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Review: Potential alternatives to high-concentration carbon dioxide stunning of pigs at slaughter

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ABSTRACT

Using carbon dioxide (CO_2) for stunning pigs at slaughter is common in Europe. The use of group stunning is a major advantage with CO_2, which is done without restraining the pigs and with minimized human contact. However, high concentrations of CO_2 have been known for decades to cause pain, fear and distress in pigs before loss of consciousness, and the stunning method is clearly associated with animal welfare concerns. This study reviewed the scientific literature to find recent developments or evaluations of alternative methods that could lead to the replacement of CO_2 for stunning pigs at slaughter. Potential alternative methods found in the literature were described and then assessed to identify specific research and development needs for their further development. Only 15 empirical studies were found in the search of peer-reviewed literature since 2004, which is less than one per year. Furthermore, half of the studies focused on evaluating methods to improve high-concentration CO_2 stunning rather than an alternative to CO_2. Since no clear alternative has emerged, nor a method to improve CO_2 stunning, there is obviously a strong need to focus research and development to find solutions for improving animal welfare when stunning pigs at slaughter.

Implications

For the past two decades, the animal welfare concerns from using carbon dioxide (CO_2) for stunning pigs at slaughter have been well known and the need to develop alternative methods that improve welfare has been clearly stated. Currently, no alternative method is available that offers the advantages of CO_2 but addresses the problems, and pigs are still forced to experience pain and suffering before loss of consciousness. This review evaluates potential alternative methods to identify specific research needs to help drive method development. It is imperative that a more humane method of ending the life of pigs is developed into a commercially viable replacement for CO_2.

Introduction

According to European Council (**EC**) Regulation No. 1099/2009, approved stunning methods for pigs at slaughter include carbon dioxide (CO_2), penetrative captive bolt devise, firearm with free projectile, electrical stunning (head-only and head-to-body) and inert gases including mixtures with CO_2. Carbon dioxide is currently one of the more common methods for stunning of pigs at slaughter in Europe; however, electrical stunning is also widely used. An advantage with CO_2 stunning is that pigs can be handled and stunned in small groups rather than individually, which minimizes human–animal contact since there is no need to separate individuals from the group or restrain them. This has been shown to greatly reduce separation anxiety and distress for pigs (Mota-Rojas et al., 2012; Steiner et al., 2019). Stunning with CO_2 has also been shown to improve meat and carcass quality compared to electrical stunning (Velarde et al., 2000b; Channon et al., 2003).

Conventional CO_2 stunning uses a dip-lift system, so the stun box descends into a pit where the CO_2 concentration gradually increases to at least 90% at the bottom. Verhoeven et al. (2016) showed that stunning of pigs with 95% CO_2 induced unconsciousness in a shorter time compared to stunning with 80% CO_2 (33 ± 7 s and 47 ± 6 s, respectively). Rodríguez et al. (2008) found that loss of consciousness, with a commercial dip-lift stunning system, occurred on average 60 s after exposure to 90% CO_2. However, several studies have shown that pigs express aversive behaviors (e.g. gasping, escaping and vocalization) when exposed to CO_2 concentrations as low as 15% (Steiner et al., 2019). High CO_2 concentrations are known to cause pain and distress due to respiratory irritation of mucous membranes and invoke behavioral responses that indicate hyperventilation and excitation (European Food Safety Authority (**EFSA**), 2004). For these reasons, EFSA (2004) concluded that CO_2 stunning is not optimal from an animal welfare perspective and that further research is needed to find non-aversive gas mixture alternatives. European Food Safety Authority recently specified that exposure to CO_2 at high concentrations (defined as >80% by volume) should be replaced by exposure to other gas mixtures that are less aversive (EFSA AHAW Panel et al., 2020).

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Seventeen years after EFSA (2004) stated that there was a need for a better stunning method than CO_2, there is still not a viable alternative commercially available. Recently, Steiner et al. (2019) published a literature review to establish research priorities to identify alternatives to CO_2 stunning for rodents, poultry and pigs. While Steiner et al. did end the review with a brief overview of some potential alternative stunning methods, the focus of their study was to prioritize research needs for establishing standardized criteria to evaluate the alternative stunning methods. They identified four main evaluation criteria needing development including: 1) standardized behavioral and physiological methods to test for aversion and to assess anxiety, fear and pain; 2) determine behavioral markers of unconsciousness, which in pigs has been suggested to occur up to 10 s after loss of posture (Rodríguez et al., 2008; Verhoeven et al., 2016); 3) determine markers of dyspnea during controlled atmosphere stunning (**CAS**); and 4) identify environmental and handling factors prior to and during stunning that reduce stress and anxiety in pigs. The aim of this review was to provide a more comprehensive evaluation of the potential alternative methods to CO_2 stunning of pigs at slaughter in order to identify specific research and development needs to further aid their development.

Methods

To conduct a systematic scan for scientific, peer-reviewed studies describing the development or evaluation of alternative methods to CO_2 stunning of pigs, parallel searches were conducted on SCOPUS and Web of Science. The search terms were (pig OR pigs OR swine OR hogs OR piglet* OR sow OR sows) AND (stunning OR euthanasia OR killing OR anoxia OR hypoxia OR slaughter OR “loss of posture”) AND (laps OR “low atmospheric pressure” OR “controlled atmosphere” OR cas OR argon OR “inert gas*” OR “gas mixture*” OR “carbon dioxide” OR monoxide OR nitrogen OR “nitrous oxide” OR foam OR electric* OR microwave) NOT (“swine model” OR porcine). Searches were limited to articles published after 2004, which is the year EFSA first published that there was a need to find an alternative to CO_2 stunning of pigs.

Results

The negative search terms “swine model” and porcine were added to remove the vast medical literature that uses pigs as a model to study disease and illness in humans. The SCOPUS search gave 538 results, so the search was further limited to areas of agricultural sciences and veterinary medicine which narrowed the list to 293 results. The Web of Science core collection search without subject limitations gave 440 results. The titles and abstracts from both searches were then reviewed to find studies on how alternative methods to CO_2, for stunning or euthanasia, affected pig welfare. Reviewing titles and abstracts narrowed the resulting list to 17 relevant papers (See Table 1). Of these, 15 were empirical studies and two 2 were reviews. The references of these papers were also reviewed to find studies not revealed in the searches, which uncovered a few student theses and non-peer-reviewed reports. When relevant, we discuss the findings of these non-peer-reviewed studies but did not include them in Table 1.

Table 1
Peer-reviewed studies found in the SCOPUS and Web of Science searches published after 2004 that evaluate alternative methods to carbon dioxide (CO_2) for either stunning of pigs at slaughter or euthanasia of pigs on farm.

Authors	Study[1]	Method	Intention	Alternatives	Conclusions[2]
Dalmau et al., 2010b	Empirical	CAS	Stunning	Ar 90%, N_2:CO_2 70:30%, N_2:CO_2 85:15%	(?) no HC CO_2, Ar 90% (+[3]) N_2:CO_2 70:30% (−[3]) N_2:CO_2 85:15% (−[3])
Llonch et al., 2012a	Empirical	CAS	Stunning	N_2:CO_2 70:30%, N_2:CO_2 80:20%, N_2:CO_2 85:15%	(?) no HC CO_2, N_2:CO_2 70:30% (+[4]) N_2:CO_2 80:20% (+[4]) N_2:CO_2 85:15% (+[4])
Llonch et al., 2012b	Empirical	CAS	Stunning	N_2:CO_2 70:30%, N_2:CO_2 80:20%, N_2:CO_2 85:15%	N_2:CO_2 70:30% (+) N_2:CO_2 80:20% (+) N_2:CO_2 85:15% (+)
Llonch et al., 2013	Empirical	CAS	Stunning	N_2:CO_2 70:30%, N_2:CO_2 80:20%, N_2:CO_2 85:15%	N_2:CO_2 70:30% (+) N_2:CO_2 80:20% (+) N_2:CO_2 85:15% (+)
Machtolf et al., 2013	Empirical	CAS	Stunning	He	He (+)
Rault et al., 2013	Empirical	CAS	Euthanasia	N_2O:CO_2 60:30%, Ar:CO_2 60:30%, N_2:CO_2 60:30%, 2-step N_2O:O_2-CO_2 60:30%–90%	N_2O:CO_2 60:30% (−) Ar:CO_2 60:30% (−) N_2:CO_2 60:30% (−) N_2O:O_2-CO_2 60:30–90% (+)
Sadler et al., 2014a	Empirical	CAS	Euthanasia	Ar:CO_2 50:50%	Ar:CO_2 50:50% (−)
Sadler et al., 2014b	Empirical	CAS	Euthanasia	Ar 100%	Ar 100% (−)
Sadler et al., 2014c	Empirical	CAS	Euthanasia	Ar 100%	Ar 100% (−)
Rault et al., 2015	Empirical	CAS	Euthanasia	N_2O:air (90:10)	N_2O (+)
Fiedler et al., 2016	Empirical	CAS	Euthanasia	Ar 100%	(?) no HC CO_2, studied effects of stocking density
Kells et al., 2018	Empirical	CAS	Euthanasia	Ar 100%, Ar:CO_2 60:40%	Ar 100% (+) Ar:CO_2 60:40% (−)
Smith et al., 2018	Empirical	CAS	Euthanasia	2-step N_2O-CO_2	N_2O-CO_2 (−)
Bouwsema and Lines, 2019	Review	CAS	Stunning	LAPS	n.a.
Steiner et al., 2019	Review	Physical and CAS	Stunning	n.a.	n.a.
Çavuşoğlu et al., 2020	Empirical	CAS	Euthanasia	N_2O 100%, CO_2b (with butorphanol)	N_2O 100% (?) CO_2b (−)
Lindahl et al., 2020	Empirical	CAS	Stunning	N_2 > 98% in foam	(?) no HC CO_2, studied aversion to foam

CAS = controlled atmospheric stunning; LAPS = low atmospheric pressure stunning, HC = high concentration, n.a. = not applicable.
[1] Studies reported either empirical research or were reviews.
[2] Symbols after gas or gas mixtures indicate animal welfare impact in relation to HC CO_2 based on authors own conclusions where: (+) positive, (−) negative or no difference or (?) unclear due to conflicting results or due to no HC CO_2 treatment for comparison.
[3] Treatment comparisons within study.
[4] Compared with HC CO_2 in Velarde et al. (2007).

The studies found in the searches were then sorted according to the type of alternative method, either gas or physical methods, and described in the section "Alternatives to CO_2 stunning" below. Nine of the 15 empirical studies evaluated alternative methods to CO_2 for stunning pigs. Seven of the papers presented results evaluating gas mixes with CO_2 that could potentially reduce aversion compared to high-concentration CO_2. It is a matter of definition whether this constitutes an alternative to CO_2 stunning or a method to reduce the aversiveness of CO_2, but for this review, it was considered an alternative to high-concentration CO_2. Two of the studies evaluated the potential for two-step multiple gas stunning, where the first step uses a gas to anesthetize the pig to reduce aversion during the second step using high-concentration CO_2 for stunning. This seemed more straightforward as a method to improve CO_2 stunning rather than an alternative to CO_2; however, they were also included in this review since the goal was to reduce aversion. Several of the published studies evaluated both alternatives to CO_2 and methods for improving CO_2 in the same paper.

Following the description of alternatives section, the alternatives were assessed in terms of their potential to offer improved animal welfare during pre-handling and slaughter, and in terms of their potential commercial viability. Suggestions for further research and development were also made.

Alternatives to CO_2 stunning

It is critical that alternative methods to CO_2 stunning are effective. EFSA defined the most important assessment criteria for evaluating the effectiveness of stunning methods include "…immediate onset of unconsciousness and insensibility or absence of avoidable pain, distress and suffering until the loss of consciousness and sensibility, and duration of the unconsciousness and insensibility (until death)." (EFSA AHAW Panel et al., 2013, p. 1).

The two main stunning methods for pigs fall under the category of CAS and physical methods. Controlled atmosphere stunning includes any method that changes gas concentrations or ambient atmospheric pressure that subsequently leads to unconsciousness, where physical methods directly affect the brain to induce unconsciousness and can be accomplished mechanically, electrically or through electromagnetic radiation.

Controlled atmosphere stunning

Controlled atmosphere stunning is an umbrella term for methods changing ambient gas concentrations, resulting in unconsciousness generally due to hypoxia (lack of O_2). Hypoxia can be attained by immersion into gas, displacing gas or by low atmosphere pressure stunning (**LAPS**) (Steiner et al., 2019). Gas methods may be used in pits, tunnels, sealed containers or rooms. If the gas has a greater density than air, it can generally be contained in a pit in which the pigs are lowered into for stunning (Dalmau et al., 2010a).

Low atmosphere pressure stunning

Low atmosphere pressure stunning is a method where the pressure in a stunning chamber is lowered by removing the air, thereby reducing the oxygen (O_2) level which results in stunning by hypoxia (Mackie and McKeegan, 2016). The LAPS method has been approved in the EU for commercial slaughter use for broilers under 4 kg, after EFSA concluded that the method is "…able to provide a level of animal welfare not lower than that provided by at least one of the currently allowed methods" (EFSA AHAW Panel et al., 2017, p. 1).

Limited studies have been conducted on the stunning effects of LAPS on pigs. Engle and Edwards (2011) compared hypobaric hypoxia (LAPS) and high-concentration CO_2 as on-farm methods for euthanasia of nursery piglets (mean weight 5.6 kg). When euthanized with CO_2, gasping was observed in 100% of the piglets, indicating that the pigs experienced suffocation before unconsciousness. When euthanized with hypobaric hypoxia, only 29% of the piglets showed gasping and therefore it was concluded that hypobaric hypoxia may cause less stress than CO_2 (Buzzard, 2012). Some signs of respiratory discomfort (dyspnea) were also reported in broiler chickens stunned with LAPS (Mackie and McKeegan, 2016); however, they point out that it was difficult to determine if these signs were part of the birds normal response to hypoxia or evidence of pain. The piglets euthanized with hy hypobaric hypoxia indicated a longer time until death compared to CO_2 (a complete isoelectric state was reached after an average of 13.4 min and 7.8 min, respectively), and more pigs were observed with lung lesions postmortem when euthanized by hypobaric hypoxia. An important result was also that hypobaric hypoxia was not effective in euthanizing every piglet, which raises questions about its effectiveness as a consistent method of euthanasia (Engle and Edwards, 2011).

Currently, there are no published studies on using LAPS for stunning adult pigs; however, there is one review paper discussing the potential use of LAPS on pigs based on previous research on humans, poultry, rats and piglets (Bouwsema and Lines, 2019). The authors concluded that LAPS could be suitable to provide irreversible stunning of healthy pigs while allowing group stunning with minimized handling and low stress. However, the authors did also point out possible welfare issues with LAPS due to potential pain and distress that may be experienced by pigs with excess intestinal gas in the alimentary canal or by those suffering from respiratory problems or inflammation of the upper respiratory tract. Lung lesions, which seem relatively common in slaughter pigs (Vecerek et al., 2020), might also cause pain and distress under LAPS and should be investigated. Pressure changes during LAPS could also cause ear pain if pigs have difficulty equalizing the pressure in their middle ear. This is a common issue for humans exposed to rapid changes in atmospheric pressure and should be ruled out as an issue for pigs.

There is ongoing, not yet published research, with the aim to establish whether pigs show any signs of poor welfare when exposed to gradual decompression (Hubrecht, 2018). A comparison of LAPS to CO_2 stunning is currently being conducted to assess pigs' physiological and behavioral responses to determine whether LAPS offers a more humane way to stun pigs. The project is led by Dr. McKeegan, who previously led the research projects which contributed to the approval of LAPS for poultry in the EU (Hubrecht, 2018).

Inert gases

Inert gases are stable gases that do not readily react with other substances, are free from smell, color and taste, and therefore in contrast to CO_2 do not irritate the mucous membranes and airway passages (Raj and Gregory, 1995; Dalmau et al., 2010b). The inert gases include not only all noble gases but also stable gas molecules with strong covalent bonds, e.g. dinitrogen (N_2). The stunning mechanism of inert gases is hypoxia resulting from exposure to an anoxic atmosphere with $<2\%$ residual oxygen (O_2) by volume. The use of inert gases for stunning is reversible with short stunning times, so the potential for pigs to regain consciousness is of concern. Three hazards for pig welfare associated with inert gas stunning were identified by EFSA (2020) including exposure to O_2 concentrations above 2%, too short exposure times and overloading the stunning area. EFSA further pointed out other hazards for pig welfare when using mixtures of inert gases and CO_2 which included exposure to high CO_2 concentrations and low temperature of the gas. Meat quality could possibly be affected negatively if time until unconsciousness is too long (Llonch et al., 2012b).

Inert gases with higher density than atmospheric air could easily be used in "dip-lift" systems where the pigs are lowered into stunning pits similar to those used with CO_2 (Dalmau et al., 2010a). The inert gases with lower density than atmospheric air are more difficult to handle, which could be contributing to the lack of research using them.

Argon. Argon (Ar) is the most common noble gas in the atmosphere. However, its presence is only 0.9%, which limits its availability and might increase cost for commercial use. Argon has higher density compared to atmospheric air and is therefore easy to contain in a stunning pit without risk of being diluted with air (Dalmau et al., 2010a).

Raj and Gregory (1995) found that pigs did not show aversive behaviors when they could choose to enter a box with 90% Ar gas for a reward and they did not show any discomfort or fear re-entering the box. Several other studies have also reported improved pig welfare when stunning with Ar compared to CO_2 (Machold et al., 2003; Kells et al., 2018). However, Dalmau et al. (2010b) reported there were indications of aversion when slaughter-weight pigs were exposed to Ar (90%). Duration of convulsions and time to loss of posture were longer for 90% Ar compared to high-concentration CO_2 (Machold et al., 2003). Raj (1999) showed that when slaughter-weight pigs were exposed to 90% Ar for 3 and 5 min, the pigs could be bled within 25 and 45 s, respectively, without regaining consciousness during bleeding. However, the pigs that were exposed to Ar for only 3 min developed convulsions during bleeding, which could pose animal welfare concerns if this indicates that unconsciousness is reversing. This is also a risk to worker safety. When exposed to Ar for 7 min, the majority of the pigs were dead (Raj, 1999).

In a study of suckling pigs determined for euthanasia, Sadler et al. (2014c) showed that 90% Ar increased time to loss of posture compared to 90% CO_2, increased duration of open mouth breathing and ataxia and increased number of righting attempts. In another study, weaned pigs (3–17 kg) euthanized in high-concentration Ar in groups of two or six paced less and made fewer escape attempts than pigs euthanized in the solitary treatment (Fiedler et al., 2016). This indicates gas euthanasia and probably stunning should be done in groups, but it also clouds interpretation of behavioral indicator results from studies with single pig treatments.

Several studies have shown mixing Ar with CO_2 reduces aversion when stunning pigs compared to high-concentration CO_2 (Llonch et al., 2012a, 2012b and 2013); however, other studies have reported no advantage of mixing Ar and CO_2 (Sadler et al., 2014a and 2014b; Kells et al., 2018). Dalmau et al. (2010b) reported that pigs stunned with gas mixes of Ar and CO_2 showed more aversion than with only 90% Ar, and furthermore, the higher the CO_2 concentration in the Ar mix, the stronger the aversive behavior by the pigs. However, mixing CO_2 with Ar reduced time to loss of posture compared to only Ar. Machold et al. (2003) also showed reduced duration of convulsions and shorter times to onset of respiratory arrest with a mix of 30% CO_2 and 60% Ar compared to 90% Ar. They conclude that stunning with a two-step Ar–CO_2 process could potentially alleviate risks of aversion while keeping the stunning and convulsions time from being excessively long.

Nitrogen. Nitrogen is the sixth most common element on earth and makes up 78% of atmospheric air. Nitrogen has lower density compared to atmospheric air, which means it cannot be used in a stunning pit as with CO_2. A study on gas stability and uniformity in a commercial dip-lift stunning system with the aim of obtaining 98% N_2 and 2% of residual O_2 showed that the minimal O_2 concentration 60 cm above the bottom of the pit did not go under 6% (Dalmau et al., 2010a). However, they did show that gas mixtures of N_2 and CO_2 were contained in a stunning pit when the CO_2 concentrations were 15% or greater.

Only one published study of stunning pigs with high concentrations (>98%) of N_2 gas was found, probably due to the technical problems in maintaining a controlled atmosphere of N_2 and preventing it from mixing with air. However, a number of studies have been made with N_2 gas mixtures. Behavioral tests with pigs in an anoxic atmosphere consisting of 80% N_2 and 20% CO_2 have shown reduced discomfort (Llonch et al., 2012b). Additionally, the sense of breathlessness seemed to be lower with N_2:CO_2 mixtures compared to high-concentration CO_2 (Llonch et al., 2012a and 2013). Mixtures of N_2:CO_2 were shown to require longer exposure times until decreased brain activity (electroencephalography (**EEG**)) than only CO_2 (Llonch et al., 2013), and the duration of unconsciousness could be reduced. The longer times to unconsciousness may negatively affect meat and carcass quality, according to Llonch et al. (2012b).

Recent studies have evaluated the use of high expansion foam filled with N_2 gas to stun pigs and poultry. The practical advantage of using gas-filled foam compared to free gas is that the foam can effectively purge the air from a container creating a high-concentration N_2 atmosphere (>98%) and avoids mixing with air (Lindahl et al. (2020); McKeegan et al., 2013). One study on poultry showed that euthanasia achieved with N_2-filled foam is effective, rapid and humane (McKeegan et al., 2013). A doctoral thesis by Pöhlmann (2018) evaluated the N_2 foam method for stunning of slaughter-weight pigs with rather poor results. Exposure times of 3.5 min to the N_2 foam were not sufficient to assure unconsciousness and insensibility. Furthermore, animals were seen gasping and regaining sensibility shortly after stunning leading to a high rate of re-stunning (22%). Aversive behaviors (escape attempts and vocalizations) were observed in 67% of the pigs. The time to loss of posture was not possible to obtain since the pigs were restrained in a hammock during the experiment. Furthermore, in 72% of the pigs, foam residue was found in the lungs after slaughter; however, the meat of N_2-foam stunned animals had a similar or even better quality compared to the CO_2 stunning.

In a recently published study (Lindahl et al., 2020; Wallenbeck et al., 2020), behavior and physiological response was assessed on pigs (approx. 30 kg) exposed to air-filled foam, N_2-filled foam or no foam (control). The pigs did not show any strong aversive behaviors when exposed to foam, regardless if it was air-filled or N_2-filled. However, when the foam levels became high, pigs seemed to avoid putting their heads and snouts into the foam and the rate of escape attempts through the lid of the box increased. Heart rate and respiratory rate increased for pigs when O_2 levels decreased during exposure to the N_2-filled foam. Mean time to loss of posture was 57 s and was followed by a short period of vigorous convulsions. After 5 min from starting N_2-filled foam production, the pigs were removed from the box and all were assessed to be in either deep unconsciousness or dead. The high expansion foam was very effective at decreasing the concentration of O_2 in the box to below 1% in a short time.

The conflicting evaluations for N_2-filled foam between Lindahl et al. (2020) and Pöhlmann (2018) are likely due to Pöhlmann using an open top container for their experiment which would allow air to mix with the N_2 gas as the foam bubbles popped from animal movements. The foam generators used by Pöhlmann were also an earlier prototype with lower capacity than those used by Lindahl et al. (2020) (Personal comment by Michiel van Mil, Anoxia BV, The Netherlands). The lower capacity of the foam generators made it difficult to cover the head of the pig with foam as the pig was simultaneously breaking the foam through its movements, which would further prolong the time where air can mix with the N_2. Continually mixing O_2 into the N_2 gas would explain longer stunning times as well as the increased risk of regaining sensibility. Lindahl et al. (2020), on the other hand, used a closed top chamber and foam generators that very quickly lowered the O_2 concentrations to below 1% and maintained this controlled atmosphere throughout the exposure time. Most likely, the negative results when using N_2-filled foam reported by Pöhlmann (2018) were a result of technical difficulties that were solved in the later study.

Helium. Helium (He) is a noble gas which is relatively rare in atmospheric air; however, it can be extracted from natural gas, which can contain as much as 7% He. As He has a lower density than atmospheric air, it makes it challenging to use for stunning purposes. In a study by Machtolf et al. (2013), a plexiglass dome filled with He was lowered over a cage holding a pig to compare He (>95%) stunning with a commercial CO_2 dip-lift system. Pigs stunned with He showed no aversive behavior, while pigs stunned with CO_2 (>90%) showed escape attempts, vocalization and hyperventilation before loss of posture. Time to loss of posture was similar between treatments, 20 s in He and 16 s in CO_2. Convulsions were observed in most pigs in both treatments with minor differences in severity of convulsions. Helium exposure time of 180 s was sufficient to ensure a state of unconsciousness and insensibility after stunning and during bleeding. Carcass and meat quality were comparable between groups.

Xenon. Xenon (Xe) is a noble gas and the rarest of the stable elements and has unique anesthetic properties at atmospheric conditions (Rylova and Maze, 2019). Xenon is also much denser than air and would therefore, like Ar and CO_2, be easy to contain in a stunning pit. However, the proportion of Xe in the geosphere and atmosphere is very low which makes the gas expensive and limits its usability (Neice and Zornow, 2016).

No studies have been found on using Xe gas for stunning of pigs. A few studies were found on euthanasia of mice using xenon gas. The results by Gent et al. (2019) suggested that Xe offers improved welfare conditions over CO_2 as a euthanasia agent and does not appear to cause any behavioral aversion or fear response in mice, like jumping or freezing. Xenon also has sedative properties with the effect of reduced locomotion ability. Gent et al. (2018) evaluated epileptiform activity (uncoordinated muscle activity synchronized with high-amplitude EEG activity) caused by inert gases and CO_2 in mice. No epileptiform activity was found in the mice exposed to Xe, whereas 100% of mice exposed to N_2, He and Ar had epileptiform activity. Carbon dioxide euthanasia also produced epileptiform activity but was shorter in duration and apparently less severe than those from N_2, He and Ar.

Other gases or gaseous agents

Other gases than CO_2 or inert gases are not currently allowed for stunning of pigs at slaughter in the EU (EC 1099/2009), but carbon monoxide (CO) is allowed for euthanasia. Lambooy and Spanjaard (1980) reported that euthanasia of small pigs with a slow flow rate of CO showed little signs of excitation before loss of posture and when CO was combined with N_2O, no signs of excitation were seen before loss of posture.

Nitrous oxide (N_2O) is considered relatively inert at room temperature and has well-known anesthetic properties. In a study of the effectiveness of N_2O to relieve pain during castration of piglets, Rault and Lay (2011) showed that N_2O induced anesthesia in piglets; however, the piglets still showed indications of castration-induced pain. In a study comparing single gas euthanasia, Rault et al. (2015) reported 90% N_2O was effective for euthanasia and piglets showed much less aversive behavior compared to the 90% CO_2 treatment. Çavuşoğlu et al. (2020) reported conflicting behavioral responses of weaned pigs but concluded that N_2O was as effective as CO_2 and possibly more humane.

Some studies suggest the potential of N_2O to reduce the aversive response in pigs to CO_2 in two-step multiple gas stunning systems where an anesthetic gas is administered in the first step to reduce aversion to a second step with CO_2. Rault et al. (2013) showed that a first step mixture of N_2O (60%) and O_2 (30%) followed by second step immersion into CO_2 (90%) offered a more humane method of euthanasia than single step euthanasia using either a mixture of N_2O (60%) and CO_2 (30%) or only CO_2 (90%). However, the time needed for the anesthetizing first step with the N_2O and O_2 mixture was more than 14 min. Smith et al. (2018) later reported, in another piglet two-step euthanasia study, that a first step with only N_2O showed more behavioral signs of stress and aversion than a single-step CO_2 treatment, but later attributed this to the prolonged time for the N_2O first step (Çavuşoğlu et al., 2020).

A number of other gaseous agents with anesthetic properties have been studied for euthanizing mice, including isoflurane, halothane, enflurane, sevoflurane and others, yet there is no consensus regarding whether the level of distress is lower with these anesthetics than it is with CO_2 (Valentim et al., 2016). We found only one study evaluating the behavioral response of pigs to an anesthetizing agent, butorphanol, but it was used to eliminate behaviors indicative of pain for comparison with CO_2, and these pigs showed significantly greater escape attempts than only CO_2 (Çavuşoğlu et al., 2020).

Physical methods

Physical methods are stunning techniques that affect the brain to induce unconsciousness. Electrical stunning is the most common physical method used commercially for pigs. Penetrating captive bolt and firearm with free projectile are not viable options to replace CO_2 stunning at slaughter as it requires intensive manual handling, targeting the brain is difficult, processing speeds are too slow and the excessive convulsions associated with them negatively affect meat quality. They are generally only used as back-up methods for inadequate stunning or for very small-scale slaughterhouses or on-farm slaughter and are therefore not further addressed here.

Electrical stunning

Electrical stunning of pigs is commercially used in several European countries and worldwide. Electrical stunning requires the animal to be restrained, which is a potential stress factor (Brandt and Dall Aaslyng, 2015). The stunning is induced instantaneously and is caused by a grand mal epileptic seizure. There are three types of electrical stunning: head-only, head-to-body cardiac arrest and head-only followed by a cardiac arrest. Five hazards for pig welfare have been identified relating to electrical stunning and include restraining, wrong placement of the electrodes, poor electrical contact, too short exposure time and inappropriate electrical parameters (EFSA AHAW Panel et al., 2020). EFSA further reported that the origin of all of these hazards for pig welfare was largely staff related.

In head-only stunning, the electrodes are placed on both sides of the head causing a reversible stun. Pigs can return to consciousness within 30 s, and it is therefore recommended to bleed the animal within 15 s to avoid that the animal returns to consciousness during bleeding (Grandin, 2013). Head-to-body, where current is transferred through both brain and heart simultaneously, and head-only followed by cardiac arrest are both methods to avoid return to consciousness. However, during head-to-body electrical stunning, proper placement of the electrodes for the heart can be difficult and poor placement could result in pain and fear for the animals during stunning (Grandin, 2013). While the use of a restraining device can help to place electrodes correctly, the restrainer itself can also be aversive and stressful to pigs. Jongman et al. (2000) found indications that one type of restraining device used for electrical stunning was equally aversive to pigs as a CO_2-stunner crate with 90% CO_2. Their results also showed that 90% CO_2 was considerably less aversive than an electric shock with a prodder which is often used to move the pigs into the restraining device. Vogel et al. (2011) compared head-only stunning to head-only followed by cardiac arrest in pigs and concluded that the head/heart electrical stunning reduced the incidence of signs of pigs regaining consciousness without significant effects on meat quality, plant operation speed or blood lactate concentration. Thus, head-only stunning followed by a cardiac arrest may be a more effective stunning method than the head-only method with regard to animal welfare.

A survey of electrical stunning in the UK showed that 15.6% of all pigs were incorrectly stunned and the stunning procedure had to be repeated (McKinstry and Anil, 2004). Stocchi et al. (2014) surveyed pig slaughterhouses in Italy and reported that wrong electrode placement was observed in 54% of the pigs. Velarde et al. (2000a) found that, despite a relatively high occurrence of wrong placement of the electrodes, there was a low occurrence of animals with an absence of grand mal seizures that regained sensibility. The stunning current used in that study was greater than the European recommendations, which indicates that using a higher current allows for a greater margin of error for electrode placement.

Epileptic contractions caused by electrical stunning increase risk of fractures and hemorrhaging, and pigs stunned with CO_2 were shown to have fewer condemned carcasses due to fractures and lesions compared to electrical stunning (Marcon et al., 2019). An alternative to conventional electrical stunning is single-pulse ultra-high current (**SPUC**), which can induce a more prolonged state of unconsciousness and also prevent a grand mal seizure. In a study by Robins et al. (2014), SPUC was evaluated on cattle. Single-pulse ultra-high current caused sustained depolarization of central nerve cells, i.e. a change in distribution in electric charge, which caused unconsciousness. The results showed that stunning of cattle with SPUC led to unconsciousness that lasted for up to 4 min and the method eliminated seizures, which commonly occurs during conventional electrical stunning. However, the presence of variable reflex activity (breathing, corneal and palpebral reflexes) soon after stunning was a concern, even though the authors propose the evidence suggested that the reflexes were dissociated from sensibility.

Electromagnetic radiation

The method utilizes microwave energy to induce a rise in temperature in the brain to a point at which the animal loses consciousness. Lambooy et al. (1989) considered microwave energy unsuitable for pigs, partly because of the capacity of the microwave generator being too low to deliver sufficient power. In recent years, however, microwave technology has developed and high-power equipment is available and shown to produce a rapid rise in temperature in cattle brains (Rault et al., 2014). The method has also been tested on anesthetized sheep (Small et al., 2013).

A novel system based on microwave energy for stunning animals prior to slaughter has recently been developed (McLean et al., 2017) and assessed on cattle (Small et al., 2019). Stunning was performed by restraining the animal and fixating the animal's head to bring the forehead into contact with the waveguide. The results showed that the method successfully rendered cattle insensible, based on loss of reflex responses and EEG changes, and the insensibility was of a sufficient duration to allow humane slaughter. Behavioral expression of distress was not observed (Small et al., 2019).

Assessment of potential viability of alternatives to CO_2 stunning and research and development priorities

Viable alternative methods to CO_2 stunning of pigs should also provide acceptable animal welfare during handling before stunning. The most promising methods to fulfill these demands are methods that allow group stunning of pigs. Aside from this, alternative stunning methods should of course provide either immediate onset of unconsciousness, or the absence of avoidable pain, distress and suffering until loss of consciousness as defined by EFSA (2013). For the purpose of this review, the alternative method should at least provide a clear reduction in distress and suffering compared to high-concentration CO_2 stunning. Currently, there are no group stunning methods that result in immediate stunning of pigs; however, there are several options that have shown reduced signs of aversion compared to CO_2 stunning. Furthermore, for any alternative method to be considered viable for commercial slaughter of pigs, it is also important they are both practical to implement and cost-efficient.

Low atmosphere pressure stunning

Research is needed on the use of LAPS for pigs as currently no studies were found in the literature regarding this. Consideration for testing LAPS on pigs is based mostly on positive response in trials for other species, but also from preliminary tests on piglets, which indicated welfare advantages over CO_2 stunning. Other potential benefits with LAPS are that the method can allow group stunning of pigs with minimal human contact and that irreversible stunning can be achieved. However, concerns that LAPS can cause physiological pain in pigs must be ruled out.

One challenge with LAPS is the stunning cycle, which will be much longer (approx. 9–14 min) than current CO_2 systems. Bouwsema and Lines (2019) suggested that large-scale use of LAPS will result in more complex pig-handling systems compared to current CO_2 stunning, since multiple LAPS systems will be needed to reach a high capacity. Large vacuum pumps, tubing and airtight seals needed for a LAPS system will require both investments, running and maintenance costs. Thus, even if LAPS might be technically possible for large-scale slaughter, its economic viability is uncertain due to the large investment and maintenance costs. Furthermore, the long stunning cycles with prolonged exposure to hypoxic conditions could pose animal welfare problems for pigs and meat quality issues.

The research and development priorities for LAPS should focus on:

- Assessment of pigs' physiological and behavioral responses when exposed to gradual reductions in atmospheric pressure. Different categories of pigs should be included, especially slaughter ready pigs.
- Determine if common issues with pigs, such as lung lesions, respiratory problems, excess intestinal gas or equalization of middle ear pressure could cause pain and suffering during LAPS.
- Pressure reduction rate and end pressure are parameters that will affect animal welfare during stunning, as well as time to unconsciousness and to irreversible stunning. Consistency of stunning needs to be established. Bouwsema and Lines (2019) suggested that trials should start with a decompression rate of 0.43–0.52 kPa/s. This decompression rate has been concluded as being pain free for humans and are lower compared to minimum decompression rates used for poultry. Time to irreversible stunning could possibly be established by, e.g., EEG.
- If LAPS aims to be irreversible, the stun to stick interval would not be a welfare issue. However, establishing an optimum stun to stick time to allow good bleed-out and preserve meat quality may be important.
- Effects of LAPS on carcass and meat quality.
- Technical development of LAPS systems for various capacities with accompanying assessments of their economic viability.

Gases

Inert gas stunning has generally been shown to be less aversive than exposure to high concentrations of CO_2. Gas stunning is beneficial as it reduces the need for handling of the pigs, i.e., no operator needs to perform the stun and the pigs can be stunned in a group without restraining. Disadvantages with inert gas stunning are that the time until unconsciousness might be longer compared to CO_2 and the time until regaining consciousness is likely shorter unless an irreversible state is reached.

Many of the studies we found using inert gases for stunning pigs have focused on the evaluation of mixing inert gases at different concentrations with CO_2 in order to reduce aversiveness compared to high-concentration CO_2. Most of these studies did show reduced aversion; however, they all still showed some signs of aversion and results from different studies are sometimes contradictory.

Argon

Argon has been shown to be less aversive than CO_2 but also seems to need longer stunning times to ensure insensibility throughout the stun to stick interval. Argon gas is more expensive than CO_2 which together with longer stun times would affect its commercial viability in comparison to CO_2. This might be why it is still not used commercially for stunning pigs. A benefit with argon, however, is that it is denser than atmospheric air, which means it could be used in existing dip-lift systems. This would also make it practical to mix with CO_2 to improve animal welfare during stunning compared to high-concentration CO_2; however, it should be confirmed which gas concentration mix offers the best ratio between reduced aversion, acceptable stun times and viable commercial economy.

Nitrogen

Nitrogen is easily accessible and relatively inexpensive compared to other inert gases and can even be produced on site. Despite this, however, limited research has been conducted using high-concentration N_2 gas (>98%) for stunning of pigs due mostly to technical difficulties containing the gas and maintaining a controlled anoxic atmosphere with N_2. Nitrogen has slightly lower density than atmospheric air, which means it is not practical to use in a dip-lift system.

To use N_2 gas for stunning, closed container systems should be designed that can rapidly establish a controlled anoxic atmosphere to quickly induce unconsciousness and assure irreversible stunning to avoid the risk of the pigs regaining consciousness during bleeding. Lindahl et al. (2020) showed that using N_2-filled high expansion foam in a closed container solved the technical issues for stunning small pigs with N_2 gas, but there are still a number of unanswered questions concerning this method. Do the foam generators have the capacity to work with larger, slaughter-ready groups of pigs? What are the potential risks for soap residues contaminating the carcass and meat and can this be avoided? Is it practically feasible to integrate this method into commercial slaughter lines and could it be economically viable? One last potential issue is that pigs stunned with N_2 gas show vigorous convulsions and there is a need to ensure that this will not have negative effects on carcass and meat quality.

Helium and xenon

Helium and xenon seem to be the least aversive gas alternatives, although only helium has been evaluated on pigs. Both He and Xe have also been shown to reduce convulsions after unconsciousness. However, He and especially Xe are rare and expensive gases and therefore not likely economically viable alternatives for large-scale commercial use unless there is a technique to prevent losses and which can efficiently recycle the gas within the system. It might, however, be possible to find economy for small-scale specialty niche applications.

Other gases, gaseous agents

Using N_2O for single gas stunning did anesthetize pigs effectively and showed slightly improved animal welfare for pigs compared to CO_2 (Rault et al., 2015; Çavuşoğlu et al., 2020). However, N_2O is an extremely potent greenhouse gas with atmospheric lifespan of over 100 years and a global warming potential 265 times that of CO_2, so even with the development of a recapturing system, the risk for negative climate impacts is too great to recommend widespread adoption of N_2O for stunning of animals at slaughter, or even for use as a first step gas to reduce aversiveness to high-concentration CO_2.

Carbon monoxide is currently not allowed for slaughter and, due to its toxicity even at low concentrations, would require strict safety equipment to protect staff.

Other gases or gaseous agents with anesthetizing properties could be advantageous in two-step multiple gas stunning, either for reducing aversion to high-concentration CO_2 in the second step, or to reduce the intensive convulsions that seem common during anoxic stunning with inert gases such as argon and nitrogen. A system for the practical application of two-step multiple gas stunning under slaughter conditions needs to be developed, and the most effective gases for each sequence step must be determined. The commercial viability of such a system will likely be determined by the cost of the first step anesthetizing gas and by whether the total stunning time is increased significantly. It would also need to be assured that whatever gas agent is used it does not introduce any food safety issues when used at slaughter.

To conclude, the research and development priorities for inert gases should focus on:

- General priorities.

  - o Comparison of relevant high concentrated inert gases on pigs to find which method is least aversive.
  - o Evaluate the difference in stunning mechanisms between the inert gases to understand differences between aversion behaviors.
  - o Evaluate the stunning method on pigs of different categories.
  - o Assess exposure time, stunning quality, stun-to-stick time and the effects of stronger convulsions on carcass and meat quality for relevant alternatives.
  - o Evaluation of the economic viability of the different inert gas alternatives to rule out alternatives not relevant for commercial use.

- *Argon*. Determine if there is commercial viability in stunning with 90% Ar considering the cost of the gas and longer stunning times needed. If not, determine which mix concentration of Ar and CO_2 is the optimum compromise between reducing aversion to CO_2, reasonable stunning times and effectiveness and economic viability.
- *Nitrogen*. Since N_2 is relatively inexpensive to produce, there is a good likelihood of finding commercial viability if an effective stunning system could be developed. Focus should be on developing a system that can quickly replace the atmospheric air in the container with N_2 gas to achieve stable anoxic conditions with minimum of 2% O_2. Nitrogen-filled foam could be a promising method to achieve this; however, further studies are needed. The capacity of the foam generators must be able to handle group stunning of slaughter ready pigs. The risk of foam residues contaminating the carcass and meat during slaughter and sticking needs to be evaluated.
- *Helium*. Finding economy with helium by development of techniques to avoid losses during use and reuse of the gas, and if this is possible, the development of a stunning system for commercial use that works with a gas less dense than atmospheric air.
- *Combination of gases*. Evaluate if mixtures of gases, or combinations of gases in a stepwise stunning process, can reduce aversive behaviors while at the same time achieving other benefits such as short time to unconsciousness, longer duration of unconsciousness or irreversible stunning. Reduced convulsions and improved carcass and meat quality should also be considered.

Physical methods

Electrical stunning

Electrical stunning is an immediate stunning method, which is beneficial from an animal welfare point of view; however, the high rate of unsuccessful stuns needs to be addressed through improved accuracy of electrode placement or using higher current. Furthermore, the frequent occurrence of pigs regaining sensibility during bleeding must be addressed with the development of methods to ensure irreversible stunning. The need to separate individual pigs from the group to a stunning pen increases the need of handling and that, in combination with social isolation, is highly distressful to pigs and labor intensive. There is therefore a need to develop methods for improved handling of the pigs prior to stunning, to avoid restraining and social isolation as well as ensure proper positioning of the stunner to avoid distress or suffering before unconsciousness. Another disadvantage with this stunning method is the strong convulsions, which can cause carcass and meat quality problems and is a safety issue for the operators.

Single-pulse ultra-high current

Single-pulse ultra-high current stunning may be an alternative to conventional electrical stunning but has not yet been evaluated on pigs. The method has benefits of inducing longer stunning effect and decreased or no epileptic seizures, which could improve carcass and meat quality and operator safety compared to conventional electrical stunning. The handheld device with only one contact point needed on the animal may allow for better precision and consistency compared to conventional electrical stunning. Even though the method does not enable group stunning, it might be possible to stun pigs one by one in a group box thus avoiding isolation of individual pigs. Disadvantages include that the method requires operators to be in close proximity to the animals, which is considered a stressor to pigs, and it will be relatively labor-intensive which can limit capacity. While SPUC may be a viable alternative for small- or medium-sized slaughterhouses, it probably is not a viable alternative for large-scale slaughter unless an automated

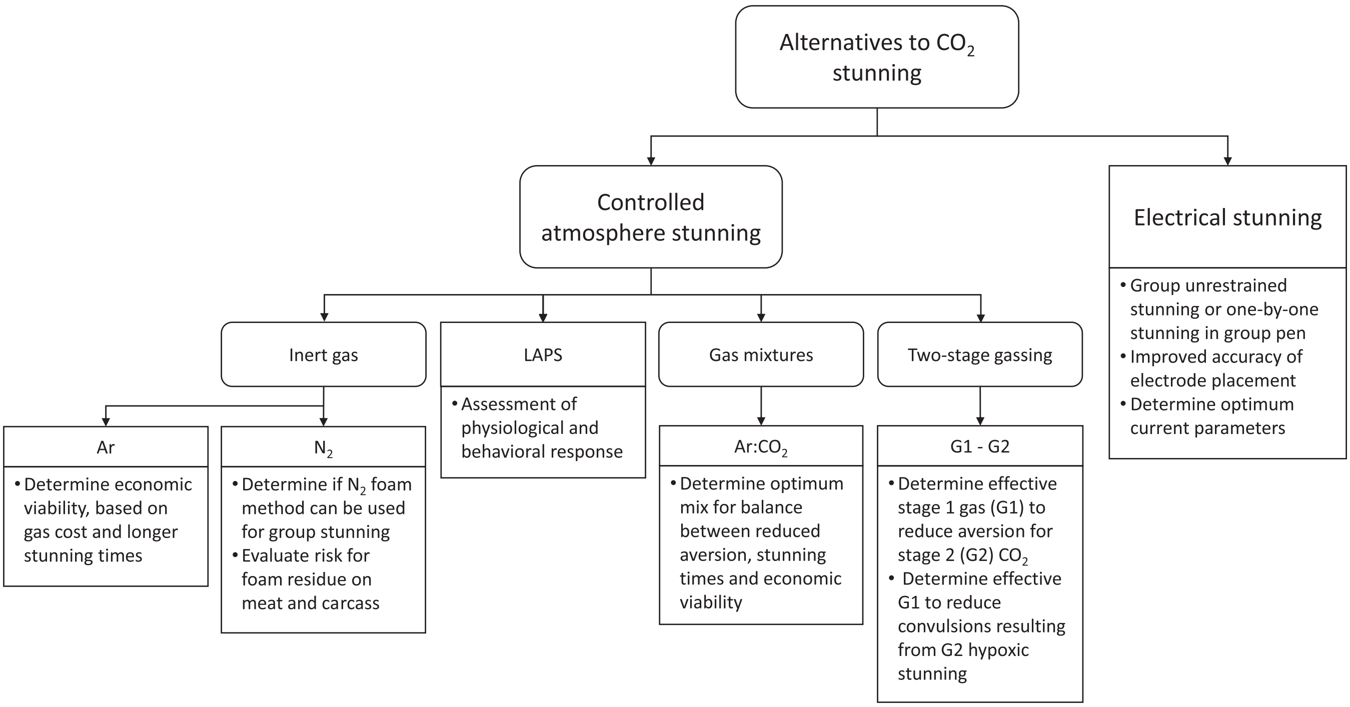

Fig. 1. Overview of most relevant potential alternatives to CO_2 stunning of pigs at slaughter found in review, and the immediate research needs for further development. LAPS = Low atmosphere pressure stunning.

system is achievable that avoids all the negative aspects associated with pre-handling of pigs that are issues for conventional electrical stunning.

Electromagnetic radiation

Microwave energy stunning has not been assessed on pigs. As the technique is currently designed, there is a need to restrain the animal and fixate the head (Small et al., 2019). This handling will be very stressful to pigs; thus, the method is not considered suitable for pigs at present. If the technique can be developed for group stunning in the future, it may be relevant to re-evaluate the method.

To conclude, the research and development priorities for physical methods should focus on:

- *General priorities*. Development of methods to enable stunning of pigs in groups without restraining or excessive handling including electrical prodding into a restraining device. Evaluation of perceived distress by pigs in a group with one-by-one stunning in the group pen compared to perceived stress by social isolation and restraining.
- *Electrical stunning*. Improve the accuracy of electrical stunning to ensure stunning consistency and improve the effectiveness of stunning to ensure irreversible stunning, even when the animal is not restrained.
- *SPUC*. The method needs to be evaluated on pigs.

Conclusion

It seems clear that high-concentration CO_2 stunning of pigs is associated with animal welfare concerns. Sixteen years have passed since 2004, when EFSA first stated that there was a need to develop non-aversive gas stunning methods for slaughter of pigs, and yet only 15 scientific peer-reviewed empirical studies were found addressing this issue. Of these, only nine studies evaluated actual alternatives to CO_2, while the others evaluated methods to improve CO_2 stunning. An overview of potential alternatives found in this review that seem reasonable to pursue development is presented in Fig. 1. We found nothing to suggest that any of the alternatives considered in these studies are currently being developed into a commercial method to replace CO_2 stunning. Considering this, we feel there is an urgent need to push forward with research and development of alternative stunning methods that will lead to improved animal welfare for pigs at slaughter. At the same time, it is also worth pursuing means to improve current CO_2 methods by eliminating or reducing aversion to CO_2. We addressed the issue of improving CO_2 stunning by mixing gases and through two-step multiple gas stunning as these options turned up in the search for alternative methods. However, we did not conduct a systematic review of possible methods for improving CO_2 stunning, so we more than likely missed some other possible avenues to achieve this. One area worth mentioning here that did not turn up in this search is the possibility of genetic differences in the response of pigs to CO_2. Some pigs seem to have a calm induction to high-concentration CO_2, while others have agitated responses with repeated escape attempts (Grandin, 1988; Velarde et al., 2007). Limited research has been done on this area, and genomic methods should be used to determine if specific genetic types do have milder reactions to high concentrations of CO_2.

To help objectively compare different studies assessing alternative methods, the four main recommendations from Steiner et al. (2019) should be carried out to establish standardized criteria to evaluate the alternative stunning methods.

From the perspective of pig welfare, viable alternatives should consider: low stress pre-handling, group stunning without restraint, immediate onset of unconsciousness or absence of avoidable pain, distress and suffering until the loss of consciousness, consistency of stunning and duration of unconsciousness and insensibility until death. However, in order to be widely adopted by industry, alternative methods must also be considered viable in terms of operator safety, production efficiency (processing speed and capacity), integration into slaughter production lines, improved meat and carcass quality, food safety and profitability.

The research and development priorities should focus on:

- Determining which single gas, or gas mixtures, can best fulfill the above stated aspects.
- Development of systems to administer the gas under industrial

slaughter conditions to ensure sufficient, controlled gas concentrations. This might possibly include gas scrubbing and reuse techniques to maintain economic viability.

- Evaluation of stepwise multiple gas stunning with an anesthetic first step gas application to reduce aversion or to minimize convulsions that could potentially affect meat and carcass quality negatively.
- Assessment of LAPS of pigs. A comparison, not only to CO_2 stunning, but also to inert gases or gas mixtures, is relevant. The possibilities for LAPS to meet animal welfare and industrial needs for pigs must be established.
- Development of electrical stunning to correct the existing, negative aspects of the method, i.e. stressful pre-handling of pigs including restraint, inconsistency of stunning due to displacement of electrodes, limited duration of unconsciousness and meat and carcass quality issues. An automated system enabling low stress pre-handling of the pigs is crucial. Single-pulse ultra-high current stunning could be an option, but the method needs to be evaluated on pigs.

Ethics approval

Not applicable.

Data and model availability statement

Search results used for the present study are not deposited in an official data repository.

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Author contributions

Erik Sindhøj: conceptualized, methodology, reviewed search hits, critical review and commented report, writing report into manuscript, revised manuscript and visualization. Cecilia Lindahl: conceptualized, methodology, writing draft report, revised report, critical review and commented manuscript, acquired financial support for original internal report. Linnea Bark: writing parts of the first draft of an internal report.

Declaration of interest

None.

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