Behavior of piglets after castration with or without carbon dioxide anesthesia

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ABSTRACT: Surgical castration of male piglets without anesthesia is a routine management practice conducted on commercial pig farms. For animal welfare reasons, it would be beneficial to develop methods of practical pain relief. The objective of this study was to evaluate the effect of providing CO2 anesthesia before castration on the behavior of piglets for up to 8 d after castration in comparison with piglets castrated without anesthesia. In 3 successive replicates, the behavior of 186 male piglets castrated with (n = 95) or without (n = 91) anesthesia was observed for up to 8 d after castration. All piglets in a given replicate were castrated on the same day, before 8 d of age. Behavioral observations were carried out in accordance with a continuous focal sampling procedure that began immediately after castration and continued for a period of 1 wk. Barrows anesthetized with CO2 before castration displayed more interactive behaviors during the overall observation period than the other barrows (P = 0.0412), which may indicate better welfare. Assessing all observation periods separately, differences in activity at the udder, lying, walking, and interactive behaviors appeared to support the beneficial effect of providing CO2 anesthesia before castration. However, these differences varied over time between treatment groups. The most important conclusion was that piglets castrated with or without CO2 anesthesia displayed behaviors indicative of pain and discomfort for up to 6 d after castration. Therefore, additional analgesia may be necessary to eliminate the long-term pain caused by castration even in piglets anesthetized with CO2 before castration.

Key words: anesthesia, behavior, carbon dioxide, castration, piglet

INTRODUCTION

Carbon dioxide gas is currently being used in several countries to stun pigs before exsanguination during slaughter. Carbon dioxide gas is usually administered at concentrations greater than 80% to minimize the aversion period experienced by the animal and reduce the risk of inadequate anesthesia (Nowak et al., 2007). Kohler et al. (1998) concluded that CO2 anesthesia with a concentration of 80% can be induced safely and rapidly in pigs and that castration can be performed without any reaction, but stress induced by handling and manipulation before castration is not reduced. Administering CO2 can cause behavioral signs of aversion in pigs. Svendsen (2006), on the other hand, stated that aversion before losing consciousness is compensated for by the fact that piglets experience complete anesthesia and analgesia during castration. Gerritzen et al. (2008) observed that heavy breathing was the only typical behavior that piglets exhibited when exposed to a mixture of 70% CO2 and 30% O2. Gerritzen et al. (2008) also concluded that the period of unconsciousness and analgesia achieved by this gas mixture was long enough to castrate pigs without them experiencing pain.

Trembling behavior has been observed in piglets, lambs, and dogs for several days after castration, which is indicative that castration causes pain (Morton and Griffiths, 1985; Wemelsfelder and van Putten, 1985; Molony et al., 1997). Other pain-specific behaviors observed in piglets after castration include spasms, tail wagging, scratching, and huddling up, as well as changes in lying and suckling behavior (McGlone et al., 1993; Taylor et al., 2001; Hay et al., 2003; Llamas Moya et
al., 2008). However, the effect of CO₂ anesthesia before castration on pain-related behaviors displayed by piglets after castration has not yet been studied.

The hypothesis of this study is that piglets will experience less pain and discomfort after castration when anesthetized with CO₂ before castration, thus improving their overall welfare.

**MATERIALS AND METHODS**

Piglets were handled in accordance with the Belgian law on the protection of animals, and the experimental protocol was approved by the Ethical Committee of the Katholieke Universiteit Leuven on the use of experimental animals.

**Animals and Housing**

Hybrid pigs (Piétrain × Hypor), heterozygous for the halothane gene, were used. Piglets were raised in the same housing conditions at the Zootechnical Centre, Katholieke Universiteit Leuven R&D (Belgium). Management of this system was based on the “all-in, all-out” principle for each room. The farrowing pen had a lying area for the piglets and a sow lying/suckling area provided with rails for piglet protection. Piglets had ad libitum access to water and a commercial diet throughout the study. Piglets were individually marked with an ear tag within the first 24 h after birth and weaned on the same day at approximately 28 d of age.

The farrowing house was temperature controlled using floor and air heating so that the piglets were kept within their thermal neutral zone. The housing environment was automatically controlled with a computerized heating and ventilation system, so that the required temperature was managed independent of the outside temperature. The selected temperature of the farrowing rooms was based on information advised in McGlone and Pond (2003) and was dependent on piglet BW.

A total of 186 male piglets from 38 litters were used in 3 successive replicates. The number of gilts and boars within a litter was more or less equal, but the total number was not standardized. Cross fostering was applied during the week of birth to balance litters for BW and number of piglets. All barrows from a single litter received the same castration treatment. Other painful interventions, such as tail docking, ear tagging, and iron injection were standardized for each piglet and applied without anesthesia or analgesia within the first 3 d of life, thus avoiding a confounding effect with the experimental treatment.

**Experimental Procedure**

Piglets within a given replicate were castrated on the same day but before 8 d of age (European Directive 2001/93/EG). The age of piglets at castration ranged from 2 to 8 d because of different dates of birth. Piglets were weighed on the day of castration. Between weighing and castration, male piglets were vaccinated for *Mycoplasma hyopneumoniae* (Stellamune Mycoplasma, Intervet, Ukkel, Belgium) and treated with 0.5 mL of amoxicillin (Duphamox, Fort Dodge, Weesp, the Netherlands). Surgical castration was performed by the company veterinarian following the common castration technique. The piglet was held in the hand of the technician (in a head-down position); a single transverse incision was made with a scalpel; and then the testicles were removed by cutting the spermatic cords. Finally, a disinfectant (Cyclospray, Eurovet, Bladel, the Netherlands) was sprayed onto the wound. After castration, the piglets were returned to their pen. Piglets were either anesthetized with 100% CO₂ for a period of 25 s before castration (experimental group I) or castrated without anesthesia (experimental group II). Carbon dioxide gas was administered via a mouth mask at a concentration of 100% to reduce the duration of aversion. Table 1 shows the experimental design, consisting of 2 treatment groups. In the first, second, and third replicates, 8, 18, and 12 pens were used, respectively, with 4, 10, and 6 pens, respectively, assigned to the CO₂ anesthesia treatment. The CO₂ group consisted of 95 piglets from 20 litters; the unanesthetized group contained 91 piglets from 18 litters.

After castration, barrows were marked visually with a colored sign on their back and returned to their pen. Individual piglet behavior was scored according to the ethogram validated by Hay et al. (2003) (Table 2).

**Behavioral Observations**

Immediately after castration, behavior of each barrow within each pen was observed continuously for a period of 10 min. Pens were observed at random within a sequence according to a scan sampling procedure. The duration of each observation period (i.e., 10 min multiplied by the number of pens) was considered adequate to achieve the objective of the present study. The main objective of the study was to observe piglet behavior for up to 8 d after castration in relation to anesthesia treatment because pain-related behaviors were observed in piglets after castration for up to 5 d by Hay et al. (2003). The fact that castration causes pain shortly after the procedure is well known because most

**Table 1. Experimental design**

<table>
<thead>
<tr>
<th>Experimental group I</th>
<th>Experimental group II</th>
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<tbody>
<tr>
<td>Anesthetized with CO₂, then castrated</td>
<td>Castrated without anesthesia</td>
</tr>
<tr>
<td>n = 95 piglets (20 pens)</td>
<td>n = 91 piglets (18 pens)</td>
</tr>
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studies made observations within 24 h after castration (McGlone and Hellman, 1988; Taylor et al., 2001; Llamas Moya et al., 2008). The observation frequency in these studies was high (e.g., every 3 min), but on a limited number of piglets (n = 80). Thirty-eight pens were included in the present study, which represented a total of 186 male piglets. Each pen was observed for a period of 10 min, 2 times a day, giving a total of 13 h of continuous behavioral observations a day for the 38 pens. Hence, to make it feasible to combine observations that spanned over 8 d and 186 animals, a sample frequency of 10 min per pen was chosen. It was assumed that pen sampling matched time sampling, so that the observed behaviors per pen were a representative sample of the evolution of pain experienced by the animals over time from castration to d 8. Moreover, the applied statistical method made it possible to consider the individual piglet as the experimental unit, so that about 5 male piglets per time frame of 10 min were available during observation of a pen.

During each 10-min observation period, the frequency of each behavior was recorded every minute for each piglet in accordance with a continuous focal sampling procedure (Fraser, 1978). It was possible that a piglet could show a sequence of different behavioral events within a given single minute; however, the number of times each defined behavioral category was displayed was taken into account by calculating the frequency of that behavioral category (i.e., per minute for the total number of observed piglets). All behavioral categories were mutually exclusive and are described in Table 2. In the event a piglet was sleeping isolated from the others, both categories were assigned to that piglet at that moment of observation. All observations [i.e., two 10-min sessions per pen per day (am and pm) for 6 d], were performed by a single observer standing in the central corridor of the pig house. It was expected that no behavioral effects due to castration treatment would continue after d 6, as reported by Hay et al. (2003). The observations started on Wednesday and ended the next Wednesday (there were no behavioral observations on Saturday and Sunday, so there were 3 d of observation followed by a 2-d break and then another 3 d of observation). The total observation time per pen, in combination with the number of pens (i.e., 38), was assumed to be sufficient to obtain an adequate understanding of the treatment effects studied (McGlone et al., 1993; Hay et al., 2003).

**Statistical Analysis**

All data were analyzed using the SAS software (SAS Inst. Inc., Cary, NC). A significance level of 0.05 was used. Behaviors were grouped for analysis to have a
sufficient number of observations within each grouped category, so that the empirical estimation could be carried out on a sufficient number of observations. A condition of the applied statistical model is that the performance of the empirical estimation passes the convergence test (SAS Inst. Inc.). Hence, lateral lying, ventral lying, and sleeping were grouped under “lying”; teat seeking, suckling, and udder massage were grouped under “udder activity”; huddled up, trembling, spasms, scratching, and tail wagging were grouped under “pain-related behaviors”; nosing, chewing, licking, playing, and aggression were grouped under “interaction behaviors”; walking and running were grouped under “walking”; and sitting, standing, and kneeling were grouped under “postures.” The complete sequence of suckling behavior was estimated to last about 2 min, consisting of 5 typical different phases, with the behavior preliminary to suckling being very variable (Fraser, 1980). This information was considered to be a biologically sound argument for grouping these behaviors into a single category representing nursing behavior, which is reported to be sensitive to castration (McGlone and Hellman, 1988). Moreover, grouping of a sufficient number of physiologically related behavioral events into a single category increased the probability of matching the power requirements of the applied statistical model. Huddling and trembling behaviors were not regarded as thermoregulatory behaviors because environmental temperature in the farrowing/nursing room was engineered according to the thermal neutral zone of the piglet. This was confirmed by the displayed temperature and by observation of piglet lying behavior (i.e., that of the female piglets being neutral for castration; Geers et al., 1986).

Data were not normally distributed and were dichotomized using the median as cut-off value. The binary data were analyzed using the logistic mixed model, with fixed effects being treatment, observation period and the interaction between treatment and observation period, as well as piglet BW and age at castration, with the piglet as random effect. Random effects accounted for the variability between the piglets within and between litters. The applied procedure made it possible to allocate a random effect to a variable (SAS Inst. Inc.), so that piglets could be regarded as the experimental units. Two factors were considered in defining this random effect: variability between litters and variability between individual piglets. When taking into account the lowest level in the model (i.e., individual piglets), the variability between piglets partially represented the variability between litters. Furthermore, Fraser (1978) analyzed behavior of group-housed piglets with the individual piglet being regarded as the experimental unit. After analysis of the data for the overall period of observations, data were also analyzed in the same way for each morning and afternoon observation period. Values of the calculated frequency are presented as means ± SEM for each behavioral category. The unit of values mentioned in the text is mean frequency per minute.

**RESULTS**

To fully understand the effect of CO₂ anesthesia on the behavior of piglets after castration, results were reported for all data over time and for each observation period separately. Attention was focused on the complementary behavioral events that are considered to be indicative of pain and discomfort, and also on the so-called positive behaviors (i.e., interactive behavior and social cohesion) that are important for animal welfare (Fraser and Broom, 1990; Blackshaw et al., 1997).

**Observation Periods Taken Together**

Assessment of all observation periods taken together found that the only behavioral category that showed a significant difference was interactive behavior (nosing, chewing, licking, playing, and aggression; Figure 1). Barrows in the anesthetized group displayed 13% more interactive behaviors than barrows from the unanesthetized group (0.0842 ± 0.00548 vs. 0.0748 ± 0.00523; \( P = 0.0412 \)).

**Observation Periods Separately**

**Lying.** During the afternoon observation periods on d 2 (\( P = 0.0004 \)) and 4 (\( P < 0.0001 \)), barrows in the CO₂ group (0.980 ± 0.0763 and 1.409 ± 0.0836, respectively) lay down about 71% more than unanesthetized piglets (0.567 ± 0.0708 and 0.834 ± 0.0836, respectively). During the morning observation periods on d 4 (\( P = 0.0008 \)) and 6 (\( P = 0.0245 \)), barrows in the unanesthetized group (1.295 ± 0.0801 and 1.089 ± 0.0843, respectively) lay down 55 and 9% more, respectively, than barrows in the anesthetized group (0.837 ± 0.0830 and 0.996 ± 0.0839, respectively; Figure 2).

**Udder Activity.** Barrows in the CO₂ group were 26% more active at the udder than piglets in the unanesthetized group during the morning observation periods on d 1 (0.0789 ± 0.0169 vs. 0.0627 ± 0.0233; \( P = 0.0259 \)), 69% more during the afternoon observation periods separately.
periods on d 1 (0.193 ± 0.0276 vs. 0.114 ± 0.0201; \( P = 0.0372 \)), 56% more during the morning observation periods on d 6 (0.231 ± 0.0326 vs. 0.148 ± 0.0242; \( P = 0.0245 \)), and 19% more during the afternoon observation periods on d 6 (0.229 ± 0.0330 vs. 0.193 ± 0.0359; \( P = 0.0149 \)). However, during the afternoon observation periods on d 3 (\( P = 0.0424 \)) and the morning observation periods on d 5 (\( P = 0.0167 \)), barrows in the unanesthetized group (0.399 ± 0.0467 and 0.309 ± 0.0396, respectively) performed more udder activity than barrows in the CO2 group (0.292 ± 0.0367 and 0.172 ± 0.0311, respectively), 74 and 80%, respectively (Figure 3).

**Interactive Behavior.** During the morning observation periods on d 1 (\( P = 0.0188 \)) and 4 (\( P = 0.0008 \)), barrows in the CO2 group (0.0116 ± 0.00330 and 0.128 ± 0.0156, respectively) performed more interactive behaviors than barrows in the unanesthetized group (0.00110 ± 0.00110 and 0.0385 ± 0.00921, respectively), 955 and 228%, respectively. During the afternoon observation periods on d 4, on the other hand, barrows in the unanesthetized group (0.406 ± 0.0390 vs. 0.166 ± 0.0296; \( P < 0.0001 \)) and d 5 (0.359 ± 0.0402 vs. 0.303 ± 0.0476; \( P = 0.0011 \)), 145 and 19%, respectively (Figure 5).

**Pain-Related Behavior.** During the morning observation periods on d 2 (0.131 ± 0.0192 vs. 0.0549 ± 0.0104; \( P = 0.0376 \)), d 3 (0.0684 ± 0.0130 vs. 0.0527 ± 0.0150; \( P = 0.0191 \)), d 4 (0.0958 ± 0.0148 vs. 0.0582 ± 0.0156; \( P = 0.0477 \)), and d 6 (0.100 ± 0.0154 vs. 0.0473 ± 0.0105; \( P = 0.0028 \)), barrows of the CO2 group displayed more pain-related behaviors (138, 30, 65, and...
113%, respectively) compared with piglets in the unanesthetized group. During the afternoon observation periods on d 5, piglets in the unanesthetized group displayed 75% more pain-related behaviors (0.0220 ± 0.00973 vs. 0.0126 ± 0.00584; \( P = 0.0109\); Figure 6).

Figure 6 shows a larger difference between the mean values of pain-related behavior during d 1, compared with the significant differences. However, these differences between the mean values on d 1 were not statistically significant. The explanation is that variability with respect to showing pain-related behavior among barrows was large.

**Postures.** During the morning observation periods on d 4, barrows in the anesthetized group displayed 139% more postures than those in the unanesthetized group (0.0684 ± 0.0107 vs. 0.0286 ± 0.00591; \( P = 0.0079\)). During the afternoon observation periods on d 4; however, barrows in the unanesthetized group performed 64% more postures (0.0637 ± 0.0104 vs. 0.0389 ± 0.00767; \( P = 0.0359\); Figure 7). Figure 7 shows a larger difference between the mean values of postures for afternoon observations on d 5, compared with the significant differences. However, this difference between mean values on the afternoon of d 5 was not statistically significant. The explanation is that individual variability with respect to showing sitting, standing, and kneeling among barrows was large.

**Social Cohesion.** Whether or not a piglet isolates itself from other piglets is considered a measure of social cohesion (Table 2). There were no differences in social cohesion between the 2 treatment groups.

**DISCUSSION**

**Effects of Treatment on Behavior: Overall Period of Observation**

Anesthetized barrows interacted more with one another than barrows in the unanesthetized group. Interactive behaviors in this study included frequency of nosing, chewing, licking, aggression, and playing. Decreased interactive behaviors, such as play, displayed by castrated piglets may indicate poor welfare (Llamas Moya et al., 2008). Blackshaw et al. (1997) specifically described playing behavior as an indication of positive animal welfare. Conversely, Hay et al. (2003) suggested that reduced oral exploration, such as licking and chewing, may be associated with the experience of pain. These conclusions suggested by other researchers agree with our findings that the decreased number of interactive behaviors (less playing, licking, and chewing) performed by unanesthetized barrows as compared with anesthetized barrows could be an indication of a different state of welfare.

**Effects of Treatment on Behavior: Per Observation Period**

The frequency of lying behavior was greater in barrows in the anesthetized group than the unanesthetized group during the afternoon observation periods on d 2 and 4, though the opposite was true during the morning observation periods on d 4 and 6 (i.e., d 6 and 8 after castration, respectively). In the present study, a beneficial effect of anesthesia during castration could be advocated, according to Torrey et al. (2009), because of a greater frequency of lying behavior the first 6 d after castration. A decreased frequency was observed from d 6 on, on the other hand, which matched the point of view of McGlone and Hellman (1988) and Hay et al. (2003). Both McGlone and Hellman (1988) and Hay et al. (2003) observed a greater frequency of lying behavior within the first hours after castration, mutually excluding suckling activity, and considered this an indication of poor welfare.

Udder activity differed between the 2 treatment groups over the observation periods. During the morning and afternoon observation periods on d 1 and 6, barrows in the CO2 group displayed more activity at

![Figure 7](image-url)
the udder than unanesthetized barrows. During the afternoon observation periods on d 3 and morning observation periods on d 5, however, barrows in the unanesthetized group were more active at the udder. Noonan et al. (1996) suggested that increased udder activity may be related to the experience of pain as endorphins (i.e., endogenous opioids) may be released during suckling, which may have an analgesic effect. McGlone and Hellman (1988) and Hay et al. (2003), on the other hand, found that piglets spent less time at the udder during the first hours after castration, when they were experiencing pain. The results from the present study seem to be in line with the latter findings; during the first days after castration barrows that were anesthetized with CO2 showed more udder activity, whereas unanesthetized barrows became more active only from d 7 (i.e., observation d 5) after castration.

The observation that the turning point in udder activity occurred during the observation periods on d 4 (d 6 after castration) is also supported by the observed changes in interactive behaviors. During the morning observation periods on d 1 and 4, barrows in the CO2 group showed more interactive behaviors. During the afternoon observation periods on d 4, however, barrows in the unanesthetized group displayed more interactive behaviors. The explanation previously given concerning the beneficial aspect (overall period of observation) can also be applied here (Torrey et al., 2009).

Llamas Moya et al. (2008) suggested that castrated piglets may avoid certain behavioral activities, such as walking, to minimize pain. During the morning observation periods on d 4 and 5, barrows in the CO2 group walked more than barrows in the unanesthetized group. During the afternoon observation periods on d 2 and 4, however, the opposite was found. These alternating observations of walking behavior could suggest that both treatment groups in fact experienced pain as result of castration and therefore adjusted their walking patterns, though at different times.

The postures performed by piglets differed significantly between the 2 treatment groups, but only during the observation periods on d 4. During the morning observation periods on d 4, barrows in the CO2 group sat, kneeled and stood more, whereas during the afternoon of that same day barrows in the unanesthetized group displayed these behaviors more frequently. Taylor et al. (2001) observed that the frequency of sitting and standing postures increased after castration in piglets. Other studies (McGlone and Hellman, 1988; McGlone et al., 1993; Kielly et al., 1999), however, found that piglets spent less time standing after castration. Concerning postures, the literature appears to be contradictory, which suggests that changes in posture are not a fully reliable indicator of pain in response to castration in piglets (Hay et al. 2003).

In the present study, barrows in the CO2 group displayed more pain-related behaviors during the morning observation periods on d 2, 3, and 4 and afternoon observation periods on d 6. Only during the afternoon observation periods on d 5 did barrows in the unanesthetized group show more pain-related behaviors. These results contradict the hypothesis of the present study that piglets anesthetized with CO2 during castration would suffer less pain after castration; however, these results confirm the finding that CO2 induces analgesia but only for a short period of time (Gerritzen et al., 2008). In other words, piglets under CO2 anesthesia did not feel pain during castration, but it appears as though the anesthetic effects of the CO2 wore off quickly, so additional analgesia may be necessary to avoid the long-term pain experienced by piglets in response to castration. Another point of view is that the variables used to assess pain-related behaviors used in this study did not discriminate between actual pain and discomfort, which originated from different sources. In other words, the specificity and sensitivity of the variables used in this study may have been too low for detection of actual pain. Nevertheless, these behaviors can be considered to be part of the normal ethogram of the pig, in which the expressions of both discomfort and pain are equally accepted as indications of reduced animal welfare.

Conclusion

The objective of the present study was to evaluate the effects of CO2 anesthesia during castration on the behavior of piglets after castration. The observed differences in behaviors were not conclusive for any of the behavioral categories studied. However, the fact that barrows castrated under CO2 anesthesia displayed more interactive behaviors during the overall observation period than unanesthetized barrows may be an indication of better welfare. The fact that the observed behavioral differences with respect to lying, suckling, and interactive behaviors continued for up to 6 or 7 d after castration support this conclusion. However, all barrows that were castrated under anesthesia also displayed behaviors indicative of pain and discomfort, which continued for at least 6 d after castration, which was longer than reported by Hay et al. (2003). Therefore, piglets may need to be provided with additional analgesia to eliminate the pain caused by castration even if piglets are anesthetized with CO2 before castration. By way of conclusion, for postoperative effects, male piglets appeared to benefit to a certain extent from the use of CO2 anesthesia for castration, though this conclusion still needs to be confirmed regarding the use of CO2 anesthesia in the performance of other painful interventions whether or not in combination with castration.

LITERATURE CITED
