was used when the piglet could not been seen within the field of view of the camera.

The following ethogram was used to describe behaviours:

Table 1: 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. Vigorous and rhythmic suckling movements.
Asleep	Eyes closed while lying down.
Playing/frolicking	Head shaking, springing (sudden jump or leap), running with horizontal and vertical bounces.

Growth Performance

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

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. Chi-squared analysis was used to analyse treatments effects on number of piglets that died or were removed between treatment and weaning.

Table 2: Number of piglet deaths and removals between treatment and weaning.

Cause of death/removal	Sham	Tail docked using clippers	Tail docked using cauteriser	Topical Anaesthetic/ Antiseptic + clipper	Meloxicam + clipper
Overlain by sow	2		2	3	1
Scours	1				
Unthrifty	1	3		1	1
Other	1			1	2
Total	5/72	3/72	2/72	5/72	4/72

There was no significant difference (X^2=1.88; P=0.864) between the number of piglet deaths and piglet removals due to illness and injury between treatments (Table 2).

Table 3: Effect of treatment on mean total cortisol concentrations (ng/ml).

Cortisol (ng/ml)	Sham	Tail docked using clippers	Tail docked using cauteriser	Topical Anaesthetic/ Antiseptic + clipper	Meloxicam + clipper	SEM	P value
15 min post-treatment	147.4^{ac}	162.7^{b}	157.7^{ab}	148.3^{abc}	140.5^{c}	2.807	0.041
30 min post-treatment	205.8	214.1	215.5	217	208.1	3.681	0.735

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

There was a significant difference (P<0.05) in cortisol concentrations between treatments at 15 min post-treatment. Cortisol concentrations were significantly lower (P<0.05) in the sham and cauterisation treatment compared to the clipper treatment. There was no significant difference (P>0.05) in cortisol concentrations at 15 min between the cauterisation and topical anaesthetic treatment compared to the clipper treatment. The meloxicam treatment had significantly lower cortisol concentrations than the clipper treatment 15 min post-treatment. There was no significant difference (P>0.05) in cortisol concentrations 30 min post-treatment (Table 3).

Table 4: Effect of treatment on behaviour of piglets during and 60 min after treatment. Mean total time (sec) spent in each posture or state during observation period. Transformed means are presented and back transformed means presented in parentheses.

	Sham	Tail docked using clippers	Tail docked using cauteriser	Topical Anaesthetic/ Antiseptic + clipper	Meloxicam + clipper	SEM	P value
Duration of vocalisations during treatment (sec)	1.06[a] (1.2)	1.89[bc] (3.6)	1.71[b] (2.9)	1.91[bc] (3.6)	1.93[c] (3.6)	0.04	0.000
Number of escape attempts during treatment	0.6[a] (0.36)	1.4[b] (2.0)	1.3[b] (1.7)	1.5[b] (2.3)	1.4[b] (2.0)	0.04	0.000
Posture (sec):							
Standing (normal)	14.8[ab] (219.0)	14.5[a] (210.3)	15.0[ab] (225.0)	13.9[c] (193.2)	15.3[bc] (234.1)	0.22	0.007
Standing (head lowered)	4.4 (19.4)	5.0 (25.0)	5.6 (31.4)	5.1 (26.0)	5.8 (33.6)	0.18	0.211
Sitting	0.4 (0.16)	0.3 (0.09)	0.3 (0.09)	0.6 (0.36)	0.1 (0.01)	0.09	0.417
Lying (with sow contact)	4.8 (23.0)	4.3 (18.5)	4.4 (19.4)	4.4 (19.4)	4.0 (16.0)	0.34	0.406
Lying (without sow contact)	18.0 (324.0)	18.1 (327.6)	17.4 (302.8)	18.6 (346.0)	17.5 (306.3)	0.32	0.055
Out of view	4.5 (20.3)	5.3 (28.1)	4.7 (22.1)	5.1 (26.0)	5.2 (27.0)	0.20	0.567
States (sec):							
Idle	8.8[b] (77.4)	8.2[a] (67.2)	8.8[b] (77.4)	7.8[a] (60.8)	9.1[b] (82.8)	0.20	0.006
Walking/Running	5.0[a] (25.0)	5.1[a] (26.0)	5.6[bc] (31.4)	5.5[b] (30.3)	6.0[c] (36.0)	0.11	0.011
Massaging udder/Nursing	11.9[a] (141.6)	12.2[ab] (148.8)	12.8[b] (163.8)	11.8[a] (139.2)	13.07[b] (170.8)	0.27	0.032
Asleep	19.5[ab] (380.3)	19.0[a] (361.0)	18.8[a] (353.4)	20.1[b] (404.0)	17.9[c] (320.4)	0.23	0.006
Playing/Frolicking	0.8 (0.64)	1.0 (1.0)	0.8 (0.64)	0.8 (0.64)	0.7 (0.49)	0.14	0.953
Out of view	5.0 (25.0)	5.4 (29.2)	4.7 (29.1)	5.2 (27.0)	5.2 (27.0)	0.20	0.638
Total active	13.6[ab] (185.0)	14.0[ab] (196.0)	14.4[ac] (207.4)	13.5[b] (182.3)	15.0[c] (225.0)	0.25	0.030
Total inactive	21.8[a] (475.2)	21.1[ab] (445.2)	21.1[ab] (445.2)	21.7[a] (470.9)	20.5[b] 420.25	0.21	0.029

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

* Data square root transformed prior to statistical analysis.

There were significantly (P<0.001) more vocalisations and escape attempts at the time of tail docking in all the treatments compared to the sham treatment (Table 4).

There was no significant difference (P>0.05) in the pain-related behaviour of standing with head lowered between treatments. Piglets in the meloxicam treatment spent significantly more time standing (P<0.05) compared to piglets in the clipper treatment. Pigs in the meloxicam treatment spent significantly more time walking, running compared to pigs in the clipper the clipper treatment and the sham (Table 4).

Table 5: Effect of treatment on growth performance of piglets.

	Sham	Tail docked using clippers	Tail docked using cauteriser	Topical Anaesthetic/ Antiseptic + clipper	Meloxicam + clipper	SEM	P value
Live weight prior to treatment (kg)	-	1.8	1.8	1.8	1.8	0.18	0.937
Weight 7 days post-treatment (kg)	-	3.3	3.4	3.5	3.4	0.04	0.742
Weaning weight(kg)	-	6.8	6.9	7.0	6.6	0.08	0.323
Rate of gain (g/day) 0-7 days	-	227	229	238	227	0.004	0.570
Rate of gain (g/day) Treatment-weaning	-	233	237	243	224	0.003	0.062

*data not included for sham treatment as these piglets had their tails docked after behaviour and physiology samples were collected. Unable to keep tails intact in commercial production system.

There was no significant difference (P>0.05) between start weight, day 7 weight and weaning weight and the rate of gain during these same time periods between the four tail docking treatments (Table 5).

Discussion

The aim of this experiment was to use the physiological and behavioural responses of piglets to assess the efficacy of cauterisation, meloxicam and a topical anaesthetic in mitigating acute responses to tail docking.

In the current experiment the administration of injectable meloxicam 60 min prior to tail docking treatment reduced the cortisol response at 15 min post-treatment compared to the clipper treatment. Although meloxicam, like other non-steroidal anti-inflammatory drugs, is believed to exert anti nociceptive effects mainly through inhibition of peripheral inflammatory responses, there is some evidence that it may also have central and pre-emptive analgesic effects (Cashman 1996, Isiordia-Espinoza et al., 2012). Hansson et al. (2011) showed that surgically castrated piglets that were given meloxicam displayed less pain-related behavior (huddled up, spasms, rump-scratching, stiffness and prostrated) on both the castration day and the 24 hours following. Meloxicam did not alter pain-related behavior of vocalization, escape attempts and standing with head lowered in the current experiment. Piglets in the meloxicam treatment appeared to be more aroused and were more active and spent significantly more time standing compared to piglets in the clipper and the sham treatment. This change in behaviour in the meloxicam treated pigs was not expected and the authors do not have an explanation for this. Pigs treated with meloxicam appeared to be aroused compared to the sham treatment. Further research is required to fully understand the impact of meloxicam on behaviour and physiology of piglets when used as a possible pain relief for tail docking.

There was no impact of a topical anaesthetic applied immediately after tail docking on acute cortisol response or pain-related behaviours.

In the current experiment there was no significant difference in the pain-related behaviour of standing with head lowered between treatments. This is contrary to what was observed in Part 1 of this project where piglets that had been tail docked with either the clipper or cauteriser spent more time standing idle with their head lowered compared to the sham treatment. In the current experiment the piglets in the sham treatment spent more time standing with their head lowered compared to those in the Experiment 2. It is speculated that the differences observed between the two experiments may be due to environmental conditions affecting the behaviour of the piglets post-treatment. The summer in which the current experiment was conducted (summer 2012/2013) was much hotter than when Experiment 2 was conducted (summer 2010/2011) which may have masked some of the treatment effects. Piglets may have been behaving differently in the current experiment to enable them to cope with the hotter conditions.

There was no impact of pain medication on growth performance of piglets in the current experiment.

In conclusion, tail docking 2 day old piglets using clipper caused a cortisol response at 15 min post-treatment, providing further evidence that tail docking using clippers causes an acute pain response. This response had diminished by 30 min post-treatment. Tail docking using clippers or cauterisation caused an increase in vocalisations and escape attempts and these behaviours were not mitigated by the use of pain relief. Injectable meloxicam administered prior to tail docking reduced the acute cortisol response, however did not influence pain-related behaviours. Further research is required to investigate physiological and behavioural responses and the use of meloxicam as a possible medication to reduce acute pain associated with tail docking.

General Discussion

In conclusion, the neurophysiological, physiological and behavioural investigations showed that tail docking by either clipper or cauterisation caused an acute pain response in piglets. The typical mammalian EEG response to noxious stimulation is a shift to high frequency low voltage activity, with a corresponding increase in F50 and decrease in P_{TOT} (Murrell & Johnson, 2006). Consistent with this nociceptive response, in Experiment 1 and 3 animals tail docked using clippers and cauterisation demonstrated an increase in F50 and decrease in P_{TOT} of the EEG following docking.

Changes in stress physiology in Experiment 2 and 4 showed that there is activation of the HPA axis after tail docking which is observed for at least 15 min and up to 30 min after treatment. There were no significant differences in the stress response or behaviour at 24 hours post-treatment in these experiments, which indicates that the stress response associated with tail docking is acute.

Piglets that were tail docked in both experiments exhibited more vocalisations and escape attempts during the tail docking treatment compared to the sham treatment. In Experiment 2, piglets that had been tail docked exhibited more pain-related behaviour (standing head lowered) compared to the sham treatment. This change in behaviour post-treatment was however not observed in Experiment 4. The sham treated pigs appeared to have a higher proportion of time spent with their head lowered in the Experiment 4 compared to Experiment 2 (5 vs. 19 sec in Experiment 2 and 4, respectively). This change in behaviour may have been due to seasonal conditions and temperature affecting piglet behaviour.

Neurophysiological and physiological examination showed that cauterisation may be less aversive in the short-term than clipper treatment to tail dock piglets. It is speculated that the intense heat associated with cauterisation may destroy nociceptors in the immediate area and may reduce the perception of pain in these areas, resulting in a lower neurophysiological and physiological response. However, there may be long-term welfare concerns with cauterisation that were not investigated n this experiment. There is some evidence in the scientific literature that after cauterisation, neuromas (a tumour or mass growing from nerve consisting of nerve fibres) develop on the tail stump when the nociceptors regenerate, which causes sensitivity of the area. Therefore, cauterisation should not be recommended in the immediate future as a practical alternative to clippers for tail docking, until the long-term detrimental effects of cauterisation are investigated.

There were no impacts of treatment on growth performance in Experiments 2 and 4. 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 Experiment 2, piglets in the surgically castrated and tail docked treatments had activation of the HPA axis 15 min and 30 min after treatment, the response was not significant enough to have a biological impact on the piglet and cause a reduction in growth performance. These data provide further evidence that tail docking causes a short-term stress response which does not impact on biological fitness of the animal.

Part 2 investigated practical strategies that could be used to reduce or eliminate the acute pain caused by tail docking procedure. Three commercially-available medications were investigated. A topical anaesthetic that was applied to the base of the tail 60 min prior to tail docking, a topical anaesthetic/ antiseptic that was applied immediately after tail docking and an anti-inflammatory (Meloxicam) administered 60 min prior to docking. Cauterisation was also included as a treatment strategy as in Part 1 it was shown to be less aversive than clipper treatment and is a cheaper alternative to the medications that were investigated.

The neurophysiological (EEG) component showed that application of a topical anaesthetic cream to the base of the tail 60–90 minutes prior to docking with clippers abolished the EEG responses observed with clippers alone. 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). 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. Further research is required to assess the physiological and behavioural responses of piglets to tail docking after treatment with a topical anaesthetic applied prior to tail docking. The practicalities of this technique should also be studied i.e. application of topical anaesthetic prior to tail docking treatment and ensuring that the medication does not become an attractant to piglets within the pen.

In Experiment 3 prior administration of oral meloxicam had little effect on acute nociceptive responses to tail docking, however in Experiment 4 a significant lower cortisol response was observed. It is speculated that the method of administration of meloxicam in the two different experiments may have influenced results. In Experiment 3 (EEG analysis) the meloxicam was administered orally so as not to interfere with EEG analysis (i.e. an injection would be a noxious stimulus that may have interfered with the EEG results). However, oral meloxicam is slower acting compared to injectable meloxicam, therefore it may have taken longer than 60 min for the

meloxicam to become effective in Experiment 3. There is also speculation that the meloxicam may not have been effective at the time of the EEG analysis (i.e. during the tail docking process) as the medication becomes effective once there is a injury in the body. 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. Experiment 4 showed there a significantly lower stress response in the meloxicam pigs (administered via injection) at 15 min post-treatment compared to the clipper treated pigs. The effect diminished by 30 min post-treatment. There was no impact of meloxicam on pain-related behaviours exhibited during or after tail docking. Interestingly the meloxicam treated pigs were more active and aroused compared to the other treatments (including the sham treatment) which cannot be explained. Further research is required on the timing and method of administration of meloxicam if it is deemed an appropriate medication to reduce the acute pain response of tail docking.

Experiment 3 also investigated the use of a topical anaesthetic applied to the tail docking wound directly after treatment. This medication did not influence stress physiology and pain-related behaviour.

Table 6: Cost of tools and medications*.

	Sham	Tail docked using clippers	Tail docked using cauteriser	Topical Anaesthetic/ Antiseptic + clipper	Meloxicam + clipper	EMLA cream
Cost/piglet treatment ($)	-	One off purchase for clippers $30	One off purchase for cauteriser $168 + $8 gas refill	$0.36	$0.53	$2.00

*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, financial arrangements with drug companies etc.

Outcomes/Conclusion

In conclusion, based on neurophysiological, physiological and behavioural responses, tail docking caused an acute pain response. This response had diminished by 24 hours post-treatment. The use of cauterisation appears to be less aversive, however further research is required to assess long-term welfare implications of cauterisation. Topical anaesthetic cream and injectable meloxicam administered prior to tail docking appear to mitigate this acute pain response, however further research is required to investigate physiological and behavioural responses to application of a topical anaesthetic cream and behavioural changes observed with the use of meloxicam. The commercial-viability of any pain relief medications needs to be addressed.

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