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The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia[1]

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ABSTRACT: Surgical castration is a common management practice performed on male pigs to prevent the occurrence of boar taint. Surgical castration is known to cause physiological and behavioral changes in pigs indicative of pain-induced distress; however, it is commonly performed without pain relief. The objective of this study was to evaluate the effectiveness of carbon dioxide gas (CO_2) anesthesia and a non-steroidal anti-inflammatory drug (NSAID) to alleviate the pain caused by castration. At 3 d of age, male pigs were either control handled (CON), castrated without pain relief (CAS), given an NSAID and then immediately castrated (CAS+NSAID), anesthetized with CO_2 and then castrated (CAS+CO2), or anesthetized with CO_2 and given an NSAID at the time of castration (CAS+BOTH). Blood samples were collected before castration, and at 30, 60, 120, and 180 min, 24 h, and 3 d after castration or handling for analysis of cortisol, C-Reactive protein (CRP), and substance-P (SP) concentrations. This study was then repeated using the same treatment groups, and the behavioral response to castration and handling were measured using a 1-min scan sampling procedure. The percentage of stress vocalizations was recorded during the administration of all treatments. Anesthesia and analgesia did not effectively reduce ($P > 0.05$) the cortisol response to surgical castration. Overall, CRP concentrations were greater ($P < 0.05$) in CAS+CO2 pigs as compared with CON pigs. Sixty minutes after castration or handling, SP concentrations were greater ($P < 0.08$) in pigs given CO_2 anesthesia (CO2, CAS+CO2, and CAS+BOTH) than CON, CAS, and CAS+NSAID pigs. Pigs castrated without pain relief spent more ($P < 0.001$) time lying without contact than all other treatments during the first 30 min after castration, but thereafter CAS+CO2 pigs spent more ($P < 0.001$) time lying without contact than other treatments. During the first 30 min after the treatments were applied, CAS+CO2 pigs spent more ($P < 0.01$) time displaying pain-like behaviors than CON, CAS, CAS+NSAID, and CAS+BOTH pigs. The percentage of stress vocalizations was greater ($P < 0.05$) in CAS and CAS+NSAID pigs than all other treatments. Neither CO_2 anesthesia nor a NSAID, given separately or combined, markedly reduced the pain-induced distress caused by castration in pigs. More research is needed to evaluate practical methods of on-farm pain relief for pigs.

Key words: behavior, castration, physiology, pig, welfare

INTRODUCTION

Castration of male pigs is a common management practice carried out on commercial swine farms to prevent the occurrence of boar taint and aggressive behaviors; however, the procedure of castration causes acute pain-induced distress, which is an animal welfare concern. Negative public perception concerning castration without analgesia or anesthesia is increasing. 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.

Surgical castration is the most common technique used to castrate pigs. Published evidence indicates that surgical castration causes physiological (White et al., 1995; Prunier et al., 2005; Carroll et al., 2006) and behavioral (McGlone and Hellman, 1988; Taylor et al., 2001; Moya et al., 2008) changes indicative of acute pain. Analgesics, such as local anesthetic, can effectively reduce the physiological and behavioral response to castration in pigs (McGlone and Hellman, 1988; White et al., 1995; Haga and Ranheim, 2005); however, giving local anesthesia requires repeatedly handling the animal. Alternative forms of analgesia/anesthesia for reducing the pain caused by castration could include gas anesthesia using carbon dioxide (**CO$_2$**) and analgesia in the form of a non-steroidal anti-inflammatory drug (**NSAID**). The advantages of these methods of pain relief include ease of use and the necessity of only needing to handle the animal once. However, little is currently known concerning the effectiveness of these methods to reduce the pain-induced distress caused by surgical castration in pigs. Therefore, the objective of this study was to evaluate CO$_2$ gas anesthesia and an NSAID, given separately or combined, to alleviate the pain-induced distress caused by castration in pigs as measured by known physiological and behavioral indices of distress in pigs.

MATERIALS AND METHODS

All animal procedures were approved by the Texas Tech University Animal Care and Use Committee (approval number ACUC 09026-06).

Animals and Housing

Pigs were PIC USA genetics using the Camborough-22 sow line. All sows were fed a diet that met or exceeded NRC (1998) nutrient requirements. Water was provided ad libitum.

The Physiological Response to Castration with Analgesia/Anesthesia

At 3 d of age (± 1 d), 70 weight-matched male pigs (2.1 ± 0.06 kg) were allocated to 1 of 7 treatment groups. Treatments included 1) sham castration (**CON**; n = 10); 2) sham castration while the pig was anesthetized with CO$_2$ (**CO2**; n = 10); 3) sham castration plus NSAID administered at the time of handling (**CON+NSAID** ; n = 10); 4) castration (**CAS**; n = 10); 5) castration while the pig was anesthetized with CO$_2$ (**CAS+CO2**; n = 10); 6)

castration plus NSAID administered at the time of castration (**CAS+NSAID**; n = 10); and 7) castration conducted while the pig was anesthetized with CO$_2$ plus NSAID administered at the time of castration (**CAS+BOTH**; n = 10). No other painful procedures, such as tail docking or ear notching, were performed on pigs before the study.

Before castration or handling, all experimental pigs were removed from the sow 1 litter at a time and taken to an adjoining room separated by a closed door, so as not to disturb the remaining sows and pigs in the farrowing room. Treatments were applied in a random order within each litter and each treatment was represented within each litter. Pigs in the CAS treatment group were restrained between the legs of the person performing the procedure to expose the anogenital region of the pig, an incision was made on each side of the scrotum using a scalpel, the testicles were freed, and the spermatic cords cut. Iodine disinfectant was sprayed onto the castration wound. The CON pigs were handled and restrained for approximately 30 s in the same manner as the CAS pigs, but without being cut. The CO2 pigs were anesthetized with CO$_2$ gas and handled in the same manner as CON pigs. Carbon dioxide was administered at a concentration of 100% for 30 s; a concentration of 100% was chosen based on a pilot study conducted by Sutherland et al. (2011). Carbon dioxide gas was administered by placing a surgical gas mask on the snout of the pig which covered the entire mouth. The handler held the pig in 1 hand while fitting the gas mask over the mouth of the pig with the other hand. The gas was turned on once the mask was firmly placed over the mouth of the pig. The CON+NSAID pigs were handled in the same manner as CON pigs and then given an intramuscular (**i.m.**) injection of Banamine (flunixin meglumine, Intervet/Schering-Plough Animal Health, Boxmeer, the Netherlands) into the rump. Pigs in the CAS+CO2 and CAS+BOTH treatment groups were anesthetized by administering CO$_2$ for 30 s using the same procedure described for the CO2 pigs. Immediately after the 30-s inhalation period, the gas mask was removed from the snout of the pig and pigs were castrated in the same manner as CAS pigs. Pigs in the CAS+NSAID and CAS+BOTH treatment groups were given an i.m. injection of Banamine into the rump and then immediately castrated in the same manner as CAS pigs.

Before castration (time 0), and at 30, 60, 120, and 180 min, 24 h, and 3 d after castration, pigs were held in a supine position and 2.5 mL of blood was obtained by anterior vena cava puncture. Blood was collected into vacutainers (BD, Franklin Lakes, NJ) containing EDTA. Blood samples were centrifuged for 15 min at 1000 × *g* at 4°C and plasma collected into 2 aliquots. The first aliquot was stored at −20°C for future analysis of cortisol and C-reactive protein (**CRP**) concentrations. All

plasma samples were analyzed to measure cortisol concentrations using an enzyme immunoassay kit (Assay Designs, Ann Arbor, MI). A subset of samples, times 0 h, 24 h and 3d only, were analyzed to measure CRP concentrations using an enzyme immunoassay kit (Tri-Delta Diagnostics, Inc., Morris Plains, NJ). The second plasma aliquot was spiked with 1 mM benzamidine hydrochloride (Sigma Aldrich, St. Louis, MO), a protease inhibitor, then stored at −20°C for future analysis. A subset of samples (times 0, 30, 60, 120, and 180 min), were analyzed for substance-P (**SP**) concentrations using a validated method as previously described (Coetzee et al., 2008). Briefly, SP was extracted from plasma by acidifying the samples with acetic acid and fractionating with reverse-phase solid-phase extraction columns. The peptide was eluted from the column using an organic-aqueous solvent mixture and concentrated by drying under nitrogen. The dried extract was reconstituted and analyzed according to the manufacturer's instructions in the SP ELISA kit (Assay Designs).

Behavioral Response to Castration with Analgesia/Anesthesia

At 3 d of age (± 1 d), 70 BW-matched male pigs (2.0 ± 0.6 kg) were allocated to 1 of 7 treatment groups (n = 10/treatment). All treatments were administered in the same manner as described above.

Sixty minutes before commencement of treatments, experimental pigs were individually marked with an aerosol spray (Prima Aeorsal Spray, QC Supply, Schuyler, NE) using color to differentiate among individual pigs for easy identification. After 60 min, all pigs from 1 sow were removed and taken to an adjoining room separated by a closed door, so as not to disturb the remaining sows and pigs in the farrowing room. Pigs were processed depending on which treatment group they were allocated. Treatments were applied in a random order within each litter and each treatment was represented within each litter.

After processing, all pigs were returned to their home pen at the same time and the behavior of individual pigs was recorded using 1-min scan samples (direct observations) for the first 30 min after processing, and then 60 to 90 min and 120 to 150 min after processing. The observer sat directly behind the sow to prevent disturbing her as much as possible, but had a complete view of all pigs in the farrowing crate. Behaviors measured are described in Table 1.

Stress Vocalizations

Camcorders (DCR-SR85, Sony, New York, NY) were used to record vocalizations before and during

Table 1. Description of behaviors recorded in pigs in response to experimental treatments

Behavior	Description
Lying without contact[1]	Maintaining a recumbent position and not in contact with other pigs or the sow
Lying with contact[1]	Maintaining a recumbent position while contacting another pig(s) or the sow
Nursing[2,3]	Rhythmic and sustained mechanical manipulation of the mammary of the sow by the pigs before, during, and after nursing
Sitting[2]	Resting on the caudal part of the body
Standing[2]	Assuming or maintaining an upright position on extended legs
Walking[2]	Relatively low speed locomotion in which propulsive force derives from the action of legs
Pain-like behaviors	Scooting (caudal part of the body being dragged across the ground or the side of the crate[1]), sitting, and huddling (lying or standing with a hunched back posture[4]) behaviors combined

[1]Sutherland et al., 2008.
[2]Hurnik et al., 1995.
[3]Nursing in this study refers to nursing and massaging, as it is difficult to differentiate between these 2 behaviors.
[4]Moya, 2008.

administration of all treatments in both the physiology and behavior experiments. Vocalizations were analyzed using an automatic stress call monitoring system (STREMODO, Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, Dummerstorf, Germany). The STREMODO system is described in detail by Schön et al. (2004). Briefly, the system calculates the percentage of stress (high-frequency) vocalizations emitted by the pigs within 10-s periods and differentiates between the high-frequency sounds emitted by the pig and high-frequency background sounds. In the present study, the percentage of stress vocalizations elicited during handling and during the performance of the treatments was averaged. Vocalization data were not able to be analyzed for some animals due to poor picture or sound quality.

Body Weight and Wound Healing

All pigs from the physiology and behavior experiments were weighed 1 d before and 24 h after castration or handling. All pigs were observed and wound healing was scored to assess any detrimental effects (e.g., abscesses) caused by any of the castration alleviation methods every second day for 14 d after castration. Castration wounds were scored from 1 to 6, with 6 being a bloody wound with no scab to 1 being completely healed as described by Sutherland et al. (2011).

Statistical Analysis

All data were tested for constant variance and departures from normal distribution using the univariate

procedure (SAS Inst., Inc., Cary, NC). Data lacking normality and transformed logarithmically included cortisol, CRP, and SP concentrations; all behavior and vocalization data; and wound-healing scores. Data were subjected to analysis of variance using the mixed model procedure of SAS. Multiple comparisons were calculated using the PDIFF option in SAS. Each litter contained all 7 treatments. Ten litters were used in the physiological response and another 10 litters were used in the behavioral response experiment. For physiological measures, the main fixed effects were treatment and time. Litter was a random effect. The interactions between treatment by time and treatment by litter were included in the model. Behavioral data also were analyzed using analysis of variance using the mixed model procedure of SAS. For behavioral measures, the main fixed effects were treatment and period. Litter was a random effect. The interaction between treatment by period and treatment by litter was included in the model. Data displayed in the graphs, tables, and text are actual data (except for SP data, which are presented as log-transformed data) summarized by least squares means ± SE. Statistical significance was determined at $P < 0.05$ and trends determine at $P < 0.10$.

RESULTS

The Physiological Response to Castration with Analgesia/Anesthesia

Cortisol Concentrations. Cortisol concentrations differed (treatment × time: $F_{36,360} = 1.51$, $P = 0.034$) among castration and control treatment groups over time. Cortisol concentrations did not differ ($P > 0.10$) among control treatment groups (CON, CO2, and CON+NSAID) at any time point, but the cortisol response to castration without analgesia or anesthesia was greater ($P < 0.05$) than CON pigs at 30, 60, and 120 min after castration (Table 2).

Anesthetizing pigs with CO_2 before castration did not reduce ($P > 0.10$) the cortisol response as compared with pigs castrated without pain relief; except 120 min after castration, cortisol concentrations tended ($P = 0.057$) to be lower in CAS+CO2 than CAS pigs. Furthermore, cortisol concentrations were greater ($P < 0.05$) in CAS+CO2 than CON pigs 30 min after castration; but thereafter, cortisol concentrations did not differ ($P > 0.10$) between CAS+CO2 and CON pigs (Table 2).

Giving pigs an NSAID before castration did not reduce ($P > 0.05$) the cortisol response as compared with pigs castrated without pain relief and cortisol concentrations were greater ($P < 0.05$) in CAS+NSAID than CON pigs, 30, 60, and 120 min after castration (Table 2).

Anesthetizing pigs before castration and giving them an NSAID immediately before castration did not reduce ($P > 0.10$) the cortisol response to castration without pain relief; except 60 min after castration, cortisol concentrations tended ($P = 0.068$) to be less in CAS+BOTH than CAS pigs. Furthermore, cortisol concentrations were greater ($P < 0.05$) in CAS+NSAID than CON pigs 30 and 120 min after castration, but not at 90 or 180 min (Table 2).

The cortisol response did not differ ($P > 0.10$) among any treatments 24 h after castration; but 72 h after castration, cortisol concentrations in CAS+NSAID and CAS+BOTH pigs was greater ($P < 0.05$) than CAS and CAS+CO2 pigs, but not CON pigs (Table 2).

For all treatment groups, the total cortisol response during the first 180 min after administration of the treatments was determined by calculating the area under the cortisol curve (**AUC**). The AUC differed (treatment: $F_{6,40} = 2.77$, $P = 0.024$) among treatments; the AUC

Table 2. Cortisol concentrations (ng/mL) in pigs (least squares means ± SE) in response to control handling or castration with or without pain relief (n = 10/treatment)

Time	CON	CO2	CON+NSAID	CAS	CAS+CO2	CAS+NSAID	CAS+BOTH	SE[2]
				Treatment[1]				
0 min	10.0	8.9	11.5	7.8	7.4	9.6	4.2	11.8
30 min	24.2[a]	26.7[a]	20.6[a]	70.2[b]	51.7[b]	49.3[b]	63.8[b]	11.6
60 min	30.6[a]	39.3[a]	34.5[abc]	98.6[d]	49.4[abcd]	79.9[c]	43.9[abcd]	11.8
120 min	6.3[a]	12.3[a]	23.3[abc]	46.0[c]	14.8[ac]	54.5[dc]	41.9[c]	11.9
180 min	12.7	10.4	11.2	11.6	6.3	20.4	33.0	11.7
24 h	11.6	10.8	13.1	18.8	16.7	24.5	8.8	11.5
3 d	11.3[ab]	10.8[ab]	29.8[ab]	7.3[a]	8.2[a]	22.9[b]	27.6[b]	11.5

[1]Treatments: sham castration (CON); sham castration while the pig was anesthetized with carbon dioxide (CO2); sham castration plus non-steroidal anti-inflammatory drug (NSAID) administered at the time of handling (CON+NSAID); castration (CAS); castration while the pig was anesthetized with carbon dioxide (CAS+CO2); castration plus NSAID administered at the time of castration (CAS+NSAID); and castration conducted while the pig was anesthetized with carbon dioxide plus NSAID administered at the time of castration (CAS+BOTH).

[2]Pooled SE.

[a-d] Within a row, means without a common superscript differ ($P < 0.05$).

was similar among castrated pigs regardless of pain relief treatment, and was greater ($P < 0.05$) in all castrated than CON pigs, except that the AUC was similar between CAS+CO2 and CON pigs (CON: 7.5 ± 0.40, CO2: 7.8 ± 0.28, CON+NSAID: 8.1 ± 0.33, CAS: 8.9 ± 0.30, CAS+CO2: 8.3 ± 0.30, CAS+NSAID: 8.8 ± 0.28, CAS+BOTH: 8.8 ± 0.30).

C-Reactive Protein Concentrations. C-Reactive protein concentrations differed among treatments (treatment: $F_{6,161} = 2.18$, $P = 0.048$), but there was no treatment by time interaction ($P > 0.10$). C-Reactive protein concentrations did not differ ($P > 0.10$) among any of the control treatment groups. C-Reactive protein concentrations tended to be greater ($P = 0.067$) in CAS, and were greater ($P < 0.05$) in CAS+CO2 pigs than CON pigs. There was no difference ($P > 0.10$) in CRP concentrations among CAS+NSAID, CAS+BOTH, and CON pigs. Furthermore, CRP concentrations were less ($P < 0.05$) in CAS+BOTH than CAS pigs. (Overall CRP concentrations for each treatment; CON: 15.2 ± 35.33 ng/mL, CO2: 46.4 ± 32.91 ng/mL, CON+NSAID: 37.1 ± 34.36 ng/mL, CAS: 76.5 ± 33.13 ng/mL, CAS+CO2: 81.3 ± 32.83 ng/mL, CAS+NSAID: 28.4 ± 32.81 ng/mL, CAS+BOTH: 0.0 ± 32.84 ng/mL).

Substance-P Concentrations. Substance-P concentrations differed (treatment × time: $F_{24,233} = 2.46$, $P < 0.001$) among castration and control treatment groups over time. Substance-P concentrations tended be greater ($P = 0.065$) in CO2 than CON pigs 30 min after the start of the treatment and were greater ($P < 0.001$) in CO2 than CON pigs at 60 min. Similarly, SP concentrations were greater ($P < 0.001$) in CO2 than CON+NSAID pigs 30 and 60 min after administration of the treatments (Table 3).

Substance-P concentrations did not differ ($P > 0.10$) between CON and CAS pigs at any time point. Anesthetizing pigs with CO_2 before castration resulted in pigs having greater ($P < 0.005$) SP concentrations than CON pigs 60 min after castration and greater ($P < 0.01$) SP concentrations than CAS pigs at 30 and 60 min after castration (Table 3). Similarly, pigs anesthetized with CO_2 before and given an NSAID after castration had greater ($P < 0.005$) SP concentrations than CON and CAS pigs 60 min after castration (Table 3). Substance-P concentrations were also greater ($P < 0.05$) in CAS+CO2 and CAS+BOTH pigs than CAS+NSAID pigs 60 min after castration.

The Behavioral Response to Castration with Analgesia/Anesthesia

The time pigs spent lying without contact differed (treatment × time: $F_{12,1245} = 2.83$, $P < 0.001$) among castration and control treatment groups over time (Figure 1). During the first 30 min after the treatments were applied, CAS pigs spent more ($P < 0.001$) time lying without contacting other pigs or the sow than CON, CAS+CO2, CAS+NSAID, and CAS+BOTH pigs. Sixty to ninety minutes after administration of the treatments, CAS+CO2 spent more ($P < 0.001$) time lying without contact than CON, CAS, CAS+NSAID, and CAS+BOTH pigs. Similarly, 120 to 150 min after administration of the treatments, CAS+CO2 spent more ($P < 0.05$) time lying without contact than CON, CAS+NSAID, and CAS+BOTH pigs. The time spent lying without contact did not differ ($P > 0.05$) among CON, CO2, and CON+NSAID pigs during any of the time periods.

The time pigs spent displaying pain-like behaviors differed (treatment × time: $F_{12,1245} = 2.35$, $P = 0.005$) among castration and control treatment groups over time (Figure 2). During the first 30 min after the treatments were applied, CAS+CO2 pigs spent more ($P <$

Table 3. Substance-P concentrations (pg/mL; log-transformed data) in pigs in response to control handling or castration with or without pain relief (n = 10/treatment)

Time, min	CON	CO2	CON+NSAID	CAS	CAS+CO2	CAS+NSAID	CAS+BOTH	SE[2]
				Treatment[1]				
0	4.7	4.8	4.8	4.9	4.8	4.8	4.7	0.1
30	4.6[abc]	4.9[c]	4.3[a]	4.4[a]	4.8[bc]	4.6[ab]	4.6[abc]	0.1
60	4.5[b]	5.2[de]	4.6[b]	4.2[a]	5.0[d]	4.4[a]	4.8[bcd]	0.1
120	4.5	4.8	4.7	4.8	4.7	4.6	4.7	0.1
180	5.0	4.8	4.8	4.8	4.9	4.9	4.6	0.1

[1]Treatments: sham castration (CON); sham castration while the pig was anesthetized with carbon dioxide (CO2); sham castration plus NSAID administered at the time of handling (CON+NSAID); castration (CAS); castration while the pig was anesthetized with carbon dioxide (CAS+CO2); castration plus NSAID administered at the time of castration (CAS+NSAID); and castration conducted while the pig was anesthetized with carbon dioxide plus NSAID administered at the time of castration (CAS+BOTH).

[2]Pooled SE.

[a-e] Within a row, means without a common superscript differ ($P < 0.05$).
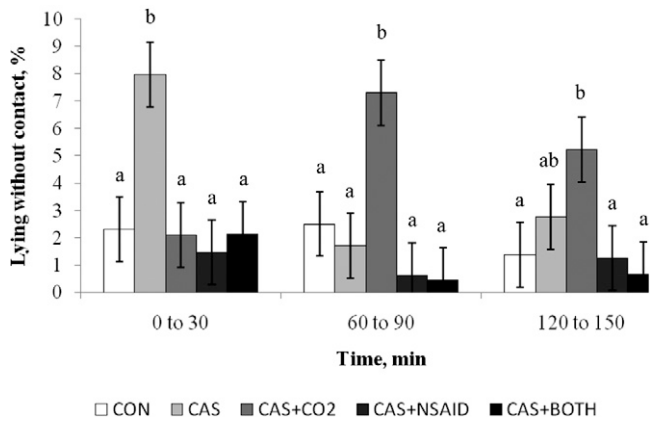
Figure 1. The percentage of time (least squares means ± SE) pigs spent lying without contact with other pigs 0 to 30, 60 to 90, and 120 to 150 min after castration or control handling. At each time period, means without common subscripts differ ($P < 0.05$). Treatments: sham castration (CON; n = 10); castration (CAS; n = 10); castration while the pig was anesthetized with carbon dioxide (CAS+CO2; n = 10); castration plus non-steroidal anti-inflammatory drug (NSAID) administered at the time of castration (CAS+NSAID; n = 10); and castration conducted while the pig was anesthetized with carbon dioxide plus NSAID administered at the time of castration (CAS+BOTH; n = 10).

Figure 2. The percentage of time (least squares means ± SE) pigs spent displaying pain-like behaviors 0 to 30, 60 to 90, and 120 to 150 min after castration or control handling. At each time period, means without a common subscript differ ($P < 0.05$). Treatments: sham castration (CON; n = 10); castration (CAS; n = 10); castration while the pig was anesthetized with carbon dioxide (CAS+CO2; n = 10); castration plus non-steroidal anti-inflammatory drug (NSAID) administered at the time of castration (CAS+NSAID; n = 10); and castration conducted while the pig was anesthetized with carbon dioxide plus NSAID administered at the time of castration (CAS+BOTH; n = 10).
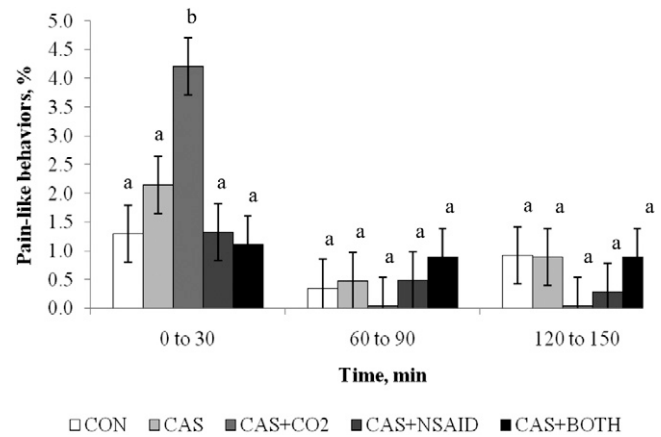
0.01) time displaying pain-like behaviors than CON, CAS, CAS+NSAID, and CAS+BOTH pigs. The time spent displaying pain-like behaviors did not differ ($P > 0.05$) among treatments 60 to 90 min or 120 to 150 min after administration of the treatments. The time spent displaying pain-like behaviors did not differ ($P > 0.05$) among CON, CO2, and CON+NSAID pigs during any of the time periods.

There were no other treatment or treatment by time interactions ($P > 0.05$) for the other behaviors measured (Table 4).

Stress Vocalizations

The percentage of stress vocalizations differed (treatment × time: $F_{6,400} = 5.39$, $P = 0.001$) among castration and control treatment groups (Figure 3). The percentage of stress vocalizations did not differ ($P > 0.05$) during handling before administration of the treatments. However, the percentage of stress vocalizations elicited by CAS and CAS+NSAID pigs during administration of the treatment was greater ($P < 0.05$) than CON, CO2, CON+NSAID, CAS+CO2, and CAS+BOTH pigs. The percentage of stress vocalizations did not differ ($P > 0.05$) among CON, CO2, and CON+NSAID pigs before or during administration of the treatments.

The percentage of stress vocalizations differed (treatment: $F_{3,410} = 26.47$, $P = 0.001$) among procedures with castration eliciting a greater ($P < 0.001$) percentage of stress vocalizations than handling, induction with CO2, and an i.m. injection. (handling: $17.0 ± 1.17$%, induction

with CO2: $27.1 ± 1.52$%, i.m. injection: $36.0 ± 1.93$%, castration: $62.5 ± 1.32$%).

Body Weight and Healing Scores

Change in BW did not differ (treatment: $F_{6,131} = 0.79$, $P = 0.579$) among treatments 24 h after castration (CON: $0.12 ± 0.044$ kg, CO2: $0.15 ± 0.044$ kg, CON+NSAID: $0.17 ± 0.044$ kg, CAS: $0.17 ± 0.044$ kg, CAS+CO2: $0.19 ± 0.044$ kg, CAS+NSAID: $0.15 ± 0.044$ kg, CAS+BOTH: $0.16 ± 0.044$ kg).

Wound healing score did not differ ($P > 0.05$) among treatments and there was no significant treatment by day effect (CAS: $3.1 ± 0.07$, CAS+CO2: $3.1 ± 0.07$, CAS+NSAID: $3.3 ± 0.07$, CAS+BOTH: $3.3 ± 0.07$).

DISCUSSION

Surgical castration is the most common method used on commercial farms to prevent boar taint and aggressive behavior amongst male pigs; however this procedure can cause physiological and behavioral changes indicative of pain-induced distress, including increased cortisol (Carroll et al., 2006) and lactate (Prunier et al., 2005) concentrations, heart rate (White et al., 1995), pain-related behaviors (Moya et al., 2008), and reduced activity at the udder (Hay et al., 2003). In the present study, surgical castration without pain relief also resulted in physiological and behavioral changes indicative of pain-induced distress, including increased cortisol and CRP concentrations, as well as increased stress vocal-

Table 4. The percentage of time pigs spent performing behaviors and postures (least squares means ± SE) after control handling or castration with or without pain relief (n = 10/treatment)

	Treatments[1]								P-value		
Behavior[2]	CON	CO2	CON+ NSAID	CAS	CAS+ CO2	CAS+ NSAID	CAS+ BOTH	SE[3]	Treatment	Period	Treatment × Period
Lying without contact	2.1[a]	2.5[ab]	1.4[a]	4.1[bc]	4.9[c]	1.1[a]	1.1[a]	0.8	< 0.0001	0.066	0.001
Lying with contact	38.9	39.7	38.9	34.4	35.7	41.0	38.0	2.8	0.141	0.000	0.891
Nursing	12.1	12.4	14.4	14.4	12.3	13.1	13.7	2.5	0.908	0.235	0.775
Sitting	0.7	0.9	0.9	1.0	0.7	0.7	0.6	0.3	0.860	0.001	0.335
Standing	7.0	5.7	5.3	6.8	6.8	5.1	7.9	1.5	0.302	< 0.0001	0.875
Walking	1.5	1.1	1.4	1.6	1.2	1.4	0.7	0.7	0.741	< 0.0001	0.805
Pain-like behaviors	0.9	1.0	0.9	1.2	1.4	0.7	1.0	0.4	0.744	< 0.0001	0.007

[1]Treatments: sham castration (CON); sham castration while the pig was anesthetized with carbon dioxide (CO2); sham castration plus NSAID administered at the time of handling (CON+NSAID); castration (CAS); castration while the pig was anesthetized with carbon dioxide (CAS+CO2); castration plus NSAID administered at the time of castration (CAS+NSAID); and castration conducted while the pig was anesthetized with carbon dioxide plus NSAID administered at the time of castration (CAS+BOTH).

[2]Behaviors are described in Table 1.

[3]Pooled SE.

[a-c] Within a row, least square means without a common superscript differ (P < 0.05).

izations and lying without contact behavior. It would be beneficial for the welfare of pigs and the swine industry to develop methods of pain relief that could be used to alleviate the pain caused by procedures such as castration, which are also practical to implement on the farm. Local anesthetic was shown to reduce the mean arterial blood pressure (Haga and Ranheim, 2005), heart rate (White et al., 1995), vocalizations (Leidig et al., 2009), and behavioral response (McGlone and Hellman, 1988; White et al., 1995) to surgical castration in pigs.
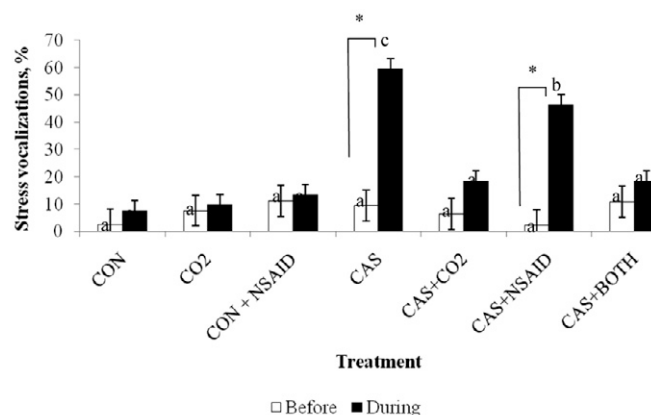

Figure 3. The percentage of stress vocalizations (least squares means ± SE) before (Before) the administration of the treatment and during (During) administration of the treatment. Treatments: control handling (CON; n = 16); sham castration while the pig was anesthetized with carbon dioxide (CO2; n = 17); sham castration plus non-steroidal anti-inflammatory drug (NSAID) administered at the time of handling (CON+NSAID; n = 17); castration (CAS; n = 17); castration while the pig was anesthetized with carbon dioxide (CAS+CO2; n = 15); castration plus NSAID administered at the time of castration (CAS+NSAID; n = 19); and castration conducted while the pig was anesthetized with carbon dioxide plus NSAID administered at the time of castration (CAS+BOTH; n = 18). *Within treatment, least squares means differed between Before and During (P < 0.05). [a-c]Means without a common superscript differ (P < 0.05).

However, administering local anesthetic on the farm has several disadvantages, including the need to handle pigs twice (i.e., once to administer the anesthetic and secondly to castrate the animal), and the necessity of having a veterinarian to perform the procedure, which has economic implications. Therefore, in the present study, we wanted to determine if general anesthesia induced by CO_2 or analgesia elicited by giving an NSAID would reduce the pain caused by castration. Anesthesia can be defined as the loss of feeling or sensation (Blood and Studdert, 1995), and general anesthetic agents achieve this by causing a state of unconsciousness, which results in an absence of pain sensation over the entire body. Inhalation of CO_2 causes anesthesia by suppressing nerve cell function and cerebral electrical activity (Martoft et al., 2003). The advantages of using CO_2 as a form of anesthesia include the speed at which CO_2 can be administered, the speed at which animals return to consciousness, the fact that CO_2 is not a restricted drug requiring administration by a veterinarian, and lastly, that there are no issues with drug residues (Gerritzen et al., 2008).

Non-steroidal anti-inflammatory drugs have analgesic, antipyretic, and anti-inflammatory properties resulting from their ability to inhibit the synthesis of PG (Blood and Studdert, 1995). Pigs that were given an NSAID immediately after castration displayed reduced pain-related behaviors and acute-phase response after castration (Hansson et al., 2011). The advantages to using an NSAID are that they can be administered intramuscularly, and there are NSAID (e.g., Banamine) approved for use in swine in the U.S. Furthermore, both of these methods can be administered by trained staff, and the animal needs only to be handled once.

Cortisol concentrations were increased in pigs for over 2 h in response to surgical castration in the present study, which is consistent with Prunier et al. (2005), Carroll et al. (2006), and Marchant-Forde et al. (2009), who also showed that surgical castration causes an increase in cortisol concentrations in pigs of several ages. In the present study, anesthetizing pigs with CO_2 before castration did not markedly reduce the cortisol response to castration. Therefore, it appears as though pigs anaesthetized with CO_2 still experienced distress as a result of castration, even though they probably did not experience the initial noxious sensory input caused by this procedure. Approximately 5 min after castration or handling, ACTH and β-endorphin concentrations were greater in pigs anesthetized with 80% CO_2/20% O_2 before castration, as compared with pigs castrated without pain relief (Kohler et al., 1998). Kohler et al. (1998) demonstrated that induction with CO_2 can cause pigs considerable distress, as shown by increased struggling, vocalizations, and strenuous breathing. Therefore, it is possible that pigs that are anesthetized with CO_2 experience distress as a result of the induction process, and then again when they regain consciousness. However, the integrated cortisol response to castration, as measured by calculating the AUC, was similar between control and CAS+CO2 pigs. This could suggest that CO_2 anesthesia may reduce the distress caused by castration. Previous studies have shown that anesthetizing rats with CO_2 at concentrations > 70% can have an anti-nociceptive effect for up to 60 min (Mischler et al., 1994, 1996). However, Mischler et al. (1994, 1996) refer to this being a stress-mediated anti-nociceptive response, implying that animals must initially experience stress (exposure to increased concentrations of CO_2) to gain these anti-nociceptive benefits. Even though anesthetizing pigs with CO_2 before castration may marginally reduce the distress caused by this procedure, it is unlikely that this outweighs the distress caused by induction. However, more research is needed to ascertain the long-term pros and cons of this method of pain relief.

Non-steroidal anti-inflammatory drugs are a group of drugs that have analgesic, antipyretic, and anti-inflammatory properties (Blood and Studdert, 1995). In the present study, pigs were given an NSAID immediately after castration to evaluate if this type of drug is effective at reducing pain-induced distress associated with castration, even though the pigs would still experience the initial noxiousness caused by surgically removing the testes. The NSAID was injected at the time of castration to eliminate the necessity of handling the pigs twice. In the present study, the cortisol response to castration was not reduced by giving an NSAID immediately after castration. Pigs given the NSAID Meloxicam i.m. 10 to 30 min before castration had numerically less cortisol and

ACTH concentrations 30 min after castration, as compared with pigs castrated without pain relief (Keita et al., 2010). Similarly, giving calves the NSAID Ketoprofen 20 min before surgical castration (Earley and Crowe, 2002), or lambs given the NSAID Diclofenac 20 min before ring castration (Graham et al., 1997), reduced the peak cortisol response to castration. In the present study, administering an NSAID immediately after castration reduced the percentage of time pigs spent lying without contact and displaying pain-like behaviors to percentages similar to those of CON pigs. Furthermore, pigs given Meloxicam immediately after castration displayed reduced pain-related behaviors and had a reduced acute-phase response after castration compared with pigs that did not receive an NSAID (Hansson et al., 2011). Therefore, administering an NSAID at the time of castration appears to have some beneficial effects on the behavioral response to castration, but to achieve the optimum beneficial effects of giving an NSAID, it may be necessary to administer the NSAID at least 20 min before the painful procedure.

In the present study, blood samples were collected at 24 and 72 h after castration or handling to determine the long-term effect of these procedures on the cortisol response in pigs. At 24 h, cortisol concentrations did not differ among any of the treatments, which is similar to the findings of Carroll et al. (2006). However, 72 h after castration or handling, cortisol concentrations were greater in pigs that received an NSAID after castration (CAS+NSAID and CAS+BOTH) than baseline concentrations, and greater than pigs castrated without pain relief. After i.m. administration of flunixin, quantifiable plasma drug concentration can be measured up to 18 h post-drug administration, according to the Banamine package insert. However, in studies submitted for regulatory approval of flunixin meglumine in swine in the U.S., 66% of animals showed signs of injection site irritation and damage 4 d after treatment, with 83% of animals having gross lesions at the injection site at 7 and 14 d after treatment (FDA-CVM, 2005). Therefore, it is possible that this increase in plasma cortisol response could be associated with pain at the injection site that occurred after the analgesic effects of the drug decreased.

The acute-phase response can occur as a result of infection, inflammation, or trauma. In the present study, CRP concentrations were greater in pigs castrated without pain relief and pigs anesthetized with CO_2 before castration, as compared with control-handled pigs, but were similar between control pigs and pigs given an NSAID at the time of castration. Conversely, the acute-phase proteins, CRP, serum amyloid A, and haptoglobin were not elevated in response to surgical castration in 5-d-old pigs at 24 or 72 h after castration in a study by Moya et al. (2008). Earley and Crowe (2002) re-

ported that surgically castrated bull calves had greater fibrinogen and haptoglobin concentrations as compared with controls for up to 7 d after castration. Furthermore, calves given Ketoprofen before castration had increased fibrinogen and haptoglobin concentrations as compared with control calves, but these concentrations were less than calves castrated without an NSAID for up to 3 d after castration. These results suggest that the NSAID used in the present study was effective at reducing the inflammation caused by surgical castration in pigs. However, wound healing scores did not differ between pigs that did or did not receive an NSAID at the time of castration, suggesting that the anti-inflammatory effects of the NSAID did not translate into improved healing.

Substance-P concentrations were measured to more accurately assess nociception in response to castration in pigs. Surgical castration did not elicit a SP response in pigs as compared with control-handled pigs. Conversely, in beef cattle, surgical castration caused increased SP concentrations as compared with sham-castrated calves (Coetzee et al., 2008). To our knowledge, this is the first study that attempted to use SP as an end point to assess pain in piglets, and as such, further research is needed to quantify the relationship between castration and SP release. Furthermore, our failure to find a detectable difference between CON and castrated pigs could be due to timing of sample collection, stability of SP in swine plasma such as collection and storage conditions, and the kinetics of SP release in older as compared with younger animals.

Substance P concentrations were greater in pigs anesthetized with CO_2 30 and 60 min after castration as compared with pigs castrated without pain relief. Furthermore, SP concentrations were increased in pigs anesthetized with CO_2 but not castrated, as compared with control-handled pigs, suggesting that it is the induction with CO_2 that caused the increase in SP concentrations, and not the combined effect of CO_2 and castration. Substance-P is reported to have stimulatory effects on respiration and is released in response to hypoxia. Repeated episodes of hypoxia have been shown to significantly increase interstitial concentrations of substance-P in maturing piglets (Waters et al., 1997). This may explain why plasma SP concentrations were increaesd in pigs exposed to CO_2 compared with unexposed pigs.

Pig behavior during the first 150 min after castration was affected by castration treatment in the present study. During the first 30 min after castration, pigs castrated without pain relief spent more time lying without contacting other pigs or the sow, as compared with pigs given anesthesia or analgesia. An increase in the time spent lying without contract also was found by Hay et al. (2003) and Sutherland et al. (2010) in pigs castrated without pain relief. Social isolation, as a result of lying without contacting another pig, may result from

a protective mechanism to avoid contacting other litter mates, and thereby, potential situations that may elicit pain (Mellor et al., 2000). It also may result from pigs prostrating their bodies due to pain (Hay et al., 2003). Regardless of the cause, this behavior could have potentially negative consequences, as pigs that spend more time lying away from a heat source, such as other pigs or a heat lamp, may become more vulnerable to hypothermia, especially as young pigs have poor thermoregulatory capacity (Herpin et al., 2002).

Interestingly, during the 60- to 90-min and 120- to 150-min observation periods, pigs anesthetized with CO_2 and then castrated spent more time lying without contact than pigs from other treatments. However, Van Beirendonck et al. (2011) did not observe a difference in social cohesion/isolation between pigs anesthetized with CO_2 before castration or pigs castrated without pain relief. However, they did find that pigs anesthetized with CO_2 before castration lay down more than non-anesthetized pigs at certain times. Pigs recover consciousness completely from CO_2 anesthesia after approximately 1 min (Gerritzen et al., 2008), but inducing anesthesia with CO_2 could have some unknown prolonged effects that could account for this increased social isolation in CAS+CO2 pigs. Possibly, CAS+CO2 pigs are experiencing an increase in noxiousness due to the anti-nociceptive effects of the CO_2 wearing off at this time. Furthermore, during the first 30 min after castration, pigs anesthetized with CO_2 and then castrated displayed more pain-like behaviors than pigs in the other treatment groups. This corresponds with findings of Van Beirendonck et al. (2011), suggesting that pigs anesthetized with CO_2 may experience more pain-induced distress as a result of castration over the entire observation period.

The measurement of vocalizations has been shown to be a reliable non-invasive measure of distress in pigs in response to different stressors (Hillmann et al., 2004; Schön et al., 2004; Puppe et al., 2005) and the STREMODO system can simplify the assessment of stress-induced vocalizations in pigs. In the present study, castrating pigs that were anesthetized with CO_2 resulted in a decreased percentage of stress vocalizations than non-anesthetized pigs, suggesting that the 30-s CO_2 induction period used in the present study was sufficient to anesthetize pigs adequately. However, a reduction in the percentage of stress vocalizations at the time of castration displayed by CAS+CO2 and CAS+BOTH pigs does not necessarily confirm that these pigs did not feel distress in response to being castrated, as these pigs still had increased cortisol concentrations and displayed increased pain-like behaviors during the first 30 min after castration.

In the present study, the vocal response to handling, induction with CO_2, giving an i.m. injection, and castration were compared. The percentage of stress vocaliza-

tions were numerically greater in response to CO_2 induction and administration of an i.m. injection as compared with control handling. It was only castration that resulted in a significantly greater vocal response, suggesting that the noxiousness of this procedure surpasses that of handling, respiratory distress caused by CO_2 induction, and an i.m. injection.

In conclusion, none of the anesthesia or analgesia treatments markedly reduced the pain-induced distress caused by surgical castration in pigs. The contradictory physiological and behavioral effects associated with CO_2 anesthesia, the reduced cortisol response, increased SP response, and increased performance of pain-like behaviors, suggests further investigation is needed before any conclusions can be made regarding the effectiveness of CO_2 anesthesia as a method of pain relief in pigs. Administering an NSAID immediately after castration appeared to reduce the percentage of time pigs spent lying without contact and displaying pain-like behaviors to percentages similar to those of control-handled pigs. Therefore, there may be a potential benefit to giving pigs an NSAID at the time of castration. Ultimately, more research is needed to find methods of pain relief that are practical to implement on the farm, or to develop alternatives to castration in pigs.

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