Australian Government
Department of Agriculture, Fisheries and Forestry

AUSTRALIAN Pork

A comparative literature review of current and potential commercial stunning methods of pigs and their effects on animal welfare

Final Report
APL Project 2024/0040

January 2024

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Acknowledgements

This project is supported by funding from Australian Pork Limited, industry levy payers and the Commonwealth.

Executive Summary

Stunning prior to slaughter aims to intentionally cause unconsciousness and insensibility without pain and suffering. Animals must remain unconscious until death occurs through loss of blood, if not killed by the stunning method itself. Stunning should reliably result in immediate loss of consciousness without suffering. When stunning does not result in immediate unconsciousness or unconsciousness is not maintained until death due to blood loss, there are potential impacts on animal welfare.

There are several methods of stunning which can broadly be classified as mechanical, electrical and gas stunning, although there are a few other alternative methods that may be developed further in the future. The most commonly used stunning methods for pigs in commercial abattoirs in Australia are electrical and CO_2 stunning. This review outlines the main advantages and disadvantages of those stunning methods and discusses the potential alternative methods of stunning that are under investigation.

Captive bolt guns are used in some small abattoirs in Australia as the main stunning method. However, this method is not always effective due to the anatomy of the pig skull. It requires firm restraint, which is highly aversive in itself.

Electrical stunning is mainly used in small and medium-sized abattoirs. It can be applied as head-only, which results in a short duration of unconsciousness, or involve a second cycle that includes the heart and results in a much longer duration of unconsciousness or even death. While both methods involve individual handling and restraint, head/heart methods minimise the risk of inefficient stuns and the possibility of animals regaining consciousness before death due to blood loss. However, head/heart electrical stunning may have some negative impacts on meat quality. The use of high frequency stunning may mitigate some of these impacts and may result in better meat quality.

The advantage of CO_2 stunning is that pigs can be handled and stunned in small groups. However, CO_2 exposure is aversive, particularly at high concentrations (>30%). While it is difficult to establish the exact timeframe under commercial conditions, when concentrations around 90% are used, pigs remain conscious for about 22 to 32s. Therefore, there is concern for the welfare of pigs from the time of first exposure until unconsciousness. Pigs show several behaviours that indicate that CO_2 exposure is aversive, but there is large individual variation in those responses. Pre-slaughter stress, including handling, genetics and factors associated with farm of origin have been identified as sources of variation. Further research in identifying the exact mechanisms of reduced aversion may improve the animal welfare impacts of CO_2 stunning. Stunning in paternoster systems with high concentrations (>90%) of CO_2 and long duration of exposure (>3min) results in long duration of unconsciousness or even death, so there is little risk of animals regaining consciousness after stunning.

Despite the animal welfare advantages of gas stunning in groups, there have long been concerns about the use of CO_2 gas stunning considering the aversive responses. This has inspired research into alternative gasses for well over 25 years, but no alternative gas is yet available commercially. The focus of much of the research has been on Argon as an alternative. However, pigs still show some aversive responses to Argon exposure and the resulting duration of unconsciousness is very short. Therefore, there is a risk of animals regaining consciousness before death due to blood loss. A gas mix of Argon with 30% CO_2 increases the duration of unconsciousness (or decreases the time to death for long

exposures >7min), however this still compares unfavourably with 90% CO_2. The gas mix is also more aversive than pure Argon, reducing the advantage of this gas mix compared to CO_2 stunning. In addition, Argon is fairly expensive, and a recycling system would need to be investigated to reduce the cost of stunning using Argon.

Other gasses and gas mixtures that have been investigated are Helium and Xenon (which are rare and more expensive that Argon) and N_2 and N_2O as well as a mixture of N_2 and CO_2. N_2 may potentially be a more economical alternative to Argon as it is comparable in cost to CO_2. However, N_2 is slightly lighter than air and is therefore hard to contain in a pit. A potential solution is to use high expansion foam filled with N_2. However, the foam itself was found to be aversive, and the duration of exposure to death may be extensive. A gas mixture of N_2 and 30% CO_2 is stable and uniform when filled in a pit and is less aversive than 90% CO_2. It is comparable in cost and may be the most viable alternative gas to CO_2. However, exposure times need to be much longer (up to 7 min) to ensure animals do not regain consciousness after stunning to result in a practical stun to stick interval.

There are several alternative methods of stunning under investigation. While LAPS is considered a humane alternative for CO_2 stunning of poultry, there are still many questions on the effects on pigs. More research is required, particularly on conscious slaughter-weight pigs as there are concerns about observations of congestion and haemorrhage in several body regions after recompression. It is not clear if these changes may have occurred after loss of consciousness if these pigs were not under anaesthesia. Microwave stunning and Single Pulse Ultra-High Current (SPUC) stunning have been studied as alternatives for reversable Halal stunning and have not been applied to pigs. Both require restraint and therefore would be aversive.

The ideal stunning method involves low stress handling in groups without restraint, causes immediate and reliable unconsciousness without fear or pain and results in death or unconsciousness for the duration of death by blood loss with a wide margin. However, none of the stunning methods identified in this review would achieve this and some compromise on animal welfare will need to be accepted. Further research on the cause of individual variation CO_2 and a reduction in pre-slaughter stress may be the most immediate way to achieve improved animal welfare during stunning while potential alternative methods of stunning are being developed. In addition, pre-slaughter stressors, such as handling and restraint, may have a larger influence on meat quality than stunning system *per se*. None of the alternatives to CO_2 stunning result in improved meat quality and some of the identified alternatives may in fact be detrimental to meat quality, which needs to be considered when developing an alternative commercial stunning method.

Table of Contents

1. Introduction

Stunning prior to slaughter aims to intentionally cause unconsciousness and insensibility without pain and suffering. Animals must remain unconscious until death occurs through loss of blood, if not killed by the stunning method itself (EFSA, 2020).

Stunning is only one aspect of the slaughtering process, and several other components may contribute to concern for animal welfare. For example, pigs may find aspects of lairage and handling unpleasant or aversive, and the design of raceways leading up to the stunning area varies with different stunning systems. Therefore, the impact of a stunning system should not be examined in isolation but include the differences in the components of the slaughter process that affect animal welfare. Prior to stunning, animals need to be moved from lairage to the stunning area and need to be restrained in a way conducive to the method of stunning. This review includes all aspects of stunning that may be inherent to particular stunning systems and includes handling up to the stunning area, stunning method, stunning efficiency and duration of unconsciousness. While the focus of this review is on the potential effects on animal welfare of different stunning systems, the potential effects on meat quality and the practical application have also been noted.

2. Function of stunning

During slaughter, animals usually die from loss of blood after arteries of the brachiocephalic trunk have been severed, causing loss of blood supply, and thus oxygen and nutrients, to the brain. The basic aim of stunning before slaughter is to render an animal unconscious prior to slaughter to minimise pain and suffering. It is a legal requirement in many countries to stun animals prior to slaughter and remain unconscious until death (EFSA, 2020; Australian Model Code of Practice for Welfare at Slaughter Establishments, 2002). While stunning renders an animal unconscious, it may or may not kill an animal outright, depending on the method of stunning used. Some methods may only induce unconsciousness for a short time before the animal fully recovers, while others may result in a long duration of unconsciousness or even death in some or all animals. Stunning should reliably result in immediate loss of consciousness without suffering and should be of sufficient duration until death due to blood loss. When stunning does not result in immediate unconsciousness or unconsciousness is not maintained until death due to blood loss, there are potential impacts on animal welfare.

Regardless of the stunning method, stunning is followed by sticking (bleeding) to ensure death prior to the slaughter process and to ensure blood loss to maximise meat quality. The stunning method must be residual free, so the meat is suitable for human consumption. While animal welfare is of utmost importance, the safety of the human operators and economics and environmental impacts also need to be considered (Steiner et al., 2019). Currently, the main methods of stunning used in Australia are electrical stunning and stunning using CO_2 gas.

3. Animal Handling

The ability of farm animals to be handled easily is affected by a range of factors including fear, exploratory and social behaviour (Hemsworth, 2014). Fear in particular is affected by the animals' contact with humans, as well as the animals' sensory characteristics and the physical environment. In addition, learning can have long-term effects on fear (Schaefer, 1968; McFarland, 1981).

Fear-provoking stimuli are likely to elicit behavioural responses by the animal that reduce the ease of handling (Hemsworth, 2014). Considerable research has shown that the interactions between stockpeople and their animals, can limit animal welfare and productivity. Handling studies, primarily on dairy cattle, pigs and poultry, show that negative or aversive handling, imposed briefly but regularly, will increase fear of humans and reduce the growth, feed conversion efficiency, reproduction and health of livestock (see reviews by Hemsworth, 2009; Hemsworth & Coleman 2011; Waiblinger et al., 2006). Studies with pigs clearly demonstrate that a chronic stress response is implicated in these adverse effects on productivity, since handling treatments which resulted in high fear levels, often produced either a sustained elevation in basal free cortisol concentrations or an enlargement of the adrenal glands (see Hemsworth & Coleman 2011; Hemsworth et al., 2018).

Although many of the interactions between stockpeople and their animals occur regularly and often appear harmless to the animal, research has shown that the frequent use of some routine behaviours by stockpeople can result in increased fear in farm animals (Hemsworth & Coleman, 2011; Hemsworth, 2014). Negative tactile and non-tactile stockperson behaviours that increase fear in pigs include mild to forceful slaps, hits, kicks and pushes, as well as yelling/shouting and fast speed of movement. This demonstrates the sensitivity of pigs to mild and moderate negative behaviours by humans (Hemsworth, 2003). Alternatively, positive stockperson behaviours such as pats, strokes, the hand resting on the pig's back, quiet talking and slow speed of movement have been shown to reduce fear in pigs (Hemsworth, 2003; Rault et al., 2021). Positive handling experience has also been found to provide benefits in terms of ease of handling and meat quality (Geverink et al., 1998, Hambrecht et al., 2005). Consequently, stockperson behaviours impact both the immediate behaviour of the animal, as well as the subsequent behavioural responses of the animal to humans. Another outcome of stockperson behaviour and the subsequent behavioural response(s) by the animals is reduced stockperson comfort (see Hemsworth & Coleman, 2011). The stockperson's frustration with difficulties in handling may lead to increased negative interactions with the animals which may further increase the animals' fear and reduce ease of handling.

Thus, understanding the animal's behavioural characteristics and sensory and cognitive capabilities is important for effective handling, as well as ensuring high animal welfare and productivity in all stages of livestock production.

3.1. Principles of pig handling

Fear and exploration are important states of motivation that affect ease of handling animals such as pigs (Hemsworth, 2014). Furthermore, the sensory and cognitive capabilities of pigs, as well as their social behaviour, will also affect their movement and thus handling, in both familiar and unfamiliar environments.

Fear can be considered as an intervening variable, linked to i). a range of stimuli that may pose some risk to the welfare of the animal, and ii). a series of behavioural and physiological responses by the animal which allow it to respond appropriately to this challenge (Hemsworth, 2014). Fear is an emotional reaction (Janczak, 2010), and is generally considered as a motivational state aroused by specific stimuli that normally result in defensive or escape behaviour (MacFarland, 1981; Hogan, 2021). Fear may be elicited by environmental stimuli that are novel, have high intensity (e.g., loud and large stimuli), have special evolutionary dangers (e.g., heights, isolation and darkness), develop from social interaction (e.g., contagious learning), or have been paired with aversive experiences (Gray, 1987). Although fear seems to be triggered predominantly by external stimuli, intrinsic factors such as breed, sex, social and strain effects have also been shown to be important (Hogan, 2021).

Limited research in pigs and other livestock has shown that animals that are highly fearful of humans are generally more difficult to handle (Gonyou et al., 1986; Grandin et al., 1987; Rushen et al., 1999; Hemsworth, 2014). For example, Hemsworth et al. (1994) found relationships between the behavioural response of pigs to humans and their ease of handling. These findings indicate that pigs showing high levels of fear of humans, based on their avoidance behaviour of, or lack of approach to, an experimenter in a standard approach test, were the most difficult pigs to move along an unfamiliar route: fearful pigs took longer to move, displayed more baulking and were subjectively scored as the most difficult to move by the handler (Hemsworth et al., 1994). Furthermore, Eldridge and Knowles (1994), Abbott et al. (1997) and Geverink et al. (1998) reported regular handling and moving of pigs may facilitate subsequent ease of handling from farm to slaughter.

Exploratory behaviour has been defined as behaviour that serves to inform the animal of the topography of its environment (Shillito, 1963). In addition to serving the animal's biological needs, exploratory behaviour can be characterised by distinct tendencies to investigate and seek new forms of stimulation (Inglis, 2010). In a broad sense, exploration involves behaviours seeking to gather information about the environment. The amount of exploration of an object that an animal performs will depend upon the characteristics of the object, including its novelty, complexity, intensity and contrast, and the deficiency of its previous environment (Berlyne, 1960). A sudden environmental change will generally elicit an orientation response, whereby the animal turns towards the disturbance source. The orientation response may then be followed by startling responses and defensive or flight responses by the animal (Hemsworth & Barnett, 1987). As fear responses decline, the animal will also approach and investigate the stimulus (Hinde, 1970). Exploration will stop once the animal is familiar with the stimulus. As such, animals' responses to novel stimuli contain elements of both fear and exploratory responses (Hemsworth, 2014).

Pigs will initially be fearful of novelty and will generally baulk when novel stimuli are encountered. Pigs are generally wary of moving towards a novel or unpredictable situation and, if they are fearful of both the environment and the stockperson, they may show exaggerated behavioural responses to handling, such as baulking or fleeing back past the handler. Therefore, housing or facility features such as floor surfaces, floor levels and wall types should be as consistent as possible throughout a race or corridor to reduce baulking. Unfamiliarity is likely to have a greater effect on ease of handling at critical points in a route such as pen exits, corners and entrances to races or corridors. If pigs become fearful in a novel location, it is important to allow them time to familiarise themselves with the environment. Trying to move pigs quickly in a novel or fear-provoking situation will reduce ease of handling, increase time taken to move animals, as well as risking injury to both pigs and stockpeople. To effectively move pigs, stockpeople should follow behind, and slightly to one side, using a solid board to prevent pigs from turning back (Grandin, 1980; Hemsworth, 2014).

Pigs' sensory and behavioural characteristics affect their movement and handling. Pigs have good colour vision (see Grandin, 1987), and as such, may respond to the novelty of a change in the colour of facilities and housing, or stockperson clothing. Although pigs have poor eyesight (Holmes et al., 2020), they have a wide angle of vision (panoramic vision of 310° and binocular vision of 35-50°; Prince, 1977). Pigs prioritise their lateral monocular vision which increases their panoramic vision (providing a greater capacity for detecting possible danger, food, other pigs, etc.) and decreases their bifocal vision (resulting in greater difficulty for calculating distances) (Dalmau et al., 2009). Optical irregularities such as shadows and rays of light can cause baulking and significantly affect pigs' willingness to move forward (Holmes et al., 2020). Like most animals, pigs have a tendency to move towards a more brightly lit area (Van Putten and Elshof, 1978), and research suggests that light should be even and diffuse (excessive light (1200 lux) caused avoidance) (see Grandin, 1980). Tanida et al. (1996) found that piglets preferred to move from dark to lighter areas and could be encouraged to move to darker areas with the provision of lighting. Therefore, lighting may be used to encourage movement into poorly lit areas, including races and dark corridors. Due to their reduced bifocal vision, pigs are only moderate judges of distance (Grandin, 1980), and are reluctant to cross changing light patterns, drain grates, steps, puddles of water, gutters, and other high-contrast objects. Additionally, if facility features such as walls of corridors or races, pen fronts and gates are not solid, pigs that are being moved may be distracted by what they see, including other people or pigs (Grandin, 1980). ig boards should also be solid to block the vision of pigs and thereby to encourage their movement away from the boards (Hemsworth, 2014).

Pigs' snouts have a net of sensitive nerves providing an excellent surface with high sensitivity and ability to smell, which provides pigs with a well-developed sense of smell (Holmes, et al., 2020). They will use these senses to investigate their surroundings, and they will look to investigate novel environments by stopping and sniffing (Holmes et al., 2020). Pigs will avoid locations containing urine from stressed pigs (Vielle-Thomas & Signoret, 1992). As a result, handling of fearful pigs or handling that creates stress in pigs may cause animals following to respond fearfully to these olfactory signals, making these pigs difficult to handle as well (Hemsworth, 2014). Pigs also have sensitive hearing and may avoid excessive and unfamiliar noise (Grandin, 1990; Geverink et al., 1998; Waynert et al., 1999).

There are several aspects of pigs' social behaviour that will affect ease of handling. Herding or flocking, where social spacing and orientation are maintained, is most evident in sheep but is also apparent in other livestock, including pigs (Grandin, 1980; Hemsworth, 2014). Following behaviour, where there is synchrony of behaviour such as walking, running, feeding and lying, is commonly seen in pigs and other livestock. Pigs show pronounced herding and following behaviour (Van Putten & Elshof, 1978). Pigs' motivation to follow other pigs and maintain body and visual contact with other pigs can be utilised to move pigs. Corridors or races should be wide enough to provide the animals with a clear view ahead and of other animals moving ahead: Grandin (1990) suggests that races for pigs should be 1 m wide. They also generally prefer to move in small groups (5-7 pigs), with evidence of a negative effect on pig welfare and handler ease when pigs are moved in larger groups (>7 animals) (Lewis & McGlone, 2007). While livestock appear to move more easily on a level surface, excessively steep ramps were avoided by pigs in a preference test: 20–24° ramps were preferred to 28–32° ramps (see Grandin, 1990).

3.2. Pre-slaughter handling of pigs

It is well recognised that the stress imposed on pigs during transport, in lairage and at slaughter is an animal welfare risk, as well as a meat quality issue (see reviews Faucitano & Goumon, 2018; Faucitano & Velarde, 2021; Holmes et al., 2020). Furthermore, handling and human-animal interactions are key factors impacting stress and therefore welfare of pigs' pre-slaughter (Herskin et al., 2020; Holmes et al., 2020). In addition, poor handling or handling related issues have been shown to negatively impact meat quality and carcass quality (Hemsworth et al., 2002; Driessen & Geers, 2001). As a result, pre-slaughter handling of pigs has received attention from both science (e.g., Hemsworth et al., 2002; Correa et al., 2010; Brandt et al., 2013; Goumon & Faucitano, 2017; Herskin et al., 2020) and industry (e.g. SEGES, 2020). In pigs, most research has been directed at finishers (e.g., Brandt et al., 2013, 2015), despite a significant number of sows slaughtered every year (Herskin et al, 2020). Sows are typically slaughtered after multiple production cycles (de Jong et al., 2014; Zhao et al., 2015), and therefore, may be in a vulnerable clinical condition (Fogsguaard et al., 2018) and thus less fit for transport than other types of pigs (Grandin, 2016).

Optimisation of lairage and slaughter conditions (particularly facility layout, environmental conditions and animal handling) is important in order to allow pigs to recover from the stress of handling and transport and to ensure optimal and uniform carcass and meat quality (Faucitano & Velarde, 2021). It is outside the scope of this review to consider the effect of handling during pre-slaughter transport on pig welfare. However, the implications of pre-slaughter transport have been reviewed recently by a number of authors, including Faucitano & Goumon (2018), Faucitano & Velarde (2021), Isbrandt et al. (2022).

3.3. Handling of pigs during lairage

Upon arrival at an abattoir and during lairage, animals are simultaneously exposed to a variety of stressors that may result in high levels of stress and thus compromise welfare (Grandin, 1997). These potential stressors include exposure to a novel environment, handling by humans, feed and water deprivation, mixing of unfamiliar individuals, noise, forced physical exercise and temperature and humidity extremes (Velarde & Dalmau, 2018). To prevent the negative effects of lairage, it is recommended that animals should be unloaded promptly after arrival (unloading should begin within 30 minutes of arrival and take no more than 60 minutes; Grandin, 2019) and subsequently slaughtered without unnecessary delay. However, abattoirs try to avoid interruption in the supply of livestock to the slaughter line by having a sufficient reserve of animals waiting in lairage (Faucitano, 2018). In addition, lairage is designed to provide animals with an opportunity to recover from the stress of transport and previous handling (Warriss, 2003), which can be beneficial for both animal welfare and carcass and meat quality (Warriss, 2003; Faucitano, 2010, 2018). However, as covered in reviews by Warriss (2003) and Faucitano (2010; 2018) the benefit of providing animals with resting time can be diminished if they experience poor handling and stressful environmental conditions in lairage. Grandin (1994) suggests that pigs should be rested 2 to 4 hours before stunning. Interestingly, Aaslyng and Barton Gade (2001) have shown that a recovery period in lairage is less important in pig without the halothane gene.

Moving animals through lairage towards the stunning point poses a significant risk to animal welfare. The combination of a higher slaughter speed, poorly designed facilities (including slippery floors, with distractions that make animals balk, dark and poorly maintained corridors, sharp edges/corners, etc.) and in some facilities, the change from a free-moving group to a single line of individuals between the exit from the lairage pen and stunning may result in a greater proportion of slips, jamming, baulking and stopping, backing up and turning around and vocalisation (Warriss et al., 1994; Edwards et al., 2010, 2011; Van de Perre et al., 2010; Vermeulen et al., 2015; Rocha et al., 2016). These conditions are likely to be detrimental to pig welfare as they can lead to increased use of negative behaviours and handling by stockpeople to maintain the speed of the slaughter line (Hemsworth, 2014; Holmes et al., 2020), including increased goad use (Rocha et al., 2016). The behavioural responses observed in pigs in these situations have been associated with indicators of both reduced welfare and meat quality: including increased heart rate (up to 240 beats/min; Chevillon, 2001; Correa et al., 2010), blood lactate and CK levels at slaughter (Hambrecht et al., 2005; Edwards et al., 2010; Rocha et al., 2015), skin lesions scores (Rabaste et al., 2007) and exudative pork (Van der Wal et al., 1999; Hambrecht et al., 2005; Rabaste et al., 2007; Dokmanović et al., 2014; Rocha et al., 2016). Furthermore, shocking pigs with electric goads cause pain and significantly increases heart rate, open mouth breathing and vocalisations (Velarde & Dalmau, 2018; Holmes et al., 2020). Faucitano & Geverink (2008) report the routine use of electric goads is related to negative attitudes towards animal handling. They found the use of a goad with the power turned off was associated with positive attitudes to animal interactions in abattoir stockpeople, whereas if the goad was turned on it was associated with a negative attitude (Faucitano & Geverink, 2008). Electric goads and prods which cause fear, pain and stress should not be used to move pigs in lairage. As an alternative, flags, paddles and plastic boards can be used to encourage pigs to move, supported by voice and gentle patting (Hemsworth, 2014; Velarde & Dalmau, 2018; Holmes et al., 2020). However, the effectiveness of these handling tools and methods will depend on the attitudes and training of the stockperson using them, as well as the design of the facilities (Hemsworth & Coleman, 2011; Velarde & Dalmau, 2018).

Moving pigs during lairage may also be facilitated by considering pigs' sensory and behavioural characteristics, such as mono- and binocular vision, the concepts of 'flight zones' and their point of balance (Grandin, 2019; Holmes et al., 2020). Pigs will move or stop depending on a stockperson's positioning within or outside the animal's flight zone, and moving in the opposite direction behind the pig's point of balance encourages it to move forward (Grandin, 2019). Stockpeople should avoid standing within the blind spot behind the animal. The layout and construction of lairage should encourage pigs to move freely within corridors and races to the stunning area. As discussed previously, factors that will likely affect pigs' ease of movement in lairage include the handling of animals, the size of groups being moved, the layout of lairage, lighting, flooring and ramps, the level of noise and airflow (Hemsworth, 2014; Holmes et al., 2020). Negative handling in lairage, as well as technical hazards, such as steep ramps, slippery and uneven floors, and unsuitable pathways may cause fear which can result in pigs stopping, turning back, slipping and falling. As discussed previously, this increases the risk of stockpeople using negative behaviours to speed up the process, thus increasing the risk of pigs experiencing fear and/or pain (Hemsworth, 2014; Holmes et al., 2020).

Reducing negative stockperson behaviour will not only reduce fear, stress and welfare risk in the pigs being handled, but also improve ease of handling in pigs following in the slaughter line. Pigs will avoid locations containing urine from stressed pigs (Vielle-Thomas & Signoret, 1992), and moving pigs through locations containing olfactory signals from stressed pigs, will make these pigs difficult to handle as well (Hemsworth, 2014).

The race or passageways in lairage should be wide enough to provide pigs with a clear view of where they are moving and allow herding and following behaviour in small groups, utilising their motivation to follow other pigs to maintain visual and body contact (Van Putten & Elshof, 1978). This can be facilitated by using races and corridors in lairage that are at least 1 meter wide to enable pigs to walk next to and behind each other in small groups (Grandin, 1990). Moving pigs in small groups allows stockpeople to maintain control over the pigs and to limit the risk of them stopping and turning back, and therefore the need for increased pressure on pigs to continue moving (Dalmau et al., 2009). In both finishers and sows, the use of small group sizes during handling and moving in abattoirs has been shown to lead to behavioural changes indicative of improved animal welfare as well as facilitated movement of the pigs (Lewis & McGlone, 2007; Herskin et al., 2020).

Pigs prefer to walk up rather than downhill, so aisles should be level or have an increasing angle of 2 to 3° (Holmes et al., 2020). Due to their vision, pigs are likely to confuse shadows and floor irregularities with physical barriers or solid objects and together with their difficulty in judging distance and depth, may make pigs reluctant to cross areas with changing light patterns, drain grates, steps, puddles of water, gutters, and other high-contrast objects (Grandin, 1980; Hemsworth, 2014; Holmes et al., 2020). Furthermore, pigs tend to move towards brightly lit areas (Van Putten & Elshof, 1978), and it is recommended that light should be even and diffuse (excessive light (1200 lux) causes avoidance) (see Grandin, 1980). Additionally, if corridor or race walls, pen fronts and gates are not solid, pigs that are being moved are likely to be distracted by their surroundings (Grandin, 1980). Light management and race systems that eliminate visual distractions and promote the animals' herding and following behaviour will therefore promote ease of handling in pigs in lairage (Grandin, 1990; Holmes et al., 2020). Grandin (1982) found that a smooth, concrete floor with a wet, slippery surface inhibits pig movement. As such, non-slip flooring such as textured concrete, grooving, and rubber mats can be used to diminish the risk of pigs slipping and falling and becoming agitated during handling in lairage (Grandin, 2019).

Pigs are sensitive to excessive and unfamiliar noise (Grandin, 1990; Geverink et al., 1998; Waynert et al., 1999), so efforts should be made to minimise wherever possible in lairage. Compared to other livestock species, lairages in pig abattoirs have been shown to be the loudest ones (Weeks et al., 2009), with the average noise level ranging from 76 dB to 108 dB and the highest peaks (120 dB) being recorded in the peri-mortem area (Talling et al., 1996; Rabaste et al., 2007). Excessive lairage noise has been shown to result in a fear response in pigs, evident by the number of pigs huddling in the pen or escaping from the source of sound (Geverink et al., 1998), increased heart rate and greater blood lactate, CK and cortisol levels at slaughter (Faucitano, 2010), all resulting in an increased production of PSE pork (Warriss et al., 1994; Van de Perre et al., 2010). Vermeulen et al. (2015) reported the risk for PSE meat was reduced by keeping sound levels lower than 85 dB in the peri-mortem area. Lairage noise appears to be mostly caused by gates clanging, operating machinery, echoes and pig vocalisation (Weeks, 2008; Weeks et al., 2009), although pigs seem to be more stressed by industrial sounds than the sounds of conspecifics (Geverink et al., 1998).

3.4. *Handling of pigs around restraint and stunning*

The final stage of pig production is the slaughter of pigs for human consumption. The slaughter process includes the restraining of the animal, the stunning application and the exsanguination (see Faucitano & Velarde, 2021). The stunning phase involves both the restraint and the stunning process (Gerritzen et al., 2021). Before pigs can be restrained for stunning, they are moved from the lairage pens to the stunning area. In this case 'restraint' is the application of any procedure to an animal that is intended to restrict its movements in order to minimise pain and fear and facilitate effective stunning and killing (Gerritzen et al., 2021). Animal handling directly associated with stunning and killing involves moving animals so that they can be restrained for stunning, and this differs between the two main methods of stunning (electrical and CAS) (Gerritzen et al., 2021).

After arriving at the stunning area, pigs are generally either manually moved into the stunner for electrical stunning or by automatic pushing gates in the CAS stunning systems (Gerritzen et al., 2021). Two critical factors in the peri-mortem area that relate to ease of handling or movement of pigs are the entrance into the stun chute and the 'stop-start' forward movement of pigs towards the stunner, which are relevant in races leading to both electrical and CO_2 stunners (Faucitano & Velarde, 2021). A key issue associated with these factors is the increased use of electric goads by stock people to encourage pig movement into the stunner (Faucitano, 2010). The batch flow system or curved raceway has been found to work well, by easing the flow of pigs into the single or double electric stun raceway (Grandin, 1982, 2017; Edwards et al., 2010). This improvement in ease of movement is demonstrated by the lower number of pigs that are reluctant to move forward and the use of electric goads by stockpeople when compared with the single file chute entrance (Edwards et al., 2010). For this type of system, it is recommended that the entrance should not be abrupt, and it should not be filled with pigs for more than 75% of its capacity (ideally 50%) to prevent jamming and overlaps in the crowd pen (Grandin, 2017b). Moving pigs into a CO_2 stunner has also been improved by utilising the group-wise stunning system (Christensen & Barton-Gade, 1997), where pigs are moved forwards in small groups (generally 10–15 pigs) using pushing gates and are then loaded in smaller sub-groups of (approximately 5 pigs) into the cradle. This system has been shown to reduce the frequency of PSE and blood-splashed pork and bruised carcasses due to a reduction in negative handling and stress (Christensen & Barton-Gade, 1997).

3.5. Handling prior to electrical stunning

For electrical stunning, pigs need to be either separated individually to enter a stunning box for individual (manual) stunning, or into a single file for automated stunning (Gerritzen et al., 2021). Moving pigs from a group into a single line and restraining them individually can be very stressful for the animals (Troeger, 1989). Social isolation has been shown to be stressful to pigs (Soler at al., 2013), however, moving in a single file along a path is a natural behaviour for pigs and stress can be reduced if animal handling and facility design are appropriate (Stolba & Wood-Gush, 1984). Forcing pigs from a group into a single line is potentially the most critical part of the process due to the high speed of slaughter, and this is one of the main reasons abattoirs are moving to CAS (Gerritzen et al., 2021).

As with lairage, the construction of the race leading to the stunning area (e.g. bottlenecks, corners or right angels), light management in the raceway and stunning area, air circulation, flooring and noise may have a significant effect on ease of handling and pigs' willingness to enter the race/stunning area (Grandin, 2010). Negative handling, including the use of too much pressure or force, shouting, hitting or the use of rattles and electric goads to force animals to move from a group into a single line raceway, and a novel environment unfamiliar including fear-related vocalisations and olfactory stimuli from other pigs will increase fear and reluctance of the animals to move towards the stunner (Gerritzen eta al., 2021). In a fully automated system, as noted earlier another critical factor is the "stop-start" motion of pigs in the raceway towards the stunner due to the flip-flop gate between two raceways allowing pigs one by one onto the restraining conveyor belt (Faucitano & Velarde, 2021;

Gerritzen et al., 2021). Another factor which may increase the stress experienced by pigs during the transition from moving in a group to the single file is being carried with their feet off the ground to the point of stunning on a V-type restrainer or a centre-track conveyor belt (Troeger, 1989). Comparative studies have shown that pigs restrained in a centre-track conveyor belt had lower heart rate and proportion of PSE pork production compared with those restrained on a V-type restrainer (Griot et al., 2000).

Depending on the facility layout and the handling they receive at the point of isolation, pigs may attempt to back out and/or turn back, or they may be reluctant to move forward into the race while vocalising at a high pitch. Pigs experiencing pain and/or fear caused by negative handling have been shown to perform high pitch vocalisation (Hemsworth & Coleman, 2011; Hemsworth, 2014; Gerritzen et al., 2021). Schrader and Todt (1998) report that pigs' vocalisations can indicate an activation of the endocrine stress response, with the rate of squeal-grunts, characterised by high frequency ranges and short duration, increasing with increased concentrations of adrenaline.

A curved raceway or carousel can be used to facilitate ease of handling and pig flow (Jones, 1999; Grandin 1990), which should reduce the level of fear and stress pigs experience when they are being moved from a group into a single line (Gerritzen et al., 2021). Furthermore, keeping the race and entrance to the restrainer or stunner clean and free from sharp edges should enable ease of animal movement by reducing the need for force and avoiding animals slipping and falling (Grandin, 2021). Reducing the loading speed and giving animals the time and opportunity to orientate themselves and to go from a group into a single line will also prevent or reduce the level of fear and stress they experience at this stage of the slaughter process (Gerritzen et al., 2021). In addition, restraint time may cause fear and as such, pigs should not enter the restrainer until equipment and personnel are ready to slaughter the animal (Faucitano & Velarde, 2021).

3.6. Handling prior to CO_2 stunning

During the CO_2 stunning process, pigs are moved in small groups into the stunner. In the lairage area, groups of approximately 15 pigs are separated using automatically operating doors and moved as a group to the stunner by hydraulic push gates. Finally, before entering the CAS stunner the group is split into smaller groups of pigs based on the size of the animals and the gondola (Gerritzen et al., 2021). Moving pigs in small groups (2-8 animals) into the stunner using automatic doors has been shown to be less stressful than the isolation of pigs prior to electric stunning (European Food and Safety Authority (EFSA), 2004; Velarde et al., 2000). Moving animals in groups also removes the need to apply restraint. Previous research has shown this reduces separation anxiety and distress for pigs (Mota-Rojas et al., 2012; Steiner et al., 2019). However, the benefit of moving the pigs in groups compared to handling into a single race may be lost if waiting pigs can hear other pigs vocalising in the stunner, if the handling pressure is too great and if the stocking density in the race is too high (Hemsworth, 2014; Holmes et al., 2020; Gerritzen et al., 2021). Pigs may also experience stress, fear and pain if automatic gates are lowered onto their backs or limbs, or they are pushed forward while not walking. As with electric stunning, perceived pressure to maintain slaughter speed may also result in stockpeople negative handling which will lead to pain, fear and suffering (Jones, 1999). Research has shown that in the presence of normal atmospheric air (absence of a high CO_2 concentration), the process of entering the gondola and the gondola with the pigs being lowered into the pit causes moderate aversion to a change of environment to which pigs can habituate if they are exposed to it repeatedly (Velarde et al., 2007; Dalmau et al., 2010).

3.7. Summary of animal handling

Human–animal interactions and animal handling are key features of modern pig production, including around slaughter. Considerable research on pigs has shown that human-animal interactions may markedly affect the behaviour, welfare and productivity of pigs at all stages of production. Furthermore, by affecting the behavioural response of the pigs to humans, these interactions can affect the ease with which pigs can be observed, handled and moved by the stockperson at the abattoir. In addition to handling, physical features of the abattoir environment will also affect animal movement and thus animal handling. Therefore, an understanding of the behavioural characteristics and sensory and cognitive capabilities of pigs is also important in effectively handling pigs pre-slaughter.

There remains a clear, ongoing need for the pig industry to both train their personnel to effectively. handle and move their animals and ensure that current knowledge on the characteristics of pigs is appropriately used in the design of handling facilities in abattoirs. The stockperson may be the most influential factor affecting pig handling and welfare, so underestimating the role and impact of the stockperson during slaughter will seriously risk the welfare and productivity of pigs. In addition, key industry stakeholders including the general community are placing an increasing emphasis on ensuring the competency of stockpeople in managing the welfare of pigs in all stages of production.

Research has shown that training of stockpeople can improve their handling of farm animals, reduce fear responses in these animals and thus improve animal welfare and productivity (see Hemsworth & Coleman, 2011). Studies in the dairy and pig industries (Hemsworth et al., 1994a; Coleman et al., 2000; Hemsworth et al., 2002b) have demonstrated a causal link between stockperson attitudes, stockperson behaviour, farm animal fear, stress and productivity. The Model for human-animal interactions in the livestock industries (Hemsworth & Coleman, 2011) explains the causal pathway between stockperson attitudes, stockperson behaviour, farm animal behaviour and stress, and farm animal productivity and welfare outcomes. This sequential human-animal relationship model is especially significant because it integrates human and animal factors in the causal pathway and targets outcomes that are relevant to both farm animal welfare and commercial profitability. Furthermore, these studies have shown that cognitive-behavioural training, in which the specific attitudes and behaviour of stockpeople are targeted, can successfully improve the attitudes and behaviour of stockpeople towards their animals, with consequent beneficial effects on animal fear and productivity (Hemsworth et al., 1994a; Coleman et al., 2000; Hemsworth et al., 2002). The cognitive-behavioural training programme used as an experimental tool during this research in the pig industry has been commercialised for on-farm use and is known as 'ProHand Pigs®' (a contraction of the 'Professional Handling of Pigs Program'). ProHand Pigs ® is widely used in Australia, New Zealand, and North America. A similar research-based cognitive-behavioural training programme was also developed for abattoir handling of pigs in Australia, called 'ProHand Pigs Abattoir®'. Research demonstrates that in order to achieve sustained stockperson behavioural change, cognitive-behavioural training is necessary to improve stockperson-farm animal interactions, and thus reduce animal fear and physiological stress (Hemsworth & Coleman, 2011).

4. Mechanical and electrical stunning methods

There are several methods of stunning which can broadly be classified as mechanical, electrical and gas stunning, although there are a few other alternative methods that may be developed further in the future. However, the most commonly used stunning methods for pigs in commercial abattoirs in Australia are electrical and CO_2 stunning. While the use of CO_2 is considered an acceptable method of stunning pigs in Australia (Model Code of Practice for the Welfare of Animals: Livestock at Slaughtering Establishments, 2001), the United States (NAMI, 2021), the European Union (Council Regulation (EC) No 1099/2009) and by the World Organisation for Animal Health (OIE, 2005), there are questions about the humaneness of exposure to high concentrations of CO_2. The European Food Safety Authority recently specified that exposure to CO_2 at high concentrations (>80%) should be replaced by exposure to other gas mixtures that are less aversive (EFSA AHAW Panel et al., 2020). In the next sections the different commercial stunning methods will be discussed as well as alternative methods, including alternative gasses, that are in different stages of development and may or may not be suitable for commercial application in the future.

4.1. Captive bolt devices

Captive bolt devices are mostly used for stunning before slaughter of cattle, but may be used in very small abattoirs as the main stunning method for pigs. However, they are mainly used as a back-up method of stunning when the primary method of stunning has failed or for emergency slaughter or euthanasia (including on-farm).

There are two types of captive bolt devices, penetrating and non-penetrating. In addition, captive bolt devices can be powered by powder-filled cartridges (usually hand-held) or compressed air (pneumatic). Only penetrating captive bolt devices are acceptable for use in pigs in the EU (EFSA, 2004), other than small piglets <9kg. Penetrating bolts penetrate the skull as well as the brain, causing immediate unconsciousness due to kinetic energy (causing concussion) and brain damage (Wallgren et al, 2021). Penetrating captive bolt stunning should be followed by a secondary killing procedure even though the stunning method itself will result in death, when applied correctly (Anderson et al, 2022).

Captive bolt stunning of pigs can be problematic due to the shape of the head, the presence of sinuses between the scull and the brain, and a brain that lies relatively deep in the head. In addition, older animals may have a central ridge of bone, preventing penetration of the bolt into the brain. When captive bolt stunning is used for pigs, a heavy cartridge should be used, and they should be bled or pithed immediately after stunning to ensure death occurs rapidly and before the animal gains consciousness. The Australian Model Code of Practice for Welfare at Slaughter Establishments (2002) does not recommend the use of captive bolt for routine slaughter of pigs other than large sows or boars. This is contrary to advise from EFSA (2004), which recommends that captive bolt guns are not to be used for stunning in older pigs such as sows and boars due to the anatomy of the head. If used, the recommended placement of the captive bolt gun is at a point midway across the forehead and about 2 cm above eye level for adult pigs, although the recommendation of the exact placement varies between different organisations (Anderson et al, 2022). The gun should be placed firmly against the forehead and aimed towards the tail to maximise the impact on the brain.

Little is known about the stun failures of pigs using captive bolt devices. however, there are several studies on stunning effectiveness in cattle. For example, Von Wenzlawowicz (2012) observed nearly

2000 cattle in 25 different abattoirs and found the effectiveness of the stun to be insufficient in 9.2% of cattle. However, there was a large variation between abattoirs, and while inaccurate placement was a major factor in stun failure, both head restraints and high powered (pneumatic) devices improved efficiency. In contrast, Vecerek et al (2020) found that ineffective stuns of cattle in a single abattoir were not related to placement of the device, even though only 20% of shots were placed within 2cm of the ideal location. However, they did not assess bolt angle and damage to brain tissue. They did observe more post-stun reflexes, indicative of a less effective stun, in bulls compared to cows, probably due to a different skull structure and heavier body weight of bulls (Vecerek et al., 2020). Stunning issues are due to poor placement, improper restraint and handling, or equipment failure. About 25 years ago Grandin (1998) reported that poor gun maintenance was the main cause of ineffective stuns in US abattoirs. These days maintenance is much improved and poor placement is the main reason for stun failures with captive bolt. In the US during routine audits 32% of FSIS humane handling enforcement actions in pig abattoirs was for ineffective stuns, needing more than 2 stun attempts, which was less than for cattle (Baier and Willson, 2020).

In summary, the structure of the skull of pigs and the challenge of correct placement may result in ineffective stunning when a captive bolt is used. Correct placement would be facilitated by restraint of the head, which in itself is highly aversive to pigs. Although captive bolt stunning is used in some small abattoirs in Australia, it is not recommended for commercial stunning of pigs at larger operations, other than for emergency slaughter.

4.2. Electrical stunning

Electrical stunning is mainly used in small and medium-sized abattoirs worldwide (Sindhøj et al, 2021) and is used as the main method of stunning in some countries where CO_2 stunning is not used. Electrical stunning involves placement of electrodes to apply an electric current. During one-cycle electrical stunning electrodes can be placed either on the head only, or at both the head and the back. Two cycle stunning usually involves an automatic system where electrodes are first placed on the head just below the ears to stun the animal. This is followed by a third electrode on the chest, to deliver a second current resulting in fibrillation of the heart and cardiac arrest (HSA, 2016). The animals need to be properly restrained during the application of the electrodes to minimise movement and ensure correct placement. In these systems pigs are moved in a single file raceway to a conveyer belt type restrainer, although static restrainer systems are sometimes used for large pigs. The conveyer belt can either be in a V-arrangement, gripping both sides of the animal, or a single belt that supports the belly of the animal. While restrained, electrodes are either placed manually or automatically. However, restraining systems are aversive to pigs (see section 3.5) and manual application allows for systems without firm restraint or even application in groups. However, there is a risk when electrodes are placed on pigs in groups without restraint that placement is not correct (Von Wenzlawowicz et al, 2012) and that other pigs within a group may be touched with active electrodes (Wallgren et al, 2021) which is considered a severe animal welfare risk.

Recommendations on minimum current (A or mA), minimum voltage (V), maximum frequency (Hz), minimum time of exposure, frequency of calibration of the equipment, optimisation of the current flow, position and contact surface area of electrodes, maximum stun-to-stick interval can vary between different jurisdictions and can be highly variable. Generally minimal statutory requirements for Europe are defined by the European Food Safety Authority (EFSA) and are considered good practice worldwide. However, individual European countries may have more extensive and stringent requirements. The Australian Model Code of Practice for Welfare at Slaughter Establishments (2002) recommends head to back electrical stunning of pigs, with a minimum of 400 V, 1.3 amps for 2s. However, electrical stunning causes violent muscle contractions and may have a negative effect on meat quality, depending on current, voltage, frequency and (Chanon et al, 2002; Channon et al, 2003; Faucitano et al, 1998; see section 7.2).

4.2.1. Head only electrical stunning

The ideal position for the electrodes during a head-only stun is between the eyes and the base of the ears, however electrodes may also be placed on each side of the head or just below the ears (EFSA, 2004). While the general aim is for the electrodes to span the brain to ensure maximum current intensity and optimum stunning efficiency, electrodes may be placed more caudally resulting in insufficient current and therefore voltage should be increased. The recommended placement of electrodes is as close as possible behind the eyes and no more than 5cm behind the ears (Anil and McKinstry, 1998). However, particularly with manual placement, it is easier to place electrodes further behind the ears, resulting in lower stunning effectiveness and greater difficulty for breeds with hanging ears (see Gerritzen et al, 2021).

A head-only stun aims to induce an epileptic seizure (Grand Mal) resulting in unconsciousness and insensibility by passing an electric current through the brain to depolarise the neural membranes in the thalamus and cerebral cortex (Raj, 2015). A successful stun results in a generalised epileptic seizure which causes muscle contraction (tonic phase). During this phase pigs are in a state of tetanus with rigidly stretched front limbs and flexed hind limbs. The eyes may be fixed or rotated and there is a lack of breathing activity. This is followed by the clonic phase where muscles alternate between contractions and relaxation, with kicking and paddling mostly of the hind legs but a more rigid trunk (HSA, 2016). To ensure death before pigs return to consciousness, pigs need to be bled before entering the clonic phase.

Correct placement of electrodes and sufficient current are the most important factors to induce an immediate and complete stun. While electrical stunning induces unconsciousness instantly, pigs can recover quickly from head-only stunning. Successful stunning relies on a combination of voltage, current, waveform and duration, which also partly determines the duration of unconsciousness. Because of the large variation in current, voltage, duration, frequency and wavelength, the duration and depth of the stun is variable, and it is hard to establish a general maximum stun to stick interval. Recommendations are for a maximum interval of 15 sec, reduced to 6 sec when high frequency low voltage stunning is used (EFSA, 2004).

While a minimum of 1.3 A and at least 240V for 3 sec is recommended, voltage, amps and duration can be quite variable and should be adjusted for weight and size of the pig as well as properties of the skull, skin and hair (EFSA, 2004). Large pigs weighing more than 150 kg live weight should be stunned with a minimum current of 1.8 to 2.0 amps (based on a frequency of 50 to 100 Hz; Stocchi et al., 2014). When low voltage is used it is recommended that stunning is followed by a timely (within 15 sec; Anil and McKinstry, 1998) and effective stick to ensure the animal remains stunned prior to death due to blood loss. A longer duration stun of up to 4 seconds results in a greater stunning depth and a longer duration of unconsciousness. A stunning duration of up to 10 seconds results in extended muscular immobilization post-stun. However, these long stunning times are a risk to animal welfare as they will reduce the possibility of identifying an inadequate stun because of the paralysing effect on the muscles (EFSA, 2013).

4.2.2. Head to body/heart

Head to body electric stunning may be used in high throughput abattoirs and with this method electrodes are placed automatically. In a one-cycle system a pair of electrodes is automatically placed just below the ears, while a third electrode is simultaneously placed on the sternum. In a two-cycle system the third electrode may be placed on the sternum, shoulder or back as soon as possible and preferably within 0.7 sec, but may be up to 15 sec, after the head electrodes. The third electrode results in cardiac ventricular fibrillation, and often in death. To induce cardiac ventricular fibrillation a current of 1-3 sec of at least 1.0A to 1.3 A at a maximum frequency of 50Hz AC (sine wave) must be applied (Wallgren et al, 2021). There is a risk to animal welfare if the first cycle (head only) has not been properly applied and the animal is still conscious during the application of the second cycle (EFSA, 2020). Care should be taken with the correct placement of electrodes to ensure current flows through both the brain and the heart and that electrodes are cleaned regularly (HSA, 2016). Incorrect placement of electrodes can result in pain and fear during stunning (Grandin, 2013). Automatic systems ensure correct placement of electrodes and often control for the application of the correct current (HSA, 2016).

4.2.3. High Frequency Electrical Stunning

High Frequency Electrical Stunning was developed as an alternative for Halal electrical stunning. As such this technology has not been used for pigs. In the USA, high frequency pulsed DC current is the most commonly used electrical stunning system for poultry (Sabow et al, 2017). Frequencies can be as high as 1000-2000Hz. It can both be used as 'head only' or 'head to body' without killing the animal and has been used for poultry, cattle, sheep and goats. While it induces seizures, it does not cause muscle contractions (Sazili et al, 2023). The high frequency causes spinal inhibition of the seizure movement without ventricular fibrillation (Simmons and Daly, 2004). This also results in less carcass damage and better meat quality (Sabow et al, 2017). In poultry, a frequency of 650 Hz was most efficient to cause unconscious and to promote greater meat quality (Siqueira et al, 2017). An early study in pigs however found that High Frequency Electrical Stunning resulted in shorter duration of unconsciousness compared to conventional electrical stunning at 50Hz (Anil and McKinstry, 1992). However, a two-cycle system with a high frequency (800Hz) head stun followed by a 100Hz heart stun may have advantages over a traditional 50Hz stun in reducing the number of re-stuns and reducing the occurrence of PSE meat in pigs (May et al, 2022).

4.3. Efficiency of stunning and return to consciousness

There are several studies that have compared the effectiveness of electrical stunning methods in commercial settings. However, they may be hard to interpret as each abattoir appears to have different methods and settings to apply electric current. Reports on the percentage of pigs that are incorrectly stunned can also vary wildly and sometimes high backup stunning rates (re-stun) have been reported for electrically stunned pigs under commercial conditions. For example, McKinstry and Anil (2004) reported on a survey in which 15.6% of pigs stunned using head-only electrical stunning were subjected to electrical re-stunning, although not all of those were because of an insufficient first stun.

A study of four abattoirs in Hungary conducted by Vegh et al (2010) on a limited number of pigs that were stunned using head-only electrical stunning found that 88.3% were effectively stunned. While effectiveness was related to current, it was important that the minimum level of current (1.3A) was achieved within the first second of application. Recommendations in minimum current should therefore be updated to include more detail of the application of current under commercial conditions (Vegh et al, 2010).

In some abattoirs stun to stick intervals may be greater than 15 sec, which may be a problem when using head-only stunning. In a small study in the US, 89 pigs in a small abattoir were subjected to either head-only stunning for 3 sec or head-only stunning followed by an application of the same wand to the chest for 3 sec to induce cardiac arrest (Vogel et al, 2011). Stun to stick intervals were around 32 sec, which resulted in a high percentage of pigs showing signs of consciousness (40.8% natural blinking and 32.7% eye tracking) when head-only stunning was used. These responses were all eliminated when the head-heart stunning method was used, after which a detectable heartbeat in all pigs was also eliminated (implying death).

Similar differences between head only and head/heart stunning methods were found in other studies. For example, Nodari et al (2014) observed pigs in 4 abattoirs in Italy and found that the only abattoir that used head to heart automatic stunning was more efficient than manual head-only stunning in 3 other abattoirs. Even so, the percentage of pigs observed as definitely unconscious was high across all abattoirs, ranging from 97.5% for the lowest head-only stunning to 99.8% for head-heart stunning. During the observations all electrodes were positioned correctly and the stun to stick interval was always between 4-8 sec.

Dalmau et al (2016) reported on observations post-stunning on 8100 pigs in 42 abattoirs (of which 23 used electrical stunning) in 5 countries (Portugal, Italy, Finland, Brazil and Spain). They found a mean of 18.2% of pigs return to rhythmic breathing for pigs subjected to electrical stunning. However, this did include one abattoir that used head-only stunning with a very long stun to stick interval, where 90% of pigs showed signs of return to consciousness.

Von Wenzlawowicz et al. (2012) conducted a total of 63 assessments of electrical stunning in abattoirs in Germany, Austria and Switzerland. Observations including 6855 pigs subjected to electrical stunning in different systems, included pen, restraining box and automatic systems. Across all systems the head/heart stunning was found to be more effective than head only stunning and effectiveness of automatic systems were more efficient than confined manual systems. Nevertheless, stun failures in automatic systems were still 3.3%, and 1.9% of pigs were observed to be awake. A wide variety in failure rates was found between different abattoirs and stunning efficiency appears to be related to several factors, including those relating to technology (system, settings, duration, maintenance), the knowledge and skills of the staff (including placement) and the conditions of the animals (Von Wenzlawowick et al, 2012; EFSA AHAW Panel et al., 2020a).
Best practice electrical stunning involving both the head and heart results in signs of recovery in 0.5% of pigs using a fully automatic system, for 1% of pigs using a semi-automatically, and for 2% of pigs using a manual stunning system (BSI Schwarzenbek, 2013).

The conditions, including breed, was found to have an effect on stunning efficiency, with backup stunning rates from head/heart stunning ranging from 1.59 to 5.01% in the five genetic lines (May et al, 2022). While placement of electrodes affected stunning failures (4.29% when electrodes for the head-cycle were positioned behind the ears compared to 3.16% when positioned in front of the ears), pigs from organic systems were also found to have a higher failure rate of stunning. It was speculated that this was mainly due to a higher fat percentage and a dirty coat. When comparing different settings across systems they recommended a reduced current, high frequency and a short duration of the head-cycle to reduce backup stunning rates and reduce the risk for PSE meat.

In summary, the main welfare concerns for electrical stunning are the isolation from the group when moved in single file and restraint necessary to apply the electric current. Failure of an effective stun may be caused by incorrect placement of electrodes (including poor contact), inappropriate electrical parameters and lack of maintenance of the equipment (including cleaning of electrodes). In addition, the short duration of unconsciousness after head-only stunning may result in animals regaining consciousness prior to death due to blood loss. However, automatic systems that apply head/heart electrodes appear to be very efficient and provide long durations of unconsciousness, if not instant death, and therefore are much less risk to animal welfare.

5. Atmospheric gas stunning (Controlled atmosphere)

There are a few advantages in using gas to stun pigs prior to slaughter. In particular, handling and stunning in small groups, rather than individually, reduces pre-slaughter stress (Mota-Rojas et al, 2012; Steiner et al. 2019; see section 3.6). While CO_2 is the most commonly (and currently the only) gas used in commercial conditions, there has been extensive research in alternative gasses due to the aversive nature of CO_2 gas. In gas stunning systems pigs enter a gondola and are lowered into high concentrations of CO_2 gas. There are two main systems, a dip-lift with a single gondola and a paternoster system with up to 7 gondolas that rotate through the CO_2 filled pit (Geritzen et al, 2021). In dip-lift systems the gondola descends to the bottom of a pit that is generally 2-4m deep, where it will stay for the duration of the exposure time. Paternoster systems are normally bigger with a pit that can be between 3-8m deep and gondolas pause at different levels in the pit that will have a gradient of CO_2 concentrations. In addition, pigs can be loaded single file in one end of the gondola, which necessitates individual handling, or as a group on the side of the gondola (backloader system). Back loader systems are often fitted with hydraulic handling systems, which has the advantage that it reduces stress associated with handling. The number of pigs per group, the time taken to reach maximum CO_2 concentrations, and total exposure times, vary.

5.1. Carbon Dioxide (CO_2) stunning

CO_2 stunning for pigs is the most common stunning method across the European Union, North America, Asia, Canada and Australia. CO_2 is a colourless, odourless gas, is slightly acidic and heavier than air, and therefore easy to contain in a pit. Pigs can be lowered in a pit that is prefilled with CO_2 gas at a high concentration resulting in loss of consciousness and insensibility. However, unconsciousness is not immediate and inhalation of high concentrations of CO_2 (>30%) is considered aversive. The highest concentrations of CO_2 (up to 95%) are the most aversive, but also induce unconsciousness quicker (Velarde et al, 2007).

CO_2 inhalation at concentrations above 30% causes irritation of the nasal mucosal membranes and lungs, where there are CO_2 -sensitive chemoreceptors. In addition, CO_2 is absorbed by the blood in the lungs and causes hypercapnic hypoxia (too much CO_2 in conjunction with lack of oxygen) and acidification of the blood and cerebrospinal fluid (Rodríguez et al, 2008). These changes depress brain activity resulting in unconsciousness and eventually death (Velarde et al, 2000a). Additionally, when chemical receptors in the brain detect the drop in pH caused by the increase in carbon dioxide it results in respiratory stimulation and can induce hyperventilation and a sense of breathlessness prior to loss of consciousness (Gregory et al 1990; Raj & Gregory, 1995). Breathlessness consists of chest tightness, increased respiratory effort and 'air hunger', which is a condition where the increased respiration rate is insufficient to provide the required ventilation (Parshall et al, 2012). In high concentrations of CO_2 air hunger will occur, which is considered unpleasant and a concern for animal welfare (Beausoleil and Mellor, 2015).

Several studies have shown behavioural aversion by pigs when exposed to CO_2 gas, however, there is some variation between studies. There is also large individual variation in reaction to CO_2 exposure, from no observable reaction to violent attempts to escape and vocalisations (EFSA, 2020). The conditions under which aversion is tested should also be considered. Some of the responses to CO_2 may be exacerbated by other stressors associated with lairage and slaughter, as reactions seen under low stress experimental conditions are often reported as milder than those seen under commercial conditions (Verhoeven et al, 2016). On the other hand, high stress under commercial conditions may increase respiration rate, with may reduce the time to loss of consciousness compared to experimental conditions. Exposure to CO_2 under experimental conditions may differ as the gradient of CO_2 in large commercial systems is generally different to those of experimental conditions. Under commercial conditions concentrations of CO_2 are much lower near the top of the pit with the highest concentrations only at the bottom (Dalmau et al, 2010).

There is also a variation in the way aversion is measured, ranging from preference testing, escape behaviours, vocalisations, and physiological indicators during voluntary and forced exposures (Steiner et al, 2019). Observing the behavioural responses of pigs exposed to CO_2 may provide some indication on the level of aversiveness of CO_2. When submersed in CO_2 gas, pigs may raise their head, sniff and sneeze and shake their head as a first response. Generally, pigs show several behavioural signs of aversion when exposed to CO_2 and escape attempts and vocalisations are some of the more violent responses that can be observed in some pigs. While gasping is often observed, this behaviour can be seen in both conscious pigs and after loss of posture and is therefore a little harder to interpret as a sign of aversion. Velarde et al (2007) reporting gasping in pigs prior to loss of posture when exposed to either 70 or 90% CO_2 but reported a stronger aversion to the higher concentration. Rodriguez et al (2008) observed gasping after pigs lost posture after 26s of exposure to 90% CO_2 but argued that at this time pigs were conscious, as this was prior to depression of brain activity based on middle latency auditory evoked potentials. Verhoeven et al (2016) also reported gasping prior to loss of consciousness based on EEG measurements. In an observational study of five commercial abattoirs in Australia gasping was observed prior to loss of posture ranging from 63-82% of pigs, with little variation between abattoirs and was considered a sign of onset of breathlessness (Jongman et al, 2021). However, gasping was also reported beyond isoelectric EEG, and occur after loss of consciousness (Rault et al., 2020), indicating a reflex rather than an active behaviour.

While there is consensus that CO_2 is aversive in high concentrations, there are some conflicting studies on the degree of aversion and the effect of concentration. For example, Raj and Gregory (1995) found that most pigs found 30% of CO_2 only mildly aversive and continued entry into a chamber to obtain a food reward. However, few pigs entered the chamber when the concentration was increased to 90%, although 12% still entered. There appeared some effect of breed, with Duroc pigs being more reluctant than Large White pigs, although individual temperament may have had a greater effect. In an experimental setting, all pigs exposed to 70% or 90% CO_2 were observed to gasp when exposed to CO_2, but a greater percentage of pigs attempted to escape from 90% CO_2 compared to 70% (Velarde et al, 2007).

While it is generally assumed that CO_2 is aversive from concentrations above 30%, a behavioural study by KC et al (2016) found that weaned pigs showed equal aversion to 10, 20 and 30% CO_2. However, this aversion was not strong enough to cause avoidance when a food reward was offered, even if long exposure led to loss of posture. Single exposures to 60% and 90% CO_2 in a learning task led to pigs willingly re-enter the empty chamber after exposure, although with some hesitation (Jongman et al, 2000). When this was compared against an electric shock with a prodder, it appeared that the latter was much more aversive than CO_2 exposure (Jongman et al, 2000), although the quality of the stimuli are quite different and may evoke different emotions. On the other hand, repeated exposure to 70 and 90% CO_2 in a similar learning task led to increased reluctance to re-enter after multiple exposures (Velarde et al, 2007).

5.1.2. Time to unconsciousness

Animals may experience distress from exposure to CO_2 from the first behavioural sign of aversion until loss of consciousness. Therefore, it is important to assess when animals lose consciousness, and therefore awareness, to determine the impact on animal welfare. However, this state is not easy to establish, and different studies have used different behavioural indicators to assume that the animal either was less aware or lost consciousness. While EEG may be the most accurate assessment of the inability of perceiving an aversive stimulus, animals may lose actual awareness before they lose the absolute capability of awareness (Verhoeven et al, 2016). Other variables may be more easily observed, although it is not always clear how they relate to unconsciousness. Loss of posture is most commonly used as a proxy for loss of consciousness, even though animals may still have some awareness. Loss of the righting response, loss of responses to external stimuli and loss of movement have also been used (Steiner et al, 2019) and the first two are often used to check for return to consciousness after stunning. Loss of movement is a less reliable indicator and may be a response based on spinal or cranial nerve reflexes without the animal being conscious (Dunnet et al, 1991).

The time to loss of consciousness does not only depend on how it is measured but also on the CO_2 concentration and the speed of descend into the pit filled with CO_2, where the highest concentration is at the bottom (Dalmau et al, 2010). Under experimental conditions animals may be exposed to CO_2 in a small pre-filled chamber, which would also affect the time to consciousness. The time to loss of posture in a pit filled with CO_2 at 40, 50, 60, 70, 80 and 90% was found to be 38, 34, 25, 17, 22 and 15 seconds after the start of exposure respectively (Raj and Gregory, 1996). These latencies were very similar to a laboratory study, where pigs showed a lack of brain activity as measured by EEG after exposure to 80 and 90% CO_2 at 21 and 15 seconds (Martoft et al, 2002). Loss of posture is expected to occur prior to lack of brain activity, however in a laboratory setting pigs are exposed to high concentrations of CO_2 immediately, while pigs lowered in a pit will be gradually exposed to increasing concentrations of CO_2. Therefore, comparisons between studies on exact times of loss of awareness is confounded by several factors and therefore not straight forward. A study by Verhoeven et al (2016) found that loss of posture was generally observed about 10s before loss of consciousness, based on

EEG measures. They compared 80% CO_2 to 95% CO_2 and onset of expression of aversion such as sniffing, retreat attempts and gasping were observed earlier in the higher concentration, but the interval between the onset of these behaviours and loss of consciousness was similar for both concentrations. Loss of posture was observed about 23s after the commencement of descent in 95% CO_2 and 37s in 80% CO_2. What is somewhat concerning is that most pigs (88-94%) showed muscle excitations before loss of consciousness and on average even before loss of posture. During observations in commercial abattoirs convulsions were noted in 60-70% of pigs after loss of posture and not before (Jongman et al, 2021). Loss of posture was on average 22.5s after the onset of exposure and it is unclear how much awareness these pigs had at the time of these convulsions.

Rodrigez et al (2008) also observed loss of posture, convulsions, and muscle excitation prior to loss of EEG and within 26s of exposure to 90% CO_2. In a learning task where pigs were repeatedly exposed to high concentrations of CO_2, Dalmau et al (2010) found that pigs that had high intensity muscle excitations on a previous exposure were more reluctant to re-enter the gondola than pigs who had not shown this muscle activity. They observed muscular excitations before they considered them unconscious and speculated that these may be painful causing reluctance to re-enter the gondola. Therefore, muscle excitations within 10s of loss of posture may add to the welfare concerns of CO_2 exposure.

Vocalisations are often reported when exposed to CO_2 and there is some debate on whether these are voluntary or unvoluntary after loss of consciousness. The types of vocalisations and the timing in relation to loss of posture may be the most important indicators, as vocalisations appear to occur both before and after the onset of unconsciousness.

Loss of posture varies with concentration and a range of times from first exposure is reported in the literature. For example, 80% CO_2 was reported to cause loss of posture ranging from 22.4 (Velarde et al, 2007) to 37s (Verhoeven et al, 2016) while concentrations of 90-95% caused loss of posture ranging from 17s (Raj, 1999) to 23s (Verhoeven et al, 2016). Generally higher concentrations cause earlier loss of posture, although much depends on individual conditions.

The larger CO_2 stunning systems aim for concentrations greater than 90% as there appears to be less physical stress compared to lower concentrations, as indicated by less pronounced metabolic acidosis (Mota-Rojas et al., 2012). Due to the gradient in CO_2 concentrations, with the highest concentration at the bottom of the pit, the time of exposure to maximum concentrations should be measured from when the gondola reaches the bottom of the pit (EFSA 2004). CO_2 concentrations at 90% in a pit were found to not be very stable under commercial conditions of gondolas being in motion. CO_2 is heavier than air and will concentrate at the bottom of the pit, but the descending gondola will add atmospheric air, which consist of 79% CO_2 and 20% O2, reducing the concentration of CO_2 (Dalmau et al, 2010). This effect may be less pronounced with a deeper pit and very high concentrations of CO_2 (>95%) and may explain some of the problems that can be seen with dip-lift systems and a shallower pit.

5.1.3. Variation in responses to CO_2

There is a large variation in responses to CO_2 exposure, which may be related to age, breed, environmental conditions and the large variation in stressors and other conditions prior to slaughter.

For example, variation in responses to CO_2 was partly explained by genotype, with halothane positive (Nn) pigs showing stronger aversion to CO_2 than halothane negative (NN) pigs (Velarde et al, 2007). However, halothane positive pigs lost posture sooner than halothane negative pigs, which may indicate that the total duration of being exposed to the aversive stimulus was reduced. The shorter duration to loss of consciousness may be explained by an increase in respiration rate due to the higher susceptibility to stress of Nn pigs. Although commercial breeds of pigs in Australia are mostly halothane negative (Channon et al, 2017), some halothane positive pigs may still exist in some of the genetic lines used in free range and boutique farms (Wait and Bryant, 2023).

Age may also affect the response to CO_2 as it was found that on average sows lost posture 11s later than slaughter weight pigs after exposure to 88% CO_2 in a dip-lift (Lechner et al, 2021). Slaughter-weight pigs lost posture about 18s after the first indication of aversive behaviour, but it took another 18s for the head to fully relax. High temperatures and high humidity can also increase the duration until loss of posture (Lechner et al, 2021). It was found that an increase of 10% in humidity may increase the time to loss of posture by 2.7s. The effect of temperature is less clear and higher temperatures above a certain threshold may increase time to loss of posture (by 1.9s with an increase of 1°C at 1 m), while temperatures below 17°C may also or increase the time to loss of consciousness (Lechner et al, 2021).

Another source of variation is found between different abattoirs, which may be a culmination of different factors such as design of the handling and stunning system, animal handling during lairage and stunning, concentration of CO_2 and many of the different factors on farm, during transport and during lairage (Jongman et al 2021). Observations across five Australian abattoirs (Jongman et al, 2021) found that escape attempts during CO_2 exposure ranged from 0.6% to 46.2%. Although many variables within each abattoir were measured, ranging from behaviour in lairage, handling by stock people and CO_2 concentrations, no single factor had a major effect on this behaviour during exposure to CO_2. However, considering that at one of the abattoirs very few escape attempts were observed, it appears that it may be possible to reduce some of the more violent responses to CO_2 seen in other abattoirs.

5.1.4. Efficiency and Stun to stick interval

Paternoster systems provide long exposure times and usually higher concentrations of CO_2 at the bottom of the pit. This ensures high stunning efficiency and causes little problems when longer stun to stick intervals are needed. When exposure times are short (90s) it is important that pigs are stuck immediately and certainly within 60s, even with concentrations as high as 95% (Bolaños-López et al, 2014). Increased exposure times to 120s are more effective than 90s in abolishing corneal reflexes (Hartmann et al, 2009). In a study by Nowak et al (2007). Exposure to 90% CO_2 for 100s enabled a stun to stick interval of 40-50s, which was reduced to 25-35s when pigs were exposed to 80% CO_2 for the same duration. Long exposures of 3-5 minutes to 90% CO_2 results in the death of most pigs and much longer stun to stick intervals are possible (Llonch et al, 2013).

Atkinson et al (2012) visited eight abattoirs in Sweden and found no stun quality issues with paternoster systems despite 65% of pigs with stun to stick intervals between 70 and 100s. In contrast, abattoirs using dip-lift systems showed problems with the stunning efficiency in 1.7 to 3.3% of pigs in abattoirs with those systems and stun to stick intervals exceeded 60s for 25% of pigs. There was a significant correlation between exposure times, concentration, and stun efficiency.

In summary, exposure to CO_2 gas is aversive to pigs until the time of unconsciousness, which on average is after about 22s (loss of posture) to 32s (loss of consciousness). The advantage of CO_2 stunning (and any other gas stunning system) is that animals can be moved in groups using low stress handling methods, although some small systems use single file entry. In paternoster systems with CO_2 concentrations above 90% pigs reliably become unconscious and are unlikely to regain consciousness after stunning, even with long stun to stick intervals.

5.2. Other gasses and mixtures

Despite the animal welfare advantages of gas stunning in groups, there have long been concerns about the use of CO_2 gas stunning considering the aversive responses. This has inspired research into alternative gasses for well over 25 years, but no alternative gas is yet available commercially (Sindhoj et al, 2021). There are some gasses and gas mixes that may have the potential to be used commercially providing some major adjustments are made to the design of the handling and stunning systems and very short stun to stick intervals are incorporated. It is unlikely that CO_2 can simply be replaced by another gas in the existing systems without affecting the throughput of pigs/h.

During gas stunning pigs lose consciousness mainly due to hypoxia (lack of oxygen). This can either be achieved by the gas itself or by excluding oxygen from the atmosphere. Inert gasses would appear to be ideal alternatives as they do not have an odour or taste and do not cause irritation of the airways (Raj and Gregory, 1995). Pigs need to be exposed to a concentration of inert gas that excludes oxygen to less than 2% by volume to cause hypoxia. However, this means that once exposed to normal air pigs regain consciousness quickly, unless the exposure time is long enough to kill all pigs. If pigs are not killed it will result in very short stun to stick intervals and the possibility of pigs regaining consciousness after sticking and prior to death from blood loss. It may be a challenge to create an atmosphere of reliable concentrations of O_2 less than 2% in a commercial setting, with pigs moving in and out of the gas-filled area. Anoxic stunning results commonly in more intensive convulsions compared to CO_2, which may potentially impact meat quality (Llonch et al, 2012).

Some inert gasses are heavier than air and can be used by filling a pit, similar to CO_2. Other gasses are lighter than air and are therefore harder to handle. When selecting a gas that can be used to fill a pit it is important to establish that the gas or gas mixture can be maintained at a concentration that displaces air so oxygen levels are below 2% and that the gas is stable and uniform throughout the different levels of the pit (Dalmau et al, 2010).

Inert gasses include all noble gasses as well as N_2 and CO_2. While all inert gasses could be considered, only easily available gasses can realistically be used, and preferably those that are heavier than air. Mixtures of gasses may improve some of their properties and are also considered. Xenon, Krypton and Argon are inert gases with anaesthetic properties, which makes them particularly attractive. Xenon is an anaesthetic gas under normobaric conditions, however Argon and Krypton have anaesthetic properties only under hyperbaric conditions (Raj, 1999). Since Krypton is extremely rare it can be excluded as an alternative for commercial stunning on cost alone. Research has been conducted on a range of gasses and some mixes that may be considered potential alternatives.

5.2.1. *Argon*

Argon is the most studied alternative gas for stunning. Argon is also heavier than air and therefore would be more practical to use in current stunning systems. When a pit was filled with Argon it was found that a level of O_2 of less than 2% was maintained at different levels inside the pit and was quite stable when animals entered the pit in a gondola. Argon was more uniform than CO_2, avoiding the gradual exposure common to CO_2 (Dalmau et al, 2010). This also means that animals are exposed to maximum levels of Argon (and therefore minimum levels of O2) from the moment they are lowered into the pit, which may reduce the duration of dwell time (as measured from time of entry) in Argon to lose consciousness. Nevertheless, it was found that time to loss of posture was about 50% longer in Argon (33.5s, Dalmau et al, 2010) compared to reported times to loss of posture for 90% CO_2 (22s). However, under experimental conditions loss of posture to 90% Argon was found to be 15s, which was similar to 80-90% CO_2 in the same setting (Raj, 1999).

There are conflicting results when the aversion of pigs to Argon exposure was measured. Raj and Gregory (1995) found that pigs readily accepted a food reward in a box filled with Argon almost until they lost consciousness. In contrast, 88% of pigs in the same learning task avoided 90% CO_2 and 25% of pigs avoided 30% CO_2. While Argon is found to be less aversive than CO_2, other studies have found that pigs still show some aversion to Argon exposure. Dalmau et al, (2010) conducted a learning trial in which pigs exposed to 90% Argon showed retreat and gasping, although less than pigs exposed to gas mixtures of CO_2 and 15-30% CO_2. The time to loss of posture was an average of 33.5 on first exposure, which was slightly longer that for gas mixtures of N_2 /CO_2 (28.9-30.7s on average). While not shown by all pigs, onset of aversion behaviours to 90% Argon ranged from 6.0s (escape attempts), 10.1 s (retreat attempts) and 13.4s (gasping) prior to loss of posture, while gagging and muscle jerks were seen mostly after loss of posture.

Anoxia can be rapidly reversed when exposed to normal air. Exposure to 90% Argon for 3 minutes results in a maximum stun to stick interval of 25s to prevent pigs from showing signs of return to consciousness (EFSA, 2014), which was observed after 50s (Raj, 1999). Even after exposure to 90% Agon for 7 minutes, most (93%) but not all pigs died (Raj, 1999) and 7% showed some signs of return to consciousness after 50s. Raj (1999) suggested a combined stunning method, where pigs that were initially stunned with Argon were subsequently exposed to an electric current across the chest, resulting in cardiac arrest and death.

5.2.2. *Argon mixed with CO_2*

Argon causes hypoxia, which can be quickly reversed when exposed to atmospheric air. In contrast CO_2 causes hypercapnic hypoxia, which has some anaesthetic properties and reverses more slowly. Since CO_2 is only mildly aversive in concentrations below 30%, a mix of Argons and CO_2 would preserve the advantage of Argon (less aversive), while overcoming some of the disadvantages. CO_2 and Argon have similar densities and therefore are stable when mixed and are more uniform than CO_2 alone (Dalmau et al, 2010).

When 30% CO_2 was mixed with 60% Argon, it produced improved depth of unconsciousness compared to 90% Argon. Nevertheless, about 10% of pigs submerged in the CO_2 /Argon mixture for 5 minutes still showed some signs of insufficient stunning but were completely unconscious after 7 minutes of being submerged (Raj, 1999). This was an improvement compared to Argon alone, but significantly longer than for 90% CO_2. An alternative mix of 60% Argon and 40% CO_2 was found to cause considerable stress in pre-weaned piglets prior to loss of consciousness (Kells et al, 2018) and is not considered a humane alternative for the use of CO_2 alone.

Although Argon is the most common noble gas in the atmosphere it has limited availability and therefore increased cost compared to CO_2. Considering the cost of industrial grade and scarcity of Argon, it is not considered a viable alternative for commercial stunning, and little research into the use of Argon for stunning has been conducted in the last 15 years. Unless a technical solution can be found to recycle Argon as it is being used, this is not likely to change.

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In summary, while Argon has been one of the most researched alternative gas to CO_2 for more than 25 years, it has not resulted in a commercial alternative. Argon is less aversive than CO_2, however some aversive responses are still observed. The main problem with using Argon to stun pigs is the short time to regain consciousness after stunning, which is a risk to animal welfare. A mixture of Argon and CO_2 at less than 30% may increase the duration of unconsciousness but is more aversive than Argon alone. The expense of Argon compared to CO_2 is also an obstacle to commercial adoption and further research in recycling used Argon would be needed if Argon stunning is considered.

5.2.3. Helium

Helium is an inert gas that has anaesthetic properties and therefore seems a humane alternative to CO_2 stunning. There has only been a single study under experimental conditions that evaluated the response to Helium exposure in pigs. Helium is lighter than air and a 98.5% mixture is needed to induce hypoxia. No behavioural signs of aversion were observed in pigs exposed to Helium, and adrenalin and noradrenalin concentrations were lower than for 90% CO_2. Loss of posture occurred 20s after exposure compared to 16s for 90% CO_2 in the same setting (Machtolf et al, 2013). Exposure for 3 minutes resulted reliably in unconsciousness, however the stun to stick interval was limited to 15-30s. The expense of Helium and the short stick to stun interval for group stunning may preclude Helium as an alternative for commercial stunning. However, longer exposure times may be result in death, similar to Argon. Since Helium is lighter than air, containment may be difficult and would rely on a specifically designed chamber.

5.2.4. Xenon

Xenon is interesting as an alternative gas for stunning as it is the only 'inert' gas which is an anaesthetic under normobaric conditions (Kennedy et al, 1992). It can be considered an ideal anaesthetic for human medicine however the high cost of the gas has prevented its use in for anaesthesia (Baumert, 2009). Therefore, it is unlikely to be considered as a commercial alternative for CO_2 stunning of pigs.

5.2.5. N_2 and N_2/CO_2 mixture

Nitrogen is widely available as it is present in high concentrations (79%) in atmospheric air and may potentially be a more economical alternative to Argon as it is comparable in cost to CO_2. However, N_2 is slightly lighter than air and is therefore hard to contain in a pit. The lowest level of O_2 that can be achieved in a pit filled with N_2 is 6% O_2 (Dalmau et al, 2010), which is insufficient to induce unconsciousness in pigs. As inert gasses rely on residual concentrations of O_2 below 2%, N_2 is not suitable as a single gas contained in a pit in current commercial stunning systems. However, N_2 may potentially be mixed with low concentrations of CO_2 as a more humane alternative to high concentrations of CO_2.

When N_2 and CO_2 are mixed in a pit, CO_2 tends to sink to the bottom and N_2 to rise, as N_2 is lighter than CO_2. This can result in uneven mixes, with a rise of CO_2, but also O_2 at the bottom of the pit. However, providing CO_2 concentrations are kept below 30% this separation can be kept to a minimum and has been shown to have good stability and uniformity. In fact, the uniformity of this mixture is greater than that of 90% CO_2, which typically results in a gradient, with the highest concentration at the bottom of the pit (Dalmau et al, 2010).

A mixture of these two gasses, with CO_2 kept at a maximum of 30% would not only reduce aversion compared to 90% CO_2, but also leads to a reduced time to unconsciousness due to the combined effect of hypercapnia and hypoxia, compared to hypoxia (Llonch et al, 2013). On the other hand, submersion of slaughter weight pigs in N_2/CO_2 mixtures with 30, 20 and 15% CO_2, although less aversive than 90% CO_2, took longer to cause loss of consciousness and was less effective in causing death (Llonch et al, 2013). While all pigs submersed in 90% CO_2 for 3 minutes had died (and 75% died after 2 minutes), none of the pigs submerged in the N_2/CO_2 gas mixtures for 3 minutes were dead and even after 5 minutes only 70% of pigs had died. After stunning brain activity returned much faster for those pigs that had been submerged in the 15% N_2/CO_2 mixture and this concentration would be considered unsuitable for commercial application due to the short resulting stun to stick interval (Llonch et al, 2013). Compared to 90% CO_2, a N_2/CO_2 gas mixture may also negatively affect meat quality. The gas mixture was found to increase pH at 45 minutes and result in more exudative meat with reducing concentrations of CO_2 (Llonch et al, 2012).

In summary, while N_2/CO_2 gas mixtures are cheap and less aversive than high concentrations of CO_2 alone, the time to unconsciousness and therefore the total exposure time to an aversive stimulus increases. Pigs return to consciousness sooner than when submerged in 90% CO_2, even after much longer dwell time in the gas mixture. In addition, there are some negative effects on meat quality of N_2/CO_2 gas mixtures compared to 90% CO_2. Therefore N_2/CO_2 gas mixtures would have limitations as an alternative gas to high concentrations of CO_2 alone, but may be the most appropriate gas to consider as an alternative to CO_2 gas.

5.2.6. N_2 in foam

While N_2 has potential as an alternative gas for CO_2 stunning, the challenge is to maintain low oxygen levels, as N_2 is slightly lighter than air. One potential method to quickly eliminate oxygen and maintain an anoxic environment may be by using high expansion foam filled with N_2. A study in poultry has shown that N_2 high expansion foam was effective in poultry while few aversive responses were observed (McKeegan et al, 2013).

A single trial was conducted with 30kg pigs in an enclosed box fitted with a lid. Pigs were observed for their response to foam alone as well as N_2 filled foam. Pigs showed similar escape responses to both types of foam, particularly when their head became submerged (Lindahl et al, 2020). Heart rate was also increased as the foam level rose. From the start of foam production until loss of posture took 57s on average. Loss of posture was immediately followed by convulsions and vocalisations. The pigs stayed submerged for 5 minutes, which resulted in the death of half the pigs while the remaining pigs were assessed as in a deep state of consciousness. Further research is needed to test the interval to return to consciousness to provide indicative stun to stick intervals. However, it appears that the foam itself is cause of stress and therefore may not be suitable as an alternative to high levels of CO_2. An additional challenge is that in a commercial setting foam generators need to be able to produce foam quickly to submerge slaughter-weight pigs (Lindahl et al, 2020).

5.2.7. *Nitrous oxide (N_2O)*

N_2O (Laughing gas) was found to be less aversive to piglets than other gasses containing CO_2, N_2 or Argon. Concentrations of 90% N_2O were found to be least aversive and produced isoelectric EEG within 71s, compared to 59s for 90% CO_2 (Rault et al, 2015). While N_2O appears to be somewhat less aversive than high concentrations of CO_2 for both piglets and weaned pigs, the behavioural reactions still indicate aversion of a similar magnitude (Çavuşoğlu et al, 2020; Smith et al, 2018).

Since N_2O is considered a potent greenhouse gas, this may limit the possibility to use it on a commercial scale for stunning of pigs. N_2O has not been tested on slaughter weight pigs, however results from younger pigs do not suggest that N_2O exposure would be considerably more humane than CO_2 exposure.

5.3. *Two-step systems*

One of the main disadvantages of gas stunning other than CO_2 is the quick return to consciousness, unless exposure is long enough to kill all pigs. It has been suggested that a two-step system may solve this issue by using a non-aversive gas to anaesthetise pigs, followed by exposure to a second gas or other method (such as electric shock to the heart; Raj, 1999) to cause long-term unconsciousness or kill pigs outright. Smith et al (2018) applied a two-step system with N_2O and CO_2 for euthanasia of piglets. They observed high levels aversive behaviour towards N_2O when piglets were euthanised in groups. Although these behaviours were observed less frequent compared to CO_2 exposure, the time to loss of posture was extended and therefore the total experience was considered stressful and not a humane alternative to CO_2 euthanasia. No research has been conducted on a two-step system for slaughter weight pigs and it would involve some major design challenges to develop a reliable and commercially viable system.

6. Alternative stunning methods

6.1. LAPS

When reducing atmospheric pressure, the available oxygen is also reduced. Therefore, Low Atmospheric Pressure Stunning (LAPS) causes loss of consciousness by inducing hypoxia. During LAPS animals enter a sealed chamber to which progressive decompression is applied (Holloway and Pritchard, 2017). The reduction in atmospheric pressure causes a proportional decrease in oxygen, inducing hypoxia. While only limited research has been conducted in pigs, more comprehensive research in poultry has concluded that it is a humane method comparable with CAS with inert gas for this species (Martin et al 2016; 2019). However, the avian respiratory system has a unique anatomical structure. Mammals have a diaphragm and extendable lungs whereas birds have no diaphragm, but have large interconnecting air sacs and their lungs are not expandable. This makes it less likely that air will be trapped in the lungs and abdominal cavity during LAPS of poultry (Vizzier-Thaxton et al, 2010). Therefore, results from studies on poultry cannot be directly applied to pigs and a slower rate of decompression may be required to allow air to escape and pressure to equalize.

Hypoxia induces euphoria and unconsciousness in people (Bouwsema and Lines, 2019), however it is unclear what the effects are of reducing ambient pressure (hypobaria). Research has been conducted on rats and chickens and there is some preliminary research on pigs, but there is still little known about the effect on the welfare of conscious slaughter-weight pigs if this method is applied for pre-slaughter stunning. Any method that results in hypoxia will result in air hunger, which has its own welfare concerns. Humans subjected to air hunger reported that they prefer pain over air hunger (Banzett & Moosavi 2001) as it causes a feeling of impending death (Lansing et al 2009). From an evolutionary point of view this makes sense because pain indicates tissue damage while hypoxia is a direct risk to life. However, humans subjected to altitude chamber training did not find exposure to hypobaric hypoxia unpleasant (Bouwsema and Lines, 2019).

In addition to air hunger, decompression may negatively affect animals exposed to LAPS. Changes in air compression in people can be felt during such activities as diving, mountaineering, and flying. The rate of change of decompression is important and is likely to have negative effects on the welfare of animals subjected to LAPS. It is well known how people climbing at high altitudes above 6,000m (at 47kPa) without oxygen become disoriented and are unaware of their condition and the lack of oxygen and the unset of unconsciousness (Gradwell, 2016). However, to reach similar levels of oxygen in the lungs to that achieved by 90% Argon (and 2% residual oxygen) pressure would need to be reduced to about 16.5kPa. In training sessions for airline personal, rapid decompression leads to few problems and are reported in less than 5% of subjects (Bouwsema and Lines, 2019).

However, there is some concern that hypobaric hypoxia may result in gas expansion in body cavities as a result of rapid decompression. The respiratory system, digestive system, sinuses of the skull, the teeth and the middle ear are all cavities containing gas that may cause pain during decompression. Data collected post slaughter indicates that pigs are particularly susceptible to lung lesions and respiratory problems (see Wallgren et al, 2021), which may also affect their response to hypoxia itself.

In LAPS trials with poultry and rats few to no signs of pain and distress have been observed (Mackie and McLeegan, 2016; Talling, 2017). Pressure dropped from 101 kPa (atmospheric air) to 33kPa over 67s before dropping further to 20kPa (after loss of consciousness) for chickens and from 101 to 20kPa in 30s for rats. In a trial of piglet euthanasia, pressure was dropped from 101 to 6.6kPa over a 10 min period. Vocalising was reported for 60% of the piglets, while more than 30% of piglets were observed gasping within 5 min of exposure (Buzzard, 2012). Nevertheless, this was favourable to exposure to CO_2 in the same trial, where all piglets were observed to vocalise and gasp when exposed to CO_2. However, time to death would have taken at least 13 min based on O_2 levels, although the authors reported convulsions up to 29 min after the start of LAPS. Either decompression was not as successful as thought or possibly young piglets are more resilient to low levels of oxygen, which has been reported for young piglets (Marahrens et al, 2017). While a trial by Edwards and Engle (2011) on LAPS noticed post-euthanasia lesions in 21% of piglets, they were most likely caused by very low pressure after loss of consciousness. On the other hand, a trial on 4kg and 22 kg pigs was aborted due to possible problems of gas-filled digestive tracks in pigs that were not fasted prior to euthanasia with LAPS (see Bouwsema and Lines, 2019).

So far, the results of only one trial on slaughter-weight pigs has been published and these pigs were under anaesthesia. Only pathological (Baxter et al, 2022) and physiological and reflex behaviours (Martin et al, 2022) of these pigs have been described. A total of 60 pigs were subjected to four decompression curves ranging from 40 to 100 ms−1 ascent equivalent, with two cycle durations of 480 and 720s. All of these compression rates resulted in the death of all pigs and faster decompression rates resulted in earlier cardiac arrest and death (Martin et al, 2022). Pathological changes after death showed a wide range of individual variation across all body regions. It is of concern that congestion and haemorrhage were observed in several body regions (Baxter et al, 2022), which may affect the welfare of conscious pigs if these changes had occurred before pigs lost unconsciousness. However, some of these changes may be hard to interpret as decompression necessarily needs to be followed by recompression to retrieve the carcasses, and it is not clear when the changes may have occurred. In humans, physiological symptoms and pathologies are mainly associated with recompression (when descending from altitude), rather than decompression (when ascending to altitude; Baxter et al, 2022) It is also not clear if active behaviours to equalize pressure, such as swallowing, chewing and stretching, that may be performed by conscious animals, would minimise some of the effects of decompression (Baxter et al, 2022). Overall, this study did not provide enough information to extrapolate to welfare outcomes of decompression in conscious pigs (Martin et al, 2022). In an earlier study by the same authors (McKeegan et al., 2020; not publicly available) on conscious pigs, it was found that the majority of pigs suffered from ruptured ear drums, haemorrhages, and congestions in the lungs. They concluded that LAPS was not a viable, humane alternative to CO_2 stunning. However, this study was not referred to in the later publications of the same authors (Baxter et al, 2022; Martin et al, 2022) and it is unclear if they still support this conclusion or whether perhaps these changes may have occurred after loss of consciousness. A future study conducted on conscious pigs may exclude the fastest decompression rates used by Baxter et al, 2022 and Martin et al, 2022, so animals are not exposed to unnecessary fast compression rates that may be very uncomfortable and compromise animal welfare.

Application of LAPS under commercial conditions would need to consider practical aspects and cost as well as animal welfare. Decompression rates would need to be as fast as possible without compromising animal welfare and needs to consider the rate at which trapped gas in body cavities can escape. Fasting of a sufficient time to empty the digestive track prior to stunning would minimise the risk of gas expansion in the gut and is already current practise for reason of hygiene. There would need to be special consideration for pigs suffering from respiratory disease, as this may also result in pain.

To achieve decompression to 16 kPa a safe rate of reduction of pressure based on human experience would take close to 6 minutes. Pigs may need to be kept at this pressure for 7 minutes to avoid recovery and ensure all pigs have dies. At least 2 minutes need to be added for loading, decompression and unloading so that a total killing cycle may take up to 15 minutes, although a minimum of 9 minutes may be possible (Bouwsema and Lines, 2019).

As described by Bouwsema and Lines (2019), this would translate to a proposed commercial system with stationary cylinders that contained groups of 15 pigs in a floor space of about 6.4m^2. A double story design would be more efficient, although handling and moving pigs would be a little more complicated. Special handling systems, based on but different from those used with CO_2 stunning, would need to be designed for such a LAPS system. An exposure time of 15 minutes would result in a line speed of about 120 pigs/hour. For high throughput processing plants multiple cylinders may need to be used. The proposed dwell time should result in the death of all pigs, so long stun to stick intervals are not a problem, as long as pigs can be bled out effectively. If such a system would be cost effective remains to be seen since many of the components are untested at this time.

In summary, while LAPS is considered a humane alternative for CO_2 stunning of poultry, there are still many questions on the effects on pigs. More research is required, particularly on conscious slaughter weight pigs.

6.2. Microwave stunning

Microwave stunning has been proposed as an alternative method for reversable halal stunning of cattle and has not been studied in pigs. Microwaves rapidly heat the brain resulting in hyperthermic syncope (fainting) leading to unconsciousness and insensibility (McLean et al, 2017). Preliminary studies in sheep showed that 20s of microwave energy could heat the brain to temperatures ranging from 43 to 48°C, enough to cause unconsciousness similar to extreme heat stress (Small et al, 2013). Depending on the energy that is being delivered, unconsciousness can be achieved in as little as 3s (Rault et al, 2014).

This technology is still experimental and at this stage may not reliably deliver energy levels that result in long-term unconsciousness. However, the aim of previous studies was to obtain reversable stunning and avoid brain damage, which would not be a consideration for pigs. Therefore, delivery for a longer duration or at a higher energy may produce a more reliable stun.

A disadvantage of this stunning method is that the head needs to be restraint during the application (Small et al, 2019). This level of restraint would be highly aversive to pigs and can not be considered a humane alternative for current stunning systems and holds no advantage over electrical stunning. However, potentially this method may be applied using a handheld device with only one contact point, as opposed to electrical stunning, so correct placement may be easier to achieve.

6.3. Single Pulse Ultra-High Current (SPUC)

Single Pulse Ultra-High Current (SPUC) is another potential alternative to head only (Halal) electrical stunning and has been studied in cattle but not pigs. SPUC involves a high voltage (5000V), 70A discharge spike that lasts 50ms. This results in sustained depolarization of central neurons, probably by physically disturbing nerve membranes. SPUC has only been tested in one study in cattle with some preliminary work in sheep (Robins et al, 2014). It does not result in grand mal seizures and convulsions, which also would have advantages for meat quality (Robins et al 2014). While SPUC resulted in increased times of unconsciousness compared to conventional electrical stunning, the duration of unconsciousness was variable, with reflex recovery between 15 and 62s. The reason for this variation was not clear and no further work on this technology has been published. As with microwave stunning, this method could potentially be applied with a handheld device and a single contact point, although consistent contact needs to be ensured. This would require firm restraint, which is in itself aversive to pigs (Sindhoj et al, 2021).

7. Discussion

The main aim of this literature review was to identify the impact of stunning methods on pig welfare. However, when considering stunning methods, it is important to recognise that there may be direct impacts on meat quality of the stunning method. For any stunning method to be applicable in a commercial setting, the practicality and economics need to also be considered. Below is a summary of the impact of stunning method on each of these considerations.

7.1. Welfare implications of stunning methods

The ideal stunning method induces instantaneous unconsciousness and/or is non-aversive. Stunning should cause unconsciousness that provides for a long stun to stick interval (or ideally death) and does so reliably (EFSA, 2020). It should also incorporate low stress handling prior to stunning and should not involve aversive restraint (Gerritzen et al, 2021). Unfortunately, none of the stunning methods discussed in this review provide those conditions.

Captive bolt devices cause instantaneous death when used appropriately and placed correctly (Wallgren et al, 2021). Due to the unique shape of the skull of pigs, which include large sinuses, captive bolt devices are not as effective as in most other species (EFSA, 2004). They also require restraint to facilitate correct placement, which in itself is aversive to pigs (Gerritzen et al, 2021).

Electrical 'head only' stunning may cause immediate unconsciousness, however there are several risks associated with this stunning method. To ensure sufficient current passes through the brain, correct placement of electrodes, good contact, sufficient exposure time and appropriate electrical settings are all critical. Regardless, even a correct stun should be followed by a stun to stick interval of a maximum of 15s, which is essential to ensure pigs remain unconscious until death is caused by loss of blood (EFSA, 2004). Electrical head/heart stunning with an automatic system appears a much more reliable stunning method, as it causes instantaneous unconsciousness and cardiac arrest and has a very high efficiency. Providing the system is adjusted to the size of the pig, placement of electrodes and the correct current are all controlled by the automatic system (Wallgren et al, 2021). In case of a two-cycle system, where the heart electrode is applied after head-only stunning, there is a risk that animals may be still conscious when the second electrode is applied (EFSA, 2020). Using higher frequencies when applying head electrodes may improve the stunning efficiency and reduce the incidence of PSE meat (May et al, 2022). However, all electrical stunning systems require animals to be handled individually in single file raceways and involve aversive restraint (Troeger, 1989).

Controlled atmosphere stunning with high concentrations of CO_2 is aversive to pigs, as it causes irritation of the nasal mucosal membranes, induces breathlessness and may directly cause stimulation of the amygdala associated with fear (AVMA, 2020). While higher concentrations are associated with increased aversion, lower concentrations increase the time until unconsciousness and therefore the duration of exposure (Velarde et al, 2007). High concentrations and long exposure duration result in long duration of unconsciousness and even death (Llonch et al, 2013). This ensures sufficient time for long stun to stick intervals when gondolas contain larger groups. While exposure to CO_2 is aversive, pigs are stunned in groups and are relatively unrestraint in gondolas, providing for better welfare than when restraint individually (Velarde et al, 2000). In addition, larger abattoirs move groups of pigs through laneways using hydraulic gates, which minimises pre-slaughter stress caused by handling (Christensen and Barton-Gade, 1997).

In order to retain the advantages of gas stunning but to avoid the aversiveness of CO_2 exposure, there has been a lot of research on alternative gasses. Most of the research focussed on inert gasses and Argon in particular, as it is heavier than air but appears to be less aversive to pigs than CO_2. However, pigs still show some aversion to Argon and take longer to become unconscious, while regaining consciousness quickly after leaving the Argon filled pit (Dalmau et al, 2010). This results in a very short stun to stick interval (Raj, 1999), which would be problematic for pigs stunned in larger groups. Similar issues were found with N_2 and N_2O, with none of these considered suitable alternatives for CO_2 stunning. Mixtures of Argon/CO_2 and N_2 /CO_2, where CO_2 is kept at or below 30% overcome some of the problems seen with pure Argon or N_2, however they are still somewhat aversive and require long exposure times to ensure pigs do not regain consciousness prior to death from blood loss. While Argon is expensive and therefore not commercially viable, a mixture of N_2 and 30% CO_2 is currently the most viable alternative gas of all alternative gasses considered in this review. It is comparable in cost to CO_2 alone, has good stability and uniformity (Dalmau et al, 2010) and is less aversive than 90% CO_2 (Llonch et al, 2013). However, using a N_2 /CO_2 mixture would require major changes in design of the stunning system, due to the longer exposure times that will be required (Llonch et al, 2013), which may be costly. In addition, meat quality after N_2 /CO_2 stunning may be reduced compared to 90% CO_2 (Llonch et al, 2012). Although a N_2 /CO_2 mixture is less aversive than 90% CO_2, the responses of pigs to this gas mixture may still not be considered acceptable.

The most promising alternative stunning system not using gas is LAPS, which is considered the most humane stunning system for poultry (EFSA, 2017). Such a system would have similar advantages as gas stunning systems, with pigs handled and stunned in groups. However, to date there has been little research in pigs, and no research on slaughter weight conscious pigs, to confirm this method as humane for pigs. Results from poultry cannot be extrapolated to pigs as the avian respiratory system has a unique anatomical structure which makes it less likely that air will be trapped in the lungs and abdominal cavity compared to pigs (Vizzier-Thaxton et al, 2010). In the only trial with conscious piglet, some expressions of distress, such as vocalisations and gasping, were observed (Buzzard, 2012). Research on slaughter-weight pigs under anaesthesia observed congestion and haemorrhage in several body regions (Baxter et al, 2022). It was not clear however if the observed lesions occurred during decompression or recompression or even before pigs would have lost consciousness (Baxter et al, 2022). Additional research needs to confirm the suitability of LAPS for pigs and technical developments are necessary to design a system that is commercially viable. There is also additional concern for pigs suffering from respiratory disease, which is quite common in commercial slaughter pigs (see Wallgren et al, 2021), which may result in additional pain during decompression.

Other alternative stunning methods such as microwave stunning and SPUC have been developed as alternative reversable stunning methods for halal slaughter of cattle (Small et al, 2013; Robins et al, 2014) and are similar in application as electrical stunning. They require individual handling and restraint (Small et al, 2019) and are therefore no more humane than electrical head/heart stunning methods. As they are not tested on pigs it is unclear if these methods may result in longer stun to stick intervals in pigs compared to head-only electrical stunning.

Every stunning method discussed in this review has some disadvantages for animal welfare, ranging from pre-slaughter handling, individual restraint and aversive stunning methods. None of the methods are instantaneous without fear or stress during the stunning procedure and without the possibility of regaining consciousness before death caused by blood loss. Both electrical stunning and CO_2 stunning, the only stunning methods for pigs currently used commercially in Australia, have clear advantages and disadvantages. The most promising humane alternatives of a N_2 /CO_2 gas mixture and LAPS are still considered somewhat aversive and will need further research to confirm their acceptability as a humane alternative. The increased duration of stunning time and the considerable investment in changing the current stunning systems may preclude the implementation of either alternative stunning system if only a small gain in animal welfare is to be expected. However, there appears to be scope to improve current stunning systems. Improved handling prior to electrical stunning (both by designing better handling systems and better training of stock people) reduces pre-slaughter stress and results in better welfare outcomes. The large variation in responses to CO_2 indicate that a reduction in aversive responses to CO_2 may be achieved through genetic selection and a reduction in pre-slaughter stressors and other on-farm factors (Jongman et al, 2021). Further research on the cause of individual variation and the reduction in pre-slaughter stress may be the quickest way to achieve improved animal welfare during stunning while potential alternative methods of stunning are being developed.

7.2. Meat quality implications of stunning methods

Different stunning methods may have different effects on muscle metabolism and other physiological parameters, which in turn affect meat quality. One of the commercial advantages of CO_2 stunning over electrical stunning is the improvement in meat quality (Marcon et al, 2019). Electrical stunning causes violent muscle contractions, which result in a faster decline of pH postmortem and may even cause bone fractures (Chanon et al, 2003). The higher current used for head/heart stunning may negatively affect meat quality more than when head-only stunning with a lower current is used (Channon et al, 2002). Not only current, but also voltage, frequency and duration can affect pH measures, and thus meat quality, through direct stimulation of muscle but also indirect stimulation of nervous pathways. For example, high voltage stunning can result in better meat quality and less incidence of PSE meat (Faucitano et al, 1998). However, while certain settings for electrical stunning may improve meat quality, the effectiveness of the stun and duration of unconsciousness should be the main concern when deciding on settings so animal welfare is not compromised.

While CO_2 stunning is generally considered to improve meat quality by reducing petechial haemorrhage and blood spots (Gregory, 2005) and reduced losses through condemnation compared to electrical stunning, it causes acidification of blood and involuntary muscle movements. Higher concentrations of CO_2 lead to a faster time to unconsciousness, which results in better meat quality of stunning with 90% CO_2 compared to 80% CO_2 (Nowak et al, 2007). There is some indication that the improvement in meat quality is mainly the result of reduced pre-stunning stress of CO_2 stunning, rather than the stunning method *per se* (Terlouw et al, 2021). Indeed, pre-slaughter handling and stress as part of a stunning system can affect meat and carcass quality (see Adzitey, 2011). A comparison of electrical and CO_2 stunning that included low stress handling prior to stunning for both methods, found only minor reductions in meat quality in a limited number of muscles (Terlouw et al, 2021). Moving pigs in a single file raceway is often associated with aversive handling and use of electric prodders which results in higher metabolic rate, increases in lactate, glucose and cortisol and a more rapid decrease in pH after slaughter (Bertram et al, 2002; Hambrecht et al., 2004). Therefore, not only the handling system inherent to a stunning system, but also the stockmanship and handling skills affect pre-stunning stress and therefore subsequent meat quality, regardless of stunning system.

Alternative gas systems rely on anoxia to produce unconsciousness and eventually death. Anoxia without the effect of CO_2 results in more violent convulsions and therefore may negatively impact on meat quality compared to high concentrations of CO_2 (Llonch et al, 2012). No studies on the effect of stunning with Argon or Argon/CO_2 mixtures could be found. When several mixes of CO_2 and N_2 were compared, duration of muscle excitation in the N_2 mixes lasted for longer compared to CO_2 alone. This resulted in poorer meat quality, with a direct negative correlation between CO_2 concentration and incidence of PSE meat (Llonch et al, 2012; Atkinson et al, 2020). In addition, 25% of pigs exposed to the N_2 mixtures had ecchymosis in their carcasses which was not seen in animals stunned with 90% CO_2 (Llonch et al, 2012). On a small sample of experimental pigs exposed to 80% CO_2 or 98% O2 in a slow fill chamber measurements of meat quality indicated a small improvement in meat quality in pigs exposed to N_2 compared to CO_2 (Alam et al, 2022), although this is not a real representation of a potential commercial stunning system. This does however indicate that pre-slaughter stress may exacerbate the effect of muscle excitation during stunning on meat quality, rather than have a major effect on meat quality per se, which was confirmed by Terlouw et al (2021), who found little effect of stunning method (CO_2, N_2 or electrical) on meat quality when pre-slaughter stress was minimised.

There have been limited findings on the possible effect of LAPS on meat quality (Baxter et al, 2022). They found after decompression, and subsequent recompression, damage such as haemorrhage and congestion in several areas of the body which would have implications for meat quality and condemnation of certain organs. Whilst it is unclear if these changes took place before or after death, and therefore have any implications for animal welfare, they will certainly have commercial implications.

Therefore, pre-slaughter stressors such as handling and restraint may have a larger influence on meat quality than stunning system *per se*. None of the alternatives to CO_2 stunning result in improved meat quality and some of the identified alternatives may be detrimental to meat quality and need to be considered when developing an alternative commercial stunning method.

8. Next steps, future research

Electrical stunning is currently mainly used in small and medium abattoirs. In order to improve electrical stunning a method should be developed where pigs can be stunned in groups without restraint. SPUC may be an alternative to head-only electrical stunning as it induces a longer duration of unconsciousness and reduced epileptic seizures. The handheld device has a single contact point and placement may be easier than the dual electrodes for handheld electrical stunning. This technology has not been tested on pigs and further research should be conducted.

Several alternative gasses to CO_2 have been studied to maintain the advantage of group stunning while reducing the aversiveness of the gas exposure. While there are several gasses that are less aversive, they all increase the time to unconsciousness and increase muscle excitation. They also result in a much shorter time of return to unconsciousness. Therefore, much longer exposure times, up to 7 minutes or more, are needed to ensure pigs do not regain consciousness, which require major changes in design to achieve commercial throughput in larger abattoirs. Argon may be a potential alternative gas, but due to the expense a method needs to be developed to recycle the gas so it can be re-used. N2 is another potential alternative gas, but is lighter than air, so cannot be used in a pit like CO_2 and an alternative delivery method would need to be investigated. Gas mixes or step-wise exposure to different gasses have been proposed, but would need further investigation.

While LAPS appears to be a humane alternative stunning method for poultry, only limited research has been conducted in pigs. Further research on conscious pigs of slaughter weight should be conducted, including different rates of decompression. In particular, the concern of gas trapped in body cavities and any resulting pain should be investigated. The stunning system would need extensive research on design and economic viability would need to be assessed. There is some concern about the effect of recompression on meat quality and this also needs further investigation.

One area that has received little research is the possibility of reducing aversive responses to CO_2. Considering the large variation between individual pigs and between abattoirs there appears to be potential to substantially reduce aversive responses. Some genetics appear to show less aversion and genomic research may assist in identifying these genotypes. Reducing stress prior and during CO_2 exposure may also reduce the aversive responses and should be further explored.

The ideal stunning method involves low stress handling in groups without restraint, causes immediate and reliable unconsciousness without fear or pain and results in death or unconsciousness for the duration of death by blood loss with a wide margin. However, none of the stunning methods identified in this review would achieve this and some compromise on animal welfare will need to be accepted.

Table 1. Overview of effects on animal welfare of the different aspects of each stunning method (Orange indicates high risk to welfare, green indicates low risk to welfare. Intensity of colour indicates relative risk).

Stunning Method	Pre-stun handling	Restraint	Stun experience	Efficiency	Stun duration	Opportunities for improvement
Captive bolt	Pigs need to be handled in single file raceway, electric prodder use	Individually restraint to assist with accurate placement	Immediate loss of consciousness if successful	Not recommended for pigs due to anatomy of the skull. High stun failures?	Results in death (stun to kill) if applied correctly	Adequate head restraint should be required to assist with accurate placement, although this is aversive to pigs. Not recommended for use in adult pigs
Head only electrical	Pigs need to be handled in single file raceway, electric prodder use	Individually restraint to assist with accurate placement	Immediate loss of consciousness if successful	Failure rates can be high with poor placement and/or contact and insufficient current	Stun duration is short. Stun to stick intervals may result in return to consciousness prior to death from bleeding	Stockperson training to minimise pre-slaughter stress. Ensure correct placement and adequate current (auto feedback). Ensure stun to stick interval of <15s.
Head to body electrical	Pigs need to be handled in single file raceway, electric prodder use	Individually restraint to assist with accurate placement	Immediate loss of consciousness if successful	Stunning efficiency is high if applied automatically with control of current	If successfully applied automatically, results in long duration of unconsciousness or death (stun to kill)	Stockperson training to minimise pre-slaughter stress. Use fully automatic system to ensure correct placement and adequate current.

CO_2 stunning	Stunning in groups allows for moving pigs in small groups using hydraulic gates, thereby reducing handling prior to slaughter	Pigs are free-standing in groups	Exposure to CO_2 is aversive for the duration until unconsciousness (22-32s)	Efficiency is high if used in a paternoster system with high concentration of CO_2 (<90%) and long duration of exposure	If high concentration with long exposure is used, it results in long duration of unconsciousness or death	Minimise pre-slaughter stress, including handling, by using hydraulic handling system and stockperson training. Genetic selection for lines less susceptible to aversion to CO_2.
Argon/ CO_2 mix	Stunning in groups allows for moving pigs in small groups using hydraulic gates, thereby reducing handling prior to slaughter	Pigs are free-standing in groups	Exposure to Argon/30% CO_2 mixture is less aversive than 90% CO_2, however the duration to unconsciousness is longer (longer duration of aversive stimulus)	Efficiency is high, providing that O_2<2% and long duration of exposure are provided (>7min)	Even with long exposure, duration of unconsciousness can be a little variable. Further research needed	Ensure that concentration of O_2<2% and exposure is >7min. Will need re-design of stunning system to account for long exposure time.
N_2/ CO_2 mix	Stunning in groups allows for moving pigs in small groups using hydraulic gates, thereby reducing handling prior to slaughter	Pigs are free-standing in groups	Exposure to N_2/CO2 mixture is less aversive than CO2, however the duration to unconsciousness is longer (longer duration of aversive stimulus)	Efficiency is high, providing that O_2<2% and long duration of exposure are provided (>5min)	Even with long exposure, duration of unconsciousness can be a little variable. Further research needed.	Ensure that concentration of O_2<2% and exposure is >5min. Will need re-design of stunning system to account for long exposure time.

LAPS	Stunning in groups allows for moving pigs in small groups using hydraulic gates, thereby reducing handling prior to slaughter	Pigs are free-standing in groups	? Observations of congestion and haemorrhage in several body regions after recompression. Concerns over gas in body cavities	? Potentially high	Animals are exposed until death (stun to kill)	Needs research on conscious slaughter-weight pigs to establish if LAPS can be applied humanely.
SPUC	Pigs need to be handled in single file raceway, electric prodder use	Individually restraint to assist with accurate placement	Immediate loss of consciousness if successful	? No data available	? Potentially longer duration than head-only electrical stunning	This method has not been studied in pigs. Potential alternative for head-only stunning and potential improvement in meat quality. No published research since 2014.

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