Animal Welfare 2020, 29: 285-292
ISSN 0962-7286
doi: 10.7120/09627286.29.3.285

A comparison of behavioural methodologies utilised to quantify deviations in piglet behaviour associated with castration

RM Park[‡], MC Cramer[§], BK Wagner[‡], P Turner[#¥], LE Moraes[¶], AV Viscardi[€], JF Coetzee[€] and MD Pairis-Garcia*[†‡]

[†] 1060 William Moore Dr, Raleigh, NC 27607, USA
[‡] Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, USA
[§] Department of Animal Sciences, College of Agricultural Sciences, Colorado State University, Fort Collins, CO 80521, USA
[#] Department of Pathobiology, University of Guelph, ON, Canada N1G 2W11
[¥] Charles River, Wilmington, MA 01887, USA
[¶] Department of Animal Sciences, College of Food, Agriculture and Environmental Sciences, The Ohio State University, Columbus, OH 43210, USA
[€] Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA
* Contact for correspondence: pairis-garcia@ncsu.edu

Abstract

*Surgical castration is a painful procedure that is routinely performed without pain relief on commercial pig (*Sus scrofa domesticus*) farms. Previous research has focused on quantifying piglet pain response through behaviours. However, to date, behavioural sampling methodologies used to quantify pain associated with castration have not been validated. Therefore, the objective of this study was to validate scan sampling methodologies (2-min, 3-min, 5-min, 10-min and 15-min intervals) to quantify piglet pain responses expressed by castrated piglets' behaviour. A total of 39 Yorkshire-Landrace × Duroc male piglets (five days of age) were surgically castrated using a scalpel blade. Behaviour frequency and duration (scratching, spasms, stiffness, tail wagging and trembling) of each piglet were continuously collected for the first 15 min of the following hours relative to castration (–24, 1–8 and 24). To determine if the sampling interval accurately reflected true duration and frequency for each behaviour, as determined by continuous observation, criteria previously utilised from other behavioural validation studies were used: coefficient of determination above 0.9, slope not statistically different from one and intercept not statistically different from zero. No scan sampling interval provided accurate estimates for any behavioural indicators of pain. The results of this study suggest that continuous sampling is the most appropriate methodology to fully capture behaviour specific to pain associated with castration. Using validated behavioural methodologies in future research can assist in the development of objective, science-based protocols for managing pig pain.*

Keywords: *animal welfare, behaviour, castration, pain, swine, validation*

Introduction

Pain is a clinically important condition that adversely affects animal welfare (Mellor 2016; Yeates 2016). Animals in pain experience a negative mental state and, when left uncontrolled, pain can result in deleterious consequences to the animal's physical health and productivity (Hellebrekers 2000; Telles *et al* 2016). Veterinarians in the United States have an ethical obligation and must take an oath to eliminate or alleviate pain when necessary, including pain as a result of standard husbandry practices, such as castration.

Physical castration is a painful procedure commonly performed on commercial swine farms in North America during the first week of life (Dzikamunhenga *et al* 2014; O'Connor *et al* 2014). From a behavioural standpoint, castration has been scientifically quantified by either observing deviations in the pig's (*Sus scrofa domesticus*) normal behavioural repertoire (eg lying, locomotion, nursing; Weary *et al* 2006) or identifying an increased frequency or expression of behavioural indicators of pain specific to castration (eg prostrated, scratching, trembling; Hay *et al* 2003). Behaviour has been used extensively in the literature to quantify pain response and determine efficacy of pain mitigation strategies (ie pharmaceutical intervention). In fact, literature evaluating behaviours induced by pain associated with castration have supported the development of standards and recommendations for castration pain management on an international scale (Primary Industries Standing Committee 2008; European Commission 2010;

Table 1 Behavioural sampling techniques for castration pain studies utilising one or more of the pain-associated behaviours defined in the present study ethogram.

Literature cited, first author (year)	Sampling interval (min)	Days observed (post-castration)	Approximate h observed per day
Scan sampling			
Burkemper (2019)	5	3	5
Davis (2017)	60	1	4
Gottardo (2016)	1	1	1
Hansson (2011)	10	1	1
Hay (2003)	10	5	3
Kluivers-Poodt (2013)	12	5	6
McGlone (2016)	15	1	2
Llamas Moya (2008)	3	4	4
Rault (2011)	5	3	4
Sutherland (2010)	1	1	3
Sutherland (2012)	1	1	1
Sutherland (2017)	1	1	3
Continuous sampling			
Hay (2003)	–	4	6
Hug (2018)	–	1	0.17
Van Birendonck (2011)	–	8	0.33
Viscardi (2017, 2018a,b, 2019)	–	1	2
Yun (2019)	–	2	0.5

American Veterinary Medical Association 2013; National Farm Animal Care Council 2014; National Pork Board 2018; New Zealand Government 2018). However, behavioural methodologies used to quantify piglet pain responses to castration vary dramatically (Table 1). This is particularly noteworthy when evaluating previous studies that have used the same behaviours to quantify pain associated with castration as in the present study. In addition, no studies to date have validated the accuracy of behavioural methodologies used in previous work.

When assessing animal behaviour, scientists select methods based on a variety of factors, including, but not limited to, sample size, behaviour of interest and total observation time (Hepworth & Hamilton 2001; Lendvai *et al* 2015). The continuous sampling methodology is considered the most accurate for behaviour data collection as the individual animal is observed for the entirety of the observation period (Altmann 1974; Czerwinski *et al* 2017). This results in a complete data set that includes total frequency and duration for each behaviour of interest (Lehner 1992). However, time and labour constraints limit the use of this methodology, particularly in studies with large populations. To mitigate this, scan sampling, a methodology in which behaviours are recorded at selected time-points, allows scientists to increase throughput and minimise labour requirements (Martin & Bateson 1993). However, if these alternatives are to be used to quantify behavioural indicators of pain associated with castration and support on-farm recommendations, validating the accuracy of each methodology is critical. Therefore, the objective of this study was to validate behavioural methodologies using five different scan sampling intervals (ie 2-min, 3-min, 5-min, 10-min, and 15-min) compared to continuous sampling in castrated piglets.

Materials and methods

Data for the present study were derived from a portion of a larger data set (Viscardi & Turner 2018a,b, 2019) and behavioural data were not collected for the primary purposes of addressing the objectives of the present study. All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). This institution is registered under the Animals for Research Act of Ontario (1990) and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

Study animals and housing

A total of 39 Yorkshire-Landrace × Duroc male piglets from 32 litters (aged five days old and weighing a mean [± SEM] of 2.20 [± 0.38] kg and with an average litter size of 13) were used in this study across three separate trials. Piglet number was based on exclusion criteria that disqualified data from any piglet that had been provided with an analgesic drug intervention. All piglets, regardless of weight, were included in the original study and no cross-fostering was implemented. Sow breed was selected since it represented the most common genetic profile used commercially in Canada and the United States and piglet age was selected for five days as it also represented typical commercial production standards.

Sows and piglets were housed in farrowing rooms at the University of Guelph Arkell Swine Research Station. Within each room, sows were housed in farrowing pens (floor space: 1.8 × 2.4 m [length × width]; farrowing crate: 0.8 × 2.3 m). Farrowing crates were utilised as these are the most commonly available systems used in Canada and the US. Light was provided daily between the hours of 0700 and 2100h. Creep areas for piglets were heated to approximately 30–35ºC using a heating pad or lamp. Sows had *ad libitum* access to feed (their diet met or exceeded National Research Council [NRC 2012] nutrient requirements for lactating sows) beginning four days after farrowing and *ad libitum* access to one water nipple.

Processing procedure

Twenty-four hours prior to each trial commencement, piglets were weighed and individually identified using a black permanent marker on the head and leg. This was carried out to ensure piglets could be identified on camera regardless of body orientation. On the day of castration, male piglets within a pen were separated from their littermates and placed into a transport cart. Piglets were surgically castrated by trained personnel using a 'two vertical incision' approach on the scrotum. Piglets were placed on the bent leg of the castrator, facing backward, and restrained to ensure minimal movement while making the scrotal incisions. A 1–1.5-inch incision was made over each testicle. The spermatic cord was torn to remove the testicles from the body (no spermatic cord was visible after castration). All castrations occurred between 0800–1000h on the same day. Once completed, all piglets were returned to their pen.

Behavioural measurements

Behavioural collection was conducted as described by Viscardi *et al* (2017), Viscardi and Turner (2018a,b, 2019). Video was recorded pre-procedure for 1 h using a high definition video camera (JVC GZ-E200 full HD Everio Camcorder, Yokohama, Japan) mounted on a tripod outside the farrowing pens. The positioning of the camera was unable to provide complete coverage of the entire pen for the trial. On average, 10% of the time, behaviours were unable to be recorded due to piglets being out of view. Immediately post-castration, piglets were recorded continuously for 8 h, and again for 1 h, 24 h post-procedure. Behavioural pain responses for each piglet were scored continuously for the first 15 min of every hour, utilising an ethogram adapted from Hay *et al* (2003) (Table 2). A total of 150 min of behaviour was analysed for each piglet.

All continuous behaviour data were collected by six trained observers using the Observer XT programme (Version 12.0, Noldus Information Technology, Wageningen, The Netherlands). Videos were randomised and assigned to observers who were masked to time-point. Inter-observer reliability was assessed at three time-points during the behaviour-scoring period in which all participants scored the same piglet in a video and an intra-class correlation coefficient (ICC) was calculated. All inter-observer reliability tests throughout the trial produced an ICC above 0.9, indicating excellent agreement between scorers and no significant drift throughout the scoring period.

Sampling methods

Utilising the continuous sampling data set, video data were divided into 1-min intervals. These intervals were used to extrapolate five scan sampling data sets (ie 2-min, 3-min, 5-min, 10-min, 15-min). For the continuous sampling method, the data were first collected by watching the piglets continuously, then, the summation of counts and the average proportion of time spent performing each behaviour were calculated. The 2-min scan used every second 1-min interval to calculate the total occurrence and average proportion of time that the behaviour was performed. The 3-min, 5-min, 10-min and 15-min scan used every third, fifth, tenth and fifteenth 1-min interval, respectively. For each methodology, all sampling began at the 0-min interval.

Table 2 Castration pain ethogram.

Behaviour	Definition
Scratching	Rubbing the rump against the floor, pen walls or littermates
Spasms	Quick and involuntary contractions of the muscle
Stiffness	Lying with extended and tensed legs
Tail wagging	Tail movement from side-to-side (or up and down)
Trembling	Shivering, as with cold

Adapted from Viscardi *et al* (2017), Viscardi and Turner (2018a,b, 2019) and Hay *et al* (2003).

Table 3 R^2, slope *P*-value and intercept *P*-value for behavioural indicators of pain durations at each scan sampling interval*.

Behaviour	2-min	3-min	5-min	10-min	15-min
Scratching					
R^2	0.87	0.58	0.52	0.41	0.15
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.10	< 0.01	< 0.01	< 0.01	< 0.01
Spasm					
R^2	0.78	0.46	0.35	0.12	0.10
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.02	< 0.01	< 0.01	< 0.01	< 0.01
Stiffness					
R^2	0.72	0.67	0.53	0.38	0.29
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.02	< 0.01	< 0.01	< 0.01	< 0.01
Tail wagging					
R^2	0.91	0.80	0.67	0.49	0.32
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Trembling					
R^2	0.91	0.48	0.62	0.16	0.46
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.09	< 0.01	< 0.01	< 0.01	< 0.01

* The sampling intervals were considered accurate if they met three criteria: $R^2 \geq 0.9$, slope was not different from one ($P > 0.05$), and intercept was not different from zero ($P > 0.05$; Chen *et al* 2016).

Table 4 R^2, slope *P*-value and intercept *P*-value for behavioural indicators of pain bouts at each scan sampling interval*.

Behaviour	2-min	3-min	5-min	10-min	15-min
Scratching					
R^2	0.84	0.68	0.53	0.24	0.14
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Spasm					
R^2	0.82	0.39	0.54	0.24	0.11
Slope	0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.01	< 0.01	< 0.01	< 0.01	< 0.01
Stiffness					
R^2	0.83	0.69	0.69	0.38	0.25
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.04	< 0.01	0.04	< 0.01	< 0.01
Tail wagging					
R^2	0.88	0.78	0.63	0.46	0.30
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Trembling					
R^2	0.94	0.80	0.69	0.28	0.34
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.04	< 0.01	0.08	< 0.01	< 0.01

* The sampling intervals were considered accurate if they met three criteria: $R^2 \geq 0.9$, slope was not different from one ($P > 0.05$), and intercept was not different from zero ($P > 0.05$; Chen *et al* 2016).

Statistical analysis

Behavioural data were analysed using SAS software version 9.3 (v9.4, SAS Institute Inc, Cary, NC, USA). Linear regression (PROC REG) was used to conduct pair-wise comparisons between the true values and the five sample intervals for each behaviour (Chen *et al* 2016). Sampling intervals that met the following criteria were considered to accurately reflect true duration and frequency for each behaviour: i) the coefficient of determination (R^2) was greater than 0.9; ii) the slope did not significantly differ from one ($P > 0.05$); and iii) the intercept did not significantly differ from zero ($P > 0.05$; Chen *et al* 2016).

Results

No behavioural indicators of pain met all three criteria for any of the scan sampling methodologies investigated in the present study when compared with continuous sampling (Tables 3 and 4). For durations, behavioural indicators of pain including tail wagging and trembling achieved an R^2 above 0.90 for 2-min scan sampling interval. However, a slope not different from one was not achieved for either behaviour and an intercept not different from zero was only achieved for trembling. For bouts, trembling was the only behaviour that achieved an R^2 above 0.90 for 2-min scan sampling interval. However, neither a slope not different from one nor an intercept not different from zero was achieved. In addition to the assessment of accuracy, duration and bouts for each behaviour by methodology can be found in Table 5.

Discussion

Throughout the previous 20 years, research has focused on identifying alternative management practices to reduce or eliminate pain associated with castration in piglets (for a thorough review of this topic, see Sutherland 2015). Behaviour is a common metric used to quantify pain associated with castration (von Borell *et al* 2009) and has been utilised to support the development of pain management protocols implemented on an international scale (Viscardi & Turner 2018a). However, no prior studies have validated the accuracy of these behavioural sampling methodologies used to quantify pain associated with castration. Therefore, the objective of the present study was to validate the use of five scan sampling methodologies, compared with continuous sampling methodology, to assess behavioural indicators of pain in castrated piglets.

In this study, no scan sampling methodology was effective for quantifying piglet pain responses through behaviour specific to piglet castration, which is likely due to the nature of the behaviours being evaluated. Behavioural indicators of pain for pigs are categorised as behaviours that are typically short in duration with variable frequency (ie event behaviour; Roughan & Flecknell 2003; Tardin *et al* 2014; Nielsen *et al* 2019). Event behaviours are often missed when the observation period is limited and continuous sampling is not utilised (Lehner 1987; Pullin *et al* 2017b; Ross *et al* 2019; Studd *et al* 2019).

In this study, behavioural indicators of pain were brief in duration, averaging less than 5 s. Short duration behaviours are difficult to detect utilising a scan sampling methodology as the opportunity for behavioural observation is limited. This often results in the underestimation of behaviours, as was demonstrated by the present study. Previous validation studies in poultry (Daigle & Siegford 2014; Ross *et al* 2019), sheep (Pullin *et al* 2017a,b) and beef (Mitlohner *et al* 2001; Madruga *et al* 2017) demonstrated similar issues when evaluating short duration behaviours, such as preening, drinking and oral manipulation.

Table 5 Mean values (± SEM) of duration percentage and frequency for each behavioural indicator of pain performed by castrated piglets across the entire behavioural data collection period.

Behaviour	Continuous	2-min	3-min	5-min	10-min	15-min
Scratching						
Duration	0.16 (± 0.04)	0.16 (± 0.04)	0.14 (± 0.05)	0.13 (± 0.04)	0.15 (± 0.06)	0.09 (± 0.04)
Bouts	3.56 (± 0.83)	1.95 (± 0.46)	1.08 (± 0.30)	0.77 (± 0.23)	0.38 (± 0.15)	0.28 (± 0.12)
Spasms						
Duration	0.10 (± 0.02)	0.09 (± 0.02)	0.05 (± 0.02)	0.08 (± 0.02)	0.03 (± 0.02)	0.05 (± 0.02)
Bouts	3.13 (± 0.75)	1.44 (± 0.38)	0.62 (± 0.17)	0.67 (± 0.24)	0.23 (± 0.10)	0.15 (± 0.07)
Stiffness						
Duration	0.33 (± 0.08)	0.25 (± 0.06)	0.40 (± 0.10)	0.39 (± 0.10)	0.50 (± 0.15)	0.34 (± 0.11)
Bouts	4.77 (± 1.08)	2.21 (± 0.54)	1.64 (± 0.38)	1.51 (± 0.34)	0.85 (± 0.21)	0.72 (± 0.21)
Tail wagging						
Duration	3.77 (± 0.51)	3.64 (± 0.51)	4.13 (± 0.61)	3.76 (± 0.51)	4.63 (± 0.71)	3.41 (± 0.61)
Bouts	73.13 (± 5.85)	38.26 (± 3.23)	25.97 (± 2.29)	19.54 (± 1.83)	12.08 (± 1.33)	9.33 (± 1.12)
Trembling						
Duration	0.22 (± 0.07)	0.24 (± 0.08)	0.11 (± 0.05)	0.25 (± 0.09)	0.12 (± 0.08)	0.32 (± 0.13)
Bouts	4.49 (± 1.57)	2.90 (± 1.07)	1.03 (± 0.44)	1.05 (± 0.37)	0.28 (± 0.11)	0.64 (± 0.24)

In addition to the short duration of these behaviours, the piglets in the present study spent less than 5% of their total time budget expressing behavioural indicators of pain. These results are not unique to this study, as previous work has demonstrated small proportions of total time budgets dedicated to expressing pain (6%: Hansson *et al* 2011; 8, 6% Viscardi & Turner 2018a, 2019). Infrequent behaviour patterns, such as these, are difficult to accurately assess through scan sampling (Martin & Bateson 2012; Ross *et al* 2019). Thus, utilising this sampling methodology for short duration, low frequency behavioural patterns yielded poor accuracy and continuous sampling is, therefore, ideal.

In contrast, studies that evaluate state behaviours (ie behaviours which occur over a quantifiable time-period; Crews *et al* 2002; Malachowski & Dugger 2018) are more successful in utilising scan sampling methodologies as the behaviour duration and frequency are consistent enough to be observed periodically. For example, scan sampling methodology validations have been successful in swine, sheep, dairy and beef cattle when evaluating lying behaviour (swine: 15-min scan, Whalin *et al* 2016; sheep: 20-min scan, Pullin *et al* 2017a,b; dairy: 30-min scan, Chen *et al* 2016; beef: 15-min scan, Mitlohner *et al* 2001, and 30-min scan, Madruga *et al* 2017). This is also true for sitting and standing behaviours in mature swine (15-min scan, Whalin *et al* 2016).

In addition to the challenge of behavioural indicators of pain being short in duration and infrequent in occurrence, the present study also did not utilise a fully exhaustive ethogram. Two fundamental behavioural indicators of pain that were not evaluated include huddled up, defined as 'lying with at least three legs tucked under body' and prostrated, defined as 'awake, standing or sitting, motionless with head down, lower than shoulder level' (Hay *et al* 2003). These behaviours are commonly evaluated in piglet castration studies (Hay *et al* 2003; Llamas Moya *et al* 2008; Yun *et al* 2019) with 14 studies to date integrating huddled up and prostrate into the ethogram to assess pain associated with castration (Table 6). Future studies should not only include the additional behaviours of huddled up and prostrated but also include normal behaviours representative of the piglet's behavioural repertoire.

The present study also did not include typical behaviours present in a piglet's behavioural repertoire, such as nursing, activity and inactivity. Although these behaviours are less specific to pain, deviations in the frequency and duration of these behaviours are often associated with painful experiences (Kluviers-Poodt *et al* 2013; de Oliveira *et al* 2014; Whalin *et al* 2016). Additionally, maintenance behaviours are often longer in duration compared with behavioural indicators of pain (Rault & Lay *et al* 2011; Kluviers-Poodt *et al* 2013; McGlone *et al* 2016; Sutherland *et al* 2017; Viscardi *et al* 2017), potentially facilitating more accurate detection of these behaviours using scan sampling.

The results from this study suggest behavioural indicators of pain specific to pain associated with castration should be evaluated using a continuous sampling methodology. Future work validating behavioural methodologies specific to pain associated with castration should utilise a more comprehensive ethogram and the authors suggest the inclusion of common, species-specific maintenance behaviours.

Table 6 Pain-associated behaviours included in piglet pain castration studies utilising one or more of the behavioural indicators of pain defined in the present study ethogram.

Literature cited, first author (year)	Huddled up	Prostrated	Scratching	Spasms	Stiffness	Tail wagging	Trembling
Burkemper (2019)	×	×	×	–	–	×	×
Davis (2017)	–	×	–	–	–	–	×
Gottardo (2016)	–	–	×	–	–	×	×
Hansson (2011)	×	×	×	×	×	–	×
Hay (2003)	×	×	×	×	×	×	×
Hug (2018)	–	–	×	–	–	–	×
Keita (2010)	–	×	–	–	–	×	×
Kluviers-Poodt (2013)	×	×	×	×	×	×	×
Llamas Moya (2008)	×	–	×	×	–	–	×
McGlone (2016)	–	×	–	–	–	–	×
Rault (2011)	×	×	×	×	×	×	×
Sutherland (2010)	×	–	×	–	–	–	–
Sutherland (2011)	×	–	×	–	–	–	–
Sutherland (2017)	×	–	×	–	–	–	–
Viscardi (2017)	–	–	×	×	×	×	×
Viscardi (2018a,b)	–	–	×	×	×	×	×
Viscardi (2019)	–	–	×	×	×	×	×
Yun (2019)	–	×	×	–	–	×	–

× Study used pain-associated behaviour;
– Study did not use pain-associated behaviour.

Animal welfare implications

Castration is recognised as a painful procedure commonly performed on swine farms. For this reason, animal welfare concerns have developed regarding surgical castration without pain relief. Research focused on the evaluation of effective pain management techniques for pain associated with castration in swine has largely utilised scan sampling methodology to collect behavioural metrics. However, the present study determined that this is not an accurate methodology to detect changes in behavioural indicators of pain in castrated piglets. Therefore, continuous sampling is the recommended sampling method to assess pain associated with castration in piglets.

Acknowledgements

This work was financially supported by funding from National Pork Board (19-065), Ontario Pork (052367), National Pork (052457), and the Ontario Ministry of Agriculture, Food and Rural Affairs (027295). RMP received a doctoral fellowship from North Carolina State University's Provost. Gratitude is extended to James Robertson for his guidance in the statistical analysis of this paper. The authors report no conflicts of interest.

References

Altmann J 1974 Observational study of behaviour: Sampling methods. *Behaviour 49*: 227-266. https://doi.org/10.1163/156853974X00534

American Veterinary Medical Association 2013 Swine castration https://www.avma.org/KB/Policies/Pages/Swine-Castration.aspx

Burkemper MC, Pairis-Garcia MD, Moraes LE, Park RM and Moeller SJ 2019 Effects of oral meloxicam and topical lidocaine on pain associated behaviours of piglets undergoing surgical castration. *Journal of Applied Animal Welfare Science 23(2)*: 209-218. https://doi.org/10.1080/10888705.2019.1590717

Chen JM, Schütz KE and Tucker CB 2016 Technical note: Comparison of instantaneous sampling and continuous observation of dairy cattle behaviour in freestall housing. *Journal of Dairy Science 99(10)*: 8341-8346. https://doi.org/10.3168/jds.2016-11351

Crews J, Braude S, Stephenson C and Clardy T 2002 *The Ethogram and Animal Behaviour Research.* Washington University: St Louis, MO, USA

Czerwinski VH, Smith BP, Hynd PI and Hazel SJ 2017 Sampling maternal care behaviour in domestic dogs: What's the best approach? *Behavioural Processes 140*: 41-46. https://doi.org/10.1016/j.beproc.2017.03.018

https://doi.org/10.7120/09627286.29.3.285 Published online by Cambridge University Press

Daigle CL and Siegford JM 2014 When continuous observations just won't do: Developing accurate and efficient sampling strategies for the laying hen. *Behavioural Processes 103*: 58-66 https://doi.org/10.1016/j.beproc.2013.11.002

Davis K, Seddon Y, Creutzinger K, Bouvier M and Brown J 2017 An investigation into the use of sucrose to reduce castration pain in piglets. *Canadian Journal of Animal Science 97(3)*: 439-447. https://doi.org/10.1139/CJAS-2016-0170

De Oliveira FA, Luna SP, do Amaral JB, Rodrigues KA, Sant'Anna AC, Daolio M and Brondani JT 2014 Validation of the UNESP-Botucatu unidimensional composite pain scale for assessing post-operative pain in cattle. *BMC Veterinary Research 10(1)*: 200. https://doi.org/10.1080/10888705.2019.1590717

Dzikamunhenga RS, Anthony R, Coetzee J, Gould S, Johnson A, Karriker L, McKean J, Millman ST, Niekamp SR and O'Conner AM 2014 Pain management in the neonatal piglet during routine management procedures. Part 1: a systematic review of randomized and non-randomized intervention studies. *Animal Health Research Reviews 15(1)*: 14-38. https://doi.org/10.1017/S1466252314000061

European Commission 2010 *Alternatives to pig castration: European declaration on alternative to surgical castration of pigs*. https://ec.europa.eu/food/animals/welfare/practice/farm/pigs/castration_alternatives_en

Gottardo F, Scollo A, Contiero B, Ravagnani A, Tavella G, Bernardini D, De Benedictis GM and Edwards SA 2016 Pain alleviation during castration of piglets: a comparative study of different farm options. *Journal of Animal Science 94*: 5077-5088. https://doi.org/10.2527/jas.2016-0843

Hansson M, Lundeheim N, Nyman G and Johansson G 2011 Effect of local anaesthesia and/or analgesia on pain responses induced by piglet castration. *Acta Veterinaria Scandinavica 53*: 34. https://doi.org/10.1186/1751-0147-53-34

Hay M, Vulin A, Génin S, Sales P and Prunier A 2003 Assessment of pain induced by castration in piglets: Behavioral and physiological responses over the subsequent 5 days. *Applied Animal Behavior Science 82*: 201-218. https://doi.org/10.3389/fvets.2016.00108

Hellebrekers LJ 2000 *Animal pain: a practice-orientated approach to an effective pain control in animals*. Van Der Wees Utigerverij: Utrecht, The Netherlands

Hepworth G and Hamilton A 2001 Scan sampling and waterfowl activity budget studies: design and analysis considerations. *Behaviour 138*: 1391-1405. https://doi.org/10.1163/156853901317367654

Hug PJ, Cap VH, Honegger J, Schupbach-Regula G, Schwarz A and Bettschart-Wolfensberger R 2018 Optimization of analgesia for piglet castration under isoflurane anaesthesia with parental butorphanol, meloxicam or intratesticular lidocaine. *Schweizer Archiv fur Tierheikunde 160(7-8)*: 461-467. https://doi.org/10.17236/sat00169

Keita A, Pagot E, Prunier A and Guidarini C 2010 Pre-emptive meloxicam for postoperative analgesia in piglets undergoing surgical castration. *Veterinary Anaesthesia and Analgesia 37*: 367-374. https://doi.org/10.1111/j.1467-2995.2010.00546.x

Kluviers-Poodt M, Zonderland JJ, Verbraak J, Lambooij E and Hellebrekers LJ 2013 Pain behaviour after castration of piglets; effect of pain relief with lidocaine and/or meloxicam. *Anima 7*: 1158-1162. https://doi.org/10.1017/S1751731113000086

Lehner PN 1987 Design and execution of animal behavior research: an overview. *Journal of Animal Science 65*: 1213-1219. https://doi.org/10.2527/jas1987.6551213x

Lehner PN 1992 Sampling methods in behaviour research. *Poultry Science 71(4)*: 643-649. https://doi.org/10.3382/ps.0710643

Lendvai AZ, Akcay C, Ouyang JQ, Dakin R, Domalik AD, St John PS, Stanback M, Moore IT and Bonier F 2015 Analysis of the optimal duration of behavioral observations based on an automated continuous monitoring system in tree swallows (*Tachycineta bicolor*): Is one hour good enough? *PLoS One 10(11)*: e0141194. https://doi.org/10.1371/journal.pone.0141194

Llamas Moya S, Boyle LA, Lynch PB and Arkins S 2008 Effect of surgical castration on the behavioural and acute phase responses of 5-day-old piglets. *Applied Animal Behaviour Science 111(1-2)*: 133-145. https://doi.org/10.1016/j.applanim.2007.05.019

Madruga A, Mainau E, Gonzalez LA, Rodriguez-Prado M, Ruiz de la Torre JL, Manteca X and Ferret A 2017 Technical note: Recording rules for behavioral studies in growing heifers fed high-concentrate diets. *Journal of Animal Science 95*: 2339-2344. https://doi.org/10.2527/jas2016.1037

Malachowski CP and Dugger BD 2018 Hawaiian duck behavioural patterns in seasonal wetlands and cultivated taro. *The Journal of Wildlife Management 82(4)*: 840-849. https://doi.org/10.1002/jwmg.21429

Martin P and Bateson P 2012 *Measuring Behaviour*. Cambridge University Press: Cambridge, UK. https://doi.org/10.1017/CBO9781139168342

Martin P and Bateson PPG 1993 *Measuring Behaviour: An Introductory Guide, Second Edition*. Cambridge University Press: New York, NY, USA

McGlone J, Guay K and Garcia A 2016 Comparison of intramuscular or subcutaneous injections vs castration in pigs: impacts on behavior and welfare. *Animals 6(9)*: 52. https://doi.org/10.3390/ani6090052

Mellor DJ 2016 Updating animal welfare thinking: Moving beyond the 'Five Freedoms' towards 'a life worth living'. *Animals 6(3)*: 21. https://doi.org/10.3390/ani6030021

Mitlohner FM, Morrow-Tesch JL, Wilson SC, Dailey JW and McGlone JJ 2001 Behavioral sampling techniques for feedlot cattle. *Journal of Animal Science 79*: 1189-1193. https://doi.org/10.2527/2001.7951189x

National Farm Animal Care Council 2014 *Code of Practice for the Care and Handling of Pigs*. https://www.nfacc.ca/pdfs/codes/pig_code_of_practice.pdf

National Pork Board 2018 US *Packing sector*. https://www.pork.org/facts/stats/u-s-packing-sector/

National Research Council 2012 *Nutrient Requirements of Swine, 11th Edition*. National Academies Press: Washington, DC, USA. https://doi.org/10.17226/13298

New Zealand Government 2018 *Code of Welfare: Animal Husbandry Procedures*. https://www.mpi.govt.nz/dmsdocument/1443/direct

Nielsen MLK, Sprogis KR, Bejder L, Madsen PT and Christiansen F 2019 Behavioural development in southern right whale calves. *Marine Ecology Progress Series 629*: 219-234. https://doi.org/10.3354/meps13125

O'Connor A, Anthony R, Bergamasco L, Coetzee J, Gould S, Johnson AK, Karriker LA, Marchant-Forde JN, Martineau GS, McKean J, Millman ST, Niekamp S, Pajor EA, Rutherford K, Sprague M, Sutherland M, von Borell E and Dzikamunhenga RS 2014 Pain management in the neonatal piglet during routine management procedures Part 2: Grading the quality of evidence and the strength of recommendations. *Animal Health Research Reviews 15(1)*: 39-62. https://doi.org/10.1017/S1466252314000073

Pig Code of Practice Scientific Committee 2012 *Code of Practice for the Care and Handling of Pigs: Review of Scientific Research on Priority Issues.* National Farm Animal Care Council: Lacombe, AB, USA

Primary Industries Standing Committee 2008 *Model Code of Practice for the Welfare of Animals Pigs, Third Edition.* http://www.publish.csiro.au/ebook/download/pdf/5698

Pullin AN, Pairis-Garcia MD, Campbell BJ, Campler MR and Proudfoot KL 2017a Technical note: Instantaneous sampling intervals validated from continuous video observation for behavioral recording of feedlot lambs. *Journal of Animal Science 95*: 4703-4707. https://doi.org/10.2527/jas2017.1835

Pullin AN, Pairis-Garcia MD, Campler MR and Proudfoot KL 2017b Validation of scan sampling techniques for behavioural observations of pastured lambs. *Animal Welfare 25*: 185-190. https://doi.org/10.7120/09627286.26.2.185

Rault JL and Lay Jr DC 2011 Nitrous oxide by itself is insufficient to relieve pain due to castration in piglets. *Journal of Animal Science 89(10)*: 3318-3325. https://doi.org/10.2527/jas.2011-4104

Ross L, Cressman MD, Cramer MC and Pairis-Garcia MD 2019 Validation of alternative behavioral observation methods in young broiler chickens *Poultry Science 98*: 6225-6231. https://doi.org/10.3382/ps/pez475

Roughan JV and Flecknell PA 2003 Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *European Journal of Pain 7*: 397-406. https://doi.org/10.1016/S1090-3801(02)00140-4

Studd EK, Landry-Cuerrier M, Menzie AK, Boutin S, McAdam AG, Lane JE and Humpries MM 2019 Behavioral classification of low-frequency acceleration and temperature data from a free-ranging small mammal *Ecology and Evolution 9*: 619-630. https://doi.org/10.1002/ece3.4786

Sutherland MA 2015 Welfare implications of invasive piglet husbandry procedures, methods of alleviation and alternatives: a review. *New Zealand Veterinary Journal 63*: 52-57. https://doi.org/10.1080/00480169.2014.961990

Sutherland MA, Backus BL, Brooks TA and McGlone JJ 2017 The effect of needle-free administration of local anesthetic on the behavior and physiology of castrated pigs. *Journal of Veterinary Behaviour 21*: 71-76. https://doi.org/10.1016/j.jveb.2017.07.003

Sutherland MA, Davis BL, Brooks TA and Coetzee JF 2012 The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia. *Journal of Animal Science 90(7)*: 2211-2221. https://doi.org/10.2527/jas.2011-4260

Sutherland MA, Davis BL, Brooks TA and McGlone JJ 2010 Physiology and behavior of pigs before and after castration: effects of two topical anesthetics. *Animal 4(12)*: 2071-2079. https://doi.org/10.1017/S1751731110001291

Tardin RH, Pinto MP, Alves MAS and Simão SM 2014 Behavioral event occurrence differs between behavioural states in *Sotalia guianensis* (*Cetarctiodactyla: Delphinidae*) dolphins: a multivariate approach. *Zoologia 31(1)*: 1-7. https://doi.org/10.1590/S1984-46702014000100001

Telles FG, Luna SPL, Teixeira G and Berto DA 2016 Long-term weight gain and economic impact in pigs castrated under local anesthesia. *Veterinary and Animal Science 1*: 36-39. https://doi.org/10.1016/j.vas.2016.11.003

Van Beirendonck S, Driessen B, Verbeke G and Geers R 2011 Behavior of piglets after castration with or without carbon dioxide anesthesia *Journal of Animal Sciences 89*: 3310-3317. https://doi.org/10.2527/jas.2010-3104

Viscardi AV, Hunniford M, Lawlis P, Leach M and Turner PV 2017 Development of a piglet grimace scale to evaluate piglet pain using facial expressions following castration and tail docking: a pilot study. *Frontiers of Veterinary Science 4*: 51. https://doi.org/10.3389/fvets.2017.00051

Viscardi AV and Turner PV 2018a Efficacy of buprenorphine for management of surgical castration pain in piglets. *BMC Veterinary Research 14*: 318. https://doi.org/10.1186/s12917-018-1643-5

Viscardi AV and Turner PV 2018b Use of meloxicam or ketoprofen for piglet pain control following surgical castration. *Frontiers of Veterinary Science 5*: 299. https://doi.org/10.3389/fvets.2018.00299

Viscardi AV and Turner PV 2019 Use of meloxicam, buprenorphine and Maxilene® to assess a multimodal approach for piglet pain management, part 1: surgical castration. *Animal Welfare 28*: 487-498. https://doi.org/10.7120/09627286.28.4.487

Von Borell E, Baumgartner J, Giersing M, Jaggen N, Prunier A, Tuyttens FAM and Edwards SA 2009 Animal welfare implications of surgical castration and its alternatives in pigs *Animal 3(11)*: 1488-1496. https://doi.org/10.1017/S1751731109004728

Weary DM, Niel L, Flower FC and Fraser D 2006 Identifying and preventing pain in animals. *Applied Animal Behavior Science 100(1-2)*: 64-76. https://doi.org/10.1016/j.applanim.2006.04.013

Whalin L, Pairis-Garcia M, Proudfoot K, Stalder K and Johnson A 2016 Validating behavioral sampling techniques for lame sows administered flunixin meglumine and meloxicam. *Livestock Science 191*: 103-107. https://doi.org/10.1016/j.livsci.2016.07.017

Yeates J 2016 Quality of life and animal behaviour. *Applied Animal Behaviour Science 181*: 19-26. https://dx.doi.org/10.1016/j.applanim.2016.04.018

Yun J, Ollila A, Valros A, Larenza-Menzies P, Heinonen M, Oliviero C and Peltoniemi O 2019 Behavioural alterations in piglets after surgical castration: Effects of analgesia and anaesthesia. *Research in Veterinary Science 125*: 36-42. https://doi.org/10.1016/j.rvsc.2019.05.009

https://doi.org/10.7120/09627286.29.3.285 Published online by Cambridge University Press