An investigation into the use of sucrose to reduce castration pain in piglets
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Oral sucrose was evaluated for its ability to reduce pain following castration. Piglets (n = 126) were assigned to one of five treatments: 1) castrated and given 3 ml of water (C); 2) castrated with 3 ml of 30\% sucrose before castration (sucB); 3) castrated and given 3 ml of 30\% sucrose after castration (sucA); 4) sham castrated and given 3 ml of water (SHAM); and 5) sham castrated and given 3 ml of 30\% sucrose (sucSHAM). Piglet navigation time (NT) through a handling chute was tested at 0, 15, 30 and 45 min after treatment as a measure of pain. Serum cortisol and pen behaviours were also recorded. At 0, 15 and 30 min, C piglets had a greater NT than SHAM and sucSHAM piglets ($P < 0.05$). The NT of sucA piglets was similar to SHAM and sucSHAM, and shorter than C at 15, 30 and 45 min. The NT of sucB piglets was similar to SHAM and sucSHAM at 30 and 45 min, and shorter than C at 15 and 45 min. Handling chute behaviour suggests
sucrose provides some degree of pain relief following castration; sucrose given before castration showing more consistent results than when given after.

**Short title:** Davis et al. Sucrose analgesia to reduce piglet castration pain

**Keywords:** Pig, castration, sugar, pain, behaviour

**Abbreviations:**

- C, Control
- sucB, sucrose before castration
- sucA, sucrose after castration
- SHAM, sham castration
- sucSHAM, sham castration with sucrose
- NT, navigation time

**INTRODUCTION**

The pain of surgical castration is of significant concern for the welfare of neonatal piglets. Canada’s Code of Practice for the Care and Handling of Pigs requires that as of July 1, 2016, castration of piglets must be performed with analgesics to help mitigate post-procedure pain (NFACC, 2014). The non-steroidal anti-inflammatory drugs meloxicam and ketoprofen, have been shown to be effective at reducing post-castration pain (Courboulay et al. 2010; Keita et al. 2010). Given to control post procedure pain, it may be likely that the most common approach practiced on farm will be to administer the analgesic to the piglet immediately before castration. Research on the pharmacokinetics of meloxicam have determined an absorption half life of 0.19h, and time to reach
maximum concentration (Tmax) of 1 hour when administered at 0.4mg/kg intravenously to two week old piglets (Fosse et al. 2010). This is similar to that observed when administered intramuscularly to 45kg pigs (EMEA, 2006, cited in Fosse et al. 2010). Accounting for the pharmacokinetics, it suggests there will be a delay from the point of administration until the piglet receives the full analgesic effect, during which time the piglet would experience significant pain from castration. To minimize pain from immediately after castration, the piglet would need to be handled twice: first to administer the drug and later to castrate, after the drug has taken effect. However, additional handling prior to castration increases labour requirements and imposes greater handling stress on the piglets (Courboulay et al. 2010; Hansson et al. 2011; Schmidt et al. 2012).

Administering sucrose at the time of castration may be an alternative or adjunct to analgesics that could help support the piglet through the initial pain of castration, until the analgesic takes effect. This would reduce the need for repeated handling of piglets. In premature and neonatal human infants, sucrose is commonly used for pain control, and has been shown to decrease pain associated with intramuscular injection (Lewindon et al. 1998), heel prick (Isik et al. 2000), and retinopathy (Gal et al. 2005), as evaluated by crying time and behaviour scores. When tested in rodents, Hogatt et al. (2010) found that eight week old mice administered sucrose prior to oral gavage showed fewer stress-related behaviours during gavage, and had reduced corticosterone levels compared to controls. Additionally, researchers were able to complete the procedure with mice given sucrose on average five seconds faster than controls (Hoggatt et al. 2010), which decreased the amount of stress associated with the procedure. In a study by Blass et al.
(1986), rats given sucrose demonstrated a greater pain threshold to a hot plate test and fewer distress vocalizations during an isolation test. The analgesic effects of sucrose were reversed when the opioid receptor antagonist, naltrexone, was given, and its actions are therefore believed to be mediated by the endogenous opioid system. If the analgesic effects of sucrose are effective in piglets, it could offer a low cost method of providing pain relief for piglets immediately after castration, and could potentially act as a buffer to reduce pain while waiting for an analgesic to take effect. Such a procedure could reduce handling stress by allowing castration and pain control to be provided at the same time.

The objective of this study was to determine the effectiveness of an orally administered sucrose solution at reducing pain in piglets immediately following castration, as determined using behavioural measures in a handling chute and in the home pen following castration, and measures of stress physiology.

**MATERIALS AND METHODS**

The following study was conducted at the Prairie Swine Centre, Inc. in Saskatchewan, Canada. This work was approved by the University of Saskatchewan’s Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

**Animals and Housing**

A total of 126 male PIC Landrace x Yorkshire piglets, between 3 and 5 days of age (mean weight ± S.D., 1.68 ± 0.33 kg), from 22 litters were used for this study. Litters were housed over five farrowing rooms, with 16 farrowing crates per room. Farrowing pens (2.44 m x 1.83 m) were fully slatted with tri-bar metal flooring, and contained a 1.98
m x 0.86 m farrowing crate. Each pen had a hooded creep area with rubber flooring, heat lamp, and an additional rubber mat outside of the hooded creep, with an additional heat lamp. Sows were fed a standard lactation diet that met National Research Council (NRC, 2012) requirements. Needle teeth were clipped on all piglets at one day of age, and piglets received no further treatments until after the experiment.

**Treatments**

Five treatments were tested: 1) castration control: piglets were castrated without analgesia and were given 3 mL of water before castration (C, n = 25); 2) sucrose before castration: piglets were administered 3 mL of a 30% w/v sucrose solution by syringe immediately before castration (sucB, n = 25); 3) sucrose after castration: piglets were administered 3 mL of a 30% w/v sucrose solution immediately after castration (sucA, n = 25); 4) sucrose sham castration: piglets given 3 mL of 30% w/v sucrose before castration and handled as if to castrate, but not castrated (sucSHAM, n = 25); and 5) sham castration control: piglets handled as if to castrate, but not castrated, and administered 3 mL of water (SHAM, n = 26). All sucrose solutions and water were administered orally by syringe. A 30% sucrose solution was used based on the study by Isik et al. (2000), in which a 30% sucrose solution was more effective at reducing crying time when term infants were heel pricked, when compared to 10% or 30% glucose solutions. Each treatment was represented in each litter, with male piglets randomly assigned to treatments at 2-4 days of age.

Castration was performed in accordance with standard commercial practice. The procedure was carried out by one person using manual restraint. The piglet was held by its hind legs in a head down position, the testes were palpated and pushed up into the
scrotum, and two vertical incisions were made through the tunicae along both sides of the scrotum with a scalpel exposing each testicle. Each testicle was pushed out of the scrotum and pulled away from the body, tearing the spermatic chords. Sham castrated piglets were handled in an identical manner to castrates without castrating, no incisions were made. All treatments only required one handling per piglet.

**Behavioural Observations**

Behaviour was observed in all piglets using two methods to help evaluate pain: i) using a specially designed handling chute and ii) behavioural observations in the farrowing pen post treatment. Piglets were individually marked on their backs for identification prior to treatment and markings were randomised to ensure the observer was blind to treatments.

**Chute Navigation Time**

A specially designed chute containing two hurdles was used as an objective behavioural measure of pain (Bilsborrow et al. 2016). The portable chute was constructed to fit in place of the back gate of the farrowing pen and measured 1.77 m in length, 0.18 m in width x 0.33 m in height (Figure 1). Two removable hurdles, 10cm in height, were inserted within the chute, requiring the piglet to perform a small jump, or lift its legs high, in order to maneuver over the hurdles. In each chute run, piglets were placed at the start position and were required to navigate the length of the chute, before exiting into the farrowing pen.

One day prior to treatment application, piglets were trained to navigate through the chute via three runs through the chute at 30 min intervals, first with no hurdles, then with the addition of 5 cm hurdles, and finally with 10 cm hurdles. This training period
was given to ensure piglets were familiar with how to traverse the chute prior to administration of treatments. If a piglet laid down in the chute on a training day, it was placed on its feet and gently encouraged to continue down the chute. All piglets navigated the chute voluntarily by the third training run.

At testing, chute navigation time (NT) was recorded by live observation using a stopwatch. A pre-treatment run was performed 10 min prior to administration of treatment. Following treatment piglets were required to navigate the chute immediately afterwards (0 min) and at 15, 30, and 45 min. Piglets were given a maximum of two minutes to navigate the chute unaided. If a piglet laid down it was assigned a NT of two minutes and was lifted and gently pushed through the chute towards the farrowing pen. Throughout the trial, the testing of litters of piglets was consistently conducted in the morning from 07:30-12:00.

**Behaviour in the Farrowing Pen**

Piglet behaviour in the farrowing pen was recorded from the point of treatment, until four hours post treatment. Behaviour in each pen was recorded by two video cameras (Sony Handycam DCR-SR68, ©Sony Corporation, Park Ridge, NJ, USA), one mounted on the wall capturing an overhead view of the farrowing pen, and one placed to capture the area under the hooded creep. Scan sampling was performed on footage at intervals of three minutes by a single trained observer and the presence (score = 1) or absence (score = 2) of suckling, standing and lying, (termed non-specific behaviours, Table 1) recorded. Intra-observer reliability testing was not conducted. At 60, 120, 180 and 240 min, piglets were also scored for the presence or absence of castration pain related behaviours, immobility, tremors and isolation, (Molony and Kent, 1997; Hay et al. 2003). All videos were
transcribed by a single trained observer. If a piglet was not visible at a scan time point, the individual piglet score was recorded as missing data.

**Serum cortisol measurement**

Blood was collected from 20 piglets per treatment following the pre-treatment chute run at 10 – 15 min before treatment by venipuncture in lateral recumbency using 2.5 cm, 21 gauge needles and 6 mL serum collection tubes (BD Vacutainer® Blood Collection Tube, Becton, Dickinson and Company, Franklin Lakes, New Jersey). Fifty percent of piglets across all treatments were re-sampled 30 min after treatment, and the remaining fifty percent at 45 min, resulting in two blood collections per piglet. This was done to minimize the amount of stress imposed on piglets by handling and blood collection.

Following collection, blood was allowed to clot at room temperature for 1-2 hours and centrifuged at 1000 g at room temperature for 15 min, serum was then extracted and frozen at -20°C until analysis. The sample size per cortisol sampling period was determined from a power calculation using data from figure 1 in Keita et al. (2010), where the blood cortisol level of non-castrated piglets is 50 ± 60ng/ml, and piglets castrated with meloxicam, 200 ± 100ng/ml at 30 minutes post procedure. Using these figures, a minimum sample size of 5 was determined assuming a mean S.D. of 80ng/ml, power of 0.8, and an error rate of 5%. Cortisol concentrations were determined using a solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite, Siemens Healthcare Diagnostics Inc., Llanberis, UK), with a diagnostics laboratory contracted to perform the work.
STATISTICAL ANALYSIS

All data were analysed using SAS, version 9.3 (SAS Inst. Inc., Cary, NC, USA).

Differences in NT among treatments were compared using Proc Mixed with fixed effects of treatment, run time, and the interaction between treatment and run time, with piglet weight and pre-treatment NT as covariates. The model accounted for repeated measures within pigs over the four time points (0, 15, 30, 45 min) using an exponential correlation matrix; similarity between observations of piglets within litter was accounted for in the random intercept.

Scores of the presence or absence of pain related behaviours displayed in the farrowing pen, summed per pig for each time point (60, 120, 180 and 240 min), creating a pain behaviour score (PBS). A maximum score of 3 was possible at each time point if all three behaviours were present. The PBS at each of the time periods were compared across treatments via the Fisher’s exact test, in the Proc Freq function in SAS.

Differences in suckling, standing and lying behaviour between treatment groups in the farrowing pen were evaluated by comparing the frequency of behaviours between treatment groups using generalized estimating equations (Proc Genmod, SAS 9.3). The numerator for each calculation was the number of time periods the piglet was observed performing the behaviour and the denominator was the number of time periods the piglet was under observation between 3 and 240 min after treatment. Data were analyzed assuming a binomial distribution. The predicted values for each treatment are reported as the mean probability of observing piglet postures and activities within four hours of
observation, with 95% confidence intervals (CI). The difference between treatment
groups is reported as an odds ratio (OR) with 95% CI.

Differences in cortisol response (change $\Delta$ calculated from 10 min before castration to
30 and 45 min after) between treatments were analysed by analysis of variance using
Proc Mixed with fixed effects of treatment and piglet weight, with litter as the random
intercept. The residuals were tested for normality using the Proc Univariate procedure in
SAS. Significance levels in all cases were determined at $P \leq 0.05$.

RESULTS

Behavioural Observations

Chute Navigation Time

All piglets navigated the chute voluntarily on the pre-treatment run at 10 min prior to
castration. At 0 min post-treatment, NT was significantly greater for C than both SHAM
and sucSHAM piglets ($P < 0.01$, Figure 2), and sucA and sucB piglets were intermediate,
being no different to any treatment.

At 15 min post-treatment, C piglets had a significantly longer NT than all other
treatments ($P < 0.05$). SucB ($P=0.015$) and SucA piglets at the level of tendency
($P=0.059$) had a longer NT than SHAM piglets, with no difference between the two sham
treatments. There was no difference between sucA, sucB and sucSHAM piglets’ NT. At
30 min post-treatment, C piglets had a significantly longer NT compared to sucA, SHAM
and sucSHAM piglets ($P<0.05$), whom were no different, and sucB piglets were no
different to any treatment. At 45 min post-treatment, C piglets had a significantly longer NT than sucA, sucB and SHAM piglets (P<0.05) and sucSHAM piglets were intermediate, being no different to any treatment (Figure 2). In total, 21 out of 126 piglets laid down (C = 8, sucB = 4, sucA = 4, sucSHAM = 2, SHAM = 3) in 30 out of 630 chute runs.

**Behaviour in the Farrowing Crate**

Results from pain behaviour scores observed at 1, 2, 3 and 4 h after treatment are shown in Table 2. Individual pigs were not able to be viewed on 32-40% of all observations. Of the observations in which piglets could be viewed, the presence of prostration, tremors and isolation behaviours was low with a PBS score of 0 occurring in 301/323 (93%) of observations. At least one pain behaviour was performed 19/323 (6%) of observations and two behaviours performed 3/323 (1%) of the time. At 4 hours post-treatment, a significant difference was found among treatments, however this result may have resulted from a higher prevalence of missing observations for sucSHAM piglets (Table 2). Reanalysed without the sucSHAM treatment, there was no significant difference between pain behaviours within groups of piglets (Table 2).

On average, piglets were observed to be suckling (present at the udder) in 12.6 ±1.2 observations, standing in 4.9 ± 0.6 observations and lying in 49.1 ±3.4 observations. Piglets were not visible (obscured by the creep hood or behind the sow) in 14.8 ±1.6 observation periods. Over the four hours following castration, treatment differences were found in the frequency of suckling and standing, but no difference was found in the frequency of lying (Table 3). Piglets in the SHAM treatment were more likely to be observed suckling than sucSHAM piglets (OR= 1.302, CI= 1.011-1.677; P = 0.04), and
sucA piglets were less likely to be observed standing than sham piglets (OR = 0.698, CI= 0.529-0.920; P < 0.01). There were no other differences in pen behaviour between treatments.

**Cortisol Concentrations**

At 30 min after treatment, the increase in cortisol by C, sucB and sucA piglets was no different, and was significantly higher than both sham treatments, however, the increase for SHAM was greater than for sucSHAM (Table 4). There was also a tendency for sucA to have a greater cortisol response than piglets given sucB (P=0.09). At 45 min after treatment, the increase in cortisol was similarly raised in C, sucB and sucA treatments. At 45 min post-treatment, C, sucA and sucB had significantly greater increase in cortisol than SHAM, with the change in cortisol of C and sucB also greater than sucSHAM. The cortisol of sucA and sucSHAM showed similar (intermediate) responses, being different at the level of tendency (P=0.07).

**DISCUSSION**

**Behavioural Observations**

**Chute Navigation Time**

Piglets in the C treatment had longer NT than SHAM piglets at all time points (0, 15, 30 and 45 min after treatment), showing that piglets castrated without any pain mitigation took the greatest amount of time to navigate the chute than piglets handled similarly but not subjected to castration. These results further validate the chute system developed by Bilsborrow et al. (2016), which showed the duration of time piglets took to navigate the
chute and return to the home pen took significantly longer in piglets castrated without pain control at 0 and 15 minutes post-castration, compared to piglets handled but not castrated. The NT of C piglets was not significantly different from sucSHAM at 45 min post-treatment, which may have been due to the confounding stress imposed by handling and blood collection. It may be that smaller piglets had more difficulty navigating the ten-centimeter hurdles, which may have contributed to variation in the results.

The chute NT of sucA piglets was shorter than that of C piglets at 15, 30 and 45 min post-castration, and was not different from SHAM or sucSHAM piglets at any time point. This suggests a positive effect of administering sucrose immediately following castration on reducing post-operative pain. In comparison, sucB piglets had less consistent results, with shorter a NT than C piglets at 15 and 45 min post-castration, but not at 30 min. Based on navigation times, these results suggest that providing sucrose after castration was more effective in providing some relief from pain than providing it before castration. However, further research is needed to optimize the treatment and examine this response.

One difference in the methodology used by Bilsborrow et al. (2016) and the present study was how piglets were treated when they laid down during a chute run. Bilsborrow et al. (2016) re-set piglets that laid down by lifting them to their feet and encouraging them to complete the chute run. In the present study, piglets were not interfered with once they were in the chute: all piglets were given a maximum of two minutes to navigate the chute and if they laid down or had not traversed the chute in that time they were gently pushed through and assigned a NT of two minutes. All piglets voluntarily navigated the chute by the third run on the training day as well as during the pre-treatment run. Following treatment, 21 piglets laid down during testing with twice as many C piglets lying
compared to sucA or sucB, therefore, lying down in the chute may be another indicator of pain and/or stress.

Piglets given sucrose at the time of castration were faster at navigating the piglet-handling chute than piglets simply castrated in the present study, which suggests the sucrose may provide a distraction from the pain and/or some degree of analgesia. Initial results suggest that sucrose administered following castration (sucA) had positive effects on reducing post-castration pain. However, piglets given sucrose immediately before castration (sucB) did not show this response consistently. Therefore, further research is needed to optimize the treatment and examine this response, and it is suggested that future research increase the sample size for the number of piglets tested in the handling chute.

*Behaviour in the Farrowing Crate*

In the current study, performance of the pain related behaviours in the combined PBS was low with none of the behaviours present in 93% of observations, taken at 1, 2, 3, and 4 h following treatment. No differences were found at 1, 2 or 3 hours, but at 4 hours after treatment sucSHAM piglets showed more pain behaviours than other treatments, however this result was based on only two animals. It is unclear as to why sucSHAM piglets showed greater pain related behaviours than other treatments because sucSHAM piglets were not castrated. It may be that such behaviours are not only indicative of pain. Alternatively, there could be observer error playing a part. It should be noted that with the data reanalysed having removed the sucSHAM group, there was no significant difference in the pain behaviours of the remaining treatment groups. In a study by Keita et al. (2010), piglets were observed for prostration, trembling, isolation and tail
movement using live observations at four time points post-treatment (1, 2, 4 and 24 hours) and scores were compared among the time points. Piglets given meloxicam in that study showed fewer pain related behaviours at two and four hours post-castration compared to those castrated without pain control. A study by Llamas Moya and colleagues (2008), similarly found that piglets castrated at 5 days of age spent more time huddling and less time walking than non-castrated controls from approximately four to eight hours after treatment. The lack of pain behaviours observed following castration in this study could be due to the time frame in which pigs were observed, or that scan sampling was used in place of live or continuous observations. Additionally, any effect of sucrose given at the time of procedure, it can be assumed would only have an effect to reduce pain in the period immediately post-castration. It is highly likely that the effects of sucrose had worn off by the time observations in the farrowing pen took place, and therefore three treatments of castrated piglets were compared.

In the current study castrated piglets, (with or without oral sucrose) and SHAM castrates did not differ in the time spent lying up to four hours following treatment administration. However, piglets castrated and receiving the sucA treatment stood significantly less than sucSHAM piglets, but not from sham castrated piglets The significance of this finding is difficult to interpret. The results may not be entirely accurate because they were obtained by scan sampling and should be interpreted with caution. A number of previous studies have not identified differences in standing time between castrated piglets with non-castrated controls (Carroll et al. 2006, Llamas Moya et al. 2008). However, McGlone et al. (1993) found castrated piglets stood for less time than control piglets when observed
over a six hour period following the castration or handling treatment. It may be that standing time is a less consistent indicator of pain. With great variability between piglets, increasing the sample size and the frequency of behaviour observations will help to confirm the usefulness of certain behaviours to indicate pain.

In the first four hours following treatments, SHAM piglets suckled more than sucSHAM piglets but all castrate treatments did not differ from either sham treatment. The difference between these two treatments was that sucSHAM piglets were given sucrose whereas SHAM piglets were given water, so it is possible that sucSHAM piglets suckled less because the sucrose provided a small amount of nutrition. However, as sucSHAM piglets did not suckle less frequently than any of the three castration treatments (sucA, sucB, C) then it is more plausible that SHAM piglets suckled more frequently for other reasons such as individual piglet variation. Previous studies of piglet behaviour following castration have shown that castrated piglets tend to be more isolated and desynchronised (i.e. behave differently than their littermates) than non-castrated piglets (Hay et al. 2003 and Llamas Moya et al. 2008), and spend more time lying without contact with other piglets or the sow (Sutherland et al. 2012). However in one study, castrated piglets were seen walking more (Hay et al. 2003), and in another walking less than non-castrated controls (Llamas Moya et al. 2008). Measurement of activity at the udder, including suckling and massaging, has also been inconsistent, with castrates reportedly spending less time at the udder (McGlone et al. 1993 and Hay et al. 2003), and more time at the udder (Taylor et al. 2001 and Llamas Moya et al. 2008). Carroll and colleagues (2006) found no difference in activity at the udder or lying, standing, and sitting behaviours between castrated and non-castrated piglets, although castrates tended to be less active.
overall. Variability in results from studies quantifying pain using the behaviour of piglets has challenged the validity of such techniques, and highlights the need for more objective behavioural measures of pain, such as the piglet handling chute. Additionally, studies that intend to observe individual piglet behaviour in the home pen would benefit from increasing sample size to help lower the variance.

**Cortisol Concentration**

Previous research has identified cortisol as a marker of castration pain in piglets, with castrated piglets having a greater increase in serum cortisol than controls following the procedure (Prunier et al. 2005; Marchant-Forde et al. 2009; Kluivers-Poodt et al. 2012; Sutherland et al. 2012). The present study supports these findings as castrated (C) piglets had a greater increase in serum cortisol concentration than both SHAM and sucSHAM piglets at 30 and 45 min after treatment administration.

Previous work has also shown a reduced cortisol response in piglets given pain control (meloxicam) before castration compared to controls given a saline solution placebo (Keita et al. 2010). In the present study, sucA and sucB piglets did not have significantly different cortisol responses at 30 or 45 min after castration compared to C piglets.

Moreover, sucA and sucB piglets had a significantly greater increase in cortisol concentration at 30 min than the two sham treatments (sucSHAM and SHAM). The sucrose solution did not seem to provide adequate short term pain relief based on the cortisol response compared to control piglets. In eight week old mice, provision of oral sucrose has been found to reduce the corticosterone response to an unpleasant procedure, oral gavage (Hoggatt et al. 2010). However, it is recognised that oral gavage will elicit
very different sensations compared to that experienced during castration. It is likely that castration elicits too great a stress response to be overcome by the sucrose, compared to the oral gavage procedure in rodents. A higher concentration of sucrose may be more effective at relieving pain due to castration or possibly a larger volume of solution. Blass et al. (1986) proposed that sucrose might overcome the negative sensation of pain by stimulating a positive affective system as the potential mechanism of action for sucrose increasing the pain threshold of rats. It is not an opioid agonist, but is rather believed to act indirectly by stimulating endogenous opioid release (Kracke et al. 2005). In the case of piglet castration, the stress response induced may be such that it cannot be significantly reduced by sucrose alone at a concentration of 30%.

Future research to refine the procedure, such as modifications of the volume and concentration of sucrose, may improve the effectiveness and consistency of results. Using a combination of sucrose and an analgesic may result in more effective pain control, and this should be established as measured by cortisol and behavioural tests. Possible combinations for future research include oral sucrose and an injectable or oral analgesic, which may provide a more effective single dose option for pain management following castration.

CONCLUSION

Providing an oral sucrose solution to piglets at the time of castration significantly reduced navigation times through a piglet handling chute, which suggests it may have provided some level of pain relief to piglets after castration. However, piglets given sucrose before or after castration did not have a significantly lower cortisol response compared to those castrated without sucrose, suggesting that the sucrose did not decrease the stress of
castration and repeated handling. Castrated piglets had a significantly greater increase in serum cortisol concentration after castration than sham castrated piglets, and also had longer navigation times through the piglet-handling chute than sham castrates. These results confirm that castration is painful for piglets and that the piglet-handling chute is capable of distinguishing between piglets that are experiencing castration pain and those that are not.

ACKNOWLEDGEMENTS

We would like to thank Megan Bouvier of the Prairie Swine Centre for her invaluable assistance in helping perform data collection for this trail.

REFERENCES


Figure 1. Piglet handling chute containing two hurdles (HD1 and HD2), one closed end (ST) and one open end (FIN). Piglets were placed in the closed end (ST) from where they navigated to the open end (FIN) where they rejoined the sow and the rest of the litter in the farrowing crate. Permission was obtained to use the diagram from Bilsborrow et al. 2016.
Figure 2. Piglet navigation times through the handling chute at run times: 0 (immediately after treatment administration) and at 15, 30 and 45 min post-treatment. Treatments were: C: castrated with 3 ml of water orally; SucA: castrated with 3 ml of 30% sucrose after castration; SucB: castrated with 3 ml of 30% sucrose before castration; Sham: handled similarly to castrated piglets, given 3 ml of water and not castrated; and sucSham: handled, given 3 ml of 30% sucrose and not castrated.
Table 1. Description of piglet behaviours recorded following castration

<table>
<thead>
<tr>
<th>Pain related behaviours:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostration</td>
<td>Immobile sitting or standing, head lower than shoulder level</td>
</tr>
<tr>
<td>Tremors</td>
<td>Shivering while sitting, standing or lying</td>
</tr>
<tr>
<td>Isolation</td>
<td>Located away from other piglets, having no contact with littermates</td>
</tr>
</tbody>
</table>

“Non-specific” behaviours:

<table>
<thead>
<tr>
<th>Suckling</th>
<th>Positioned with mouth in contact with teat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing</td>
<td>Body weight supported by all four legs</td>
</tr>
<tr>
<td>Lying</td>
<td>Immobile, in lateral (weight supported by side, shoulder in contact with the floor) or ventral (weight supported by belly, sternum in contact with the floor) lying position</td>
</tr>
</tbody>
</table>

**Note:** Farrowing crate behaviours were recorded from zero to four hours following the treatments. Video was then transcribed by a single trained observer using scan sampling every three minutes.
Table 2. Distribution of pain behaviour scores (PBS) recorded at 1, 2, 3 and 4 h after treatment (N and % of observations) and number of missing observations in each treatment

<table>
<thead>
<tr>
<th>Time after castration (hours)</th>
<th>Treatment</th>
<th>Pain Score</th>
<th>Missing observations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>C</td>
<td>14 (93.3)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td></td>
<td>SucA</td>
<td>17 (94.4)</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td></td>
<td>SucB</td>
<td>15 (93.8)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SHAM</td>
<td>14 (87.5)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td></td>
<td>SucSHAM</td>
<td>13 (92.9)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>16 (84.2)</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td></td>
<td>SucA</td>
<td>11 (100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SucB</td>
<td>15 (88.2)</td>
<td>2 (11.8)</td>
</tr>
<tr>
<td></td>
<td>SHAM</td>
<td>17 (89.5)</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td></td>
<td>SucSHAM</td>
<td>15 (93.8)</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>16 (94.1)</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td></td>
<td>SucA</td>
<td>16 (84.2)</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td></td>
<td>SucB</td>
<td>12 (100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SHAM</td>
<td>15 (93.8)</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td></td>
<td>SucSHAM</td>
<td>12 (100)</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>20 (95.2)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SucA</td>
<td>16 (100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SucB</td>
<td>17 (100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SHAM</td>
<td>19 (100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SucSHAM</td>
<td>11 (84.6)</td>
<td>2 (15.4)</td>
</tr>
</tbody>
</table>

Note: Treatments: C: castrated with 3 ml of water orally (n=25); SucA: castrated with 3 ml of 30% sucrose after castration (n=25); SucB: castrated with 3 ml of 30% sucrose before castration (n=25); SHAM: handled similarly to castrated piglets, given 3 ml of
water and not castrated (n=25); and SucSHAM: handled, given 3 ml of 30% sucrose and not castrated (n=26).

\textsuperscript{a}PBS calculated as the sum of scores comprised of the presence (score = 1) or absence (score = 0) of prostration, tremors and isolation behaviours. Missing data for each time point due to pigs not being observable on video footage ranged from 32-40%.

\textsuperscript{b}Statistical significance compares whether the treatment groups differ in each pain score scale.

\textsuperscript{c}Reanalysed with the SucSHAM group removed, there is no significant difference in pain behaviour score between groups, P=1.000.
Table 3. Mean probability of observing piglet postures and activities (and 95% CI) over 4 hours following treatment

<table>
<thead>
<tr>
<th>Item</th>
<th>Castrated</th>
<th>Castrated</th>
<th>Sham-Castrated, no Sucrose</th>
<th>Sham-Castrated, + Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Castrated</td>
<td>Sucrose</td>
<td>Before</td>
<td>+ Sucrose</td>
</tr>
<tr>
<td>Suckling</td>
<td>1.51ab</td>
<td>1.61ab</td>
<td>1.54ab</td>
<td>1.66a</td>
</tr>
<tr>
<td></td>
<td>(1.38-1.70)</td>
<td>(1.48-1.74)</td>
<td>(1.45-1.70)</td>
<td>(1.48-1.91)</td>
</tr>
<tr>
<td>Standing</td>
<td>1.20ab</td>
<td>1.17b</td>
<td>1.17ab</td>
<td>1.20ab</td>
</tr>
<tr>
<td></td>
<td>(1.17-1.23)</td>
<td>(1.12-1.20)</td>
<td>(1.15-1.23)</td>
<td>(1.15-1.26)</td>
</tr>
<tr>
<td>Lying</td>
<td>5.50</td>
<td>5.37</td>
<td>5.50</td>
<td>5.01</td>
</tr>
<tr>
<td></td>
<td>(4.90-6.17)</td>
<td>(4.90-5.75)</td>
<td>(4.90-6.03)</td>
<td>(4.47-5.62)</td>
</tr>
</tbody>
</table>

Note: Values within a row not sharing a lowercased italic letter differs significantly at the $P < 0.05$ level.
Table 4. Cortisol levels (mean nmol\(^1\) ± S.D) in blood serum measured over five treatments before (baseline) and after application of treatment. Least square means for changes in cortisol concentration between points of measurement following application of different treatments

<table>
<thead>
<tr>
<th>Measure</th>
<th>Treatment</th>
<th>Castration Control</th>
<th>Sucrose After</th>
<th>Sucrose Before</th>
<th>Sham Control</th>
<th>Sham control + sucrose</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (n = 20/treatment)</td>
<td>120 ± 79</td>
<td>100 ± 68</td>
<td>104 ± 72</td>
<td>96 ± 53</td>
<td>104 ± 99</td>
<td>31</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>430 ± 116</td>
<td>448 ± 108</td>
<td>382 ± 101</td>
<td>258 ± 116</td>
<td>186 ± 57</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 min</td>
<td>432 ± 134</td>
<td>377 ± 138</td>
<td>471 ± 203</td>
<td>197 ± 90</td>
<td>273 ± 175</td>
<td>41</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Delta 30min - Baseline</td>
<td>328(a)</td>
<td>349(a)</td>
<td>284(a)</td>
<td>178(b)</td>
<td>78(c)</td>
<td>31</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Delta 45min - Baseline</td>
<td>305(a)</td>
<td>271(ab)</td>
<td>354(a)</td>
<td>101(c)</td>
<td>160(bc)</td>
<td>41</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Note: Where means within a row do not share a lowercased italic letter it denotes significance \((P<0.05)\). At 30min there was a tendency for Sugar After to differ from Sugar Before \((P=0.09)\). At 45min there was a tendency for Sugar After to differ from Sham control + sucrose \((P=0.07)\).