

**Title:** Methods to reduce the pain associated with castration – **NPB #08-084**

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### Industry Summary:

Surgical castration of male piglets is a common management practice carried out on commercial swine farms to prevent aggressive behavior and the occurrence of boar taint. However, the procedure of surgical castration causes acute pain induced distress which is an animal welfare concern. The objective of this study was to examine novel methods (needle-free injection systems or topical) to potentially alleviate the pain induced distress caused by castration in piglets as measured by known physiological and behavioral indices of castration stress in piglets. Physiological and behavioral measures of distress were not reduced by any of the analgesic methods used in this study. Furthermore, wound healing was delayed by applying short acting topical anesthetic to the wound. The needle-free system was as effective as the needle injection method. In the current study, the use of a short or long acting topical anesthetic was not effective in reducing the pain induced distress caused by castration in piglets. Further research is needed to evaluate alternative practical methods to reduce the pain caused by castration in piglets' on-farm.

### Scientific Abstract:

Surgical castration of male piglets is a common management practice carried out on commercial swine farms to prevent aggressive behavior and the occurrence of boar taint. However, the procedure of surgical castration causes acute pain induced distress which is an animal welfare concern. The objective of this study was to examine novel methods (needle-free injection systems or topical) to potentially alleviate the pain induced distress caused by castration in piglets as measured by known physiological and behavioral indices of castration stress in piglets. At 3 d of age, seven weight matched piglets from 10 sows were allocated to one of seven treatment groups. Treatments included: 1) Sham castration (CON; n = 10); 2) Surgical castration (CAS; n = 10); 3) Castration plus local anesthetic administered 10 minutes prior to castration using a conventional needle and syringe (LA-10; n = 10); 4) Castration plus local anesthetic administered just prior to castration using a conventional needle injection (LA-0; n = 10); 5) Castration plus local anesthetic administered just prior to castration using a needle-free injection system (Pulse; n = 10); 6) castration plus long acting topical

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anesthetic applied to the castration wound (LONG; n = 10), and 7) castration plus short acting topical anesthetic applied to the castration wound (SHORT; n = 10). Blood samples were collected from pigs prior to and 30, 60, 120, and 180 min after castration to measure leukocyte and differential counts and cortisol concentrations. The above experiment was repeated without blood collection and behavior was recorded using 1-min scan sampling for 60 min prior to and 180 min after castration with or without analgesia and control handling. All piglets were weighed prior to and 24 h after castration and wound healing was recorded daily for 14 d after castration. Cortisol concentrations were elevated ( $P < 0.06$ ) in CAS, LA0, LA10, Pulse, SHORT, and LONG piglets compared with CON pigs 30 and 60 min after castration. Cortisol concentrations were elevated ( $P < 0.05$ ) in LONG piglets 30, 60, 120, and 180 min after castration compared with CON piglets. Piglets injected with local anesthetic 10 min prior to castration vocalized less ( $P < 0.005$ ) than piglets castrated without analgesia. Body weight change did not differ ( $P > 0.05$ ) among treatments 24 hours after castration or control handling and wound healing scores were greater ( $P < 0.05$ ) in SHORT piglets compared with all other treatments. In the current study, the use of local anesthetic administered at the time of castration or topical anesthetic was not effective in reducing the pain induced distress caused by castration in piglets. While long-lasting analgesic application may reduce short-term pain, it induced a longer-term elevation in blood cortisol concentrations. Long-lasting analgesics given with castration are not advised. Further research is needed to evaluate alternative practical methods to reduce the pain caused by castration in piglets' on-farm.

### **Introduction:**

Surgical castration of male piglets is a common management practice carried out on commercial swine farms to prevent aggressive behavior and the occurrence of boar taint. However, the procedure of surgical castration causes acute pain induced distress which is an animal welfare concern. Negative public perception concerning castration without the use of analgesics or anesthetics is increasing. Norway has banned castration of piglets from January 2009. Animal activists are currently suing the state department of agriculture in New Jersey, stating castration is an act of cruelty and is in violation of their standards for humane care of farm animals. Alternatives to castration include a costly approach to slaughter pigs before they reach sexual maturity (Dunshea et al., 2001) or using immunocastration techniques, however slaughtering pigs at less than 200 pounds is not economically viable in the USA and currently there are no FDA approved immunocastration products in the USA. Therefore it would be beneficial to the welfare of the pig and the swine industry to develop commercially viable ways to reduce the pain induced distress caused by castration in piglets.

Surgical castration is the most common technique to castrate piglets in the USA. Surgical castration involves making one or two incisions on either side of the scrotum, removing the testes, and severing the spermatic cords, usually by pulling. However, surgical castration causes physiological and behavioral changes indicative of acute pain. Physiological indicators of stress including cortisol and adrenocorticotropic hormone secretion, lactate (Prunier et al., 2005), mean arterial blood pressure, electroencephalography (Haga and Ranhein, 2005), and heart rate (White et al., 1995) have been shown to change significantly in response to surgical castration of piglets. Surgical castration in piglets causes behavioral changes such as vocalization, and reduced activity, and nursing (McGlone and Hellman, 1988; Taylor et al., 2001; Hay et al., 2003; Carroll et al., 2006; Moya et al., 2007). Age of the pig at castration does not appear to influence the acute behavioral or physiological response to castration (McGlone et al., 1993; Taylor et al., 2001; Carroll et al., 2006). Therefore, surgical castration causes significant physiological and behavioral changes indicative of acute pain and distress in piglets.

The major reasons to castrate male piglets are to prevent boar taint and to reduce aggressive behavior. The main molecules responsible for boar taint are androstenone, a testicular steroid that accumulates in the fat tissue exhibiting a urine-like odor, and skatole, a product of tryptophan

breakdown in the gut that exhibits a fecal-like odor (Bonney, 1998). Androstenone and skatole concentrations are caused by testicular steroids and are influenced by weight, age, nutrition, and genetics (Dunshea et al., 2001; Zamaratskaia et al., 2004). Androstenone and skatole concentrations and aggressive behavior increase in intact male pigs at puberty (Cronin et al., 2003; Zamaratskaia et al., 2004). Alternative methods to castration include (a) immunocastration, (b) slaughtering pigs before they reach sexual maturity, or use of anesthetics/analgesics. Immunocastration involves immunizing boars against gonadotropin releasing hormone (GnRH), which uses the boars own immune system to suppress GnRH consequently shutting down the stimulus to the testes resulting in a temporary inhibition of testicular function (Thun et al., 2006). Pfizer Animal Health is currently developing an immunocastration product labeled Improvac™, but this product is currently not approved by the FDA for use in the USA. Another alternative method to castration is leaving male pigs intact and harvesting them at a lower body weight to reduce the incidence of boar taint. At a body weight of 176-200 pounds only 5 % of carcasses exhibited boar taint (Bonney, 1987). However, average weight of pigs at slaughter is increasing and light carcasses are less profitable for commercial swine processors. Due to the anatomy of the pig alternative methods of castration that have been shown to reduce the pain induced distress caused by castration in other species, such as the rubber ring in lambs and cattle, are not options with pigs. Another solution to this issue would be to develop a commercially viable method to alleviate the pain induced distress caused by castration in pigs.

Local and general anesthetics, and analgesics have been used as methods to reduce the physiological and behavioral response to surgical castration in pigs. Neither aspirin (a non-steroidal anti-inflammatory drug) nor butorphanol (a synthetic opioid analgesic) reduced the behavioral response to surgical castration in pigs (McGlone et al., 1993). General anesthetics including xylazine, ketamine hydrochloride and glyceryl guaiacolate administered intravenously (McGlone et al., 1993), isoflurane (Hodgson, 2006), and sevoflurane (Hodgson, 2007) have been used to prevent the pain caused by castration in pigs. McGlone et al., (1993) observed significant mortality rate in piglets anesthetized using a general anesthetic and piglets that survived showed suppressed nursing behavior – which indicates general anesthetics are not a wise choice for very young pigs. Local anesthetic (Lidocaine 2%) was shown to reduce the mean arterial blood pressure and electroencephalography (Haga and Ranheim, 2005), heart rate (White et al., 1995), and behavioral response (McGlone and Hellman, 1988; White et al., 1995) to surgical castration. Disadvantages to using local anesthetic include the time and stress caused by needing to repeatedly handle piglets; once to administer the local anesthetic and the second time to castrate the animal (it takes approximately 10 minutes for the local to take full effect). A second disadvantage is the use of needles. There is currently a trend for certain large commercial swine producers to move away from administering injections using needles to a needle-free program for food safety/integrity reasons. Therefore, developing a method of alleviating the pain induced distress caused by castration without the use of needles or repeated handling would be positive for pigs and producers.

The cortisol and behavioral response to rubber ring castration in lambs was significantly reduced by injecting local anesthetic into the neck of the scrotum using either a high pressure needled injection or a convention needle and syringe just prior to rubber ring castration, there was no difference between these two methods of application (Kent et al., 1998). Fisher et al. (2007) reduced the cortisol response to surgical castration and tail docking of lambs by administering (spraying) a topical anesthetic to both the scrotum and the tail stump. Therefore, in this study we are proposing to alleviate the pain induced distress caused by castration by giving local anesthetic just prior to castration using either a needle-free injection system or a topical spray (two novel methods that require evaluation). The objectives of this study were to determine different methods to alleviate the pain induced distress caused by castration in piglets as measured by physiological and behavioral measures of stress and welfare.

## Objectives:

- 1) To examine novel methods (needle-free injection systems or topical) to potentially alleviate the pain induced distress caused by castration in piglets as measured by known physiological indices of castration stress in piglets
- 2) To examine selected methods (needle-free injection systems or topical) to alleviate the pain induced distress caused by castration in piglets as measured by known behavioral indices of castration stress in piglets.

(The measurement of physiological and behavioral indices of pain induced distress caused by castration has been separated into two experiments so that the continuous blood sampling does not confound the behavior data).

## Materials and Methods:

Pigs used in this study were PIC USA genetics using the Camborough-22 sow line. All animals were fed a diet to meet or exceed NRC nutrient requirements (1998). Water was provided ad libitum. All animal procedures were approved by the Texas Tech University Animal Care and Use Committee.

### Experiment 1: Physiological response to castration

Seven weight matched piglets from 10 sows were allocated to one of seven treatment groups. Treatments included: 1) Sham castration (CON; n = 10); 2) Surgical castration (CAS; n = 10); 3) Castration plus local anesthetic administered 10 minutes prior to castration using a conventional needle and syringe (LA-10; n = 10); 4) Castration plus local anesthetic administered just prior to castration using a conventional needle injection (LA-0; n = 10); 5) Castration plus local anesthetic administered just prior to castration using a needle-free injection system (Pulse; n = 10); 6) castration plus long acting topical anesthetic applied to the castration wound (LONG; n = 10), and 7) castration plus short acting topical anesthetic applied to the castration wound (SHORT; n = 10). Piglets from 10 litters were used in this study.

At 3 d of age ( $\pm 2$  d), seven male piglets from one litter were allocated to one of the seven treatment groups. Piglets were removed from the sow and taken to an adjoining room separated by a closed door, so as not to disturb the remaining sows and piglets in the farrowing room. Piglets in the CAS treatment group were restrained between the legs of the person performing the procedure to expose the anogenital region of the piglet. A scalpel was used to make an incision on each side of the scrotum, the testicles were then freed from the surrounding tissue and the testicles pulled. Iodine disinfectant was sprayed onto the castration wound. Sham castrated piglets were handled and restrained for approximately 30 seconds in the same manner as the CAS piglets, but without any cutting. Piglets in the LA-10 treatment group were restrained and local anesthetic (Lidocaine, 2%) administered via a conventional metal needle and plastic syringe subcutaneously into the scrotal sac of the piglet just above the testes, piglets were then returned to a holding pen for 10 minutes to allow the local anesthetic to take full effect, then the piglets were castrated in the same manner as the CAS piglets. Piglets in the LA-0 treatment group were handled in the exact same manner as LA-10 piglets, except that piglets were castrated immediately after administration of the local anesthetic. Piglets in the Pulse treatment group were handled in the exact same manner as LA-0 piglets, except that local anesthetic was administered using a needle-free injection system (Pulse NeedleFree Systems, Inc., Lenexa, KS) immediately prior to castration. Lidocaine was selected for use in this study because it is a fast acting commonly available local anesthetic. Piglets in the LONG treatment group were castrated in the same manner as the CAS piglets and then a long acting topical anesthetic (Tri-Solfen, Animal Ethics, VIC, Australia) was applied into the castration wound. Tri-Solfen consists of a mixture of fast acting (lidocaine hydrochloride) and long acting (bupivacaine hydrochloride) anesthetic

to quickly numb the wound, beginning within 1 to 3 minutes and lasting for several hours, a vasoconstrictor (adrenaline tartrate) which acts on the cut blood vessels to stop the bleeding, and an antiseptic agent (cetrimide). Finally, piglets in the SHORT treatment group were castrated in the same manner as the CAS piglets and then a short acting topical anesthetic (Cetacaine®, Cetylite Industries, Inc., Pennsauken, NJ) was applied into the castration wound. Cetacaine® consists of Benzaine a fast acting local anesthetic that lasts approximately 30 to 60 minutes and is readily available in the USA.

Prior to (baseline), and 30, 60, 120, and 180 minutes after castration, piglets were held in a supine position and 2.5 mL blood obtained by anterior vena cava puncture. Blood was collected into vacutainers containing EDTA. Whole blood was analyzed to determine white cell counts and differential leukocyte counts (Cell-Dyn® 3700, Abbott laboratories, Abbott Park, IL) and the neutrophil to lymphocyte (N:L) ratio was calculated by dividing the percent of neutrophils by the percent of lymphocytes. Blood samples were then centrifuged and plasma collected for analysis of cortisol using an enzyme immunoassay kit (Assay designs, Ann Arbor, MI).

### Experiment 2: Behavioral response to castration

At 3 d of age ( $\pm 2$  d), seven male piglets from one litter were allocated to one of seven treatment groups; 1) Sham castration (CON; n = 9); 2) Surgical castration (CAS; n = 9); 3) Castration plus local anesthetic administered 10 minutes prior to castration using a conventional needle and syringe (LA-10; n = 9); 4) Castration plus local anesthetic administered just prior to castration using a conventional needle injection (LA-0; n = 9); 5) Castration plus local anesthetic administered just prior to castration using a needle-free injection system (Pulse; n = 9); 6) castration plus long acting topical anesthetic applied to the castration wound (LONG; n = 9), and 7) castration plus short acting topical anesthetic applied to the castration wound (SHORT; n = 9). Piglets from 10 litters were used in this study. Treatments were administered in the exact same manner as described in experiment 1 (see above).

Sixty minutes prior to castration, experimental piglets were individually marked with a heavy duty marking pen (Super mark pen, Fearing International Ltd, Northampton, UK) using a series of lines in the cross sectional plan or colors to differentiate among individual pigs for easy identification. After 60 min of recording piglet behavior, all piglets were removed from the sow and taken to an adjoining room separated by a closed door, so as not to disturb the remaining sows and piglets in the farrowing room. Piglets were then castrated or handled depending on which treatment group they were allocated to. After castration, piglets were returned to the sow and the behavior of each individual pig was recorded using 1 min scan-samples (live observations) for 180 min. The observer sat directly behind the sow to prevent disturbing her as much as possible, but still giving the observer a complete view of all piglets in the farrowing crate. Behaviors and postures measured included lying without-contact, lying with-contact, nursing/massaging, aggressive encounters, standing, sitting, and walking and pain-like behaviors including scooting and huddling (Table 1).

Piglet vocalizations in response to castration and Sham treatments were recorded using Handycam camcorders (DCR-SR85; Sony, New York, NY). Vocalizations were analyzed using STREMOD (Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, Germany) acoustic analysis system software.

### Performance data

All piglets were weighed prior to and 24 h after castration. All piglets were observed daily for the first 14 days after castration and wound healing scored to assess any detrimental effects (ex. abscesses) caused by any of the castration methods assessed. Wounds were scored from 1 to 5, with 1 being completely healed (no scab) and 5 still showing signs of fresh blood (Table 2).

## Statistical Analyses

All data were tested for constant variance and departures from normal distribution. Data lacking normality were transformed logarithmically using log<sub>10</sub>. Data were subjected to analysis of variance using the mixed model procedure of SAS version 9.1 (SAS Inst., Inc., Cary, NC). The piglet within each block was the experimental unit. Litter was the block. The study was a randomized complete block design with four treatments. For physiological measures, the main fixed effects were treatment, and time. The random effects were litter. The interaction between treatment and time and treatment and litter were included in the model. The model had a repeated structure on time allowing incorporation of heterogeneity of variances across time. Behavioral data were also analyzed using analysis of variance using the mixed model procedure of SAS. The 210, 1-min behavioral observations were divided into seven, 30 min periods. For behavioral measures, the main fixed effects treatment and period. The random effects were litter. The interaction between treatment and period and treatment and litter were included in the model.

## **Results:**

### *Cortisol*

Cortisol plasma concentration was elevated ( $P < 0.05$ ) in LA-0, LA-10, and Pulse pigs 30 and 60 min after castration compared with sham handled pigs (Fig. 1). At no time point was the cortisol response to castration with local anesthetic (regardless of time or method of application) reduced ( $P > 0.05$ ) compared with pigs castrated without analgesia (Fig. 1).

Cortisol was elevated ( $P < 0.05$ ) in pigs surgically castrated compared with sham handled 30 and 60 min after castration (Fig. 2). Cortisol was elevated ( $P < 0.05$ ) in SHORT pigs 30 and 60 min after castration compared with sham handled pigs (Fig. 2). Cortisol was elevated ( $P < 0.05$ ) in LONG pigs 30, 60, 120, and 180 min after castration compared with sham handled pigs and greater ( $P < 0.05$ ) at 120 min after castration compared with CAS pigs (Fig. 2). At no time point was the cortisol response to either castration with short or long acting topical anesthetic reduced ( $P > 0.05$ ) compared with pigs castrated without analgesia.

### *Blood leukocyte counts and differentials*

Leukocyte counts and differentials did not differ ( $P > 0.05$ ) in pigs castrated with analgesia compared with sham handled pigs or pigs castrated without analgesia (Table 3). There was no time by treatment interaction ( $P > 0.05$ ) for any of the leukocyte counts or differentials measured.

### *Behavior*

The percentage of time piglets spent performing all postures and behaviors changed ( $P < 0.05$ ) over time, regardless of treatment. The percentage of time piglets spent Lying with-contact, nursing, standing, sitting, and performing pain-like behaviors differed ( $P < 0.05$ ) in the first 30 min after application of the treatment compared with the 30 min prior to castration and handling. The percentage of time piglets spent lying without-contact, nursing, standing, and performing pain-like behaviors 151 to 180 min after castration and handling was similar ( $P > 0.05$ ) to baseline values. There was no time by treatment interaction ( $P > 0.05$ ) for any of the behaviors measured.

### *Vocalization*

Piglets injected with local anesthetic 10 minutes prior to castration tended ( $P = 0.067$ ) to squeal less during the scrotal incision and struggled less ( $P < 0.05$ ) during the removal and severing of the testes than piglets castrated using other pain alleviation methods.

### *Body weight and wound healing*

Body weight gain did not differ among treatments 24 hours after castration or control handling (fig. 3).

Wound healing scores were greater ( $P < 0.017$ ) in SHORT pigs compared with all other castration treatments overall (Fig. 4). Wound healing scores decreased ( $P < 0.001$ ) overtime regardless of treatment.

### **Discussion:**

Surgical castration is the most common technique to castrate piglets in the USA. Surgical castration involves making one or two incisions on either side of the scrotum, removing the testes, and severing the spermatic cords, usually by pulling. In the present study, castration caused elevated cortisol concentrations in piglets for up to 120 min after this procedure was performed. Physiological indicators of stress including cortisol (Prunier et al., 2005; Carroll et al., 2006), adrenocorticotropic hormone secretion (Prunier et al., 2005), lactate (Prunier et al., 2005), mean arterial blood pressure (Haga and Ranhein, 2005), electroencephalography (Haga and Ranhein, 2005), heart rate (White et al., 1995; Haga and Ranhein, 2005; Axiak et al., 2007), and respiration rate (Axiak et al., 2007) have been shown to change significantly in response to surgical castration of piglets. Therefore our results agree with previous studies showing that surgical castration causes significant physiological changes indicative of acute pain and distress in piglets.

Local anesthetics have been used as methods to reduce the physiological and behavioral response to surgical castration in pigs. The disadvantages to using local anesthetic include the time and stress caused by needing to repeatedly handle piglets; once to administer the local anesthetic and the second time to castrate the animal (it takes approximately 10 minutes for the local to take full effect). A second disadvantage is the use of needles. There is currently a trend for some large commercial swine producers in the US to move away from administering injections using needles and to a needle-free program for food safety/integrity reasons. Therefore, in the present study we wanted to determine if administering local anesthetic immediately prior to castration or administering local anesthetic using needle-free technology would reduce the pain-induced distress response caused by castration in pigs. The cortisol and behavioral response to rubber ring castration in lambs was significantly reduced by injecting local anesthetic into the neck of the scrotum using either a high pressure needling injection or a convention needle and syringe just prior to rubber ring castration, there was no difference between these two methods of application (Kent et al., 1998). In the present study, local anesthetic administered 10 minute prior to or immediately prior to castration (with a needle or needle-free system) did not reduce the cortisol response to castration at any of the time points measured. Previous research has shown local anesthetic (Lidocaine 2%) to reduce the mean arterial blood pressure and electroencephalography (Haga and Ranheim, 2005), heart rate (White et al., 1995), and behavioral response (McGlone and Hellman, 1988; White et al., 1995) to surgical castration in piglets. In the present study, local anesthetic administered subcutaneously into the scrotal sac was not effective in eliminating the pain caused by castration as measured by cortisol, therefore this is not a recommended method to alleviate the pain caused by this procedure.

In the present study we wanted to determine if administering a topical anesthetic to the castration wound would be a practical solution to reduce the pain-induced distress caused by castration in piglets. Several nerves are severed during castration in piglets including, the genitofemoral, superior spermatic, scrotal branch of the pudendal and distal cutaneous branch of the sacral plexus nerves. Local anesthetic agents act by blocking the conduction of signals along the nerve fibers and can even maintain these properties when administered after the incision is made, which is what we were hoping to achieve in the present study. Cetacaine® was chosen because it consists of a fast acting local anesthetic that lasts approximately 30 to 60 minutes and is readably

available in the USA. Tri-Solfen was chosen because it consists of a fast acting and long acting anesthetic as well as a vasoconstrictor, and antiseptic. However, in the present study neither the short or long acting topical anesthetics were effective at reducing the cortisol response to castration in piglets. In fact, the long acting topical anesthetic increased the cortisol response to castration as compared to castration alone. Paull et al. (2009) reduced the cortisol response to surgical castration and tail docking of lambs by administering (spraying) Tri-solfen to both the scrotum and the tail stump. Tri-solfen was also found to be effective at reducing pain-related behaviors in sheep after mulsing (Lomax et al., 2008). In the study conducted by Paull et al. (2009), blood samples were collected at 0.5, 6, 12, 24, and 48 hours after castration, but a reduction in cortisol concentrations among lambs castrated with Tri-solfen was only observed 30 min after castration. In the present study, the difference in the cortisol response among pigs that were castrated with and without Tri-solfen was not observed until 2 h after castration, therefore Paull et al. (2009) may have missed this increase in cortisol concentrations. The piglets in the present study showed no other physiological or behavioral signs indicative of pain compared with piglets castrated without anesthetic, therefore the increase in cortisol in LONG piglets' maybe a physiological artifact in response to Tri-solfen. Tri-solfen contains adrenaline, which can directly stimulate the hypothalamic-pituitary-adrenal (HPA) axis. In the present study, the increase in cortisol concentration observed in LONG piglets as compared with piglets castrated without anesthetic could be due to the direct action of the adrenaline in the Tri-solfen stimulating the HPA axis and may not actually be an indication that these animals were experiencing more distress than piglets castrated without anesthetic. Furthermore, the scrotal anatomy of the pig differs from the lamb which may affect how the topical anesthetic diffuses through the tissues and interacts with the nerve fibers thereby influencing the blocking effects of the anesthetic. Regardless of the explanation, the short or long acting topical anesthetic used in this study did not appear to be effective at reducing the pain-induced distress in piglets cause by castration.

Wound healing was scored in all piglets daily after castration to determine if either of the anesthetic treatments would have an effect on wound healing that could possibly lead to complications if these agents were used on-farm. Wound healing scores were similar among piglets castrated without anesthetic and piglets castrated and then given Tri-solfen, however wound healing appeared to be slightly delayed in piglets that were given Cetacaine<sup>®</sup> after castration. Local anesthetic agents have been shown to reduce wound healing after a surgical incision, possibly due to altering mechanisms involved with collagen production and also by causing tissue necrosis at the site of the injection (Morris and Tracey, 1977; Chvapil et al., 1979; Vasseur et al., 1984). In other studies, infiltration of local anesthetic (lidocaine) into a surgical wound produced significant histopathologic changes (Drucker et al., 1998), but did not substantially alter wound healing (Sinclair et al., 1988; Drucker et al., 1998). Procaine was shown to retard wound healing but this response appeared to be related to the concentration of anesthetic administered (Morris and Appleby, 1980). Bupivacaine showed reduced epithelial growth and increased cytotoxicity of fibroblasts and keratinocytes *ex vivo* in a dose-dependent manner with the highest concentrations having the greatest effect (Harris et al., 2009). In the present study, delayed wound healing in SHORT piglets could be related to the amount/concentration of anesthetic applied to the wound. Tri-solfen, which contains two different types of local anesthetic, did not appear to cause a delay in wound healing. Tri-solfen also contains a vasoconstrictor and an antiseptic, which may account for the differences in wound healing among piglets given Cetacaine<sup>®</sup> and Tri-solfen after castration. Tri-solfen did not cause any delay in wound healing in lambs after mulsing, in fact Tri-solfen increased wound contraction rates in lambs after mulsing compared with lambs' mulsed without Tri-solfen (Lomax et al., 2008). Even though the delay in wound healing was only slight in SHORT piglets compared to the other castration treatment groups, more research would be required to determine the practicality of using Cetacaine<sup>®</sup> to alleviate the pain caused by castration in piglets.

None of the behaviors measured were affected by castration with and without analgesia as compared to control handled piglets for up to 180 min, in the present study. In other studies, surgical castration in piglets was shown to cause behavioral changes such as vocalization, reduced nursing and lying, and increased activity (McGlone and Hellman, 1988; Taylor et al., 2001; Hay et al., 2003; Carroll et al., 2006; Moya et al., 2007). It has been suggested that animals at younger ages possibly experience less distress due to negative procedures, such as castration or tail docking, than older animals. In the present study, piglets were  $3 \pm 2$  d of age at the time of castration so this may account for the lack of a behavioral response to castration. However, in studies using pigs from 3 d to 8 wk of age, age of the pig at castration did not appear to influence the behavioral response to castration (McGlone et al., 1993; Taylor et al., 2001; Carroll et al., 2006). Castration with or without anesthetic did not appear to influence the behavior of piglets compared to control handled piglets, but behavior in all piglets was affected by being disturbed due to the administration of the treatment, regardless of the treatment. These results suggest that the act of separating piglets from the sow, placing them in a novel environment, and handling them in a similar manner as piglets being castrated may disturb the piglet as much as being castrated when using behavior as a measure of distress. It is apparent from this study that there is a need for more sensitive measures of pain-specific behaviors in pigs.

The needle-free system was evaluated relative to the more traditional needle system. For all measures of behavior and physiology, piglet responses were similar for needle-free and needle systems. Therefore, pig welfare was similar for needle or needle-free system in this animal model.

In the present study, anesthetic (topical or injected) was not effective at eliminating or even reducing the pain response to castration as measured by physiology and behavior, therefore more industry-relevant research is needed to evaluate other methods of analgesia that can be used to alleviate the pain caused by castration.

**Table 1. Description of behaviors**

Behavior	Description
Maintenance behaviors	
Walking <sup>1</sup>	Relatively low speed locomotion in which propulsive force derives from the action of legs
Sitting <sup>1</sup>	Resting on the caudal part of the body
Standing <sup>1</sup>	Assuming or maintaining an upright position on extended legs
Lying-without contact	Maintaining a recumbent position and not in contact with other piglets or the sow
Lying-with contact	Maintaining a recumbent position while contacting another piglet/s or the sow
Massaging <sup>1</sup> and Nursing <sup>1</sup>	Rhythmic and sustained mechanical manipulation of the mammary of the sow by the piglets prior to and after nursing
Nursing <sup>1</sup>	The act of releasing milk to suckling young
Aggressive interactions <sup>2</sup>	Attacks between two or more piglets including bites and pushes, primarily of the ears and neck
Pain specific behaviors	
Scooting	Caudal part of the body being dragged across the ground or the side of the crate
Huddling up <sup>3</sup>	Lying or standing with a hunched back posture

<sup>1</sup>Hurnik, 1995, <sup>2</sup>McGlone, 1985, <sup>3</sup>Moya, 2008

**Table 2. Description of wound healing score**

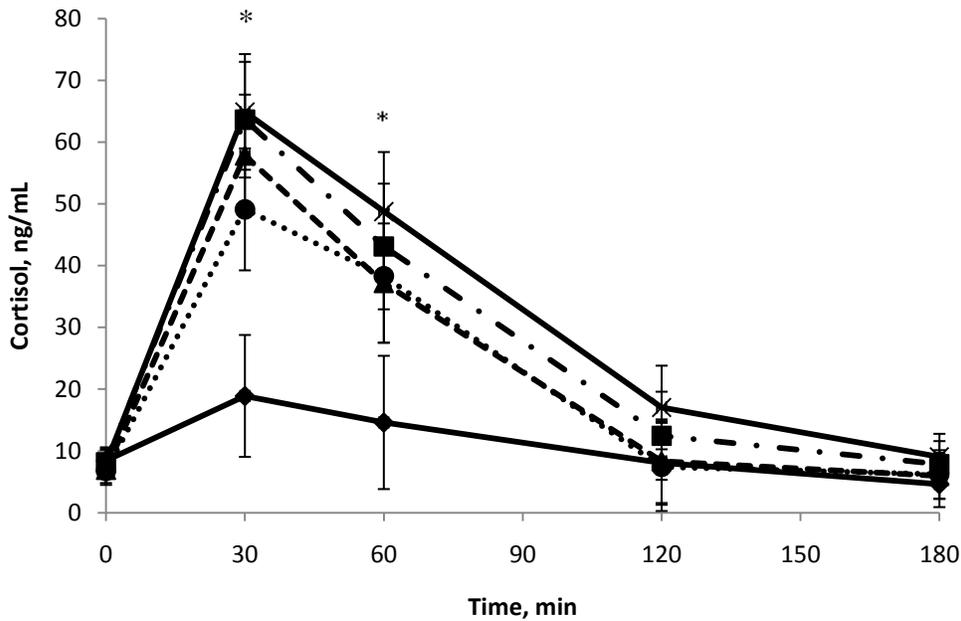
Score		Description
1		Completely healed (no scab)
2		A slight scab still present at the site of the incision
3		Fully formed scab over the wound (thick and bumpy in appearance)

4		Fully formed scab over the wound (thin in appearance)
5		Scabbing has begun, but there are still signs of raw tissue
6		Wound is still open and still looks raw

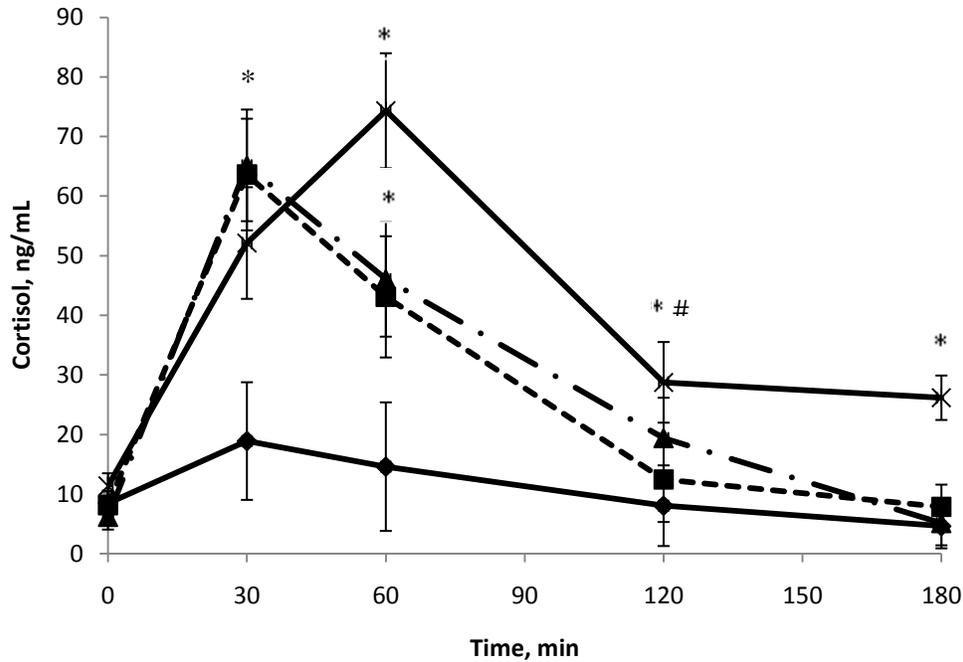
**Table 3.** Comparison of leukocyte counts and percentages of piglets castrated with and without analgesia. Treatments: Sham castration (CON; n = 10); Surgical castration (CAS; n = 10); Castration plus local anesthetic administered 10 minutes prior to castration using a conventional needle and syringe (LA-10; n = 10); Castration plus local anesthetic administered just prior to castration using a conventional needle injection (LA-0; n = 10), Castration plus local anesthetic administered just prior to castration using a needle-free injection system (Pulse; n = 10), Castration plus short acting topical anesthetic applied to the castration wound (SHORT; n = 10), and castration plus long acting topical anesthetic applied to the castration wound (LONG; n = 10).

Measure	Treatment							SE (Pooled)	P-value
	CON	CAS	LA-10	LA-0	Pulse	SHORT	LONG		
N	10	10	10	10	10	10	10		
WBC, 10 <sup>3</sup> /μL	9.0	8.6	8.6	8.3	8.7	7.5	8.0	0.77	0.836
Neutrophils, 10 <sup>3</sup> /μL	3.6	3.5	3.0	3.5	3.6	2.9	3.3	0.62	0.917
Lymphocytes, 10 <sup>3</sup> /μL	5.1	4.9	5.5	4.6	4.8	4.4	4.3	0.69	0.746
Monocytes, 10 <sup>6</sup> /μL	0.1	0.2	0.1	0.1	0.3	0.2	0.6	0.11	0.495
Neutrophils, %	36.5	36.6	34.0	37.5	42.0	35.3	40.1	6.95	0.947
Lymphocytes, %	58.7	61.2	64.8	59.4	54.8	63.4	53.8	5.79	0.868
Monocyte, %	1.7	1.7	1.4	1.3	2.3	1.6	6.4	1.08	0.430
N:L	1.4	1.3	1.2	1.3	1.5	1.1	1.5	0.27	0.892

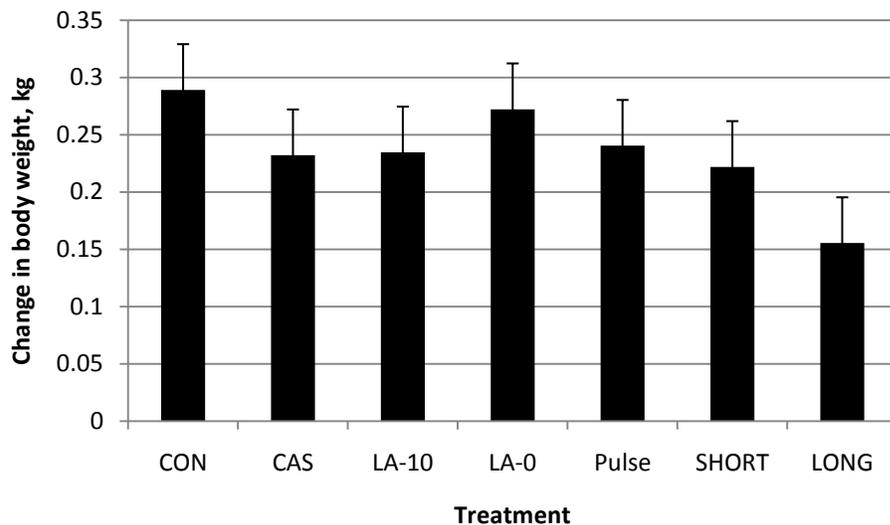
WBC: Total white blood cell count  
N:L: Neutrophil to lymphocyte count



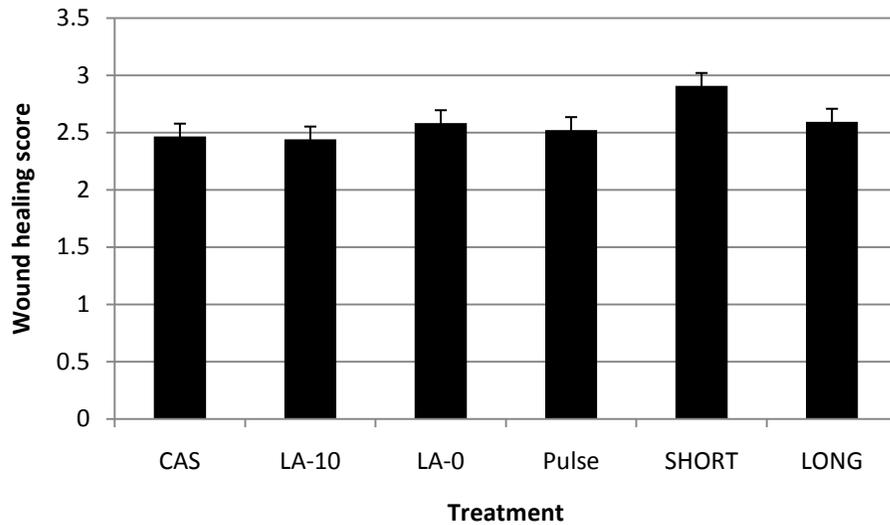
**Figure 1:** Cortisol concentrations (ng/mL) prior to and 30, 60, 120, and 180 min after sham castration or castration with and without topical analgesia in pigs. Treatments: Sham castration (CON [◆]; n = 10); Surgical castration (CAS [■]; n = 10); Castration plus local anesthetic administered 10 minutes prior to castration using a conventional needle and syringe (LA-10 [▲]; n = 10); Castration plus local anesthetic administered just prior to castration using a conventional needle injection (LA-0 [●]; n = 10), and castration plus local anesthetic administered just prior to castration using a needle-free injection system (Pulse [★]; n = 10). At each time, least square means accompanied by a \* are different from CON and least square means accompanied by a # are different from CAS at  $P < 0.05$ .



**Figure 2:** Cortisol concentrations (ng/mL) prior to and 30, 60, 120, and 180 min after sham castration or castration with and without topical analgesia in pigs. Treatments: Sham castration (CON [◆]; n = 10); Surgical castration (CAS [■]; n = 10); Castration plus short acting topical anesthetic applied to the castration wound (SHORT [▲]; n = 10), and castration plus long acting topical anesthetic applied to the castration wound (LONG [★]; n = 10). At each time, least square means accompanied by a \* are different from CON and least square means accompanied by a # are different from CAS at  $P < 0.05$ .



**Figure 3:** Body weight change (kg) 24 hours after castration or sham castration in pigs. Treatments: Sham castration (CON; n = 20); Surgical castration (CAS; n = 20); Castration plus local anesthetic administered 10 minutes prior to castration using a conventional needle and syringe (LA-10; n = 20); Castration plus local anesthetic administered just prior to castration using a conventional needle injection (LA-0; n = 20), Castration plus local anesthetic administered just prior to castration using a needle-free injection system (Pulse; n = 20), Castration plus long acting topical anesthetic applied to the castration wound (LONG; n = 20), and Castration plus short acting topical anesthetic applied to the castration wound (SHORT; n = 20).



**Figure 4:** Wound healing scores of pigs castrated with and without and without analgesia. Treatments: Sham castration (CON; n = 20); Surgical castration (CAS; n = 20); Castration plus local anesthetic administered 10 minutes prior to castration using a conventional needle and syringe (LA-10; n = 20); Castration plus local anesthetic administered just prior to castration using a conventional needle injection (LA-0; n = 20), Castration plus local anesthetic administered just prior to castration using a needle-free injection system (Pulse; n = 20), Castration plus short acting topical anesthetic applied to the castration wound (SHORT; n = 20), and castration plus long acting topical anesthetic applied to the castration wound (LONG; n = 20).

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## Appendix



1. The needle-free system being used to administer local anesthetic prior to castration.



2. Tri-Solfen (Long acting topical analgesia) being administered immediately post-castration.



3. Local anesthetic being administered using a conventional needle and syringe subcutaneously into the scrotal sac.



4. Cetacaine (Short acting topical analgesia) being administered immediately post-castration.