RESEARCH REPORT
ANIMAL WELFARE

Title: Towards an improved method of piglet castration to reduce pain: the use of one incision in combination with the use of a Vapocoolant and Metacam™ **(C-17-037)**

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Industry Summary: Two studies were conducted to evaluate the merits of alternative castration methods including: (1) 1 vs 2 cuts during castration; (2) use of topical vapocoolant as an anesthetic, and (3) use of Metacam® NSAID to reduce the pain of castration. Using 1 cut during castration was less painful than when 2 cuts were administered. Vapocooling the scrotum did not reduce signs of pain associated with castration. Metacam® caused only resulted in a small reduction in pain behavior and bruising associated with castration, but on the whole, Metacam® did not reduce overall expression of pain behaviors and physiology in a biologically relevant manner..

Scientific Abstract: Castration is a stressful and painful procedure that can negatively impact the welfare of pigs. Castration is performed to avoid boar taint in the meat of sexually mature male pigs and to reduce aggression. The objectives of this study were to: 1) evaluate the effect of two incisions compared to one incision and the use of a topical vapocoolant (VAPO; ethyl chloride; a topical anesthetic) applied before castration, on measures of performance, behavior and physiology and 2) evaluate the effect of the best method found in objective 1 and the use of Metacam® (an injectable non-steroidal anti-inflammatory drug; NSAID) before castration on measures of performance, behavior and physiology.

The first phase consisted of six treatment groups (N = 27 pigs per treatment) and included: nothing [NO]; sham castrated [SH]; one incision castration [C1]; one incision castration plus VAPO [C1+VAPOAPO]; two incision castration [C2]; two incision castration plus VAPO [C2+VAPO]. Body weights were taken at baseline (TO), at 24, 48, and 72 h, and 7 and 14 d (at weaning) after castration. Behavior measures were collected every 10 min for 1 min for an entire hour and then using 10 min scan samples for 24 h after castration. Blood samples were collected to assess physiological measures at baseline (TO), 15 min (T15), 30 min (T30), 180 min (T180) and 24 h after castration. Vocalizations were measured using STREMODO software from the start to the end of castration. Wound scores were collected daily during 10 d for phase 1 and 14 d for phase 2. In phase 1, performance measures were significantly different among treatment groups for weaning weight (P = 0.0222). C1 pigs C1+VAPOAPO pigs were significantly heavier than the other castrated treatment groups (8.44 ± 0.30 kg and 8.32 ± 0.30 kg, respectively), but not different than NO and SH pigs. Differences in behavior were not significant among treatment groups in phase 1 nor phase 2. Vocalizations were significantly higher for C1 and C1+VAPOAPO pigs (464.20 ± 51.0 db and 333.21 ± 51.0 db, respectively; P = 0.0015). Several physiological measures (hematology and blood chemistry) were

significantly different among treatment groups. Hematological measures mainly identified iron deficiencies and were not clearly due to the effects of castration. These included, mean corpuscle volume (MCV; P = 0.0002), mean corpuscle hemoglobin (MCH; P = 0.0053), mean corpuscle hemoglobin concentration (MCHC; P = 0.0065), and red cell distribution width (RDW; P = 0.0001). Several blood chemistry measures were significant among treatment groups, such as total protein (TP; P = 0.0001.), blood urea nitrogen (BUN; P = 0.0413), and glucose (GLU; P = 0.0233). Treatment differences in cortisol concentrations or wound scores were not observed.

Phase 2 consisted of three treatment groups (N = 40 pigs per treatment) and included: nothing [NO]; one incision castration [C1]; one incision castration plus Metacam® administered 15 min before castration [C1M]. The same measures were collected as in Phase 1. Performance measures did not differ among treatment groups. The behavioral data did not show differences among treatment groups in the first hour post-castration nor during the 24 h post-castration period. Vocalization data did not differ among treatment groups. Blood measures were not significantly different among treatment groups, except for red blood cells (RBC; P = 0.0304). Cortisol values were different among treatment groups (P < 0.05). C1 and C1M cortisol concentrations were greater than the NO treatment group at 15 min post-castration (74.48 ± 9.50 ng/ml, 63.65 ± 8.57 ng/ml and 33.99 ± 9.89 ng/ml, respectively). After 15 min C1M cortisol concentrations were not different than the NO group, while C1 peaked at 30 min and then decreased over time. Wound scores, specifically bruising in the scrotal area was significantly different among treatment groups Metacam® (P < 0.001). On post-castration day (PCD) 1, bruising at or around the site of castration was higher in the C1M group compared to C1 (1.00±0.11 and 0.029±0.10, respectively). On PCD 2, C1 has higher bruising compared to C1M (0.78±0.10 and 0.33 ± 0.09). On PCD 3 the C1M pigs had higher bruising than C1 pigs (2.67± 0.15 and 0.0 ± 0.13). It was speculated that the appearance of delayed bruising on C1M pigs may have been caused by Metacam®.

In conclusion, the data collected gives sufficient insight on the benefits of one incision castration compared to 2-incision castration. However, the data does not support the idea that Metacam® meaningfully reduced the acute pain of castration, as it is evident that pigs still experience stress associated with castration at 15 min post-castration with or without the use of Metacam®. Further research could potentially identify the correct timing for administration and thus, Metacam® could mitigate the pain associated with one incision castration.

Keywords: Pigs, Castration, Behavior, Physiology, Performance

Introduction:

Physical castration is a common procedure performed on young piglets usually at 2-3 days of age and is a standard practice in many countries, including the United States. Castration is a procedure that causes prolonged pain and is detrimental to the welfare of piglets. Therefore, some countries (such as EU and Israel) are incorporating analgesia during castration and other mutilations (Pozzi et al., 2016), or are considering banning castration (some EU member states) completely by 2018 (Spoolder et al., 2016).

Pig are castrated early in life to prevent boar taint in the meat of sexually mature males and to reduce aggressive behavior. During castration one incision can be made or two incisions, one on each side of the scrotum (Prunier et. al., 2006), the testicles are externalized, grasped and pulled out, tearing the spermatic cord or the spermatic cord is cut with the scalpel blade (Hay et al., 2003). Literature is limited on the benefits of one incision versus two incisions, but there is speculation that one incision would be less painful than two, considering that the skin of the scrotum and tissues have sensory and motor innervations (Prunier et al., 2006). Additionally, there is antidotal evidence to suggest wounds heal quicker when only one incision is

made because the amount of tissue damage is less, so pain would be less. However, there is no scientific evidence that suggests which method may be preferable from an animal welfare perspective.

Castration is usually performed without any type of analgesia (Lumb 2007; Hewson et al., 2007; Rault et al., 2011; Hannson et al., 2011) and therefore, it is painful (McGlone and Hellman 1988; Lumb 2007; Fredriksen et al., 2011) and a stressful procedure in young pigs. Various studies have shown that physical castration leads to changes in behavior, which may be indicative of pain (Wemelsfelder and van Putten, 1985; McGlone and Hellman 1988; McGlone et al., 1993; Taylor and Weary 2000; Taylor et al., 2001). Additionally, physical castration can lead to acute changes in physiology including the activation of the hypothalamic-pituitary-adrenal axis (HPA) and activation of the sympathetic nervous system (SNS) (White et al., 1995; Prunier et al., 2001). Physical castration in piglets also induces strong vocal responses (White et al., 1995; Weary et al., 1999; Taylor and Weary 2000; Taylor et al., 2001). Screams (classified as having a higher frequency than a squeal) have been reported as being more predominant during castration without anesthesia (Marx et al., 2003). These high frequency calls are indicative of pain, mainly associated with pulling and severing of the spermatic cord (Horn et al., 1999). Behavioral observations over a period of 5 days showed that physically castrated piglets spent significantly less time at the udder, had decreased activity while awake during the first 2.5 hours, and overall tended to walk more throughout the 5 days post castration (Hay et al., 2003). Behaviors such as stiffness, prostration, and trembling are common for the first few hours after physical castration but in the days following, piglets show other pain-like behaviors like scratching their rump and tail wagging (Hay et al., 2003).

The use of general anesthetics and local anesthetics may be limited by regulations and economics of the swine industry. Currently, the most commonly used local anesthetic is lidocaine (Prunier et al., 2005; Fredriksen and Nafstad 2006). However, lidocaine injected into the scrotum and testicles can also cause additional pain and distress to piglets. Furthermore, lidocaine concentration is highest 3 min after injection and thus, piglets would have to be castrated within that time period, which may be impractical at farms because of the increased time and stress caused by repeatedly handling of pigs to administer the injection and then to castrate them (Sutherland et al., 2010). Vapocoolant sprays are topical anesthetics with immediate skin anesthesia by rapid evaporation of volatile liquid, which produces a drop in skin temperature, resulting in temporary and swift interruption of skin sensation (Dalvandi et al., 2017). Vapocoolants have previously been used for breast needle biopsies, during intravenous cannulation (Reis et al., 1997; Costello et al., 2006), and prior to immunizations (Farion et al., 2008; Mawhorter et al., 2009; Hijazi et al., 2009) in humans. In livestock, they have been used to reduce pain of ear tagging and ear notching in unweaned calves (Lomax et al., 2016). Although, the use of vapocoolants in humans is widely reported to reduce pain prior to painful procedures, its use in farm/livestock animals is vaguely reported, but could reduce the pain associated with castration incisions.

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) that has analgesic effects due to its peripheral anti-inflammatory actions (Keita et al., 2010). Meloxicam has been researched extensively for its post-operative analgesic effects in multiple species, including humans and pigs. Meloxicam has marketing authorization for use in non-infectious locomotor disorders to reduce symptoms of lameness and inflammation, but more recently for relief of post-operative pain associated castration in piglets (Metacam™, 5 mg mL-1 solution for injection) (Keita et al., 2010; Pozzi et al., 2016). Heinritzi et al. (2006b) reported that 2mg/kg BW meloxicam given intramuscularly 15 minutes before castration prevented a rise in cortisol concentration after castration, suggesting that it was effective in reducing pain. Therefore, once incision castration with the use of a Vapocoolant in combination with Metacam™ (0.4mg/kg BW, according to the manufacturer label) could possibly reduce some of the pain associated with castration compared to not using anything at all.

Objectives:

1) To evaluate the effect of two incisions compared to one incision and the use of a Vapocoolant (ether chloride) applied before castration, using measures of behavior, performance and physiology.
2) To evaluate the effect of the best method found in objective 1 and the use of Metacam™ before

castration using measures of behavior, performance and physiology.

Materials & Methods:

All animal procedures were approved by the Texas Tech University (TTU) Animal Care and Use Committee. The experiment was conducted partially at the Texas Tech University New Deal Swine Facility and a commercial site.

Study 1

All male piglets were identified after farrowing and weighed the day prior to the study to assign them to one of six treatment groups (N = 27 pigs per treatment). Treatment groups consisted of nothing [NO]; sham castrated; SH; one incision castration ± a vapocoolant, C1 or C1+VAPO; two incision castration ± a vapocoolant (VAPO), C2 or C2+VAPO. The NO pigs did not undergo any surgical procedures (but were handled and blood sampled in the same way as the other treatments). SHAM pigs were handled in the same manner as all the other treatment groups, they were blood sampled, underwent simulated castration (with a touch on the scrotum with a scalpel handle), and applied a stream of cooled saline.

On the day of the study, pigs (3 d of age) were removed from their farrowing crate, weighed, and marked with livestock chalk according to their treatment group (also identifiable by ear notch). Randomly selected focal pigs (15 pigs/treatment group) were placed in a V trough where a baseline blood sample was taken. Pigs in the C1+VAPO and C2 + VAPO group were sprayed with the vapocoolant 10 cm away from each scrotum for 3 secs and given 10 sec for it to take effect. The vapocoolant was then wiped off the skin with alcohol and the castration procedure performed within 10-12 sec of application (for two incisions spraying the open incision was avoided by holding a hand over the incision while holding the opposite testicle and spraying). Pigs in the SH group were sprayed in the same manner but with cooled saline. Pigs were handled once if in the NO treatment for identification, weighing, and blood sampling. All other treatment groups were handled twice for identification, weighing, and blood sampling and then a second time for application of VAPO or SHAM and castration. Castration (treatments C1, C2, C1 and C2 + VAPO) was conducted by placing the pigs upside down between the handler's legs, exposing the anogenital region and making either one or two vertical incisions on the scrotum to extract the testicle/s. Once the testicles were extracted, pigs were ear notched and tail docked (as requested by the facility), and an antiseptic (iodine spray) was topically administered to the incision site/s and tail. Piglets were then return to the farrowing crate with the sow.

Study 2

Based on the findings from Study 1, all male pigs (3 d of age) were identified and weighed the day prior to beginning the study. Pigs (N = 40 pigs per treatment) were assigned one of three treatment groups (nothing, NO; one incision castration, C1; one incision castration plus an injection of Metacam®, C1M). On the day of the study, pigs were removed from their farrowing crate, weighed, marked with livestock chalk according to their treatment group (also identifiable by ear notch) and randomly selected focal pigs (20 pigs/treatment group) were placed in a V trough where a baseline blood sample was taken. Pigs in the C1M group were injected with Metacam® (0.4mg/kg BW; depending on the treatment group) intramuscularly behind the ear in the order in which they were picked up (15 min prior to castration; and castrated in the same order they were first injected to castrate in the order of time lapse since injection). All pigs were handled twice for identification, weighing, blood sampling, intramuscular injection (only if in the C1M group) and then a second time for castration. Castration was conducted by placing the pigs upside down between the handler's legs, exposing the anogenital region and making one vertical incision on the scrotum to extract the testicles. Once the testicles were extracted, pigs were each notched and tail docked, and an antiseptic (iodine spray) was topically administered to the incision site and tail. Piglets were then return to the farrowing crate with the sow.

Performance Measures

Pigs were weighed by placing the piglets in a tub on top of a digital scale. Weights were collected prior to beginning any procedures (baseline), 24 h, 48 h and 72 h (because 24 h may only represent gut fill) post treatment and at weaning (14 days). Weight change was calculated to determine the effect of the treatments.

Behavior Measures

Pig vocalizations and behaviors during castration were recorded using a camcorder. STREMODO (Stress-Scream Monitor and Documentation Unit), which uses linear prediction analysis to extract features of calls and classifies them as stress calls was used for analysis of vocalizations during castration (Schön et al., 2001; Manteuffel, G. & Schön, P. C. 2002). After castration, piglet behaviors were observed every ten minutes for one minute for one hour. After the first hour, behavior was recorded via 10-minute scan samples for 24 hours using digital video recorders (DVR). Six trained and validated observers recorded walking, sitting, nursing, fighting, tail wagging, rump scratching, chomping, thrashing and other pain-like behaviors as described by Sutherland et al., 2012.

Physiological Measures

Blood was collected via jugular venipuncture (approximately 2 ml of blood per piglet was collected) from randomly selected focal pigs (15 pigs/treatment group). Blood collection was performed prior to any procedures (baseline), and at the time in which cortisol is reported to peak (Prunier et al., 2006), at 15, 30, and 180 min and at 24 h. Blood was examined for changes hematological and blood chemistry changes that could occur during acute stress, such as white blood cell (WBC) counts, differential counts for WBC populations, Neutrophil: Lymphocyte ratio (NL normally increases with stress), hematocrit (HCT), red blood cells RBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW), glucose (GLU), total protein (TP), alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine (CRE).

Wound Healing

Wounds were assessed to determine possible detrimental effects due to castration or analgesic treatment according to Sutherland et al. (2010). Pigs were numbered with colored livestock chalk to be able to identify them through video recordings. This also aided in preventing any bias while assessing wound scores/bruising. Pictures were taken and wounds were scored later with only the wound visible to the observer.

Wounds after castration were scored daily for incision healing, using a 1 - 6 scale (1 = completely healed with no scab and 6 = fresh blood still present at the wound) and bruising, using a scale of 1- 4 (developed by this group; where 1 = one fourth of the scrotal area bruised, 2 = half of the scrotal area bruised, 3 = three fourths of the scrotal area bruised, 4 = the entire scrotal area bruised. Wounds and bruising were recorded daily over a period of 10 d for Study 1 and 14 d for Study 2.

Data Analyses

Study 1

The study was a completely randomized design. Each treatment had 27 pigs, with each treatment represented within each litter, for a total of 162 pigs. The experimental unit was sow or crate. The model had a repeated structure over time. For all measures, the main fixed effects were treatment, time, and crate. The interaction between treatment and time were included in the model with litter included as a random effect.

Study 2

The study was a completely randomized design. Each treatment had 40 pigs, represented within each litter, for a total of 120 pigs. The experimental unit was sow or crate. The model had a repeated structure on time. For all measures, the main fixed effects were treatment, time, and crate. The interaction between treatment and time were included in the model with litter included as a random effect.

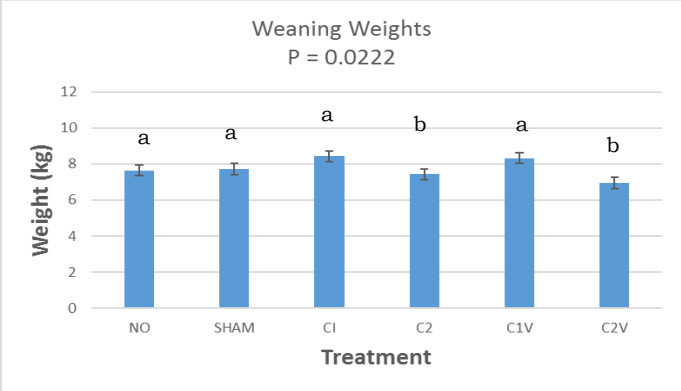
Results

Study 1

1.1 Performance

Castration method and the use of a vapocoolant had a significant effect on weaning weights (Figure 1; $P < 0.0222$). Pigs castrated with one incision (C1) and one incision plus vapocoolant (C1+VAPO) had significantly higher weaning weights than C2 and C2+VAPO castration groups (8.44 ± 0.30 and 8.32± 0.30, respectively), but were not different than NO and SH pigs.

Figure 1. LS means ± SEM for weaning weights of pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 2 incision, C2; or simulated castration, SHAM; or not castrated, NO) with or without the use of a vapocoolant (V). N= 12 pigs per treatment group.



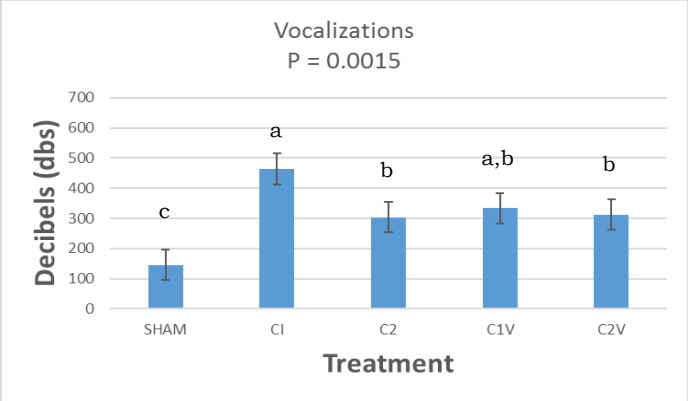
1.2 Behavior

Pig behavior did not significantly differ among pigs, regardless of the treatment.

1.3 Vocalizations

Stress vocalizations recorded during castration were significantly different among treatment groups (Figure 2: $P = 0.0015$). Pigs in the C1 treatment groups had higher pitched stress vocalizations than all the other treatment pigs but did not differ from C1+VAPO (464.20 ± 51.0 and 333.21 ± 51.0, respectively). C1+VAPO did not differ from C2 and C2+VAPO.

Figure 2. LS means of the vocalizations of pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 2 incision, C2; or simulated castration, SHAM) with or without the use of a vapocoolant (V). N= 12 pigs per treatment group.



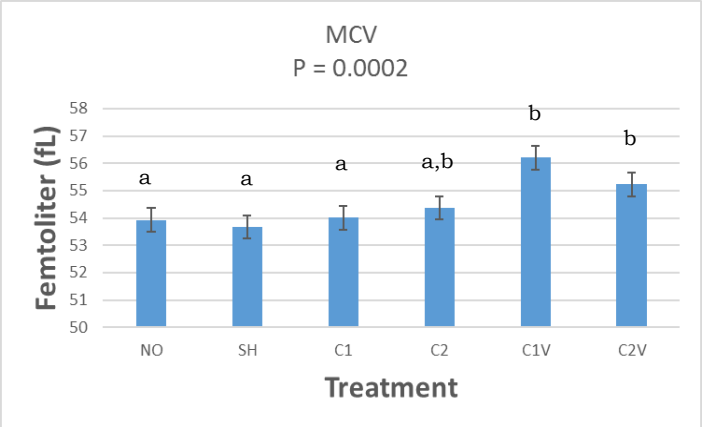
1.4 Hematology and Blood Chemistry

The overall P-values for hematological and blood chemistry values are listed on Table 1.

1.4.1 Mean Corpuscle Volume

Mean corpuscle volume (MCV) was significantly different among treatment group (Figure 3; P = 0.0002). MCV levels were higher in C1+VAPO and C2+VAPO pigs compared to other pigs (56.02 ± 0.43 and 55.23 ± 0.43, respectively; P < 0.05). However, C2 pigs (54.36 ± 0.43) were not significantly different that the C1+VAPO or C2+VAPO pigs nor other treatment groups.

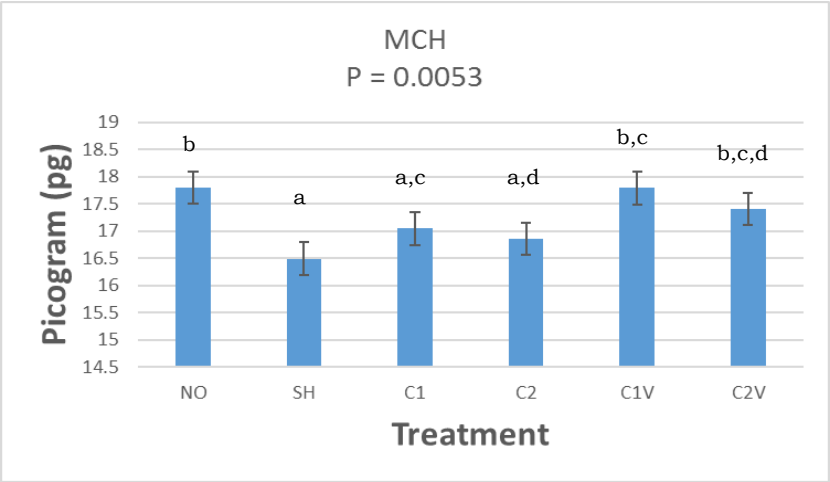
Figure 3. LS means ± SEM for mean corpuscle volume (MCV) of pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 2 incision, C2; or simulated castration, SHAM; or not castrated, NO) with or without the use of a vapocoolant (V). N = 6 pigs per treatment group.



1.4.2 Mean Corpuscle Hemoglobin

Mean corpuscle hemoglobin (MCH) was significantly different among treatment groups (Figure 4; P = 0.0053). SH pigs had the lowest values but they were not different than C1 and C2 pigs (16.49 ± 0.30, 17.05 ± 0.30 and 16.86 ± 0.30, respectively).

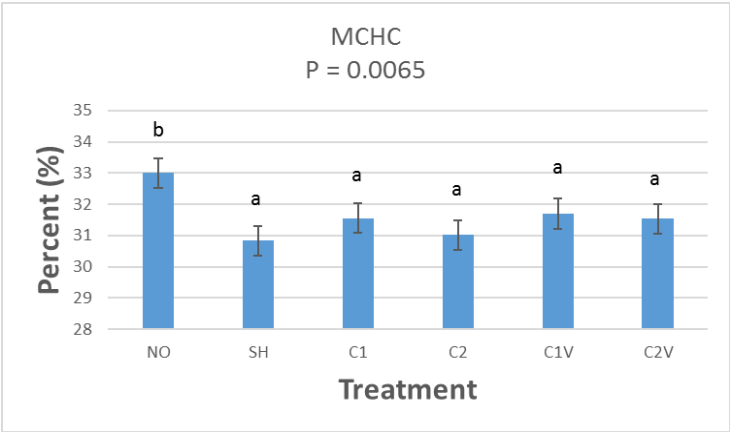
Figure 4. LS means ± SEM for mean corpuscle hemoglobin (MCH) of pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 2 incision, C2; or simulated castration, SHAM; or not castrated, NO) with or without the use of a vapocoolant (V). N = 6 pigs per treatment group.



1.4.3 Mean Corpuscle Hemoglobin Concentration

Mean corpuscle hemoglobin concentration (MCHC) was significant higher in NO pigs compared to all other treatment groups (Figure 5; P = 0.0065).

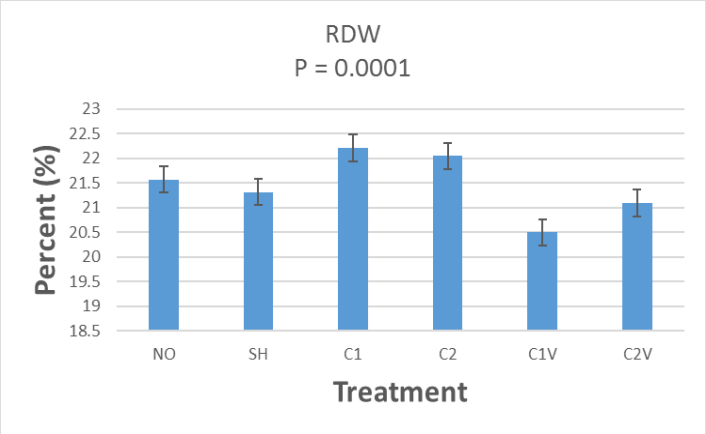
Figure 5. LS means ± SEM for mean corpuscle hemoglobin (MCH) of pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 2 incision, C2; or simulated castration, SHAM; or not castrated, NO) with or without the use of a vapocoolant (V). N = 6 pigs per treatment group.



1.4.4 Red Cell Distribution Width

Red cell distribution width (RDW) was significantly different among treatment groups (Figure 6; P = 0.0001). C1+VAPO pigs had the lowest value but were not different than C2+VAPO pigs (20.50 ± 0.27 and 21.09 ± 0.27, respectively).

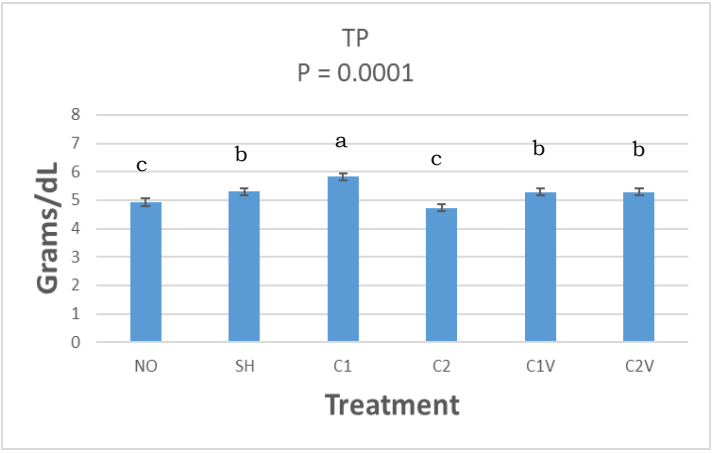
Figure 6. LS means ± SEM for red cell distribution width (RDW) of pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 2 incision, C2; or simulated castration, SHAM; or not castrated, NO) with or without the use of a vapocoolant (V). N = 6 pigs per treatment group.



1.4.5 Total protein

Total protein (TP) was significantly different among treatment groups (Figure 7; P = 0.0001). C2 treatment pigs had a lower value than the other treatment pigs but was not different than the NO pigs (5.29 0.13 ± and 4.92 ± 0.13, respectively). The C1 treatment group had the highest TP levels compared to all other treatment groups (P < 0.05).

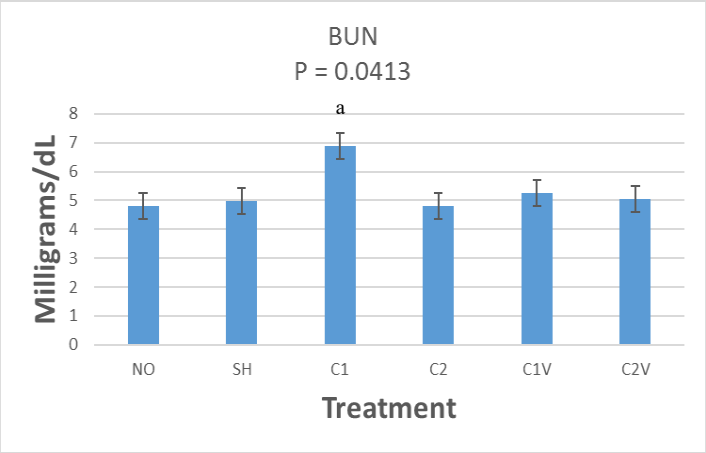
Figure 7. LS means ± SEM for total protein (TP) for pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 2 incision, C2; or simulated castration, SHAM; or not castrated, NO) with or without the use of a vapocoolant (V). N= 6 pigs per treatment group.



1.4.6 Blood urea nitrogen

Blood urea nitrogen was significantly different among treatment groups (Figure 8; P = 0.0413). The C1 treatment group had the highest BUN value compared to all other groups (6.89 ± 0.45; P < 0.05). There was no difference among the other treatment groups.

Figure 8. LS means ± SEM for blood urea nitrogen (BUN) for pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 2 incision, C2; or simulated castration, SHAM; or not castrated, NO) with or without the use of a vapocoolant (V). N = 6 pigs per treatment group.



1.4.7 Glucose

Glucose (GLU) was significantly different among treatment groups (Figure 9; P = 0223). GLU was the highest for NO and C2 pigs (123.90 ± 4.10 and 123.14 ± 4.10, respectively). Although both of these groups were not significantly different than SH pigs. C1, and C1+VAPO and C2+VAPO were not significantly different than each other (107.35 ± 4.10, 116.77 ± 4.10, and 116.77 ± 4.10, respectively).

Figure 9. LS means ± SEM for glucose (GLU) for pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 2 incision, C2; or simulated castration, SHAM; or not castrated, NO) with or without the use of a vapocoolant (V). N= 6 pigs per treatment group.

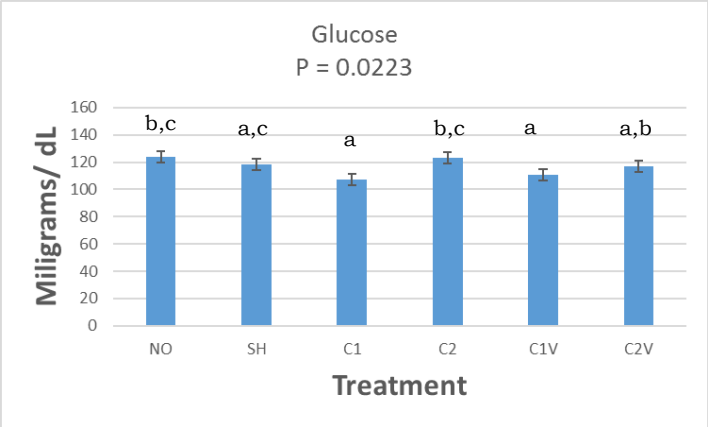


Table 1. P values and SE for hematological and chemistry values of pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 2 incision, C2; or simulated castration, SHAM; or not castrated, NO) with or without the use of a vapocoolant (V). N= 6 pigs per treatment group.

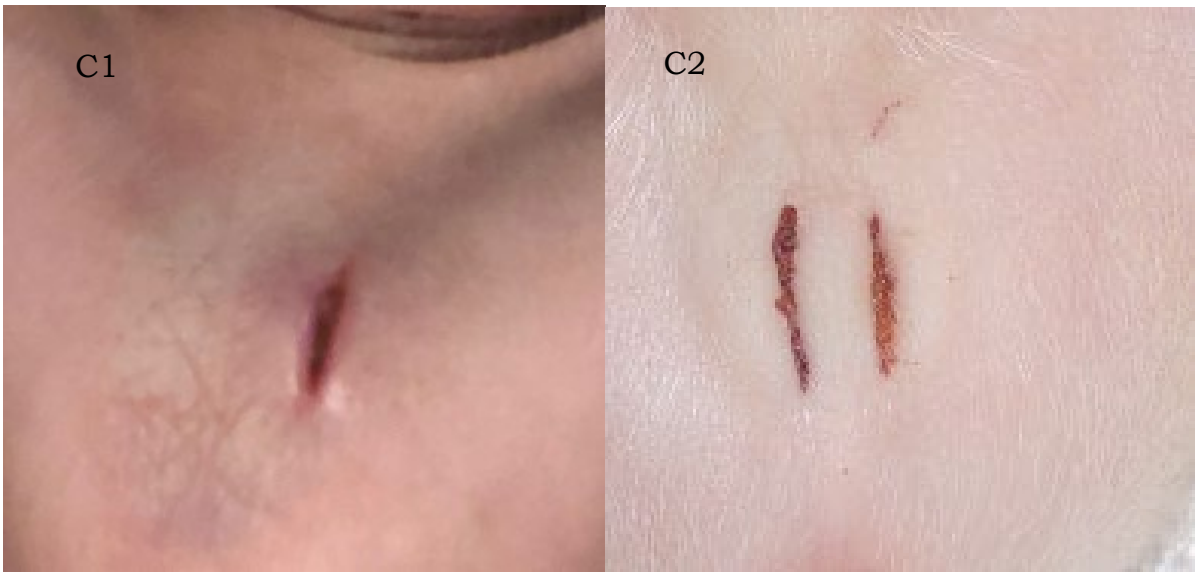
HEMATOLOGY		
Measure	**SE**	**P Value**
WBC	0.543	0.1317
LYM	3.008	0.1039
MON	0.494	0.9849
NEU	3.087	0.1448
RBC	0.175	0.0981
HGB	0.299	0.3264
HCT	0.886	0.1202
MCV	0.433	**0.0002**
MCH	0.301	**0.0053**
MCHC	0.475	**0.0065**
RDW	0.266	**0.0001**
PLT	32.930	0.0933
PCT	0.328	0.0616
MPV	0.197	0.2266
PDW	0.549	0.0600
BLOOD CHEMISTRY		
ALP	147.166	0.6457
ALT	1.367	0.3368
BUN	0.452	**0.0413**
CRE	0.026	0.5286
GLU	4.111	**0.0223**
TP	0.130	**0.0001**
Cortisol	12.6	0.5333

1.5 Cortisol

Cortisol values were not significant different among treatment groups.

1.6 Wound Scores

Post-castration wound scores did not significantly differ among treatment groups. Figure 10 shows the placement and size of incisions.



Study 2

2.1 Performance

Body weight did not significantly differ among treatment groups at baseline, 24 h, 72 h, 7 d, nor 14 d post castration (P = 0.9283).

2.2 Behavior

Pig behavior did not significantly differ among pigs, regardless of the treatment.

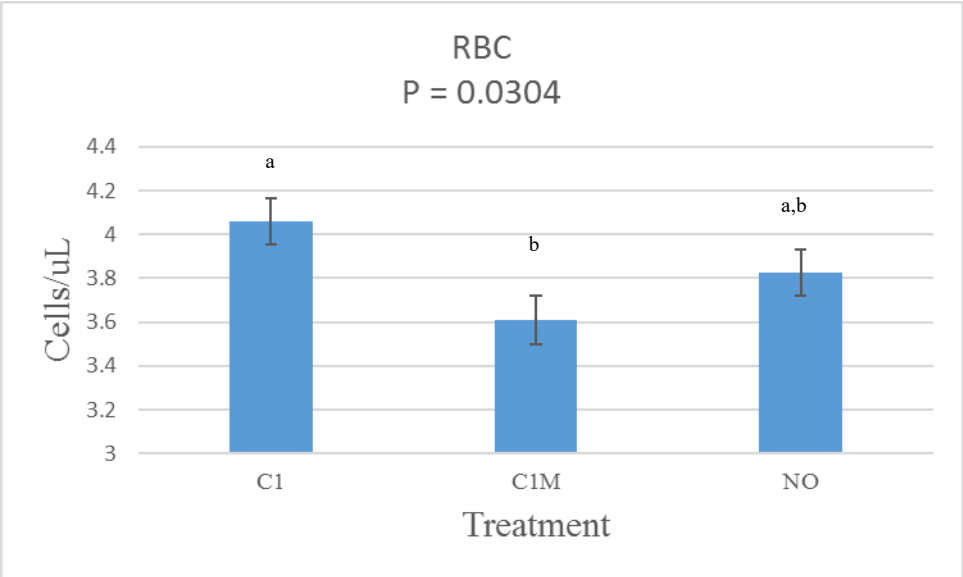
2.3 Vocalizations

Vocalizations among treatment groups were not significantly differ among treatment groups during castration (P = 0.9369).

2.4 Hematology and Blood Chemistry

Blood measures were not significantly different among treatment groups, except for red blood cells (P = 0.0304; Figure 11). Pigs in the C1 group had higher levels of RBC's compared to the C1M group (4.06±0.11 and 3.61±0.11, respectively). However, C1 and C1M groups were not different than the NO group treatment group (3.82±0.10).

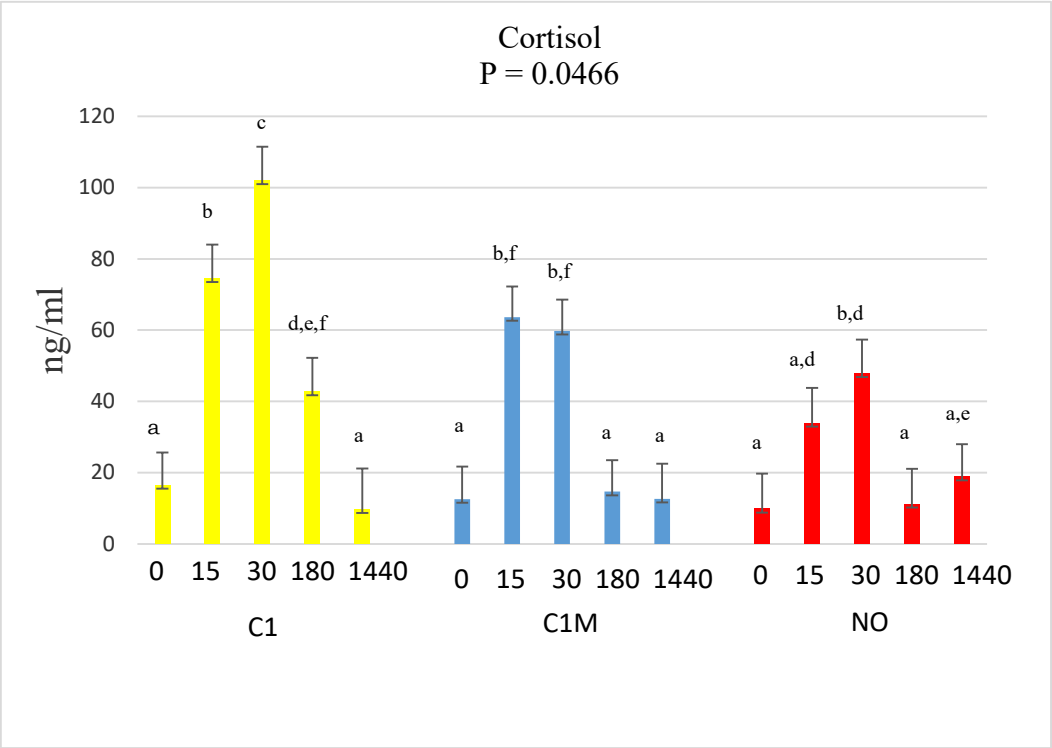
Figure 11. N= LS means ± SEM for red blood cells (RBC) for pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 1 incision plus Metacam®; C1M, or not castrated; NO). N= 12 pigs per treatment group.



2.5 Cortisol

Cortisol values were significantly different among treatment groups over time (Figure 12; P = 0.0466). At time 0 (baseline) all treatment groups had similar blood cortisol values. At 15 min post castration (T15) both C1 and C1M experienced an increase in cortisol concentrations significantly greater than the NO group but not different than each other (74.48 ± 9.50 ng/ml , 63.65 ± 8.57 ng/ml and 33.99 ± 9.89 ng/ml, respectively). At T30, the C1 group had a greater cortisol concentration (74.47±9.5) than both the C1M and NO group (59.76±8.85 and 49.11±9.5, respectively). At T180, C1 had a higher cortisol concentration than C1M and the NO group (42.69±9.50, 14.65±8.85 and 11.18±9.90, respectively) but the C1M group was not significantly different than the NO group.
At T1440 (24 h post-castration) there was no significant difference among treatment groups.
The cortisol findings support the use of Metacam® in reducing stress associated with castration at T30 and T180 post-castration. At T15, the stress of castration was high regardless of the use of Metacam®. This may indicate that administration of the product was not effective at the time given and may need to be administered with more than 15 min prior to castration.

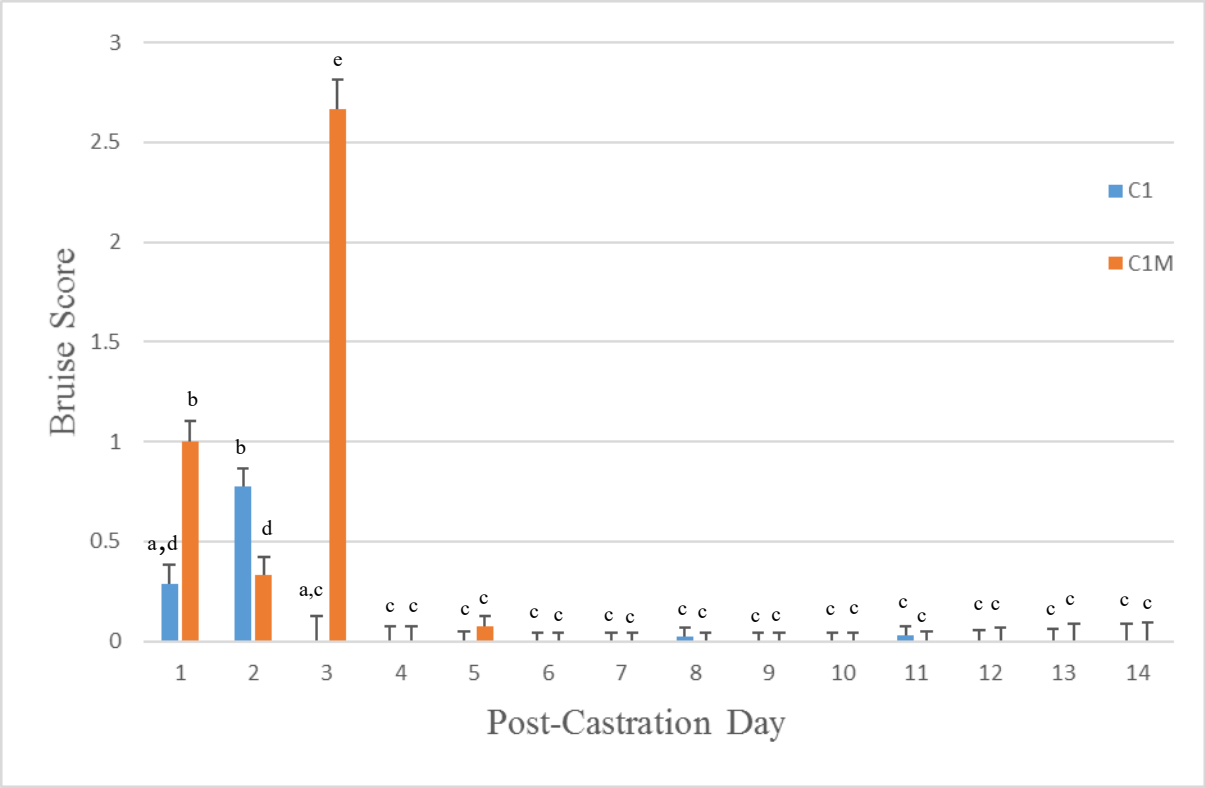
Figure 12. LS means ± SEM for cortisol for pigs castrated at 3 days of age using two methods of castration (1 incision, C1; 1 incision plus Metacam®; or not castrated, NO).
N= 12 pigs per treatment.



2.6 Wound Scores/Bruising

Wound scores, specifically bruising was significantly different among C1 and C1M treatment groups over time (post-castration day; PCD; Figure 13; $P < 0.001$). On PCD 1, bruising at or around the site of castration was higher in the C1M group compared to C1 (1.00±0.11 and 0.029±0.10, respectively). On PCD 2, C1 has higher bruising compared to C1M (0.78±0.10 and 0.33 ± 0.09). On PCD 3 the C1M pigs had higher bruising than C1 pigs (2.67± 0.15 and 0.0 ± 0.13). Within the C1 treatment group, bruising was observed to be lower on PCD 1 than PCD 2 (0.29±0.10 and 0.78±0.09). Within the C1M treatment group, bruising was observed to be higher on PCD 1, was lower on PCD 2, and then increases on PCD 3 (1.0±0.11, 0.33±0.09, and 2.67±0.15, respectively).

Figure 13. LS means ± SEM for wound scores/bruising for pigs castrated at 3 d of age using two methods of castration (1 incision, C1; 1 incision plus Metacam®; or not castrated, NO). N = 14 pigs per treatment.



Discussion and Conclusion

Castration is a stressful and painful procedure for pigs. However, finding a product that can reduce the pain of castration has proven to be challenging, as it can be time consuming to apply and expensive. The main goal of this study was to identify if modification of castration technique such as one incision versus two incisions would improve pig welfare, based on measures of performance, behavior and physiology. An additional goal was to determine if analgesics, such as a topical vapocoolant or an injectable analgesic, such as Metacam®, would mitigate the pain during or after castration or both.

This research was split into two studies. The first study evaluated whether one or two incisions made at the time of castration and the application of a vapocoolant effected the pain of castration based on measures of performance, behavior and physiology.

Based on performance measures, pigs castrated with one incision (C1) and one incision plus vapocoolant (C1+VAPO) had significantly higher weaning weights than the pigs castrated with two incisions with or without vapocoolant (C2 or C2+VAPO). However, it was less than 0.5 kg.

The behavioral data showed no differences among treatments in the hour post castration nor during the 24 h period the pigs were observed. This is in contrast to other studies that have shown that there are behavioral changes after castration (McGlone et al., 1993; Hay et al., 2003; Sutherland et al., 2010). All of the pigs were handled multiple times after castration for weighing and blood sampling, which is stressful and may have affected their behavior by masking some of the effects of castration among treatment groups. Vocalization data did show a difference among treatment groups. Pigs in the C1 and C1+VAPO treatment groups had higher pitched squeals but unlike C1, C1+VAPO did not differ from the C2 and C2+VAPO treatment groups. It was observed that it is more difficult to grasp the second testicle out of one incision versus two incisions. Although the person castrating was properly trained on how to conduct the procedure and the person remained the same throughout the study, they communicated that one incision castration is more tedious and it seemed that manipulation of the testicle was greater than in two incision castration. This

may have affected the vocalization data as the pigs would have squealed at a greater intensity for a longer period than the other treatment groups.

Physiological measures assessed included blood hematology and chemistry. Mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH), mean corpuscle hemoglobin concentration (MCHC), and red cell distribution width (RCDW) were different among treatment groups. Based on Szudzik et al. (2018), anemia in suckling pigs can be characterized by a decrease in red blood cell (RBC) parameters such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Normal MCV values range from 50-68 fL with an average of 60 fL (Clark et al., 2009). MCHC is the average weight of the hemoglobin based on the volume of RBCs. Low levels of MCHC, can be related to a decreased level of hemoglobin, again possibly due to anemia. The normal values range from 30-34% with an average 32% (Clark et al., 2009). MCH refers to the average amount of hemoglobin present in the red blood cells (RBCs). It can reflect some of the same deficiencies as MCV. The normal values range from 17-21 pg with an average of 19 pg (Clark et al., 2009). RDW can also be an indicator of iron deficiency. The normal values range between 16-33% with a mean of 24% (Clark et al., 2009). All pigs were within normal values or slightly below the normal values. These values should not have been affected by castration.

Blood chemistry measures such as total protein (TP), blood urea nitrogen (BUN), and glucose (GLU) were found to be different among treatment groups. Total protein was higher in the C1 treatment group, which may indicate they were possibly not nursing as much, or they experienced more stress and protein degradation . Total protein and albumin concentrations are markers for protein homeostasis, which increase with dehydration (Sutherland et al., 2013). However, reduced nursing behavior is not supported by the behavior data collected, as it showed no difference in nursing behavior among treatment groups. Additionally, the stress of handling and bleeding may have affected these values. This can be seen in the difference in TP values for NO and SH pigs. SH pigs were exposed to handling of bleeding and weighing plus sham castration (which may have added more stress), possibly leading to the TP difference between NO and SH pigs. BUN values were also higher for the C1 treatment compared to the other treatment groups. Blood urea nitrogen is a metabolic waste product in the blood generated from the breakdown and probably due to muscle and protein break down caused by muscle exertion, and has been reported during transport (Sutherland et al., 2013). GLU was significantly different among treatment groups. GLU was the highest for NO and C2 pigs but both of these groups were not significantly different than SH pigs. C1, C1+VAPO and C2+VAPO were not significantly different than each other. There was not a time effect. This may indicate that pigs continued to nurse during the 24 post-castration. A difference in cortisol values among treatment groups was not identified.

Wound scores were also not different among treatment groups. This is in contrast to Sutherland et al. (2010) who used a topical anesthetic (lidocaine) to reduce the pain of castration and observed differences in wound healing at 9 and 14 d.

Based on measures of performance, behavior, and physiology it was determined that one incision castration would be used versus C1+VAPO because the differences were not all that great between the two treatments and that data was not sufficiently clear to truly conclude that C1+VAPO was better than C1. C1+VAPO did not have a significant effect on reducing the pain of the incisions made during castration. In fact, when the castration incisions were made, pigs were not observed to squeal but mainly grunt. When the testicles were gripped and the spermatic cord was cut, pigs squeal at high frequencies and intensities. This is in agreement with other studies that have suggested that cutting the spermatic cord is painful (Horn et al., 1999), significantly more than the incision (Taylor and Weary, 2000). Thus, C1 was chosen to be part of Study 2.

Study 2 consisted of evaluating if one incision castration and the use of an anti-inflammatory 15 min prior to castration would reduce the pain of castration based on behavior, performance, and physiology. Performance measures did not differ among treatment groups. The behavioral data showed no difference among treatment groups in the hour post castration nor during the rest of the 24 h period the pigs were observed. Vocalization data also did not differ among treatment groups. Blood measures were not significantly different among treatment groups, except for red blood cells ($P = 0.0304$; Figure 10).

Physiological measures included blood hematology and chemistry. Blood measures were not significantly different among treatment groups, except for red blood cells. C1 pigs had higher values than C1M pigs and No pigs. This may be due to a disruption in RBC synthesis, and blood clotting that may be impacted negatively by Metacam®. Cortisol values were different among treatment groups. The cortisol findings support the use of Metacam® in reducing stress associated with castration at T30 and T180 post-castration. At T15, the stress of castration was high regardless of the use of Metacam®. This may indicate that administration of the product was not effective at the time given and may need to be administered more than 15 min prior to castration.

Wound scores, specifically bruising in the scrotal area was significant among treatment groups. The differences in bruising may have been due to the poor visibility of the bruising due to swelling. On PCD 1 there seemed to be more swelling on C1 pigs and therefore, it was easier to score bruising on C1M pigs. This may have been due to the effect of the anti-inflammatory, Metacam®, but it seemed that swelling may have just been delayed because on PCD 2 it was difficult to score C1M pigs due to swelling. Once the swelling went down, bruising was more evident in the pictures. Pictures were taken instead of live assessments because the person assessing the wound scores was able to identify pig treatment groups by the color on the pig's back.

In conclusion, the data collected gives sufficient insight on the benefits of one incision castration compared to two incision castration. The use of a VAPO has not effect on reducing the pain associated to castration incisions. Additionally, the data collected does not give sufficient insight on whether Metacam® completely reduced the acute pain of castration, as it is evident that pigs still experience stress associated with castration at 15 min post castration with or without the use of Metacam®. Further research could potentially identify further positive and negative effects of Metacam®, the correct timing for administration to truly identify if it could mitigate the pain associated with one incision castration.