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Animal Welfare 2019, 28: 487-498
ISSN 0962-7286
doi: 10.7120/09627286.28.4.487

Use of meloxicam, buprenorphine, and Maxilene® to assess a multimodal approach for piglet pain management, part 1: surgical castration

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Abstract

Surgical castration of piglets is a routine procedure on commercial pig farms, to prevent boar taint and reduce aggression. This procedure is known to cause pain, yet piglets are often not provided appropriate analgesia for relief. The objective of this study was to assess a multimodal approach to managing post-castration pain in piglets, using 0.4 mg kg^{-1} meloxicam (MEL), 0.04 mg kg^{-1} buprenorphine (BUP), and Maxilene® (MAX). Efficacy was evaluated using behavioural indicators, vocalisation, and facial grimace analysis. Male piglets were randomly assigned to one of ten possible treatments (n = 15 piglets per treatment group): MEL + BUP + MAX (castrated or uncastrated); MEL + BUP (castrated or uncastrated); BUP + MAX (castrated or uncastrated); MEL + MAX (castrated or uncastrated); saline (castrated control); or sham (uncastrated control). Castrated piglets in the MEL + BUP + MAX, MEL + BUP, and BUP + MAX treatment groups displayed significantly fewer pain behaviours than piglets administered saline. MEL + MAX was insufficient in reducing surgical castration pain behaviours. At 24 h post-procedure, saline and MEL + MAX-castrated piglets displayed significantly more pain behaviours than all other treatment groups and time-points. Facial grimace analysis indicated that MEL + MAX-castrated piglets had significantly higher grimace scores than MEL + BUP (castrated and uncastrated) and BUP + MAX-uncastrated. There were no significant differences in emitted vocalisations between the analgesia-treated and saline-castrated piglets. All treatment groups with buprenorphine were effective in alleviating castration-associated pain behaviours, suggesting that opioid administration is beneficial for managing piglet castration pain.

Keywords: *analgesia, animal welfare, castration, multimodal, pain assessment, piglet*

Introduction

Surgical castration of boar piglets is performed on commercial pig farms in North America to prevent boar taint and reduce aggressive behaviour (Rault *et al* 2011). It is known to cause acute pain, based on specific behaviour and physiologic alterations, such as rump scratching, increased blood cortisol, and high frequency vocalisations, that can persist beyond 24 h post-procedure (Hay *et al* 2003; Moya *et al* 2008; Sutherland *et al* 2012). Both Canada and the EU have animal care guidelines that require analgesia administration to alleviate piglet castration pain (EU Commission 2010; National Farm Animal Care Council [NFACC] 2014). Non-steroidal anti-inflammatory drugs (NSAIDs) are recommended for use on-farm; however, recent research exploring meloxicam and ketoprofen use found them both to be ineffective at alleviating post-procedural pain in piglets (Kluivers-Poodt *et al* 2012; Viscardi & Turner 2018a). Combining an NSAID with a more potent analgesic, such as an opioid, is common practice in companion animal medicine for post-operative pain management (Shih *et al* 2008; Epstein *et al* 2015). The efficacy of such an approach to control pain in piglets following castration has not been assessed.

A previous study demonstrated that the opioid buprenorphine was highly effective at alleviating surgical castration pain in piglets without causing any adverse side-effects (Viscardi & Turner 2018b). Most drug combinations and inhalants used for general anaesthesia, which renders an animal insensible, would be inappropriate to administer to piglets on-farm, as recovery times can be prolonged (eg 3 h for ketamine-azaperone) (Schmidt *et al* 2012), and piglets would have to be separated from the sow until fully sensible to avoid crushing risks. Anaesthesia administration may also require specialised equipment, as is the case with inhalants (eg isoflurane), which are impractical to use in a farm setting. A topical anaesthetic, used to numb and temporarily reduce the sensation of pain, is more practical and could be used to alleviate the initial pain of castration (eg the scrotal incision) (Sutherland *et al* 2010). Combining this with an NSAID and opioid may provide piglets with longer-term pain control (up to 12 h), improving their post-operative well-being (Keita *et al* 2010; Thiede *et al* 2014).

The objective of this study was to assess a multimodal approach to managing surgical castration pain in piglets, using 0.4 mg kg^{-1} meloxicam, 0.04 mg kg^{-1} buprenorphine, and Maxilene® (topical lidocaine). The efficacy of each drug regime was evaluated using behavioural indicators, vocalisation, and facial grimace analysis. We hypothesised that piglets receiving meloxicam, buprenorphine, and Maxilene® would have the greatest reduction in pain behaviours and facial grimacing post-castration and would emit lower frequency vocalisations at the time of the procedure compared to all other treatment combinations used.

Materials and methods

Ethics statement

All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). The institution is registered under the Animals for Research Act of Ontario and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

Study animals and treatments

A total of 150 Yorkshire-Landrace × Duroc male piglets (five days old, mean [± SEM] BW = 2.15 [± 0.04] kg) from 25 different litters were used in this study. Sows and piglets were housed in farrowing pens at the University of Guelph Arkell Swine Research Station (Arkell, ON, Canada). The floor space for each pen was 1.8 × 2.4 m (length × width) and the farrowing crate measured 0.8 × 2.3 m. The farrowing rooms were maintained at ambient temperature (23 [± 0.5]ºC) with lights on/off at 0700/2100h, and natural light was provided by windows in each room. Sows were fed *ad libitum* beginning four days post-farrowing. The creep areas for piglets were heated to approximately 30–35ºC by means of a heating pad or lamp. For this study, we selected litters of piglets that had remained with their biological sow.

Ten treatments were used and each treatment group was identified by a unique letter or symbol ('H', 'T', 'V', 'X', ∞, asterisk, circle, triangle, square or squiggle) written on the piglet's forehead and back with a black marker prior to castration. This was to ensure that individuals involved in behaviour and facial grimace scoring remained blind to treatment. For individual animal identification, a number was written on the back leg of each piglet. Fifteen piglets were assigned to each treatment group. Group size was based on a sample size estimate, using $\alpha = 0.05$, population $\sigma = 0.1$ (determined from a pilot study) and 5% precision (Suresh & Chandrashekara 2012; Viscardi *et al* 2017). Within each litter, piglets were randomly assigned to one of the following treatments: 0.4 mg kg^{-1} meloxicam + 0.04 mg kg^{-1} buprenorphine + Maxilene®-castrated, 0.4 mg kg^{-1} meloxicam + 0.04 mg kg^{-1} buprenorphine + Maxilene®-uncastrated, 0.4 mg kg^{-1} meloxicam + 0.04 mg kg^{-1} buprenorphine-castrated, 0.4 mg kg^{-1} meloxicam + 0.04 mg kg^{-1} buprenorphine-uncastrated, 0.04 mg kg^{-1} buprenorphine + Maxilene®-castrated, 0.04 mg kg^{-1} buprenorphine + Maxilene®-uncastrated, 0.4 mg kg^{-1} meloxicam + Maxilene®-castrated, 0.4 mg kg^{-1} meloxicam + Maxilene®-uncastrated, saline (castrated control), or sham (uncastrated control). Meloxicam (MEL) (Metacam 20 mg ml^{-1}; Boehringer Ingelheim Ltd, Burlington, ON, Canada), buprenorphine (BUP) (Vetergesic 0.3 mg ml^{-1}; Champion Alstoe Animal Health Inc, Whitby, ON, Canada; extra-label use), and saline were injected intramuscularly (IM) and drug doses were derived from the literature (Flecknell 2015). Maxilene® (MAX) (Maxilene® 4% lidocaine; RGR Pharma Ltd, Windsor, ON, Canada; extra-label use) was applied topically to the scrotal surface.

Processing procedures

All piglets were weighed 24 h prior to the start of the study for drug dose calculations. They were then marked with the symbol that corresponded to their treatment group. On the day of castration, male piglets were removed from their litter, placed in a transport cart, and administered their assigned treatments 20 min pre-procedure. One individual restrained each piglet and another gave the IM injection(s) in the neck muscle and applied the topical to the scrotum using a swab. Treatments were administered in the same order for each litter of pigs based on treatment group assignment: 1) BUP + MEL + MAX; 2) BUP + MEL; 3) BUP + MAX; 4) MEL + MAX; 5) Saline; and 6) Sham (handled). Piglets were then surgically castrated in the same order that treatments were given using two vertical incisions and tearing of the spermatic cord before being immediately returned to their home pen. Castrations occurred between 0800 and 1000h and were conducted by one individual (AVV). All handling and technical procedures were carried out by female researchers, to eliminate the potential risk of piglets altering their pain response due to stress of exposure to male researchers, as has been demonstrated in mice (Sorge *et al* 2014). Piglets in the sham treatment group were the only non-castrated pigs that underwent a simulated castration. The scalpel handle was used to simulate the scrotal incision and piglets were held in the same position and for the same length of time (approximately 20 s) as those surgically castrated.

Behaviour recording and scoring

Video cameras (JVC GZ-E200 full HD Everio Camcorder, Yokohama, Japan) were placed on tripods outside of each farrowing pen. Piglets were video-recorded pre-procedure for 1 h, immediately post-castration for 8 h, and for another hour at 24 h post-procedure (ie, 10 h of video data were collected in total from each litter of pigs). The videos were randomised across litters and time-points using a random number generator (random.org) prior to being scored. Each individual piglet was behaviour scored continuously by two trained observers for the first 15 min of every hour of video data collected using the Observer XT program (Version 12.0, Noldus Information Technology, Wageningen, The Netherlands) and a detailed ethogram adapted from Hay *et al* (2003) (Table 1) to generate time budgets. The observers were blind as to treatment, time-point, and litter; however, they were able to see which piglets had been castrated. Inter-observer scoring reliability was assessed at three times during the behaviour scoring period (once

https://doi.org/10.7120/09627286.28.4.487 Published online by Cambridge University Press

monthly), by having both individuals score the same piglet in a video and then calculating the intra-class correlation coefficient (ICC). All reliability tests produced an ICC above 0.9, indicating excellent correlation between scorers. A total of 22,500 min (375 h) of behaviour recordings were scored and analysed for this study.

Piglet behaviours were analysed separately and then grouped into active, inactive and pain categories, to assess the activity level of piglets and the total proportion of pain behaviours displayed. Active behaviours and postures included playing, running, walking, suckling, nosing, chewing, sitting, and standing. Inactive behaviours and postures included lying, sleeping, and awake inactive. Sitting was placed in the active category, as most piglets assumed this posture when suckling or scratching the rump (both considered active behaviours). Pain behaviours included stiffness, spasms, trembling, tail wagging and rump scratching (Hay *et al* 2003).

Piglet grimace scale scoring

Still images of piglet faces were captured from the first 30 min of every hour of video data collected by an individual blinded as to piglet treatment, litter, and time-point using the Everio MediaBrowser 4 program (Pixela Corporation, Osaka, Japan). Whenever a piglet face was in view and clear, the video was paused, and the image collected (excluding times when piglets were lying with their head down or sleeping). An attempt was made to take one facial image of each piglet per time-point during the study. A total of 1,118 images were captured (Table 2). The symbol marked on each piglet's forehead was blurred prior to scoring using Photoshop (Adobe Systems Incorporated, San Jose, CA, USA), to ensure volunteer scorers were blinded to treatment. Faces were randomised by litter, treatment, and time-point for scoring using a random number generator (random.org).

Four individuals were taught how to use the piglet grimace scale (PGS) (Viscardi *et al* 2017) at an interactive 30-min training session before scoring study images. The PGS score was calculated for each image by summing the scores assigned to the three facial action units (ear position, cheek tightening/nose bulge, and orbital tightening). If more than one image was taken from the same piglet at the same time-point, PGS scores were averaged prior to analysis to produce one score per piglet per time-point, eliminating the potential for pseudo-replication. The final PGS score of each piglet per time-point was calculated as a mean of the scores from the four individuals.

Vocalisations

Piglet vocalisations were measured at three points in the study: at initial handling when they were marked with a symbol (marking; all treatment groups); when they received an intramuscular injection (injection; all treatment groups except sham); and when they were surgically castrated (incision and castration; surgically castrated piglets + sham treatment group). A video camera was placed on a tripod and positioned as close to the focal piglet's face as possible to record each procedure. The resulting video files were converted to audio files and vocalisations were analysed using the sound analysis software Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, NY, USA) by two individuals who were blinded as to piglet treatment and procedure. From the spectrograms, maximum frequency (Hz), maximum amplitude (μ), maximum power (dB) and energy (dB) of each call was determined (Taylor & Weary 2000; Marx *et al* 2003).

Table 1 Ethogram used to score piglet behaviour, grouped into feeding, locomotion, non-specific behaviours, pain-related behaviours, posture and social cohesion (adapted from Hay *et al* 2003).

Behaviours	Description
Suckling[a]	Teat in mouth and suckling movements
Nosing udder[a]	Nose in contact with udder, up and down head movements
Playing[a]	Springing, bouncy movements with littermates
Agonistic[a]	Biting or fighting other littermates
Walking[a]	Moving forward at a normal pace
Running[a]	Trot or gallop
Awake inactive[b]	No special activity, but awake
Sleeping[b]	Lying down, eyes closed
Nosing[a]	Snout in contact with a substrate
Chewing[a]	Nibbling at littermates or substrates
Trembling[c]	Shivering, as with cold
Spasms[c]	Quick and involuntary contractions of the muscles
Scratching[c]	Rubbing the rump against the floor, pen walls, or littermates
Tail wagging[c]	Tail's movements from side-to-side (or up and down)
Stiffness[c]	Lying with extended and tensed legs
Lying[b]	Bodyweight supported by side or belly
Sitting[a]	Bodyweight supported by hindquarters and front legs
Standing[a]	Bodyweight supported by four legs
Kneeling[a]	Bodyweight supported by front carpal joints and hind legs
Isolated[a,b]	Alone or with one littermate at most, distance of 40 cm separates the animal(s) from the closest group of littermates
Desynchronised[a,b]	Activity different from that of most littermates (at least 75%)

[a] Active behaviour;
[b] Inactive behaviour;
[c] Castration-related pain behaviour.

Table 2 Total number of piglet faces captured for piglet grimace scale scoring.

	Treatment										
Time-point (h)	**1** 2.04 (± 0.0) kg*	**2** 2.05 (± 0.0) kg	**3** 2.24 (± 0.1) kg	**4** 2.18 (± 0.2) kg	**5** 2.18 (± 0.1) kg	**6** 2.23 (± 0.2) kg	**7** 2.16 (± 0.1) kg	**8** 2.10 (± 0.2) kg	**9** 2.27 (± 0.1) kg	**10** 2.13 (± 0.1) kg	**Total**
pre	7	7	6	5	9	7	10	5	15	11	**82**
0	15	11	18	7	22	13	16	8	18	15	**143**
1	19	10	20	10	22	14	14	4	15	14	**142**
2	19	14	20	10	16	11	9	5	10	4	**118**
3	14	11	17	9	14	14	5	2	7	8	**101**
4	14	12	14	6	14	10	6	3	12	12	**103**
5	15	7	18	5	17	15	11	5	7	7	**107**
6	15	7	15	4	18	8	8	6	9	5	**95**
7	12	7	14	9	13	9	12	6	6	3	**91**
24	18	10	19	9	19	12	13	11	13	12	**136**
	148	**96**	**161**	**74**	**164**	**113**	**104**	**55**	**112**	**91**	**1,118**

[1] Meloxicam + Buprenorphine + Maxilene® , castrated;
[2] Meloxicam + Buprenorphine + Maxilene® , uncastrated;
[3] Buprenorphine + Meloxicam, castrated;
[4] Buprenorphine + Meloxicam, uncastrated;
[5] Buprenorphine + Maxilene® , castrated;
[6] Buprenorphine + Maxilene® , uncastrated;
[7] Meloxicam + Maxilene® , castrated;
[8] Meloxicam + Maxilene® , uncastrated;
[9] Saline, castrated;
[10] Sham, uncastrated;
* Mean (± SEM) weight of piglets (n = 15) in each treatment group.

Statistical analysis

The total duration of behaviours was converted into proportions of time prior to analysis (to remove periods of time when piglets were out of view and unable to be scored). Normality was evaluated using the univariate procedure in SAS (Statistical Analysis System 9.4, SAS Institute Inc, NC, USA). Data were analysed with a GLIMMIX procedure with a beta distribution, including time, treatment, litter, and the time × treatment interaction. Litter was included as a random effect and time was a repeated measure with piglet as the experimental unit. *Post hoc* tests were conducted on significant factors using the Tukey-Kramer adjustment, to control the false-positive rate (ie, incidence of Type I error) for multiple comparisons (Ranganathan *et al* 2016). Statistical significance was set at $P < 0.05$.

The grimace scale scores were analysed using a mixed model procedure, including litter, time, treatment, and time × treatment interaction. Litter was included as a random effect, time was a repeated measure, and piglet was the experimental unit. A *post hoc* Tukey's test was conducted for significant outcomes.

The treatment variable was first set as each treatment combination included in the study. When no significant treatment and treatment × time interaction was found on any behaviour variable between BUP + MEL + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated, they were pooled into a 'BUP-castrated' group for further analysis. Similarly, no significant treatment and treatment × time interaction was found between MEL + BUP + MAX-uncastrated, MEL + BUP-uncastrated, and BUP + MAX-uncastrated, and they were pooled into a 'BUP-uncastrated' group. These groups were compared to MEL + MAX-castrated and saline-castrated for treatment and treatment × time effects for both behaviour and PGS analysis.

Vocalisation data were analysed using a mixed procedure, including litter, treatment, and procedure in the model. Litter was included as a random effect and piglet was the experimental unit. Significant outcomes were further analysed using a *post hoc* Tukey's test. Behaviour, PGS, and vocalisation data were used to assess each treatment's effectiveness in reducing surgical castration pain.

https://doi.org/10.7120/09627286.28.4.487 Published online by Cambridge University Press

Table 3 Proportion of time piglets were engaged in specific behaviours (n = 150 total; n = 15 per treatment group) across all litters and time-points. Values presented represent the proportional mean (± SEM).

	Treatment										
Behaviour[†]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	***P*-value**
Awake inactive	0.62 (± 0.03)^{a}	0.62 (± 0.03)^{a,}	0.65 (± 0.03)^{a}	0.56 (± 0.04)^{ab}	0.61 (± 0.03)^{ac}	0.57 (± 0.03)^{ab}	0.52 (± 0.03)^{bc}	0.57 (± 0.03)^{ab}	0.48 (± 0.03)^{b}	0.50 (± 0.03)^{b}	< 0.0001
Lying	0.45 (± 0.04)^{a}	0.43 (± 0.06)^{ac}	0.40 (± 0.04)^{a}	0.48 (± 0.04)^{ac}	0.45 (± 0.04)^{a}	0.51 (± 0.04)^{ab}	0.62 (± 0.03)^{bc}	0.59 (± 0.04)^{ab}	0.66 (± 0.03)^{b}	0.62 (± 0.03)^{bc}	< 0.0001
Nosing	0.09 (± 0.01)^{a}	0.08 (± 0.02)^{ab}	0.11 (± 0.02)^{ab}	0.07 (± 0.01)^{ab}	0.10 (± 0.01)^{a}	0.08 (± 0.02)^{ab}	0.04 (± 0.00)^{b}	0.05 (± 0.01)^{ab}	0.04 (± 0.00)^{b}	0.04 (± 0.00)^{b}	< 0.0001
Nosing udder	0.22 (± 0.04)^{ac}	0.22 (± 0.04)^{ab}	0.23 (± 0.04)^{ac}	0.24 (± 0.05)^{ab}	0.20 (± 0.03)^{a}	0.23 (± 0.05)^{ab}	0.30 (± 0.04)^{bc}	0.34 (± 0.05)^{ab}	0.27 (± 0.04)^{ab}	0.33 (± 0.05)^{b}	< 0.0001
Sleeping	0.45 (± 0.04)^{a}	0.44 (± 0.05)^{ab}	0.47 (± 0.05)^{ab}	0.49 (± 0.06)^{ab}	0.51 (± 0.05)^{ab}	0.44 (± 0.05)^{ab}	0.55 (± 0.04)^{ab}	0.49 (± 0.05)^{ab}	0.60 (± 0.04)^{b}	0.58 (± 0.04)^{b}	0.0015
Standing	0.52 (± 0.03)^{a}	0.55 (± 0.05)^{a}	0.57 (± 0.03)^{a}	0.49 (± 0.04)^{ac}	0.54 (± 0.03)^{a}	0.48 (± 0.04)^{ac}	0.37 (± 0.03)^{bc}	0.41 (± 0.04)^{ab}	0.32 (± 0.03)^{b}	0.37 (± 0.03)^{bc}	< 0.0001
Tail wagging	0.00 (± 0.00)^{a}	0.01 (± 0.00)^{ac}	0.02 (± 0.00)^{a}	0.02 (± 0.00)^{a}	0.02 (± 0.00)^{a}	0.00 (± 0.00)^{ac}	0.04 (± 0.00)^{c}	0.02 (± 0.00)^{a}	0.06 (± 0.01)^{b}	0.03 (± 0.00)^{a}	0.0002
Walking	0.09 (± 0.01)^{ac}	0.11 (± 0.02)^{a}	0.09 (± 0.01)^{ac}	0.12 (± 0.02)^{a}	0.10 (± 0.01)^{ac}	0.11 (± 0.02)^{a}	0.04 (± 0.00)^{b}	0.05 (± 0.01)^{bc}	0.04 (± 0.00)^{b}	0.04 (± 0.00)^{b}	< 0.0001
Active[‡]	0.55 (± 0.03)^{a}	0.56 (± 0.05)^{ac}	0.60 (± 0.04)^{a}	0.52 (± 0.04)^{ac}	0.55 (± 0.04)^{a}	0.49 (± 0.04)^{ab}	0.38 (± 0.03)^{bc}	0.41 (± 0.04)^{ab}	0.34 (± 0.03)^{b}	0.38 (± 0.03)^{bc}	< 0.0001
Pain[§]	0.00 (± 0.00)^{c}	0.01 (± 0.00)^{ac}	0.02 (± 0.00)^{ab}	0.02 (± 0.00)^{ab}	0.02 (± 0.00)^{a}	0.01 (± 0.00)^{ac}	0.05 (± 0.00)^{bd}	0.02 (± 0.00)^{ab}	0.07 (± 0.01)^{d}	0.03 (± 0.0)^{ab}	< 0.0001

[1] Meloxicam + Buprenorphine + Maxilene® , castrated;
[2] Meloxicam + Buprenorphine + Maxilene® , uncastrated;
[3] Buprenorphine + Meloxicam, castrated;
[4] Buprenorphine + Meloxicam, uncastrated;
[5] Buprenorphine + Maxilene® , castrated;
[6] Buprenorphine + Maxilene® , uncastrated;
[7] Meloxicam + Maxilene® , castrated;
[8] Meloxicam + Maxilene® , uncastrated;
[9] Saline, castrated;
[10] Sham, uncastrated;
[†] Only significant behaviour variables are presented;
[‡] Active behaviours include: nosing, suckling, walking, chewing, playing, running;
[§] Pain behaviours include: stiffness, trembling, spasms, tail wagging and rump scratching;
[a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$.

Results

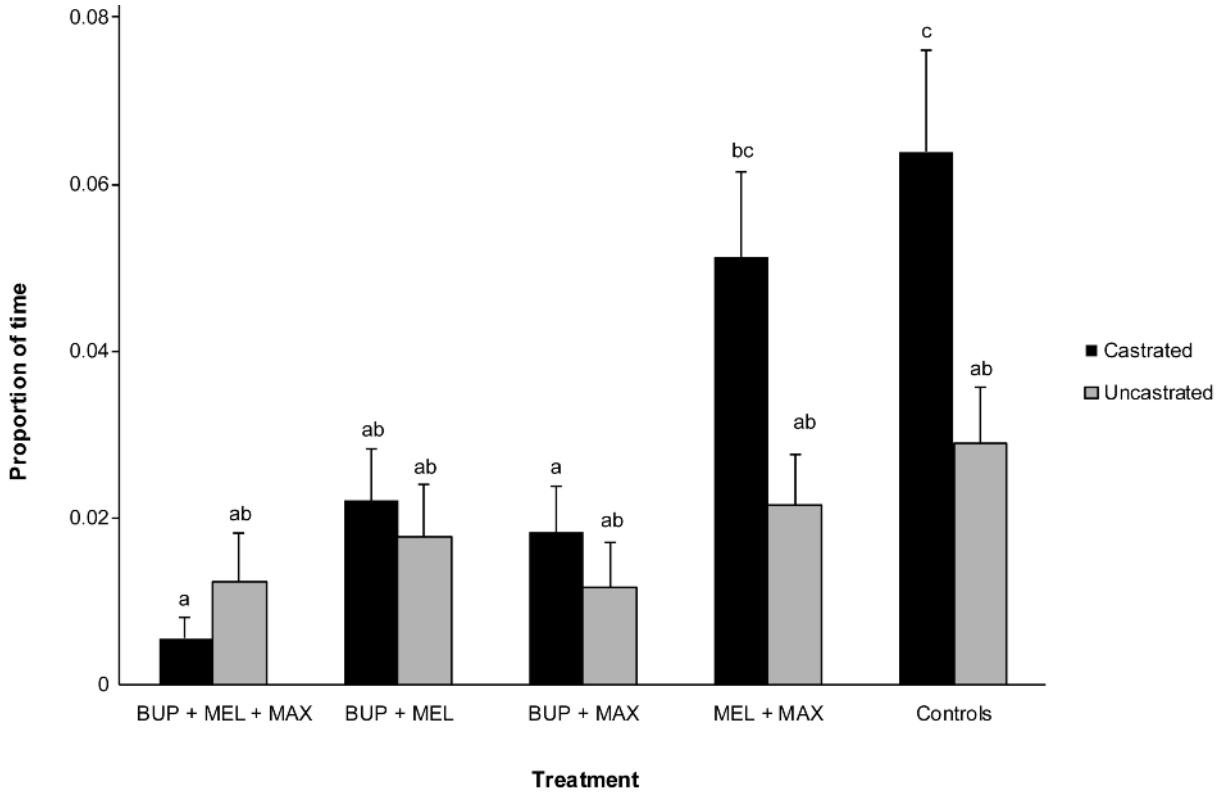
Behavioural observations

Comparison between analgesia-treated and control piglets

There were eight individual behaviours and two grouped behaviours (active and pain) significantly affected by treatment across the whole observation period: awake inactive ($P < 0.0001$), lying ($P < 0.0001$), nosing ($P < 0.0001$), nosing udder ($P < 0.0001$), sleeping ($P = 0.0015$), standing ($P < 0.0001$), tail wagging ($P = 0.0002$), walking ($P < 0.0001$), active ($P < 0.0001$), and pain ($P < 0.0001$) (Table 3). Saline-castrated piglets wagged their tails significantly more than all other treatment groups ($P < 0.05$). MEL + MAX-castrated piglets also wagged their tails significantly more than all treatment groups, except MEL + BUP + MAX-uncastrated, BUP + MAX-uncastrated, and saline-castrated piglets ($P < 0.05$). Saline-castrated piglets displayed significantly more pain behaviours than MEL + BUP + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated piglets ($P < 0.0001$) (Figure 1).

There was no significant difference in pain behaviour between MEL + MAX-castrated and saline-castrated piglets ($P = 0.1269$). Saline-castrated piglets spent significantly more time lying and less time walking, standing, and engaged in fewer active behaviours than piglets in the MEL + BUP + MAX (castrated and uncastrated), MEL + BUP (castrated and uncastrated), and BUP + MAX (castrated and uncastrated) treatment groups ($P < 0.05$). MEL + MAX-castrated piglets spent significantly more time lying and less time standing than MEL + BUP + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated piglets ($P < 0.05$). Saline-castrated and sham piglets spent significantly less time awake inactive than piglets in the MEL + BUP + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated treatment groups ($P < 0.01$). MEL + MAX-castrated, saline-castrated, and sham piglets spent significantly less time nosing than MEL + BUP + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated piglets ($P < 0.01$). Sham piglets spent significantly more time nosing the udder than MEL + BUP + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated piglets ($P < 0.05$). Saline-castrated and sham piglets spent significantly more time sleeping than MEL + BUP + MAX-castrated piglets ($P < 0.05$). There were no significant behavioural differences between any of the treatment groups pre-castration ($P > 0.05$).

Figure 1



Mean (± SEM) proportion of time piglets demonstrated pain-related behaviours (trembling, stiffness, spasms, tail wagging and rump scratching) in each treatment group (n = 15 piglets per treatment group). MEL = 0.4 mg kg^{-1} meloxicam, BUP = 0.04 mg kg^{-1} buprenorphine, and MAX = Maxilene® . Control groups include saline-castrated and sham-uncastrated piglets. Individuals (n = 2) were unaware of piglet treatment, litter, and time-point when scoring. Different superscripts indicate significant differences between treatments ($P < 0.05$).

Comparison between buprenorphine-treated, non-buprenorphine-treated and control piglets

After analysing the effect of each treatment combination with buprenorphine on behaviour and identifying no significant treatment-related effects, data were collapsed into two groups: BUP-castrated and BUP-uncastrated piglets to facilitate analysis of time × treatment interactions. The comparison focus was between BUP-castrated, BUP-uncastrated, MEL + MAX-castrated, and saline-castrated piglets. There were significant time × treatment differences found for awake inactive ($P < 0.0001$), lying ($P < 0.0001$), nosing ($P < 0.0001$), nosing udder ($P = 0.0133$), sleeping ($P = 0.0061$), standing ($P < 0.0001$), tail wagging ($P = 0.0197$), walking ($P < 0.0001$), active ($P < 0.0001$), and pain ($P < 0.0001$) (Table 4). At 0 h post-castration, BUP-castrated piglets spent significantly less time lying and more time awake inactive, standing, and engaged in active behaviours than BUP-uncastrated piglets at 4 to 7 h, MEL + MAX-castrated piglets at 0, 3, 4, 6, and 7 h, and saline-castrated piglets from 0 to 5 h ($P < 0.05$). BUP-castrated piglets also spent significantly more time nosing at 0 h than MEL + MAX-castrated and saline-castrated piglets at the same time-point and walked significantly more than MEL + MAX-castrated piglets at 3 h ($P < 0.05$). Activity level of BUP-castrated piglets did not decrease 1 h post-castration, with piglets spending significantly less time lying and more time awake inactive, standing, and engaged in more active behaviours than BUP-uncastrated piglets at 6 h, MEL + MAX-castrated piglets at 3, 5, and 6 h, and saline-castrated piglets at 1, 3 and 5 h ($P < 0.05$). BUP-castrated piglets also spent significantly less time sleeping at 1 h than BUP-uncastrated piglets at 4 h post-procedure ($P < 0.05$). At 5 h post-castration, BUP-castrated piglets spent significantly less time nosing the udder than MEL + MAX-castrated piglets at 1 and 4 h ($P < 0.05$). At 24 h post-castration, MEL + MAX-castrated piglets demonstrated significantly more pain behaviours than BUP-castrated piglets at 6, 7, and 24 h, BUP-uncastrated piglets at 24 h, and saline-castrated piglets at 0, 1 and 5 h. At 24 h post-castration, saline-castrated piglets also displayed significantly more pain behaviours than BUP-castrated piglets at 0, 3, 6, 7, and 24 h, BUP-uncastrated piglets at 6 and 24 h and MEL + MAX-castrated piglets from 0 to 7 h ($P < 0.0001$) (Figure 2). Across all time × treatment interactions, there were no significant differences in any behavioural variable at the same time-point between MEL + MAX-castrated and saline-castrated piglets ($P > 0.05$).

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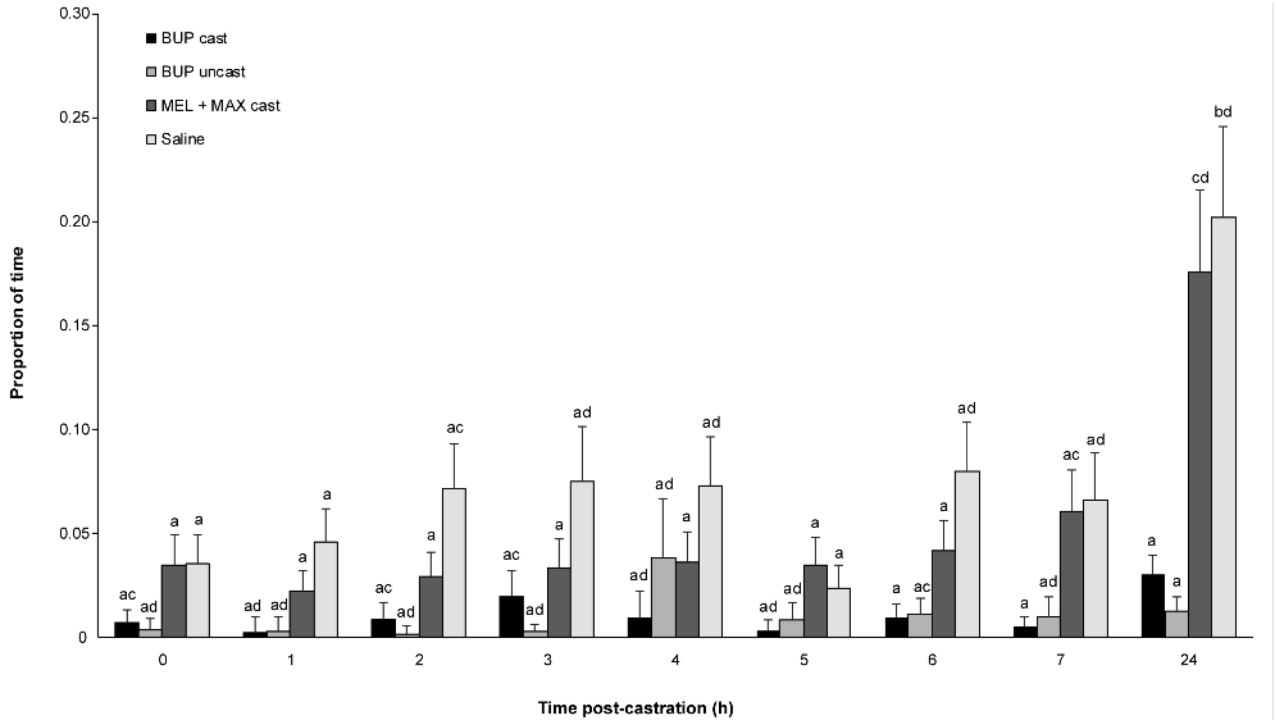
Table 4 Proportion of time piglets were engaged in specific behaviours (n = 150 total) pre- and post-treatment across all litters and time-points. Values represent the proportional means (± SEM).

Behaviour[1]	Pre-castration		Post-castration						
	Treatment *P*-value	Pre-treatment[4]	Treatment *P*-value	Time *P*-value	Time × Treatment *P*-value	BUP castrated[5]	BUP uncastrated[5]	MEL + MAX castrated[6]	Saline[6]
Awake inactive	0.2475	0.58 (± 0.09)	< 0.0001	< 0.0001	< 0.0001	0.62 (± 0.02)^{a}	0.58 (± 0.03)^{ab}	0.51 (± 0.03)^{b}	0.48 (± 0.03)^{b}
Lying	0.8864	0.52 (± 0.05)	< 0.0001	< 0.0001	< 0.0001	0.46 (± 0.02)^{a}	0.50 (± 0.03)^{a}	0.62 (± 0.03)^{b}	0.66 (± 0.03)^{b}
Nosing	0.0833	0.07 (± 0.01)	< 0.0001	< 0.0001	< 0.0001	0.11 (± 0.02)^{a}	0.09 (± 0.02)^{ab}	0.05 (± 0.01)^{b}	0.05 (± 0.01)^{b}
Nosing udder	0.1324	0.25 (± 0.04)	< 0.0001	0.0472	0.0133	0.22 (± 0.03)^{a}	0.23 (± 0.04)^{ab}	0.30 (± 0.04)^{b}	0.27 (± 0.04)^{ab}
Sleeping	0.3436	0.48 (± 0.10)	0.0001	< 0.0001	0.0061	0.45 (± 0.04)^{a}	0.40 (± 0.06)^{a}	0.55 (± 0.04)^{b}	0.59 (± 0.04)^{b}
Standing	0.8515	0.46 (± 0.05)	< 0.0001	< 0.0001	< 0.0001	0.55 (± 0.02)^{a}	0.51 (± 0.02)^{a}	0.36 (± 0.03)^{b}	0.32 (± 0.03)^{b}
Tail wagging	0.2967	0.04 (± 0.00)	< 0.0001	0.0014	0.0197	0.00 (± 0.00)^{a}	0.00 (± 0.00)^{ab}	0.04 (± 0.00)^{a}	0.06 (± 0.01)^{b}
Walking	0.1126	0.07 (± 0.02)	< 0.0001	< 0.0001	< 0.0001	0.10 (± 0.01)^{a}	0.12 (± 0.02)^{a}	0.04 (± 0.00)^{b}	0.04 (± 0.00)^{b}
Active[2]	0.8349	0.48 (± 0.05)	< 0.0001	< 0.0001	< 0.0001	0.57 (± 0.03)^{a}	0.53 (± 0.03)^{a}	0.38 (± 0.03)^{b}	0.33 (± 0.03)^{b}
Pain[3]	0.2414	0.04 (± 0.00)	< 0.0001	< 0.0001	< 0.0001	0.00 (± 0.00)^{a}	0.00 (± 0.00)^{a}	0.04 (± 0.0)^{b}	0.06 (± 0.01)^{c}

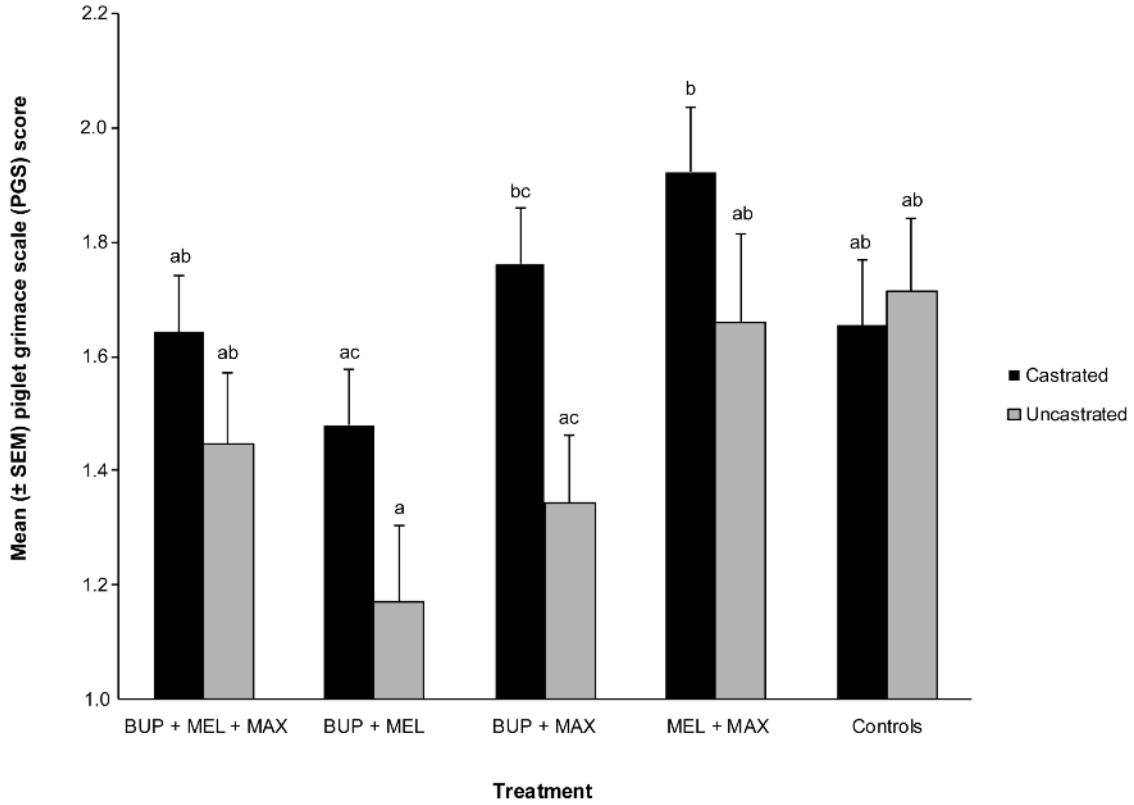
[1] Only significant behaviour variables are presented;
[2] Active behaviours include: nosing, suckling, walking, chewing, playing, running;
[3] Pain behaviours include: stiffness, trembling, spasms, tail wagging and rump scratching;
[4] n = 150 piglets included in pre-treatment group;
[5] n = 45 piglets included in treatment group;
[6] n = 15 piglets included in treatment group;
[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

Figure 2



Mean (± SEM) proportion of time piglets demonstrated pain-related behaviours (trembling, stiffness, spasms, tail wagging and rump scratching) within each treatment group and time-point. MEL = 0.4 mg kg^{-1} meloxicam, BUP = 0.04 mg kg^{-1} buprenorphine, and MAX = Maxilene® . BUP cast represent all the piglets administered buprenorphine in their treatment regimes (MEL + BUP + MAX, MEL + BUP, and BUP + MAX) and castrated (n = 45 piglets in BUP cast group). BUP uncast represent all the piglets administered buprenorphine in their treatment regimes and uncastrated (n = 45 piglets in BUP uncast group). MEL + MAX, cast and saline treatment group each included 15 piglets. Individuals (n = 2) were unaware of piglet treatment, litter, and time-point when scoring. Different superscripts indicate significant differences between treatment groups within a time-point ($P < 0.05$).

Figure 3



Mean (± SEM) piglet grimace scale (PGS) scores in each treatment group (n = 15 piglets per treatment group). Higher PGS scores indicate increased pain expression. MEL = 0.4 mg kg^{-1} meloxicam, BUP = 0.04 mg kg^{-1} buprenorphine, and MAX = Maxilene® . The control groups were saline-castrated and sham-uncastrated. Individuals (n = 4) were unaware of piglet treatment, litter, and time-point when scoring images. Different superscripts indicate significant differences between treatments ($P < 0.05$).

Piglet grimace scale

Comparison between analgesia-treated and control piglets

There was a significant treatment effect on PGS score ($P = 0.0013$) (Figure 3). Piglets in the MEL + MAX-castrated group grimaced significantly more than MEL + BUP-castrated, MEL + BUP-uncastrated, and BUP + MAX-uncastrated piglets ($P = 0.0394$, 0.0011 and 0.0184, respectively). BUP + MAX-castrated piglets also grimaced significantly more than MEL + BUP-uncastrated piglets ($P = 0.0150$).

Comparison between buprenorphine-treated, non-buprenorphine-treated and control piglets

Collapsing data into BUP-castrated and BUP-uncastrated groups resulted in a significant effect of treatment on PGS score ($P = 0.0029$), with MEL + MAX-castrated piglets having significantly higher grimace scores than BUP-uncastrated piglets (1.9 vs 1.3, respectively; $P = 0.0010$).

Vocalisation

Castration (pulling and tearing the spermatic cord) resulted in piglet vocalisations significantly higher in frequency, amplitude, energy, and power compared to all other procedures measured ($P < 0.01$). Marking piglets resulted in vocalisations that were significantly lower in frequency, amplitude, energy, and power compared to the intramuscular injection and scrotal incision ($P < 0.001$).

Piglets in the sham group emitted vocalisations significantly lower in frequency than MEL + BUP + MAX-uncastrated, MEL + BUP (castrated and uncastrated), and MEL + MAX (castrated and uncastrated) piglets ($P < 0.05$) (Figure 4). Sham piglets also produced vocalisations significantly lower in power than all treatment groups, except BUP + MAX-uncastrated ($P < 0.05$). None of the analgesic combinations reduced piglet vocalisations at the time of castration; all castrated piglets produced vocalisations similar in frequency, amplitude, energy, and power.

Discussion

This study examined a multimodal approach for mitigating surgical castration pain in piglets. Buprenorphine treatment (ie, MEL + BUP + MAX, MEL + BUP, BUP + MAX) resulted in significantly reduced pain behaviours in piglets up to 24 h post-castration. Buprenorphine has proven efficacy in alleviating pain in piglets and growing swine without causing any adverse effects (Hermansen *et al* 1986; Rodriguez *et al* 2001; Meijer *et al* 2015; Viscardi & Turner 2018b). Results from the treatment control groups (ie, piglets administered drugs and not castrated) also

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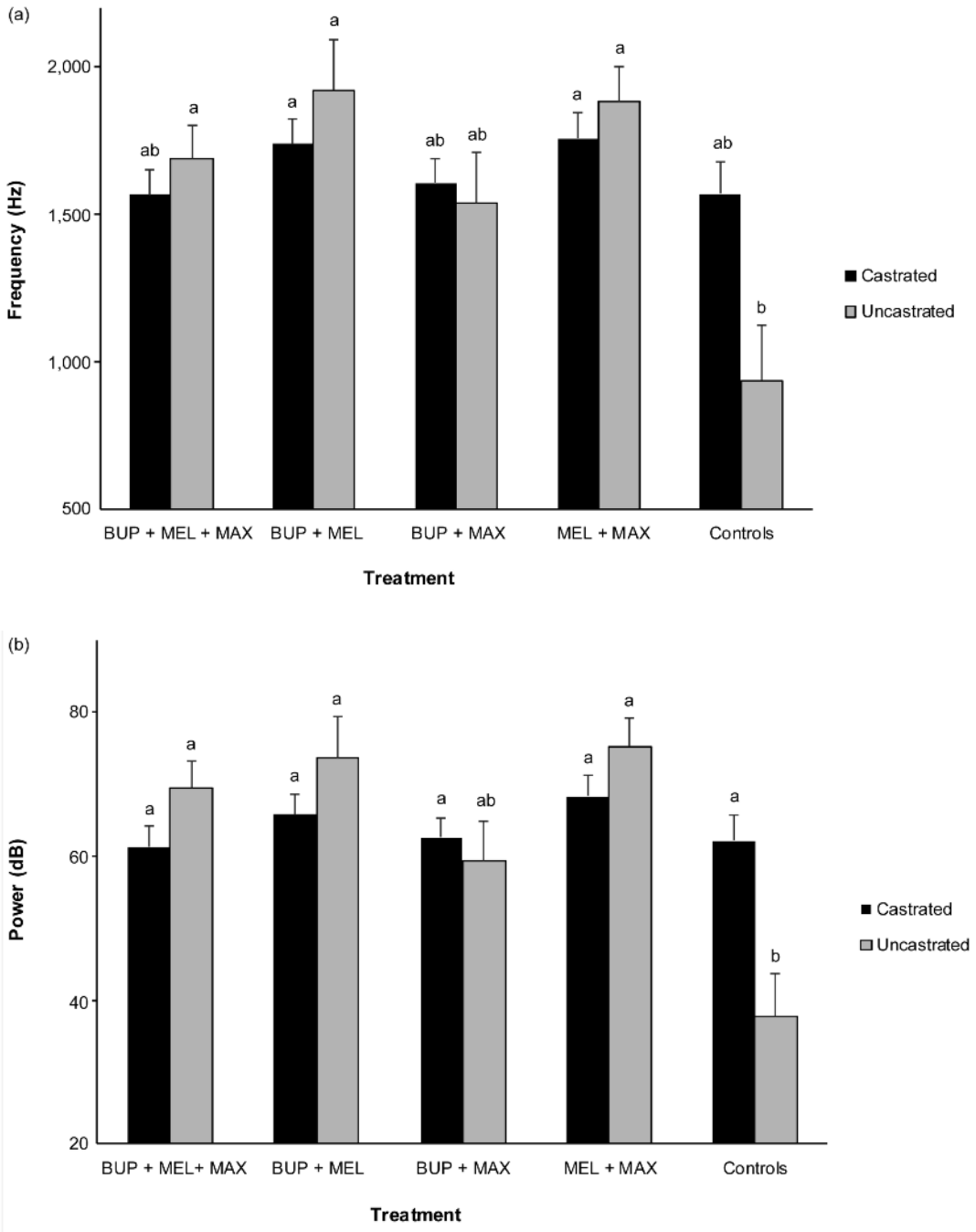
Figure 4



Mean (± SEM) vocalisation (a) frequency (Hz) and (b) power (dB) of piglets across all procedures in each treatment group (n = 15 piglets per treatment group). MEL = 0.4 mg kg^{-1} meloxicam, BUP = 0.04 mg kg^{-1} buprenorphine, and MAX = Maxilene® . The control groups were saline-castrated and sham-uncastrated. Individuals (n = 2) scoring data were unaware of piglet treatment, litter, and procedure when analysing vocalisation measurements. Different superscripts indicate significant differences between treatments ($P < 0.05$).

confirmed there were no behavioural side-effects associated with providing a single dose of buprenorphine, meloxicam, and Maxilene® to piglets; however, the addition of meloxicam and Maxilene® did not appear to provide any significant benefit to the pigs. Saline-castrated piglets were significantly less active than most other treatment groups. Animals often show a decrease in general activity level when in pain (Berger & Eeg 2006). No reduction in activity of MEL + BUP + MAX-, MEL + BUP-, and BUP + MAX-castrated piglets was observed, further supporting the analgesic efficacy of these drug combinations. The addition of buprenorphine itself may have also caused a reduction in inactive behaviour, as has been noted in other species (Wright-Williams *et al* 2013). Saline-castrated piglets also wagged their tails significantly more than all other treatment groups. An increase in tail

wagging has been observed in piglets, lambs, and calves after castration or dehorning when analgesia is lacking or inadequate (Robertson *et al* 1994; Graf & Senn 1999; Hay *et al* 2003; Rault & Lay 2014; Jongman *et al* 2016; Viscardi *et al* 2017; Viscardi & Turner 2018a,b). Thus, tail wagging may be a useful and specific indicator of piglet pain for future studies or assessments.

At 24 h post-castration, saline and MEL + MAX-castrated piglets demonstrated significantly more pain behaviours than all other treatment groups. Acute progression of the post-surgical inflammatory process may have caused this increase in pain (Kumar *et al* 2015). Previous research has indicated that meloxicam alone was insufficient in providing piglets post-castration pain relief (Kluivers-Poodt *et al* 2012; Viscardi & Turner 2018a). The addition of topical Maxilene® to meloxicam did not reduce the pain behaviours displayed. A more invasive application of lidocaine (the active ingredient in Maxilene®) via intra-testicular injection with meloxicam IM has been shown to effectively reduce castration pain in piglets (Hansson *et al* 2011); however, this route of administration may be painful and requires specialised technique, limiting its on-farm practicality (Leidig *et al* 2009). MEL + BUP + MAX-, MEL + BUP-, and BUP + MAX-castrated piglets were expected to display more pain behaviours at 24 h post-castration, as the maximum duration of action of buprenorphine in swine is thought to be 12 h (Thiede *et al* 2014). This was not observed, suggesting that a single dose of buprenorphine may provide sufficient post-operative analgesia for piglets undergoing castration. Future work should assess piglet pain beyond 24 h post-castration to determine whether pain recurs outside of this period of assessment.

Facial grimace analysis is used to assess pain in animals, such as mice and rabbits, and non-verbal humans (Langford *et al* 2010; Herr *et al* 2011; Keating *et al* 2012). A piglet grimace scale developed by Viscardi *et al* (2017) evaluates changes in ear position, cheek tightening/nose bulge, and orbital tightening to assess piglet facial expressions of pain. These facial expressions correspond well to observed pain behaviours when experienced scorers were used (Viscardi & Turner 2018b). In this study, there was no strong correspondence between displayed pain behaviours and facial grimacing in piglets. PGS scoring did not detect any significance in facial grimacing between saline-castrated piglets and piglets in any other treatment group. When trained observers conduct pain assessments, behavioural analysis appears to be a more sensitive tool. Modifications to the PGS training session may be needed to improve individual scoring success.

Piglets undergoing surgical castration emit distinct vocalisations associated with procedural pain (Marx *et al* 2003; Leidig *et al* 2009). None of the drug combinations studied reduced the frequency, amplitude, power or energy of these vocalisations at the time of castration. Tearing of the spermatic cord is thought to be the most painful aspect of the castration procedure, eliciting the strongest vocal response from piglets (Leidig *et al* 2009). This suggests that piglets may need to be anaesthetised (locally or generally) to eliminate vocalisations and fully mitigate pain associated with castration (Sutherland *et al* 2012). Piglets administered Maxilene® were expected to vocalise less at incision, but this was not observed. Maxilene® is recommended for use with a 30-min application time (Eichenfield *et al* 2002) but was administered only 20 min prior to surgical castration in this study. Therefore, it is possible that the full topical anaesthesia potential of Maxilene® was not reached. As expected, the castration procedure caused the greatest vocal response from piglets compared to all other procedures measured. The IM injection also elicited a strong vocal response, suggesting it caused acute pain. Two of the four drug combinations evaluated in this study required two injections (meloxicam and buprenorphine). As buprenorphine provided sufficient pain relief when administered with Maxilene®, the acute pain caused by the second injection of meloxicam was deemed to be unnecessary. This also eliminates the associated cost of a second drug and reduces piglet stress caused by an increase in handling time to administer multiple injections. Future work assessing a multimodal approach to alleviating piglet castration pain should focus on relieving the immediate pain of castration, as sufficient post-operative pain relief may be provided by buprenorphine alone.

In veterinary clinical practice, it is common for dogs and cats undergoing ovariohysterectomy or castration to receive multimodal analgesia to alleviate peri-operative pain (Hewson *et al* 2006). This approach is difficult to replicate in a farm setting, primarily because of the cost, time, effort, and equipment required for each surgery (Rault *et al* 2011). This study found minimal benefit to providing piglets meloxicam and Maxilene® pre-castration. While buprenorphine was determined to be most effective at alleviating post-operative castration pain in piglets, it is the least practical drug to use on-farm. Currently, buprenorphine is a controlled substance that can only be administered by a veterinarian and it is not licensed for use in pigs or other food-producing animals by the US Food and Drug Administration (FDA 2014). However, buprenorphine is a highly effective option for piglet pain management and measures to make it practical for use on-farm could be explored further.

In conclusion, buprenorphine was highly effective at reducing pain behaviours after surgical castration in piglets. A multimodal approach with meloxicam and Maxilene® did not provide significant pain-relieving benefits to the piglet to justify the added cost and time required for their administration. None of the analgesia-treatment groups reduced vocalisations that occurred at the time of castration. The PGS may be used to compliment pain scoring in piglets but should be used in combination with other pain-specific behavioural assessments until the tool is optimised.

https://doi.org/10.7120/09627286.28.4.487 Published online by Cambridge University Press

Animal welfare implications

Surgical castration is a painful procedure for piglets to undergo. Analgesia administration is required in animal care guidelines for countries in the EU and Canada for post-operative pain relief. Identifying an analgesic drug (or drug regimen) that is most effective at mitigating post-surgical pain is important for appropriate recommendations to be made to pig producers. This study has increased our knowledge on pain-relieving strategies in the swine industry, most notably, that a more potent drug class than NSAIDs (eg opioids) is needed to alleviate piglet post-surgical castration pain. This is important to improve piglet welfare on-farm, a topic of increasing societal concern.

Acknowledgements

The authors wish to thank Stephanie Cervi, Anna Maystrenko, Tim Thalen, and personnel at the Arkell Swine Research Station for technical assistance. Funding was provided by Ontario Pork (052367), National Pork (052457), and the Ontario Ministry of Agriculture, Food and Rural Affairs (027295). AVV received a doctoral scholarship from the Ontario Veterinary College as well as an Ontario Graduate Scholarship.

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https://doi.org/10.7120/09627286.28.4.487 Published online by Cambridge University Press