



Stunning methods for pigs at slaughter

Bedövningsmetoder för gris vid slakt

Torun Wallgren, Anna Wallenbeck, Charlotte Berg

Sveriges Lantbruksuniversitet
Institutionen för husdjurens miljö och hälsa
Avdelningen för miljö, omsorg och djurhälsa

Skara 2021

Rapport 56

*Swedish University of Agricultural Sciences
Department of Animal Environment and Health
Section of Environment, Care and Herd Health*

Report 56

ISSN 1652-2885

Stunning methods for pigs at slaughter

Bedövningsmetoder för gris vid slakt

Torun Wallgren, Anna Wallenbeck, Charlotte Berg

Report to the Swedish Board of Agriculture

Table of Contents

| | |
|---|----|
| Definitions | 4 |
| Introduction | 4 |
| Aim | 4 |
| Background | 5 |
| Mechanical stunning | 9 |
| Penetrating captive bolt | 10 |
| Free projectile | 11 |
| Gas stunning | 13 |
| Carbon dioxide | 13 |
| Nitrogen | 16 |
| Argon | 18 |
| Helium | 19 |
| Xenon | 19 |
| Low atmospheric pressure stunning (LAPS) | 20 |
| Gas mixtures | 21 |
| Electrical stunning | 24 |
| Electrical head-only stunning | 25 |
| Electrical head-to-body or head-to-back stun/killing – one-cycle method | 26 |
| Electrical head-to-body or head-to-back stun/killing – two-cycle method | 27 |
| Discussion | 29 |
| Scientific basis | 29 |
| Driving | 32 |
| Handling and restraint | 32 |
| Risk of unsuccessful stun | 32 |
| Experience of the stun | 32 |
| Reliability of the stun | 32 |
| Comparisons between stunning methods and stunning systems | 33 |
| Other factors | 36 |
| Previous assessments | 37 |
| Knowledge gaps and future research | 37 |
| Conclusions | 37 |
| References | 39 |

Definitions

Anoxia: Complete absence of oxygen supply.

Aversive: Negative stimulation.

Stunning: Intentionally induced process which causes loss of consciousness and sensibility, without pain.

Death: Physiological state in which respiration and blood circulation cease and the corresponding regions of the brain are irreversibly inactive (EFSA, 2013). In this context, the main clinical signs of death are absence of respiration (and no gasping), absence of pulse and dilated pupils (EFSA, 2004; EFSA, 2006). In Sweden, cardiac death, that is to say ceased rhythmic cardiac activity, is applied as the criterion to determine that an animal is dead.

Hypoxia: Inadequate oxygen supply.

Clonic seizure: Rhythmic, symmetrical contractions/spasms throughout the entire body.

LOP: Loss of posture. When the pig is no longer standing and continues in a lying position during stunning.

Residual oxygen: Remaining oxygen.

Stun-to-stick time: Time between stunning and bleeding.

Tonic seizure: Muscle spasms in the form of rigidity of the body, collapse of the body.

Introduction

Aim

The aim of this literature review was to summarise relevant animal welfare research on all methods for stunning pigs at slaughter. The review, which was conducted on behalf of the Swedish Board of Agriculture, was intended to provide an accurate picture of current scientific knowledge with regard to these methods and address the advantages and disadvantages of different methods, including all factors that may affect animal welfare in any way during stunning and slaughter. Areas identified as having a lack of knowledge regarding certain relevant aspects were also considered. Based on the findings, assessments were made of the potential for improvement of the different methods, where possible.

Background

The Swedish Animal Welfare Act (2018:1192, Chapter 5, Section 1) requires animals to be spared unnecessary suffering and discomfort and to be stunned before slaughter by exsanguination (bleeding). Stunning means any intentionally induced process which causes loss of consciousness and sensibility without pain (Regulation (EC) No 1099/2009). The purpose of stunning prior to killing is to prevent pain and suffering in the animal due to be killed, by inducing unconsciousness until death occurs (Steiner et al., 2019).

An ideal stunning method induces loss of consciousness without causing stress or pain (Steiner et al., 2019). However, it is known that stress and pain can occur when using various stunning methods, relating to both the induction of stunning and/or the handling of the animal prior to induction of stunning. Handling prior to stunning, the stunning process itself and the effectiveness of the stun are therefore very important when assessing animal welfare in connection with slaughter (Brandt & Aaslyng, 2015).

The purpose of this report was to review existing scientific knowledge relating to animal welfare during stunning in connection with the slaughter of pigs using various stunning methods and to identify knowledge gaps. The report is based on scientific studies and reports.

The approach taken and the limits of the work

Handling of live animals at the slaughterhouse starts when they are unloaded from the vehicle transporting them to the slaughterhouse and continues until the animals are dead. Handling of animals at the slaughterhouse therefore includes unloading, lairage, moving of animals, restraint, stunning and bleeding (EFSA, 2019). The effect of handling on animal welfare is hence relevant to all these stages. This report focuses on those parts of the handling process that relate to stunning and their effect on animal welfare. It therefore only addresses the effect on animal welfare relating to driving prior to stunning and to stunning and killing, as animal handling in other stages normally does not differ between different stunning methods.

As the aim of this report was to describe the animal welfare aspects of different stunning methods, the focus was specifically on animal welfare. Other aspects that are affected by the stunning method, such as meat quality, working environment and economic considerations, are not dealt with in this report. A number of studies have investigated the connection between animal welfare and the properties of the carcass. This topic was considered to be outside the scope of this review and was therefore not included. It is worth mentioning in this context, however, that reduced stress during handling generally has a positive effect on meat quality, which is also positive from an animal welfare point of view (Warner et al., 2007).

Moreover, this report does not address factors outside the slaughterhouse that may affect animal welfare during stunning, such as genetic aspects or transport, as they are not specifically affected by the stunning method. As the report deals only with the stunning of pigs at slaughterhouses, stunning methods intended to be used on animals that are not ready for slaughter (<100 kg), such as a percussive blow to the head, which can be used for killing neonatal piglets on the farm, are not covered in the report.

Assessing animal welfare

There are three important elements to animal welfare: the animal's subjective experience of its situation, the animal's biological function and the animal's capacity to adapt to the environment in which it is kept (see e.g. Broom, 1986; Fraser et al., 1997; Keeling et al., 2011). Animal welfare is often defined by the five freedoms: freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behaviour and freedom from fear and distress (FAWC, 2009). At slaughter, and more specifically at stunning prior to slaughter, freedom from pain and injury and freedom from fear and distress are particularly relevant. When driving the animals to be stunned, it is therefore important that they have the opportunity to move in a way that is normal and natural for their species and that the driving system is designed to facilitate this (Grandin, 2003).

Assessing animal welfare is complex. As the concept of animal welfare includes the animal's subjective experience, it is considered difficult to measure and indicators of animal welfare are commonly used instead. Assessment of animal welfare requires information on several different aspects of animal welfare, including both resource-based indicators (referred to as input indicators, such as the design of raceways and handling of the animals at the slaughterhouse) and animal-based indicators (referred to as output indicators, such as the animal's physiological and behavioural responses). Scientific assessments of how these different assessments

relate to each other are rare, so it is difficult to conduct a simple and complete animal welfare assessment. Due to the complexities involved in animal welfare assessments, there is a lack of specificity, for example revealing *how* aversive an animal experiences something to be (Mason & Mendl, 1993). Specific comparisons between systems are therefore difficult to evaluate scientifically. How animal welfare is assessed, and in particular the aspects of animal welfare that are assessed, also differ between different reports (Weary & Robbins, 2019), which further hampers comparisons between assessments of different stunning methods.

Welfare is often assessed by examining animals' physiological and behavioural responses in a situation. Observing behaviour can provide a direct indicator of stress level in the animals, whereas physiological tests, such as blood tests, need to be analysed before they can provide information on stress level. Behaviours, such as vocalisation, crowding or slipping, are often used as indicators of animals under stress or negatively affected by their environment. New environments, such as the slaughterhouse or stunning box, can often cause stress in themselves (Becerril-Herrera et al., 2009). Absence of behaviours can also be used for animal welfare assessment, for example the absence of regular breathing or lack of reflexes can indicate unconsciousness. Physiological measurements can be invasive (require an intervention), for example blood sampling, or non-invasive, for example observation of breathing rhythm. Another example of a non-invasive physiological measurement is cardiac rhythm, which can act as an indicator of the functioning of the autonomic nervous system, but can also be used as an indicator of stress (von Borell & Veisser, 2007). Analysis of the blood from bleeding can provide information on physiological indicators relating to the stress level of the animal prior to bleeding (Nowak et al., 2007).

Driving to and initiation of stunning

Handling prior to stunning can also affect the welfare of the animals (EFSA, 2004). Animal welfare while being driven to stunning can be assessed by studying the animals' behaviour, where behaviours such as falls, crowding, backing up, turning and vocalisation are regarded as negative indicators of animal welfare (Brandt & Aaslyng, 2015). Physiological indicators, such as blood glucose, lactate and body and blood temperature in the live animal, or pH measurements of the carcass, can also indicate the stress level of the animal (Brandt & Aaslyng, 2015).

A number of hazards that are specifically affected by different stunning methods have been identified. These relate to the handling and moving of pigs at the slaughterhouse and include: inappropriate handling, inappropriate design of gateways, use of electric prods, rushing, mixing of unfamiliar animals and inability to move side by side (EFSA, 2019). Improper handling of animals can result in animals experiencing fear or pain, and this is also the case at the slaughterhouse (Brandt & Aaslyng, 2015). Handling is therefore very important for animal welfare.

After unloading at the slaughterhouse, the pigs are usually placed in a waiting pen, where they are kept until it is time to initiate stunning. Pigs from different groups can be mixed in the waiting pen, which can lead to aggression. From the waiting pen, the pigs are moved via raceways to the stunning pen, either manually by people or via automatic gates (Brandt & Aaslyng, 2015). In some systems, the raceways are designed so that only one pig at a time can move, whereas others are designed so that the pigs can be driven in groups (Brandt & Aaslyng, 2015). Pigs are social animals and generally experience stress when separated from their group. For that reason, systems in which the animals can move together are preferable. Isolation from other pigs can in itself act as an aversive stimulus and result in attempts to escape (Raj & Gregory, 1996). Movement of pigs in small groups so that the driver can reach all pigs is preferable from an animal welfare point of view (EC, 2002). Large groups are difficult to handle and increase the risk of bruising in the carcass (Dalla Costa et al., 2019). In smaller groups, the pigs are likely to move less, including when a large group is split into smaller units, which means that the pigs suffer fewer bruises or fractures. In general, the handling of pigs in smaller groups is considered to be less negative from an animal welfare point of view. In systems in which pigs are forced to move one by one, harder driving is often required, for example using electric prods (Brandt & Aaslyng, 2015). The use of electric prods is aversive for pigs and increases heart rate, vocalisations and crowding, and increases the number of pigs which slip and fall compared with driving using for example a paddle. Electric prods also increase the incidence of bruising in the meat (Correa et al., 2010). The use of electric prods should therefore be avoided (EC, 2002). In fact, driving often does not actually proceed any quicker using electric prods, as the pigs can become so stressed that they try to turn back instead of moving forward (EC, 2002). In a Canadian study, electric prods were found to be used on between 0% and 80% of pigs, despite the prod only being used when pigs refused to move and despite them being handled by well-trained handlers (Grandin, 2003). Even when pigs are driven in large groups, an electric prod is often required to get the pigs to go into the stunning pen one at a time (Støier et al., 2001). According to European Council Regulation (EC) No 1099/2009 of 24 September on the protection of animals at the time of killing, electric prods are permitted for used on adult pigs only, which in

Sweden and most other European Union (EU) Member States is interpreted to mean that such equipment must not be used on fattening pigs.

Individual stunning, such as the use of a captive bolt or electric stunning, usually means that the animal must be firmly restrained, which normally requires individual handling. Restraint, particularly when the animal is held firmly in place, can cause both fear and distress, partly because natural instincts such as the instinct to escape are impeded. Improper restraint can also cause pain. Restraint has been identified as one of the most stressful and painful stages of the slaughtering process (EFSA, 2004). A number of hazards relating to the restraint of pigs at the slaughterhouse that are influenced specifically by different stunning methods have been identified. These include: inappropriate restraint causing injury, inappropriate design of restraint for animals too large/too small, inappropriate handling during restraint, too long restraining time, improper maintenance of the restraining system, excessive number of animals in crate and the necessity for immobilisation (EFSA, 2019). To minimise stress arising during restraint, animals should be stunned immediately after being restrained (SJVFS 2019:8 Chapter 7 Section 4 L22).

Dealing with animals in groups and minimising handling and restraint are therefore considered advantageous from an animal welfare point of view (EFSA, 2004). Stunning in groups often allows the animals to be handled as a group and this is rarely preceded by firm restraint prior to stunning. How the animals are driven and how stunning is initiated can therefore play an important role with regard to the flow of animals in the facility and animal welfare.

Stunning

Stunning prior to slaughter is a statutory requirement in the EU and aims to enable animals to be slaughtered without unnecessary suffering, fear, anxiety, pain or stress (EFSA, 2004). Stunning affects basal brain functions and prevents the animal from experiencing pain and stress. Effective stunning methods disrupt the normal mechanisms of the neurons or neurotransmitters in the brain, rendering the animals unconscious and insensible (EFSA, 2004). Methods of slaughter that are acceptable from an animal welfare perspective must therefore induce unconsciousness directly. When direct induction of unconsciousness is not possible, indirect induction of unconsciousness must be non-aversive and must not in itself cause anxiety, pain, stress or suffering to conscious animals. Today, there are three main types of stunning method for pigs: mechanical, electrical and gas stunning (EFSA, 2004), which are described in more detail in later sections of this report. In Sweden, approved stunning methods for pigs are captive bolt, free bullet firearm, shotgun, electric current and carbon dioxide (SJVFS 2020:22). The most common methods used for stunning pigs today are electrical stunning and carbon dioxide stunning (Becerril-Herrera et al., 2009). Stunning can be reversible, which means that in theory the animal can regain consciousness after the stunning has worn off, or irreversible, which means that an animal cannot regain consciousness after stunning is correctly carried out (Becerril-Herrera et al., 2009). After stunning, the animals must be stuck and bled, at which point death occurs or is ensured (regardless of whether the stunning was reversible, irreversible or directly lethal). Killing itself is usually achieved by cutting arteries (and veins) in the neck, thereby preventing blood flow to the brain, at which point the animal dies (Becerril-Herrera et al., 2009; Mota Rojas et al., 2012). In general, mechanical stunning is irreversible, whereas electrical and gas stunning are reversible. However, the reversibility of effect depends, among other things, on the method used and the length of time for which it is applied. Some stunning methods, for example gas stunning, can kill the animal without bleeding if continued for long enough. During and after stunning, the behaviour of the animal (including reflexes) can be used to determine the effectiveness of the stun (EFSA, 2004). Animal welfare during stunning can be investigated for example by examining blood glucose, lactate and haematocrit, blood temperature, carbon dioxide concentration in the stunning system, aversive responses to pure carbon dioxide or vocalisation (Brandt & Aaslyng, 2015). Pigs that are not stunned prior to bleeding become unconscious around 25 seconds after sticking (EFSA, 2004).

If the stunning is inadequate, the animal's awareness is not inhibited and the animal is not prevented from experiencing pain and distress in connection with killing. It is therefore important that the stunning is carried out correctly (Brandt & Aaslyng, 2015). Impairment of consciousness is defined as the inability to respond normally to exogenous stimuli (EFSA, 2004). In order to ensure a good level of animal welfare, stunning must be sustained until death occurs, which therefore includes both the interval between stunning and bleeding and the interval between bleeding and death (Atkinson et al., 2012). The precise time between stunning and bleeding is not regulated by EU or Swedish legislation but must be stated in the slaughterhouse's standard operating procedures (SOP) (Regulation (EC) No 1099/2009). Symptoms of inadequate stunning can arise even after the pig has been bled and hoisted (Atkinson et al., 2012). The start and duration of stunning can be assessed in different ways depending on the circumstances. The time until the animal collapses (LOP = loss of posture) is often used as an initial indicator of loss of consciousness (Velarde et al., 2007). In experiments, the start of

stunning is often assessed using measurements of brain activity (e.g. electroencephalography (EEG)). However, stunning can also be assessed by means of behaviour and other physical reflexes, which can be easier to assess in circumstances such as during commercial slaughter, where EEG measurement is not possible. Different types of behaviour can be assessed, such as vocalisation, breathing, collapse and spasms, epileptiform seizure and absence of reflexes. In practice, it is recommended that, for example, the occurrence of eye reflexes, rhythmic breathing and righting reflexes should always be examined, to ensure that the animal is not conscious after stunning (EFSA, 2004; EFSA, 2013).

Signs of inadequate stunning include corneal reflex (eye reflex) or regular breathing (Atkinson et al., 2012). The absence of reflexes is often used as a sign of loss of consciousness, but it is not a definite indication of the level of consciousness, even though absence of eye reflex probably only occurs in unconscious animals. Absence of breathing or irregular breathing is a sign of stunning. If breathing returns to a normal rhythm, this is a sign that the stunning is starting to wear off. Vocalisation at the induction of stunning is in general always treated as a sign of pain or suffering, although the absence of vocalisation should not be viewed as a sign of absence of pain or suffering (EFSA, 2004). Collapse and spasms are often seen with mechanical stunning and electrical stunning, but generally occur more gradually with gas stunning. Animals that have not been adequately stunned experience poor animal welfare, as they experience pain, fear and other adverse effects of slaughter (EFSA, 2004). Among other things, bleeding causes a rapid reduction in blood pressure, which results in fear and panic if the animal is not unconscious.

Bleeding

Stunning induces a temporary loss of consciousness and it is therefore very important that bleeding, which results in death, is carried out before consciousness returns (EFSA, 2004). Death is defined as a physiological state where respiration and blood circulation have ceased, as the respiratory and circulatory centres in the medulla oblongata (brain) are no longer active. As nutrients and oxygen are no longer supplied to the brain, consciousness is irreversibly lost (EFSA, 2004). Bleeding is carried out by cutting arteries (and veins) in the neck or chest, which prevents oxygen and nutrients from reaching the brain (Mota-Rojas et al., 2012). Pigs are usually bled by chest sticking, which severs the major blood vessels from the heart (EFSA, 2004; EFSA, 2020). Signs that death has occurred include absence of respiration and pulse and presence of pupillary dilation (EFSA, 2004). How long the effect of stunning lasts depends primarily on the stunning method used, but there are also individual variations between animals (EFSA, 2004). The stun-to-stick time must therefore be tailored so that death occurs before the animal regains consciousness after stunning (EFSA, 2004). Stunning methods that themselves result in death are not dependent on the time to bleeding from an animal welfare point of view, provided that stunning is successful. Stunning methods which result in death should therefore be preferred when available and proven to be effective (EFSA, 2004).

Mechanical stunning

In mechanical stunning, the animal should be shot so that the brain is hit and damaged in such a way that the animal immediately loses consciousness (SJVFS 2020:22, Chapter 7 Section 3 L22). Successful mechanical stunning should result in the animal collapsing, followed by convulsions, after which breathing should cease and the eyes become unfocused (Table 1). There should be a loss of corneal reflex and the animal should not vocalise. Animals must be stunned at the first attempt, mainly because one shooting attempt reduces the chances of stunning being successful in a subsequent attempt (EFSA, 2004).

There are various types of mechanical stunning methods. The most common of these is the captive bolt, which can be penetrating or non-penetrating (EFSA, 2004). For the stunning of pigs, however, only the penetrating captive bolt is approved within the EU. In addition to the captive bolt, free bullets are also included among the mechanical methods approved for stunning pigs within the EU (above 100 kg live weight). At the present time, mechanical stunning is not commonly used for pigs under commercial conditions, but is instead used as a back-up method when other stunning methods fail (EFSA, 2004).

A number of hazards have been identified relating to mechanical stunning of pigs on farm and may also be relevant at the slaughterhouse: untrained personnel, incorrect application of percussive blow, maintenance of equipment, incorrect shooting position, no SOP, incorrect bolt parameter and animals being able to see other animals being stunned (EFSA, 2019).

Table 1. Checks and indicators for monitoring of stunning using mechanical methods (Algers et al., 2012)

| Normal checks | Additional indicators |
|---------------------------------------|---|
| Immediate collapse | Relaxed muscles in neck/throat, tongue and ears |
| Convulsions followed by leg movements | No vocalisation |
| No sign of righting | Dilated pupils |
| Absence of normal respiration | No corneal reflex |
| Open eyes, unfocused gaze | No rotation of eyeballs |
| | Absence of response to pain stimuli |

Penetrating captive bolt

The penetrating captive bolt is usually not used as a stunning method for pigs in commercial slaughter, as it requires individual handling and firm restraint of the animal. The captive bolt must be tailored to the animal to be stunned in terms of captive bolt mass, velocity, diameter and length, in order to ensure correct stunning (EFSA, 2004).

Driving

Use of a penetrating captive bolt requires the pig to be firmly restrained to enable the captive bolt gun to be aimed correctly. It is therefore necessary for the pigs to be handled and driven individually.

Induction of stunning

Stunning is initiated by the bolt penetrating the skull and causing damage to the brain and brain stem resembling severe concussion (EFSA, 2004). The brain damage affects nerve function and causes loss of consciousness and absence of reflexes (Shaw, 2002; EFSA, 2004).

The damage that occurs from using a penetrating captive bolt is irreversible. The captive bolt gun is normally applied to the forehead (Anderson et al., 2019). If the captive bolt gun is not applied at the correct angle, there is a risk that the bolt will not penetrate the skull in the correct way, which can result in inadequate stunning (EFSA, 2004). Pigs are difficult to stun with a captive bolt because the target area for the bolt is small. Application can be further exacerbated by the shape of the head in some breeds and in aged pigs (HSA, 1998; EFSA, 2004). Aged pigs (boars and sows) can have thick bone where the bolt is intended to be placed, which makes it more difficult for the captive bolt gun to penetrate the bone, thus increasing the risk of inadequate stunning (HSA, 2001). Large boars in particular can be very difficult to stun using a captive bolt gun due to the size of the sinuses and the fact that the brain lies deeper in the skull (Blackmore et al., 1995; HSA, 1998; EFSA, 2004).

Application of the captive bolt gun behind the ear has been investigated, as application to the forehead is complicated by the pigs' curiosity and their common inability to stand still when handled from the front (Anderson et al., 2019). When a captive bolt is used behind the ear, the passage of the bolt is extended. Shooting in the forehead is therefore preferable, as this results in a greater degree of contact between bolt and brain and therefore correct stunning. There is also reason to assume that the power transferred from the bolt to the head is less if the bolt hits soft tissue instead of bone, which means that there is a risk of the stunning being inadequate and/or of shorter duration. Shooting the captive bolt behind the ear has proven to be more dependent on getting the correct placement exactly right, and this method is considered to require further research before it can be used on a commercial basis (Anderson et al., 2019).

Duration, reliability and monitoring

Immediately after correct shooting with a captive bolt, the animal collapses and experiences a short tonic seizure (for around 3-5 seconds), followed by disappearance of corneal reflexes and a decrease in respiration (EFSA, 2004). After the tonic seizure, the pig then has a clonic seizure that lasts for several minutes, at which point pupillary dilation occurs. Correct captive bolt stunning is irreversible but, to minimise the risk of recovery, the pigs must be bled as quickly as possible, preferably within 15 seconds (HSA, 2001; EFSA, 2004). For domestic slaughter in Sweden, the requirement is a maximum stun-to-stick time of 60 seconds.

Animal welfare aspects

The welfare hazards involved in using a penetrating captive bolt gun include correct application of the bolt and the difficulty in correctly stunning aged animals and certain breeds, depending on the anatomy of the head (Table 2). Use of a penetrating captive bolt gun can be a suitable stunning method, provided that the animals to be stunned are not older pigs such as sows and boars (EFSA, 2004). There is currently no automatic method available for captive bolt stunning of pigs. Therefore, efficiency and handling in relation to this stunning method are entirely dependent on the training and skills of the individual who performs the stunning (EFSA, 2004). Stunning using a captive bolt gun currently involves individual handling and restraint. As previously mentioned, individual handling is associated with stress and harder driving of pigs (Raj & Gregory, 1996; Brandt & Aaslyng, 2015), which is considered a negative aspect from an animal welfare perspective. In addition, individual restraint prior to stunning has been identified as the single most stressful and painful element of the slaughter process (EFSA, 2004). However, immediate, irreversible stunning is considered to be positive from an animal welfare point of view.

Table 2. Animal welfare aspects and hazards with regard to stunning using a penetrating captive bolt gun

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|---------------------------------|---------------------------------|
| Immediate stunning | Individual handling | Correct application |
| Irreversible stunning | Restraint | Aged animals and certain breeds |

Free projectile

Use of a free projectile is currently permitted within the EU as a method for stunning pigs. This includes weapons firing bullets or shot, including safety rifles for slaughter, that is to say adapted rifles that are only fired on direct contact. All use requires the ammunition to be adjusted to the animal type and size. Free bullets are not considered a suitable method for killing pigs at slaughterhouses, but are used mainly on-farm when dealing with disease outbreaks (EFSA, 2004). For pigs, free projectile stunning is in principle only used as a back-up method when the usual stunning method has not worked adequately (EFSA, 2004).

Driving

Free projectile stunning is used mainly as a back-up method at slaughterhouses and therefore the driving method is dependent on the main method of stunning. Use of safety rifles requires the pig to be restrained to enable the captive bolt to be aimed correctly and it is therefore necessary for the pigs to be handled and driven individually. When stunning using a bullet rifle or a shotgun, the animal can be unrestrained in the pen or stun box if it is still possible to hit the head correctly.

Induction of stunning

Induction of stunning is similar to stunning using a penetrating captive bolt, except that the weapon is not in direct contact with the animal when fired (unless a safety rifle is used).

Duration, reliability and monitoring

Duration, reliability and monitoring for stunning by free projectile are the same as for penetrating captive bolt.

Table 3. Animal welfare aspects and hazards with regard to stunning using a penetrating free projectile

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|---------------------------------|-----------------------------------|
| Safety rifle | | |
| Immediate stunning | Individual handling | Correct application |
| Irreversible stunning | Restraint | Aged animals and certain breeds |
| Bullet/shot | | |
| Immediate stunning | Individual handling | Correct application |
| Irreversible stunning | Restraint | Aged animals and certain breeds |
| | | Injury caused by free projectiles |

Animal welfare aspects

The welfare hazards in stunning using a free projectile are the same as in stunning using a penetrating captive bolt. They include a negative effect on animal welfare as a result of individual handling and restraint, while the positive indicators include immediate and irreversible stunning (Table 3). One hazard with stunning using a free bullet is that if the bullet misses, it can ricochet off the interior of the slaughterhouse and risk causing injury to both personnel and animals.

Gas stunning

For stunning by gas, the pigs are usually lowered into an enclosed crate environment to which the gas is introduced (EFSA, 2004). Stunning is normally induced by the pig developing a high concentration of carbon dioxide (hypercapnia) or low concentration of oxygen in the blood (hypoxia) (EFSA, 2004). Inert gases cause anoxia, that is to say a complete lack of oxygen, by replacing oxygen in the air (Kells et al., 2018). As a rule, stunning is reversible, but prolonged exposure results in death. Six hazards have been identified in relation to gas stunning of pigs at the slaughterhouse: inappropriate lowering procedure of stunning crates, inappropriate design of crates, improper maintenance of the restraining system and the fact that animals can see other animals being stunned, inappropriate stunning assessment parameters, and too short time of exposure (EFSA, 2019). The latter two aspects can be affected specifically by different gas mixtures. Stunning is monitored by, among other things, absence of normal respiration, successive loss of balance and collapse, open eyes and strong spasms (Table 4).

Table 4. Checks and indicators for monitoring of stunning using carbon dioxide (Algers et al., 2012)

| Normal checks | Additional indicators |
|---|--|
| Absence of normal respiration | Relaxed body |
| Open eyes | Strongly dilated pupils, in most cases no corneal reflex |
| Successive loss of balance and collapse | No sign of righting |
| Strong spasms | Subside after onset of stunning Absence of response to pain stimuli |

For gas stunning, the pigs are usually driven into an enclosed space (a crate often called a “butina”, after a manufacturer), where the gas is introduced. The design of the opening of the crate affects the willingness of pigs to enter. An opening that allows several pigs to enter at the same time is preferable, as the pigs can be driven in a group and thus their natural group behaviour can be exploited and handling is minimised. Once properly inside the crate, no restraint is necessary (EFSA, 2004). In addition to exposure to the gas, the drive to the stunning crate and the wait in the crate (darkness, sound, smell, movement) can cause distress (Atkinson et al., 2015; Dalmau et al., 2010). However, it has been shown that pigs become habituated (get used) to dip-lift systems that contain atmospheric air only, which indicates that the dip-lift system is not in itself aversive if the right gas mixture is used (Velarde et al., 2007). In practice in commercial slaughter, however, it is always both the first and last time the pigs encounter this unfamiliar environment.

In general, stunning with gas is considered to have high potential for acceptable stunning of pigs from an animal welfare perspective, as it is associated with a low level of stress related to handling in connection with the stunning. In order for gas stunning to be humane, however, it is necessary for the gas used to be non-aversive (EFSA, 2004). When stunning with gas, pigs should be stunned in a group, kept in stable social groups and restrained as little as possible (EFSA, 2004). It is also recommended that all pigs reach an irreversible state of unconsciousness before bleeding is initiated.

Carbon dioxide is currently the only gas approved for commercial slaughter of pigs in Sweden (the EU permits several different gases and combinations of gases, but carbon dioxide is predominantly used throughout the EU). However, carbon dioxide has been the subject of debate, as inhalation of a high concentration of carbon dioxide is aversive and therefore associated with a great deal of distress (Raj & Gregory, 1996; Grandin, 2003; Velarde et al., 2007; Llonch et al., 2011; Atkinson et al., 2015). Other gases have been proposed as suitable for enabling the positive aspects of gas stunning to be retained (e.g. minimal handling and driving in groups), but reducing the negative aspects (discomfort on inhalation) (Raj & Gregory et al., 1995). With gas stunning, the initiation of stunning is dependent on the gas mixture used. Three technical aspects should be taken into account when evaluating the suitability and use of the gas are the ability of the gas to replace oxygen in a closed space, the stability of the gas and the uniformity of the gas (possibility of maintaining an even concentration) (Dalmau et al., 2010).

Carbon dioxide

Carbon dioxide is one the most common stunning methods for pigs, both in Sweden and in other parts of the world (EFSA, 2004; Llonch et al., 2012a). In Sweden, all major slaughterhouses use carbon dioxide stunning in

a Butina® stun system (Atkinson et al., 2012). Carbon dioxide is a colourless, odourless gas that has a slightly acid taste and anaesthetic properties and does not leave any residue in the meat (Mota-Rojas et al., 2012). Carbon dioxide is easy to obtain and therefore relatively cheap to produce (Mota-Rojas et al., 2012).

Carbon dioxide is heavier than air (Dalmau et al., 2010) and in practice pigs are often lowered into a pit containing a high concentration of carbon dioxide (EFSA, 2004). There are two main types of carbon dioxide stunning methods: dip-lift (direct lowering into maximum carbon dioxide concentration) and paternoster (lowering successively to the maximum carbon dioxide concentration). The paternoster system works like a Ferris wheel, which lowers the crates gradually into an increasing concentration of carbon dioxide. When they reach the bottom, they also reach the maximum concentration of carbon dioxide. The paternoster system is filled/emptied continuously and several crates are in motion at the same time, whereas the dip-lift system only has one crate (EFSA, 2004).

Driving

One of the advantages of carbon dioxide stunning is considered to be the fact that the pigs can be handled and stunned in a group without needing to be restrained individually (Atkinson et al., 2012; Bouwsema & Lines, 2019). This reduces the occurrence of separation anxiety, refusal to move and the use of electric prods (Atkinson et al., 2015). Driving to group stunning can also be done with mechanical gates that separate the pigs into small groups, which eliminates the use of electric prods (Atkinson et al., 2012). Minimising handling reduces stress and the risk of stunning being initiated in the wrong place, and therefore promotes animal welfare (Bouwsema & Lines, 2019). Pigs exhibit refusal to enter a space that contains carbon dioxide (Velarde et al., 2007), which can make it more difficult to drive them into the stunning box if it is not emptied of carbon dioxide before stunning is initiated.

Induction of stunning

When carbon dioxide is inhaled, carbonic acid is formed, leading to elevated levels of hydrogen ions and creating acidosis at cellular level, which inhibits the functioning of neurons and produces an anaesthetic effect (Woodbury & Karer, 1960, cited in EFSA, 2004; Mota-Rojas et al., 2012). Before stunning occurs, lactic acidosis, hyperglycaemia, hyperkalemia, hypercalcaemia and respiratory and metabolic acidosis are induced (Becerril-Herrera et al., 2009). Stunning is not immediate and the time it takes for stunning to occur depends on the concentration of carbon dioxide in the air: the higher the carbon dioxide concentration, the shorter the induction time (Raj & Gregory, 1996; EFSA, 2004; Velarde et al., 2007; Dalmau et al., 2010; Atkinson et al., 2015).

Carbon dioxide induces loss of consciousness from a 40% mixture in air (Raj & Gregory, 1996). The duration of the respiratory distress that the pig experiences is reduced when the quantity of carbon dioxide in the air is increased, as LOP is induced sooner (Raj & Gregory, 1996). Some studies indicate that with 40% carbon dioxide the pigs are subjected to respiratory distress for approximately 30 seconds, compared with approximately 12 seconds with 90% carbon dioxide (Raj & Gregory, 1996). Other studies show that the first sign of loss of consciousness when exposed to 90% carbon dioxide occurs after 22.4 seconds, whereas the equivalent figure at 70% carbon dioxide is 34.4 seconds (Velarde et al., 2007). At 80-90% carbon dioxide in the air, it can take up to 36 seconds for the pigs to lose consciousness (Raj et al., 1997). Both the number of pigs per group and the time it takes to reach the maximum carbon dioxide concentration vary between different stunning systems (Atkinson et al., 2012). For piglets, it has been reported that it is less aversive for them to be gradually exposed to a gas mixture compared with being introduced to a prefilled high concentration of the gas (Rault et al., 2013). However, some studies have concluded that the dip-lift system results in better welfare for the animals (Velarde et al., 2007).

The length of time that the pigs are exposed to the carbon dioxide depends on whether they are to be stunned or killed, and with prolonged exposure the pigs die. The recommendation is for pigs to be exposed to as high a concentration of carbon dioxide as possible as quickly as possible (EFSA, 2004). However, Velarde et al. (2007) found that pigs show more avoidance behaviour the higher the carbon dioxide concentration.

Duration, reliability and monitoring

The duration of the stunning effect depends on the concentration of carbon dioxide and the exposure time, as this determines the severity of acidosis, and therefore the anaesthetising effect (EFSA, 2004). Therefore, the stun-to-stick time needs to be adapted to the carbon dioxide concentration and exposure time. Prolonged exposure to high carbon dioxide concentrations leads to death (EFSA, 2004). Short exposure time and low concentration entail a higher risk of inadequate stunning (Atkinson et al., 2012). A 90% carbon dioxide concentration for 100 seconds enables a stun-to-stick time of 40-50 seconds, whereas the equivalent figure for

an 80% carbon dioxide mixture is 25-35 seconds. The reason for this is that it takes longer for the brain activity to decline at 80% carbon dioxide compared with 90% carbon dioxide (Nowak et al., 2007). In general, carbon dioxide is considered to result in both good stunning effect and good meat quality (Llonch et al., 2012b).

In experiments, stunning with 90% carbon dioxide produced 100% successful stunning, probably because many pigs had already died from the carbon dioxide as they exited the butina (Atkinson et al., 2015). Paternoster systems have been shown in experiments to have a higher proportion of correctly stunned animals compared with dip-lift systems (99.9% compared with 98.2%) (Atkinson et al., 2012). Stunning using a relatively low carbon dioxide content (80%) for a relatively short time (70-100 seconds) has been shown to result in a large number of animals with signs of unsuccessful stunning (positive reflexes) (Nowak et al., 2007). Successful stunning was seen at 70 seconds of exposure to 90% carbon dioxide in that study. A higher concentration of carbon dioxide is also reported to reduce the number of muscle spasms in pigs (Llonch et al., 2012b).

Animal welfare aspects

Stunning using carbon dioxide allows group handling and minimum restraint of the animals prior to slaughter, which is seen as a positive animal welfare aspect, whereas the initiation of stunning is considered to be very aversive and therefore negative in terms of animal welfare (Table 5). With carbon dioxide stunning it is possible to handle and move pigs in groups, which makes driving easier and minimises stress prior to stunning. The negative effects of carbon dioxide concern the induction of stunning and could outweigh the benefits with their effect on stress prior to stunning (Grandin, 2003). Pigs show resistance to entering stunning boxes containing carbon dioxide (Atkinson et al., 2015) and may therefore also require harder driving.

The lungs of mammals contain chemoreceptors and irritant receptors that are acutely sensitive to carbon dioxide (Manning & Schwartzstein, 1995; EFSA, 2004). Inhalation of carbon dioxide is therefore aversive to pigs and causes respiratory distress prior to loss of consciousness at a mixture of over 30% in air (EFSA, 2004). Stunning with 90% carbon dioxide, which is currently used in commercial slaughter, is considered to be extremely unpleasant and causes suffering to pigs (Atkinson et al., 2015). The lack of oxygen also causes breathlessness, which is associated with stress (Atkinson et al., 2015). Grandin (2003) argues that carbon dioxide is unacceptable as a stunning method, as pigs try to escape even on first contact with the gas. However, there are individual differences in how pigs respond to carbon dioxide and genetic factors relating to responses to carbon dioxide require further investigation (Grandin, 2003; Atkinson et al., 2015). Among other things, it is likely that carriers of the halothane gene suffer more on inhalation of carbon dioxide compared with non-carriers (Velarde et al., 2007). Pigs that carry the halothane gene are not currently used in commercial production in Sweden.

Pigs try to avoid contact with high concentrations of carbon dioxide, which indicates an experience of distress or pain (Raj & Gregory, 1995; EFSA, 2004; Nowak et al., 2007; Velarde et al., 2007; Atkinson et al., 2015). The higher the level of carbon dioxide in the air, the more aversion and escape attempts are shown by the pigs, which is likely to be due to irritation of the mucous membranes in the nose increasing with increased carbon dioxide content (Raj & Gregory 1996; Velarde et al., 2007). Some researchers believe that the lower the carbon dioxide concentration, the less aversive it is and the less animal welfare is affected (Mota-Rojas, 2012). Other researchers believe that the intensity of the respiratory distress is no different regardless of carbon dioxide content (Raj & Gregory, 1996). Studies show that carbon dioxide becomes aversive at 15-30% in air, but pigs do not appear to be able to distinguish between 15% and 30% concentrations of carbon dioxide (Llonch et al., 2012a). From a concentration of 20% carbon dioxide onwards hyperventilation can be seen, the frequency of which increases with increased carbon dioxide concentration and exposure time (Raj & Gregory, 1996). Concentrations of up to 30% carbon dioxide have been assessed as tolerable for pigs, as they do not induce escape attempts or serious respiratory disturbance for the majority of pigs (Raj & Gregory, 1995, 1996). Exposure to higher concentrations of carbon dioxide is also associated with a number of behaviours, such as escape attempts, anxiety, vocalisation, sneezing, coughing, head shaking and gasping (Manning & Schwartzstein, 1995; Grandin, 2003; EFSA, 2004; Nowak et al., 2007; Atkinson et al., 2015). The pigs' eyes are also usually wide open, which can indicate fear (Atkinson et al., 2015). Escape attempts are considered to be an emotional response to fear or pain (Velarde et al., 2007). The higher the carbon dioxide content, the sooner the animals begin to gasp for breath and attempt to escape (Velarde et al., 2007).

At 90% concentration of carbon dioxide in the air, the majority of pigs withdraw from contact with the gas, even if offered treats (apples) in the carbon dioxide crate after 24 hours of fasting (Raj & Gregory, 1995). Pigs stunned using 80 or 90% carbon dioxide have 800-1000 times higher adrenalin and noradrenalin levels in their blood compared with calm pigs, which is a clear sign that they are experiencing stress (Nowak et al., 2007). Plasma cortisol levels (a physiological sign of stress) also increase after the pigs have been exposed to carbon dioxide (Kells et al., 2018). Compared with stunning with 90% carbon dioxide, pigs have higher lactate values

when stunned using 80% carbon dioxide, which indicates that the pigs experience more stress when stunned with the lower concentration of carbon dioxide, although the lactate values at 90% may still be 6- to 8-fold the normal level (Nowak et al., 2007). However, the differences between 80% and 90% carbon dioxide do not influence behaviour to such a degree that there is reason to believe that the two carbon dioxide concentrations differ very much from an animal welfare point of view (Verhoeven et al., 2016). At 80-90% carbon dioxide, however, some studies have concluded that the pigs do not have time to attempt to escape before LOP occurs (Raj & Gregory, 1996). Other studies show that pigs exposed to 90% carbon dioxide attempt to escape to a greater degree than pigs exposed to 70% carbon dioxide (Velarde et al., 2007). In stunning using 70-90% carbon dioxide, pigs exhibit escape attempts before LOP occurs, while pigs exposed to the lower concentration of carbon dioxide experience breathing difficulties for longer (Velarde et al., 2007). Together, these behaviours indicate that the survival instinct in pigs is triggered when they find themselves in the carbon dioxide-filled crate, which probably induces the highest level of fear and distress possible (Atkinson et al., 2015). The loud noise in the crate (>100 db) can also have a negative effect on the pigs (Atkinson et al., 2015).

Stunning with carbon dioxide is not immediate (Atkinson et al., 2015) and until loss of consciousness occurs the pigs are subjected to suffering (Raj & Gregory, 1996). In a paternoster system, the carbon dioxide level at the first stop is usually too low to induce loss of consciousness, but sufficiently high for the pigs to experience distress (Dalmau et al., 2010). The pig is subjected to discomfort and stress from the point at which it enters the crate until it loses consciousness, which in total can be up to 3 minutes and 39 seconds (Atkinson et al., 2015).

Table 5. Animal welfare aspects and hazards associated with carbon dioxide stunning

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|---------------------------------|-----------------------------------|
| Group handling | Very aversive on inhalation | Stunning is reversible |
| | Stunning is not direct | Individual difference in response |

Nitrogen

Nitrogen is not currently used commercially as a stunning method, but has been evaluated under non-commercial conditions (EFSA, 2004). Since nitrogen is present in high concentrations (80%) in the atmosphere, it is a cheap gas to produce (Llonch et al., 2012b; Bouwsema & Lines, 2019). Nitrogen gas is colourless and odourless. In contrast to carbon dioxide, however, the relative density of the gas is lower than that of air, which complicates the process of retaining the gas in enclosed spaces without it being replaced by oxygen, which would make the stunning process impossible (Dalmau et al., 2010; Llonch et al., 2012b; Bouwsema & Lines, 2019). Nitrogen can be used both on its own (in air) and in gas mixtures, for example with carbon dioxide. However, studies have revealed difficulties in retaining high (>94%) concentrations of pure nitrogen gas, which is why nitrogen is often mixed with other gases for stunning pigs (Dalmau et al., 2010; Atkinson et al., 2015). In order to avoid the nitrogen mixing with air, experiments have also been carried out on binding nitrogen gas in foam (Bouwsema & Lines, 2019; Pöhlmann, 2019; Lindahl et al., 2020). The foam prevents the nitrogen mixing with the rest of the air in the space by eliminating oxygen, and it eliminates oxygen on average 2.7-fold faster than pure gas (Lindahl et al., 2020).

Driving

As nitrogen is not currently used commercially, no studies have investigated driving to stunning. However, as it is a gas stunning method, it should enable pigs to be driven and stunned in a group and restraint and individual handling could be eliminated.

Induction of stunning

Nitrogen induces hypoxia at normal pressures, but can also induce anoxia where less than 2% oxygen remains in the air. Nitrogen is considered to be less aversive than high concentrations of carbon dioxide (Raj & Gregory, 1995; Dalmau et al., 2010b; Llonch et al., 2012a). Hypoxia is also considered to be non-aversive (Raj, 1999; Raj et al., 1997).

In stunning experiments using nitrogen in carbon dioxide (60% nitrogen, 20% carbon dioxide), the pigs showed clear signs of distress in the form of urinating and defecating, attempts to escape and gasping for air, among others (Atkinson et al., 2015). In general, the pigs exhibited struggling before collapse.

In experiments with nitrogen-filled foam, the foam itself has been found to induce investigative behaviour,

which indicates that neither the foam nor the nitrogen gas is aversive to the pigs (Lindhahl et al., 2020). However, when the foam starts to fill the stunning crate, the pigs try to avoid it and, among other things, exhibit slipping on the foam, gasping, attempts to escape and vocalisation (Pöhlmann, 2019; Lindahl et al., 2020). However, the function of nitrogen-filled foam has not been evaluated for group stunning of pigs (Bouwsema & Lines, 2019; Pöhlmann, 2019; Lindahl et al., 2020), and it is therefore difficult to assess its possible function under commercial conditions.

Duration, reliability and monitoring

In stunning experiments using nitrogen in carbon dioxide (60% nitrogen, 20% carbon dioxide), approximately 8% showed inadequate stunning with a stun-to-stick time of 1 minute 23 seconds, probably due to a combination of too short an exposure time and difficulty in achieving a maximum of 2% residual oxygen in the crate (Atkinson et al., 2015).

Stunning experiments using nitrogen foam with slaughter pigs (with 1% residual oxygen) have shown that a 3.5-minute exposure time after the pig is covered with foam does not provide adequate stunning, resulting in 22% of the pigs having to be stunned again (Pöhlmann, 2019). For pigs with a live weight of around 30 kg, LOP is induced after 57.9 seconds, and after a total exposure time to the gas of 5 minutes the pigs are either dead or deeply unconscious (Lindhahl et al., 2020).

Animal welfare aspects

Nitrogen is less aversive than carbon dioxide and has therefore been proposed as a good alternative for stunning (EFSA, 2004; Dalmau et al., 2010). The stunning method allows group handling, which is considered to be a positive aspect in terms of animal welfare, while the aversive nature of the gas is considered to be negative from an animal welfare point of view (Table 6). Stunning slaughter pigs with nitrogen in high-expansion foam does not cause high stress levels reflected in high levels of catecholamines or glucose in the blood, but the pigs exhibit behaviours such as gasping and attempts to escape, which indicates that the gas or the foam is perceived as aversive (Pöhlmann, 2018). Atkinson et al. (2015) concluded that more research is needed before nitrogen and carbon dioxide can be a potential alternative to carbon dioxide stunning, due to the risks of inadequate stunning. The relative aversiveness of the gas should also be investigated further, as there are contradictory results on how aversive it is.

Table 6. Animal welfare aspects and hazards with regard to stunning using nitrogen gas

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|---------------------------------|------------------------|
| Group handling | Somewhat aversive on inhalation | Difficult to handle |
| | Stunning is not direct | Stunning is reversible |

Argon

The inert gas argon has been proposed as an alternative to carbon dioxide stunning from an animal welfare perspective (Raj & Gregory, 1995; Brandt & Aaslyng, 2015; Kells et al., 2018). Argon is not currently used commercially as a stunning method for pigs, but has been evaluated under non-commercial conditions (EFSA, 2004). It is a stable, non-flammable and non-explosive gas that is odourless and tasteless (Raj & Gregory, 1995; Dalmau, 2010; Llonch et al., 2012a). Like carbon dioxide, argon is heavier than air and is therefore relatively easy to isolate in an enclosed space (Raj, 1999; Dalmau et al., 2010). Less than 0.01% of the atmosphere consists of argon, making the gas expensive to produce, which may affect its potential to be used commercially (Raj & Gregory, 1995; Dalmau et al., 2010; Llonch et al., 2012b; Bouwsema & Lines, 2019). Argon can be used both on its own (in air) and in gas mixtures, for example with carbon dioxide.

Driving

Since argon is not currently used commercially, no studies have investigated driving to stunning. However, since it is a gas stunning method, it should enable pigs to be driven and stunned in a group and restraint and individual handling could be eliminated.

Induction of stunning

Argon has anaesthetic properties under hyperbaric conditions (Raj, 1999). Stunning is induced by the argon inducing hypoxia, that is to say lack of oxygen (Kells et al., 2018). Argon has proven to be less aversive than carbon dioxide, but more aversive than air (Dalmau et al., 2010). Pigs do not find hypoxia aversive even at 90% concentration and do not avoid spaces containing argon (Raj & Gregory, 1995, 1996; Raj, 1996; EFSA, 2004). At 90% argon, LOP occurs after 10 seconds and is preceded only by investigative behaviour, which indicates that the pigs are not negatively affected by the gas (Raj & Gregory, 1995; Raj, 1999). However, physiological signs of stress, such as increased plasma cortisol levels, have been recorded in pigs exposed to argon (Kells et al., 2018). At 5 and 2% residual oxygen for a period of one minute, no LOP or escape attempts are seen and argon is considered to induce mild respiratory distress (Raj & Gregory, 1996).

Duration, reliability and monitoring

Stunning using argon does not last as long as stunning with carbon dioxide, which may result in the pig regaining consciousness if bleeding is not carried out sufficiently rapidly (Brandt & Aaslyng, 2015). On exposure to 90% argon for three minutes, pigs must be bled within 25 seconds in order to prevent them from regaining consciousness, as they are unconscious for less than 50 seconds (Raj, 1999; EFSA, 2014). After five minutes of exposure, pigs do not regain consciousness within 45 seconds (Raj, 1999). On exposure to 90% argon for seven minutes, the majority of pigs die (Raj, 1999).

Animal welfare aspects

In theory, stunning using argon should allow group handling, which is seen as positive from an animal welfare point of view, whereas the aversive nature of the gas is seen as negative (Table 7). As things stand, however, the gas is difficult to handle, which is deemed to be a welfare hazard. Argon is considered to be more aversive than air, but less aversive than carbon dioxide. Pure argon causes stress prior to inducing loss of consciousness (Kells et al., 2018). Since the period of unconsciousness is shorter than in stunning using carbon dioxide, there is a risk of a negative impact on animal welfare unless the stun-to-stick time is short (EFSA, 2004).

Table 7. Animal welfare aspects and hazards with regard to stunning using argon

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|---------------------------------|------------------------|
| Group handling | Stunning is not direct | Difficult to handle |
| | Somewhat aversive | Stunning is reversible |
| | | Short-lasting effect |

Helium

The inert gas helium has anaesthetic properties on inhalation and has therefore been proposed as an alternative to carbon dioxide stunning from an animal welfare perspective (Machtolf et al., 2013). Helium is lighter than air. Helium is not currently used commercially as a stunning method, but has been evaluated under non-commercial conditions (Machtolf et al., 2013). Mixtures of helium and nitrogen have also been proposed as an alternative to carbon dioxide that is beneficial from an animal welfare perspective, but they have not been evaluated. Helium stunning is deemed to be expensive, however (Steiner et al., 2019).

Driving

Since helium is not currently used commercially, no studies have investigated driving to stunning. However, since it is a gas stunning method, it should enable pigs to be driven and stunned in a group and restraint and individual handling could be eliminated. As helium is lighter than air, pigs in experimental conditions been stunned individually through driving them into a cage enveloped in a balloon containing helium (Machtolf et al., 2013).

Induction of stunning

Helium induces anaesthesia through hypoxia (lack of oxygen) on stunning using a 98.5% mixture (Machtolf et al., 2013). Helium is less aversive than carbon dioxide and pigs stunned in helium do not exhibit escape attempts or other signs that the gas is experienced as aversive (Machtolf et al., 2013). On stunning with 98.5% helium LOP occurs after 20 seconds, compared with 16 seconds when stunning with 90% carbon dioxide. Compared with carbon dioxide stunning, pigs also show significantly lower levels of adrenalin and noradrenalin, which indicates that they experience less stress with helium stunning (Machtolf et al., 2013).

Duration, reliability and monitoring

Individual stunning with 98.5% helium for 180 seconds induces a good level of stunning that is sustained, with a 15- to 30-second stun-to-stick time (Machtolf et al., 2013).

Animal welfare aspects

In theory, stunning using helium should allow group handling, which is seen as positive from an animal welfare perspective, and the gas is also not shown to be aversive (Table 8). Since helium and other inert gases rarely or never react with other molecules, they therefore do not induce painful or unpleasant reactions with chemoreceptors in the body (Machtolf et al., 2013). In comparison with carbon dioxide, which induces a high level of vocalisation, attempts to escape and breathlessness, helium stunning does not induce these behaviours (Machtolf et al., 2013). Very few studies have been performed on helium stunning, but it has been deemed by one research group to be a possible alternative to carbon dioxide purely from an animal welfare perspective (Machtolf et al., 2013). No studies have been carried out under commercial conditions, however.

Table 8. Animal welfare aspects and hazards with regard to stunning using helium

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|---------------------------------|------------------------|
| Not aversive | Stunning is not direct | Difficult to handle |
| Group handling | | Stunning is reversible |

Xenon

Xenon is an inert gas that has anaesthetic properties and induces hypoxia at normal pressure, but the mechanisms are still not fully understood (Raj, 1999; Baumert, 2009). The density of xenon is 4.5 times greater than that of air and it has inert properties (Baumert, 2009). Many of the positive properties assigned to xenon are associated with recovery after stunning or reduced effect on the nervous system (Baumert, 2009). There are no studies investigating the stunning of pigs in the context of slaughter. Xenon stunning is deemed to be expensive, however (Steiner et al., 2019).

Driving

Since xenon is not currently used commercially and has not been studied in relation to slaughter, there have been no studies on driving to stunning. However, as it is a gas stunning method, it should enable the pigs to be driven and stunned in groups and eliminate the need for restraint and individual handling.

Induction of stunning

There have been no studies relating to stunning using xenon on pigs prior to slaughter. In general, proposed

benefits of stunning with xenon are its rapid induction and that it is considered to be a safe and well-tolerated gas (Baumert, 2009).

Duration, reliability and monitoring

There have been no studies relating to stunning using xenon on pigs prior to slaughter. The stunning effect from xenon has in general been proven not to last as long as that of many other stunning methods (Baumert, 2009).

Animal welfare aspects

Since no studies have investigated stunning using xenon on pigs prior to slaughter, animal welfare has not been investigated either. Xenon is an inert noble gas, so it can be assumed that it will not react with its surroundings or, for example, interact with chemoreceptors that can induce aversion, which is positive from an animal welfare perspective (Table 9).

Table 9. Animal welfare aspects and hazards with regard to stunning using xenon

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|---------------------------------|------------------------|
| Rapid induction | Stunning is not direct? | Stunning is reversible |
| Not aversive? | | |

Low atmospheric pressure stunning (LAPS)

With low atmospheric pressure stunning (LAPS), stunning is brought about by inducing hypoxia as a result of low atmospheric pressure (Mackie & McKeegan, 2016). LAPS is not currently used commercially as a stunning method in pigs, but has been partly evaluated under non-commercial conditions, both in grower pigs and slaughter weight pigs (Martin et al., 2020; McKeegan et al., 2020). LAPS is currently permitted as a stunning method for broiler chickens. LAPS is considered equivalent to stunning with inert gases for broilers, but due to the physiological differences between birds and mammals it is not possible to draw conclusions for pigs based on results from studies on broiler chickens (McKeegan et al., 2020).

Driving

Since LAPS is not currently used commercially, no studies have investigated driving to stunning. The intention, however, is to allow the pigs to be driven in groups without the need for restraint. Bouwsema & Lines (2019) speculate that LAPS could enable stunning in larger groups of 15-30 pigs. As a result, pigs could possibly be stunned in the same group in which they were transported to the slaughterhouse, which would therefore minimise the mixing of animals and its consequences.

Induction of stunning

Stunning is induced by successively reducing the ambient pressure until the partial pressure of oxygen in the atmosphere is no longer sufficient to oxygenate the brain and hypoxia is induced (Bouwsema & Lines, 2019). In humans, hypoxia induces euphoria and reduces the level of consciousness, which is why LAPS has been suggested to be less stressful compared with the current commercial stunning methods (e.g. carbon dioxide or electrical stunning) (Bouwsema & Lines, 2019). However, there is a lack of research on how the reduction in ambient pressure affects humans and animals, and research on animals has mainly been performed using rats and chickens, with a complete absence of studies on slaughter pigs (Bouwsema & Lines, 2019). Results from studies on both growers and finishers show that pigs suffer negative experiences of LAPS, as indicated by head shakes, head tilts and grimaces and escaping as the stunning is induced (McKeegan et al., 2020). Provision of anti-anxiety medication can reduce signs of respiratory stress and analgesic treatment can reduce signs of pain, indicating that these emotions are induced by the stunning method (Martin et al., 2020; McKeegan et al., 2020). The pain is believed to be caused by biotrauma, for example in the ears of the pigs as indicated by ruptured ear drums, although this has not been completely clarified (McKeegan et al., 2020). Further, LAPS stunning can result in incidences of severe haemorrhage in slaughter pigs, which would lead to condemnation of the carcass at meat inspection in the abattoir (McKeegan et al., 2020). Bouwsema & Lines (2019) estimate that stunning of slaughter pigs would take seven minutes at an oxygen content of 2%, which is achieved at a pressure of 16.5 kPa. One stunning cycle would therefore take around 15 minutes, including loading and unloading. At a pressure of approximately 10 kPa, which is used for poultry, the time could potentially be shortened to around 9.5 minutes. According to studies by Martin et al. (2020) on growers, LOP is reached at 118.4±1.8 seconds. The possibility to refine the time of the procedure has been determined to be limited, however, as slower induction would lead to prolonged pain and distress because it would take longer for the pigs to become unconscious (McKeegan et al., 2020). Faster induction, on the other hand, would intensify the perceived pain from the

bio-trauma and increase the amount of general pathological concerns.

Duration, reliability and monitoring

The practical studies carried out for LAPS on pigs at slaughter weight have not reported findings regarding the duration or reliability of the method, so it is difficult to draw conclusions.

Animal welfare aspects

In the studies conducted to date, LAPS is not considered a viable stunning method for pigs based on findings that it causes stress and pain during induction (Martin et al., 2020; McKeegan et al., 2020) (Table 10). During the relatively slow induction, pigs are reported to show signs of stress, from a protracted period of ataxia and disorientation, and pain, as indicated by head shakes, head tilts and face grimaces (Martin et al., 2020; McKeegan et al., 2020). Air hunger is an unpleasant and increasingly acute feeling that humans know from holding their breath for a long time (Bouwsema & Lines, 2019). Air hunger is relevant in relation to controlled atmosphere stunning methods, as it can occur when the proportion of oxygen in the air is reduced (Bouwsema & Lines, 2019), as indicated by results reported by McKeegan et al. (2020). Pathological findings also indicate that pigs suffer rupture of the ear drums, which if caused during consciousness is likely to be very painful (McKeegan et al., 2020). In theory, stunning using LAPS should allow group handling, which is seen as positive from an animal welfare point of view.

It has also been suggested that any pig suffering from upper respiratory tract problems, tooth decay or excess gas in the alimentary canal may experience pain under LAPS, due to the low pressure (Bouwsema & Lines, 2019). Respiratory tract diseases, such as pleurisy (inflammation of the pleura) or pneumonia (lung inflammation), are among the most common health conditions observed at slaughter in Swedish pig slaughterhouses. A herd can have many signs of disease at the slaughterhouse without there being any clinical disease present in the herd (Ehlorsson et al., 2016), which suggests that it is not possible with certainty to exclude pigs with respiratory tract problems prior to slaughter. In addition, semi-closed orifices, such as ears and lungs, can be affected by pressure changes during stunning, in roughly the same way that humans can suffer blocked ears during aircraft landings. However, since the pigs are stunned when they return to normal pressure, the remaining pressure changes are not expected to have a negative effect on pig welfare. In humans, aeroembolism can also occur with rapid pressure changes. However, for the same reason as before, this is not considered to be a problem, as the pigs will be unconscious on the return to normal pressure (Bouwsema & Lines, 2019).

Table 10. Animal welfare aspects and hazards with regard to using low atmospheric pressure stunning (LAPS)

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|---------------------------------|---------------------------|
| Group handling | Stunning is not direct | Difficult to handle |
| Stunning not reversible | Slow Induction | Health status of the pigs |
| | Aversive | |
| | Painful | |

Gas mixtures

Due to the fact that stunning using carbon dioxide, the gas currently used commercially, is believed to have a negative effect on animal welfare because it is considered to be aversive at concentrations above 30%, experiments have been carried out with different types of gases mixed with carbon dioxide. The intention with mixing in other gases is to increase the stunning effect without increasing the aversive nature of the gas mixture (Raj & Gregory, 1995; Raj, 1996; EFSA, 2004). The stunning effect of carbon dioxide is dependent on the carbon dioxide concentration: the lower the carbon dioxide concentration, the longer it takes before stunning is initiated and the longer the exposure required for the stunning to be lasting. In order to increase the stunning effect, therefore, attempts have been made to replace carbon dioxide with other anaesthetising gases and thereby reduce the proportion of carbon dioxide and the aversive effect that it has. The animal welfare aspects of different carbon dioxide mixtures are summarised in Table 11.

No gas mixture is currently commercially available and none has been evaluated with regard to driving prior to stunning, but driving to stunning would presumably work in the same way as in carbon dioxide stunning. Induction of stunning would proceed as described previously for the individual gases and therefore, in theory, would not involve individual handling or restraint.

Table 11. Animal welfare aspects and hazards associated with gas mixtures

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|---------------------------------|------------------------------------|
| All gas mixtures | Aversive | Quantity of carbon dioxide |
| Group handling | Stunning is not direct | Stunning is reversible |
| | | Individual difference in response |
| | | Difficulties in filling the system |

Nitrogen and carbon dioxide

It has been suggested that stunning using a mixture of nitrogen and carbon dioxide reduces aversion if the carbon dioxide content is less than 30% (Llonch et al., 2012a). Previous studies have indicated difficulties in filling existing commercial systems with the gas mixture within a reasonable time frame and suggest that the existing paternoster system should be fitted with a more powerful pumping system, a measuring system and a heater, in order to be able to maintain the right conditions for the gas mixture (Atkinson et al., 2015).

Experiments with different compositions of gas (70% nitrogen and 30% carbon dioxide; 80% nitrogen and 20% carbon dioxide; 85% nitrogen and 15% carbon dioxide) have shown that the gas mixture is more aversive than air, but that the aversion is less than that seen with high concentrations of carbon dioxide (Dalmau et al., 2010; Llonch et al., 2012a). The higher the carbon dioxide content, the more unwilling pigs are to enter a space containing the gas mixture (Dalmau et al., 2010). In studies involving repeated visits to a gas-filled box, attempts to escape have been found to increase with the number of visits (Llonch et al., 2010). The different gas mixtures did not affect the time of the first attempt to escape in that study, but gasping increased with increasing carbon dioxide concentration. The time to LOP was not affected by the gas mixture, but was longer for those animals that did not enter the box willingly. The proportion of animals that had spasms and muscle contractions was lower at 80% nitrogen compared with the other mixtures (Llonch et al., 2010).

When stunned with 80% nitrogen and 20% carbon dioxide, pigs show signs that they are feeling the effects of the lack of oxygen (champing with open mouth, attempts to avoid the gas, aggressive body movements) (Atkinson et al., 2015). They are also described as having their eyes wide open, which may indicate fear (Atkinson et al., 2015). Together, these behaviours indicate that the pig's survival instinct is triggered to the maximum capacity, which probably induces the highest level of fear and distress possible (Atkinson et al., 2015). Compared with pigs stunned using 90% carbon dioxide, pigs stunned with 80% nitrogen and 20% carbon dioxide demonstrate violent struggling behaviour after LOP, so stunning with this gas mixture is therefore deemed to be extremely unpleasant (Atkinson et al., 2015). Pigs show individual variations in their responses to gas mixtures. Compared with carbon dioxide, the noise level in the crate is lower when a nitrogen and carbon dioxide mixture is used (80% nitrogen, 20% carbon dioxide) compared with carbon dioxide alone (Atkinson et al., 2015). With a stun-to-stick time of a maximum of 141 seconds, 92.4% of pigs in the study by Atkinson et al. (2015) were adequately stunned, with inadequately stunned pigs showing corneal reflex (4.8%), corneal reflex and regular gasping (2.9%) or regular gasping (2.4%) at bleeding. Some of the problems (6.2%) with the stunning were reported to be due to oxygen levels above 2%, while others were probably a result of too short an exposure time. After the crate had been sprayed with warm water, the number of inadequately stunned pigs decreased (Atkinson et al., 2015).

Argon and carbon dioxide

Mixtures of carbon dioxide and argon have been proposed for stunning pigs (Raj & Gregory, 1995, 1996; Raj, 1996), as this may be quicker than stunning with argon alone. The carbon dioxide concentration in this case is so low that it does not become aversive (<30%), which therefore reduces stress compared with carbon dioxide stunning (Grandin, 2003; Dalmau et al., 2010).

When stunning with 30% carbon dioxide and 60% argon, pigs must be bled within 25 seconds to prevent them regaining consciousness after three minutes of exposure to the gas (Raj, 1999). In experiments with five minutes of exposure (30% argon, 60% carbon dioxide), pigs did not regain consciousness within a 45-second stun-to-stick time, whereas seven minutes of exposure resulted in the death of the pigs (Raj, 1999).

A mixture of 60% argon and 40% carbon dioxide is considered to cause stress prior to loss of consciousness

(Kells et al., 2018). On exposure to gas mixtures of 30% carbon dioxide and 60% argon, pigs in one study displayed hyperventilation (Raj, 1999). At 30% carbon dioxide in argon with 2 or 5% residual oxygen in another study, no escape attempts were seen during the first minute (Raj & Gregory, 1996), which indicates that the gas mixture is not aversive.

Electrical stunning

Electrical stunning is one of the most common methods of stunning pigs at slaughter throughout the world and within the EU (EFSA, 2004; Dalmau et al., 2010; Llonch et al., 2010). There are essentially two types of electrical stunning methods: head-only, which induces stunning, and head-to-body, which both stuns and kills the animal (EFSA, 2004). The aim of electrical stunning is to induce an epileptiform seizure (grand mal) by passing an electrical current through the animal's head and causing loss of consciousness (McKinstry & Anil, 2004). The extent of the seizure is important for whether or not the animal loses consciousness. The electrodes must therefore be positioned so that the organs (brain or heart and brain) that are to be affected are in between the electrodes conducting the electrical current. Effective stunning occurs when an electric current of sufficient strength passes through the brain. The amount of electric current required depends, among other things, on the animal's size and shape and how clean the equipment is (EFSA, 2004). It is important that the electrodes are in good contact with the animal in order for the strength of the current to be sufficient to induce loss of consciousness and to minimise the risk of, for example, burns to the animal's skin.

The epileptic seizure that causes stunning comprises three phases: the tonic, clonic and recovery phase (McKinstry & Anil, 2004). In electrical stunning, the animal should collapse immediately and initially display rigidity (tonic seizure) and then muscle spasms (clonic seizure), followed by cessation of normal respiration, eyes becoming unfocused and pupils becoming strongly dilated. Exactly how the mechanisms of brain function operate during application of electric current to the head is not fully understood (EFSA, 2004).

Prior to electrical stunning using a manual tong, it is often necessary to handle the pig individually and to restrain it, which are stressors in themselves (EFSA, 2004; Brandt & Aaslyng, 2015). If the animal is restrained (which causes stress), the tong can also be placed automatically. The animal can be restrained by a belt restrainer, in which the pig is hung on a belt that restrains it and moves it along, and this can then be combined with automatic application of electrodes (EFSA, 2004). However, belt restrainers (V-restrainers or centre-track restrainers) are not permitted under Swedish animal welfare legislation, as excessive coercion and use of electric prods are often necessary to make the pigs enter such a device. Automatic placement is made more difficult by individual anatomical differences between pigs and stunning is therefore less precise, which can affect the induction of stunning (EFSA, 2004). If the animals are not handled individually at the initiation of stunning, there is a risk that the electrodes will also brush against animals other than the individual being stunned, resulting in painful shocks without stunning. Electric shocks can also cause stress and discomfort on handling and application of the electric tong (Bouwsema & Lines, 2019). In an American study, 19 in 20 slaughterhouses used correctly placed electrical stunning on 99% of pigs (Grandin, 2003). A later study in the same country showed that the electrodes were placed correctly on 100% of pigs in the slaughterhouses investigated, irrespective of whether the method used was head-only or head-to-body stunning (Grandin, 2012). In these cases, however, a V-shaped restrainer was used, which prevents the pigs from moving. Such restrainers are not permitted in Sweden. In Sweden, electrical stunning is carried out at only smaller slaughterhouses and without the same option for firm restraint.

The quality of the stun in electrical stunning can be difficult to assess and monitor. If the electrodes are incorrectly placed, there is a risk that the electric current will not pass through the brain, which will result in the animal being immobilised without being stunned. A similar result can be obtained if the stunning is carried out using a current strength that is too low. Immobilisation may mean that the animal is wrongly assessed to be correctly stunned. Despite legislation requiring electrical stunning to produce immediate insensibility, it is common for electrical stunning to need to be applied several times, due to a stunned animal falling before sticking or a conscious pig landing on the floor instead of on the belt (McKinstry & Anil, 2004). If the electrodes are not sufficiently clean, the current may be conducted less well through the electrodes due to increased resistance, which can result in inadequate stunning. Dirty electrodes can also cause heat to develop, which can cause pain at placement. In order to improve contact and reduce electrical resistance, either the area of application or the electrodes should be wetted. Incorrectly placed electrodes can also cause inadequate stunning and pain and result in poor animal welfare (EFSA, 2004). The stunning apparatus should show what voltage and current strength is given to the animal at each phase. Good electrical contact is required between the electrodes and the body and the equipment needs to be maintained. Among other things, the electrodes must be clean in order to enable flow. The equipment requires maintenance and must be calibrated (EFSA, 2004). Monitoring parameters for correct stunning are given in Table 12. Three hazards linked to electrical stunning at slaughterhouses have been identified: inappropriate stunning parameters, wrong positioning of the tongs and poor electrical contact (EFSA, 2019). One of the advantages of electrical stunning is that induction of unconsciousness is direct, while a disadvantage is that it often requires individual handling and restraint of the animal (EFSA, 2004).

Table 12. Checks and indicators for monitoring of stunning using electricity (Algers et al., 2012)

| Normal checks | Additional indicators |
|---|-------------------------------------|
| Immediate collapse in tonic seizure, followed by clonic seizure | Relaxed muscles in neck/throat |
| Absence of normal respiration | No sign of righting |
| Open eyes | No corneal reflex |
| Strongly dilated pupils | Absence of response to pain stimuli |

Electrical head-only stunning

Electrical stunning applied to the head produces a short period of unconsciousness and tonic-clonic seizures (EFSA, 2004).

Driving

Stunning often requires restraint and individual handling and driving. In automatic stunning, the pig must be restrained in order to enable correct application of the electrodes (EFSA, 2004). Automatic restrainer systems are not permitted in Sweden, however. Firm restraint enables automatic application of the electrodes. Manual application of the electrodes allows them to be applied without firm restraint and in groups. However, application in groups poses the risk of the electrodes brushing against other pigs and causing pain without inducing stunning.

Induction of stunning

Electrical head-only stunning has been shown to result in metabolic acidosis (accumulation of acidic metabolites in the blood), hypocapnia (abnormally low levels of carbon dioxide in the blood), hypernatraemia (abnormally high levels of sodium in the blood), hyperglycaemia (abnormally high blood sugar levels), hyperphosphataemia (abnormally low levels of phosphates in the blood), lactic acidosis (acidosis caused by lactic acid in the blood) and increased haematocrit (increased proportion of red blood cells) (Becerril-Herrera et al., 2009).

In head-only application, the current passes through the head and through the brain (EFSA, 2004). Voltage and amperage determine how quickly the animal loses consciousness (EFSA, 2004). The amperage must be sufficiently high to pass through all tissues in order to be able to induce loss of consciousness. The positioning of the electrodes must ensure that the electric current passes through the brain and must be adjusted to the individual pig's size, weight and skull porosity, density, thickness, fatness and coat hair density (EFSA, 2004). In electrical stunning, electrodes are placed on either side of the head, and a current strength of 1.3 A with at least 240 V is recommended (Raj & Gregory, 1995; EFSA, 2004). It is important that the electrodes have contact during the entire stunning process in order for it to work correctly (EFSA, 2004). Inadequate maintenance or contact with the head (e.g. due to dirt) can also cause damage to the skin (EFSA, 2004). The best placement of the electrodes is between the eyes and the base of the ears, but this position is difficult to achieve in all pigs and therefore the electrodes are often placed at the base of the ears or on the forehead/lower mandible (Anil & McKinstry, 1998). The latter position, vertically, is not recommended (Anil & McKinstry, 1998).

Duration, reliability and monitoring

Electrical head-only stunning is reversible and the pigs must therefore be bled before reaching the clonic phase (Grandin, 2003). The stun-to-stick time is related to the frequency and wavelength of the electric current and therefore the duration and depth of the stun, but what constitutes an appropriate stun-to-stick time under all conditions is not known (EFSA, 2004). The duration of the stun cannot be extended by increasing the exposure time. The stun-to-stick time should not be longer than 15 seconds and should be shorter (approximately 6 seconds) under high-frequency low-voltage stunning (Anil, 1991; Anil & McKinstry, 1992; EFSA, 2004; Vogel et al., 2011).

After stunning, signs of consciousness must be monitored at three points during the slaughter process: directly after stunning, during sticking and during bleeding (EFSA, 2013). Pigs that have been stunned should collapse immediately and show signs of tonic seizure and apnoea. After the tonic seizure, the clonic seizure starts, which produces a gradual relaxation of the body (EFSA, 2004). Pigs that have not been adequately stunned do not have a tonic or clonic seizure and may display rhythmic breathing, focused eye movements, constricted pupils, vocalisation during stunning and presence of reflexes (EFSA, 2004). Pigs that have been inadequately stunned

may be immobilised, which can be mistaken for stunning (EFSA, 2004). EFSA (2013) recommends particular monitoring points at the different monitoring times during the slaughter process. After stunning, tonic and clonic seizures should be demonstrated, along with absence of breathing and corneal reflex. In addition, signs such as spontaneous blinking, righting reflex and vocalisations may be used. During sticking, absence of breathing, tonic and clonic seizures and muscle relaxation should be demonstrated. Signs such as corneal reflex, spontaneous blinking and vocalisations may also be used. At bleeding, absence of breathing and muscle relaxation should be evident. Vocalisation, corneal reflex and spontaneous blinking may be used as signs of consciousness (EFSA, 2013).

Table 13. Animal welfare aspects and hazards in connection with electrical head-only stunning

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|--------------------------------------|--|
| Direct stunning | Individual handling | Stunning is reversible |
| Pain-free if stunned correctly | Restraint | Incorrect assessment of stunning |
| | Not pain-free if incorrectly stunned | Involuntary shocks from the electrodes |
| | | Short period of unconsciousness |

Animal welfare aspects

Electrical head-only stunning provides a pain-free, directly induced stun if carried out correctly, which is positive from an animal welfare perspective (Table 13). However, individual handling and firm restraint of the animals are often required, which is a negative aspect from an animal welfare point of view. Electrical head-only stunning induces loss of consciousness for a relatively short time (approximately 15 seconds), which can lead to bleeding not always taking place within that short time frame (Vogel et al., 2011). In experiments, electric tongs have been demonstrated to be very aversive in cases where they do not induce immediate loss of consciousness and may therefore constitute an animal welfare problem (Jongman et al., 2000).

Repeated stunning with electrical head-only stunning has been deemed acceptable if absolutely necessary to ensure that pigs do not regain consciousness (McKinstry & Anil, 2004). Regaining consciousness after stunning is deemed to be an animal welfare problem. Re-stunning (if necessary) should be carried out within 60 seconds from the first stun and should be followed by bleeding within 15 seconds.

Electrical head-to-body or head-to-back stun/killing – one-cycle method

Electrical head-to-body stunning was developed during the 1920s, but did not start to be used commercially until the 1980s. However, many slaughterhouses have phased out the method, as it involves breaking the spine, which is not good from a production point of view (Gregory, 1994). The method is not used in Sweden.

Driving

Electric head-to-body stunning usually requires the pigs to be restrained and handled individually. In automatic systems, such as V-shaped and belt restrainers, the pigs are required to be restrained one by one, so that the electrodes can be applied properly (EFSA, 2020). Automatic restrainer systems are not permitted in Sweden. Manual application of the electrodes allows them to be applied without firm restraint and in groups. However, application in groups poses the risk of the electrodes brushing against other pigs and causing pain without inducing stunning.

Induction of stunning

Stunning is usually induced with tongs or electrodes placed on either side of the animal's head (over the animal's brain) and on the body (over the animal's heart), so that the electric current passes through both the brain and the heart (EFSA, 2004). Stunning is caused by the current passing through the head and behind the heart or across the chest simultaneously, which passes electricity throughout the whole body (EFSA, 2004).

The minimum current strength required is 1.3 A and this must be applied for at least one second to induce loss of consciousness. At least 240 V are required to achieve this current strength for the shortest time possible. Good electrical contact is required between the electrodes and the body (EFSA, 2004).

Duration, reliability and monitoring

Electrical head-to-body stunning is irreversible. The electric current cycles induce cardiac arrest, which

produces rapid loss of brain function and prevents the animal from regaining consciousness. The method is therefore not dependent on the animal being bled within a short time after stunning is induced (Gregory, 1994).

If stunning is performed correctly, the pigs should collapse immediately, followed by a tonic and then clonic seizure. The clonic seizure can sometimes be absent or very weak. Corneal reflex and gasping may occur, but should cease quickly. In addition, pupils should be strongly dilated and the body should be relaxed (EFSA, 2004). Incorrect stunning is indicated by recovery of rhythmic breathing and convulsions during sticking (EFSA, 2004).

Table 14. Animal welfare aspects and hazards in connection with electrical head-to-body stunning – one-cycle method

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|--------------------------------------|--|
| Direct stunning/death | Individual handling | Correct assessment of stunning |
| Pain-free if stunned correctly | Restraint | Involuntary shocks from the electrodes |
| Irreversible stunning | Not pain-free if incorrectly stunned | |

Animal welfare aspects

This stunning method provides a pain-free, directly induced irreversible stun if carried out correctly, which is positive from an animal welfare perspective (Table 14). However, the method often requires firm restraint and individual handling, which is negative from an animal welfare perspective. The equipment requires maintenance and must be calibrated (EFSA, 2004). In order to establish good contact between skin and electrodes, it is necessary for the electrodes to be clean. If contact is insufficient there is a risk that the stun will not be induced correctly and that the introduction of the electric current will cause injury. In experiments, electric tongs have been demonstrated to be very aversive in cases where they give a shock without stunning the animal and may therefore constitute an animal welfare problem (Jongman et al., 2000).

Electrical head-to-body or head-to-back stun/killing – two-cycle method

In the two-cycle method, a current is first passed through the brain, as in head-only stunning. This is immediately followed by a current from the head to the body or across the chest, in order to induce cardiac ventricular fibrillation. This is carried out in two stages, instead of the single stage in the one-cycle method. Apart from the stunning taking place in two stages, the method works in the same way as the one-cycle method. This type of two-stage stunning is not currently permitted at slaughter in Sweden, but with a permit from the regulatory authority it may be used in on-farm depopulation.

Driving

Electric head-to-body stunning requires the pigs to be restrained and handled individually. In automatic systems, such as V-shaped and belt restrainers, the pigs are required to be restrained one by one, so that the electrodes can be applied properly (EFSA, 2020). Automatic restrainer systems are not permitted in Sweden. Manual application of the electrodes allows them to be applied without firm restraint and in groups. However, application in groups poses the risk of the electrodes brushing against other pigs and causing pain without inducing stunning.

Induction of stunning

Stunning is induced with two separate electric current cycles (EFSA, 2004). The current first passes through the brain for stunning and then through the heart to induce cardiac arrest, which also results in death (EFSA, 2004; Gregory, 1994). The front electrode must be placed on the forehead and the cardiac ventricular fibrillation electrode must be placed on either side of the heart. The minimum current strength required is 1.3 A and this must be applied for at least one second to induce unconsciousness. At least 240 V are required to achieve this current strength for the shortest time possible and a current strength of at least 1.0 A at 50 Hz AC (sine wave) is required to induce cardiac ventricular fibrillation. The current must be applied for at least three seconds. The second cycle must be applied as quickly as possible (within 15 seconds) after the first cycle.

Duration, reliability and monitoring

Electrical head-to-body/back stunning is irreversible. The electric current cycles induce cardiac arrest, which produces immediate loss of brain function and prevents the animal regaining consciousness. It is therefore not

dependent on the animal being bled within a short period of stunning being induced (Gregory, 1994). If stunning is performed correctly, the pig should collapse immediately, followed by a tonic seizure. There is also a gradual relaxation of the body and the pupils dilate. If stunning is performed incorrectly, rhythmic breathing returns and convulsions occur during sticking.

Table 15. Animal welfare aspects and hazards in connection with electrical head-to-body stunning – two-cycle method

| Positive animal welfare aspects | Negative animal welfare aspects | Welfare hazards |
|---------------------------------|--------------------------------------|---|
| Direct stunning/death | Individual handling | Correct assessment of stunning |
| Pain-free if stunned correctly | Restraint | Involuntary shocks from the electrodes |
| Irreversible stunning | Not pain-free if incorrectly stunned | Risk of inducing cardiac arrest in conscious animals, if first cycle is insufficient and second cycle is across the chest |

Animal welfare aspects

Electrical head-to-body/back stunning has the potential to provide a pain-free, directly induced irreversible stun if carried out correctly, which is positive from an animal welfare perspective (Table 15). However, the method often requires firm restraint and individual handling, which is negative from an animal welfare perspective. The equipment requires maintenance and must be calibrated (EFSA, 2004). In order to establish good contact between skin and electrodes, it is necessary for the electrodes to be clean. If contact is insufficient there is a risk that the stun will not be induced correctly and that the introduction of the electric current will cause injury. In experiments, electric tongs have been demonstrated to be very aversive in cases where they give a shock without stunning the animal, and may therefore constitute an animal welfare problem (Jongman et al., 2000). If proper stun quality is not achieved during the first (head-only) cycle, there is an obvious risk of cardiac arrest being induced on a conscious animal during the second cycle, especially if the second cycle does not involve the head.

Discussion

When evaluating animal welfare associated with a stunning method, the whole system should be considered (Grandin, 2003). Therefore, all parts of the slaughter process that affect the live animal and which may be affected by that particular stunning method must be taken into account when systems and methods are evaluated and must be weighed against each other.

Table 16 summarises the function of the different stunning methods described in this report. The data presented include initiation and duration of the stun, whether the stunning method also kills the animal and whether it is in commercial use. The animal welfare comparisons that can be made are fully dependent on available published literature. Those methods that have been used commercially for a long time, for example carbon dioxide stunning and electrical stunning, are better documented than methods that are not used commercially. This means that there are more supporting data on which to base considerations concerning animal welfare for the established methods. Newer or less well-documented stunning methods may need more research before definite conclusions regarding their impact on animal welfare can be drawn.

Table 17 presents relative animal welfare comparisons for the different methods, based on available relevant literature, with regard to driving, handling and restraint, risk of unsuccessful stunning, the experience of stunning and reliability of the stun. The relative significance of different types of stress and how these should be recorded and assessed in relation to different aspects of animal welfare are not entirely clear (Brandt & Aaslyng, 2015), which complicates comparisons of different stunning methods with respect to animal welfare. Different aspects can also affect animal welfare in different ways, which makes an overall animal welfare rating impossible. For example, it has been suggested that very stressful handling prior to slaughter cannot be compensated for by minimal stress in induction of stunning (EFSA, 2004). An overall ranking of the methods is therefore not included in the comparisons in Table 17.

Scientific basis

The specific details used in the animal welfare assessments in this report are based on scientific data, which means that when the data are limited, the animal welfare assessment is also less precise. A greater amount of data exists for more established stunning methods and therefore the animal welfare assessment could be more specific and certain. For example, there is a great deal of scientific data for carbon dioxide, enabling more detailed assessment. For many of the methods that have been tested but not practised on a commercial scale, much of the information required to assess their effect on animal welfare is lacking (Table 17). Many of the experiments that have been carried out have been on a small scale, and they also often lack a description or evaluation of, for example, driving methods and how they would work in a commercial setting, despite it being well known that this can affect welfare. It is also well known that different individuals respond differently to different stunning methods depending on individual differences in, for example, metabolism, respiration, handling of stress and genotype, which in turn may affect the impact of different gases on individual pigs (Velarde et al., 2007; Atkinson et al., 2015). It has been shown, for example, that the pig which reacts first to a particular gas mixture (80% nitrogen and 20% carbon dioxide or 90% carbon dioxide) is often the last one to achieve LOP (Atkinson et al., 2015).

Where non-commercial methods are concerned, there is sometimes a lack of experiments on slaughter pigs (live weight around 115 kg). In order to be certain of the effect on the welfare of slaughter animals under commercial slaughter conditions, experiments on these particular animals under similar conditions to commercial slaughter are required. In certain respects, there is no reason to assume that larger or adult pigs would respond any differently than younger pigs. However, a study by Kells et al. (2018) showed that 17-day-old pigs respond more negatively to carbon dioxide stunning than to stunning with argon. These results are confirmed by the majority of other relevant studies (e.g. Raj & Gregory, 1996; Dalmau et al., 2010), which indicates that they are also valid for older pigs. Studies have shown that receptors in the lungs that are sensitive to carbon dioxide are the main cause of distress (Kells et al., 2018). Since carbon dioxide in the blood helps to regulate respiration and carbon dioxide damages cells, similar carbon dioxide sensitive mechanisms are likely to be present in all pigs, irrespective of age.

Some of the literature cited in this report was published in the journal *Meat Science* or focused on meat quality, which indicates that one of the driving forces for investigating the effects of stunning has been quality of the final product, rather than animal welfare. In general, methods in which animal welfare is poor also produce poor meat quality and therefore improving the stunning method can also have a positive impact on several factors that are important for production and economics (EFSA, 2004).

Table 16. Summary of the function of the different stunning methods described in this report, where: “Function” means how stunning is initiated physiologically by the method, “initiation” means the time taken from initiation of the stun until loss of consciousness, “permanence” means whether or not the stun is reversible, “killing” means whether or not the stunning method also kills the animal under commercial conditions and “commercially available” means whether or not the stunning method is commercially available

| Method | Function | Initiation | Permanence | Killing | Commercially available |
|-----------------------------------|--------------------------------------|----------------------------|---------------------------|------------------|------------------------|
| Mechanical stunning | | | | | |
| Penetrating captive bolt | Brain damage | Immediate | Irreversible | No ¹ | Yes |
| Free projectile | Brain damage | Immediate | Irreversible | No ¹ | Yes |
| Gas stunning | | | | | |
| Carbon dioxide | Hypoxia, hypercapnia | Not immediate ² | Reversible ³ | No ⁴ | Yes |
| Low atmospheric pressure stunning | Hypoxia ⁵ | Not immediate | Irreversible ⁵ | Yes ⁵ | No |
| Argon | Hypoxia | Not immediate ² | Reversible ³ | No ⁴ | No |
| Nitrogen | Hypoxia | Not immediate ² | Reversible ³ | No ⁴ | No |
| Carbon dioxide, argon | Hypoxia | Not immediate ² | Reversible ³ | No ⁴ | No |
| Carbon dioxide, nitrogen | Hypoxia | Not immediate ² | Reversible ³ | No ⁴ | No |
| Nitrogen, argon | Hypoxia | Not immediate ² | Reversible ³ | No ⁴ | No |
| Nitrogen, argon, carbon dioxide | Hypoxia | Not immediate ² | Reversible ³ | No ⁴ | No |
| Helium | Hypoxia | Not immediate ² | Reversible ³ | No ⁴ | No |
| Xenon | Hypoxia | Not immediate ¹ | Reversible ³ | No ⁴ | No |
| Electrical stunning | | | | | |
| Head-only stunning | Epileptiform seizure | Immediate | Reversible ⁶ | No | Yes |
| Head-to-body/head-to-back | Epileptiform seizure, cardiac arrest | Immediate | Irreversible | Yes | Yes |

¹The injuries that occur in mechanical stunning are usually ultimately fatal, but it is not in itself a killing method.

²Depends on the concentration: the higher the concentration, the faster stunning is induced.

³Depends on the concentration and exposure time: the higher the concentration and the longer the exposure time, the more sustained the effect of the stun.

⁴If exposure is markedly extended, gas stunning leads to death.

⁵No experiments have been performed on pigs that have reached slaughter weight (~115 kg) and therefore it is difficult to draw conclusions.

⁶In general, electrical stunning produces a stun that lasts a shorter time than gas stunning.

Table 17. Comparison of the effects of the different stunning methods described in this report from an animal welfare perspective. Assessment¹ based on a three-grade scale, where 1 represents a low negative impact on welfare and 3 represents a high negative impact. “Driving” means driving from the waiting pen to stunning, “handling and initiation” means handling at stunning, “risk of unsuccessful stunning” means the risk of the animals not being correctly stunned by the stunning method, “experience of the stun” means the time between initiation of the stun until loss of consciousness occurs and “reliability of the stun” means the quality and impact of the stunning. The basis for the assessments was stunning carried out using best practice. For methods in which the human factor has a large impact, this was taken into account but with the assumption that stunning is initiated correctly. DD: data deficit, i.e. insufficient data for assessment

| Method | Driving | Handling and restraint | Risk of unsuccessful stun | Experience of the stun ¹ | Reliability of the stun ³ |
|-----------------------------------|----------------|------------------------|---------------------------|-------------------------------------|--------------------------------------|
| Mechanical stunning | | | | | |
| Penetrating captive bolt | 3 | 3 | 3 | 1 | 2 |
| Free projectile | 3 | 3 | 3 | 1 | 2 |
| Gas stunning | | | | | |
| Carbon dioxide | 1 | 1 | 1 | 3 | 1 |
| Low atmospheric pressure stunning | 1 ³ | DD | DD | 3 | DD |
| Argon | 1 ³ | 1 | DD | 2 | 1 |
| Nitrogen | 1 ³ | 1 | DD | 2 | 2 |
| Carbon dioxide & argon | 1 ³ | 1 | DD | 2-3 ⁴ | 1 |
| Carbon dioxide & nitrogen | 1 ³ | 1 | DD | 2-3 ⁴ | 1 |
| Helium | 1 ³ | 1 ³ | DD | 2 | 1 |
| Xenon | 1 ³ | 1 ³ | DD | DD | DD |
| Electrical stunning | | | | | |
| Head-only stunning | 3 | 3 | 3 | 1 | 2 |
| Head-to-body/head-to-back | 3 | 3 | 3 | 1 | 2 |

¹Assessment of the experience of stunning included the length of the initiation of stunning. If the stunning effect is not instantaneous, the degree of aversion during the induction phase was also assessed.

²An assessment was made based on a three-grade scale, where 1 represents a high degree of reliability of the stun and 3 represents a low degree of reliability. Reliability includes both the fact that the animals are stunned and that the stunning lasts until the animal dies.

³No experiments have been performed on pigs that have reached slaughter weight (~115 kg) and therefore it is difficult to draw conclusions. The assessment was based on the assumption that the pigs can be driven in a group in the same way as in commercial carbon dioxide stunning.

⁴Stunning is likely to be experienced as more aversive the more carbon dioxide the gas mixture contains.

Driving

Driving encompasses the movement of animals between the lairage and stunning. In general, stunning systems that allow pigs to be driven in a group were assessed as having a less negative impact on animal welfare than if pigs are driven individually (Table 17), as handling in a group generally induces less stress in pigs compared with individual handling (e.g. EFSA, 2004). However, even when animals are driven in a group, the design of the raceway affects the pigs. A well-designed system uses the pigs' natural behaviour, does not require hard driving by people or electric prods and therefore has relatively little impact on animal welfare.

Handling and restraint

Handling and restraint encompasses handling during the induction of stunning. Methods where pigs need to be handled individually and restrained in order to be able to initiate the stun (electrical and mechanical stunning) were generally assessed as having a higher negative impact on animal welfare than systems which do not require individual handling and restraint (Table 17). Restraint has previously been identified as the single most stressful stage during the slaughter process (EFSA, 2004). For those stunning methods that require restraint, it is of the utmost importance that suitable restraint systems are developed to minimise the negative impact on animal welfare associated with restraint (EFSA, 2004).

Risk of unsuccessful stun

Risk of unsuccessful stun refers to the risk that a stunning method does not result in the pig being fully stunned until death occurs. Generally speaking, mechanical and electrical stunning were assessed as entailing a greater risk of unsuccessful stun compared with gas stunning (Table 17). The higher risk is partly due to the human factor and the fact that mechanical and electrical stunning is most often dependent on a person applying the captive bolt pistol or electric tongs, for example, in the correct way, and on the equipment working perfectly. The application also requires precision, which can be made more difficult if the pig is not fully restrained, and incorrect placement results in incomplete stunning.

Experience of the stun

Experience of the stun encompasses experience of the stunning process from initiation until the animal is stunned, i.e. unconscious. In general, stunning methods that induce direct stunning (with correct application) were assessed as having a less negative impact on animal welfare than methods which do not induce direct stunning (Table 17), unless the drawn-out induction itself has absolutely no impact on animal welfare, which is seldom the case. In gas stunning, which does not produce direct stunning, the effect of the gas on animal well-being and behaviour influenced the assessment. Gases that are aversive were therefore assessed as having a negative impact on animal welfare, with increased negative impact the greater the aversiveness was deemed to be. One measure of the aversiveness of a gas is whether pigs willingly enter a space filled with the gas. It was thus assumed that pigs will enter a gas-filled space willingly if the gas is not unpleasant to inhale. The willingness to enter a crate has been shown to decrease when the crate is filled with gas (90% argon; 70% nitrogen-30% carbon dioxide; or 85% nitrogen-15% carbon dioxide) instead of air (Dalmau et al., 2010).

Reliability of the stun

The assessment of reliability of the stun encompassed both the animals being correctly stunned and whether the stun lasts until the pig is killed. Generally speaking, stunning methods that produce a shorter period of unconsciousness were assessed as having a greater negative impact on animal welfare (Table 17), as this potentially increases the risk that the stun will wear off before the animals are bled and killed.

Risks involved with assessment of unconsciousness

In assessments of the stunning process, the moment when the animal loses the ability to stand (LOP) is often used as an initial indicator that the animal is losing consciousness. In experiments, pigs have been found to return to a standing position after LOP has occurred, which indicates that the pigs may be conscious even after LOP (Atkinson et al., 2015). Despite animals being stunned and bled before they have regained consciousness, animals stunned with argon or a mixture of argon and carbon dioxide can exhibit muscle spasms, which can be mistaken for signs of consciousness and can affect meat quality (Raj, 1999).

Risks linked to stun-to-stick time

Different stunning methods result in unconsciousness for different periods of time. The interval between stunning and sticking is important because the pigs must be stuck and die of blood loss before the stun has time to wear off. A longer interval between stunning and sticking can increase the risk of sticking or death occurring after the stun has worn off, or begun to wear off. Methods that induce a short-lasting stun therefore have a higher requirement for rapid bleeding.

The corneal reflex has been found to return earlier in animals stunned in gas mixtures containing 15% carbon dioxide compared with mixtures with a higher concentration of carbon dioxide, which indicates that the stun is more short-lived (Llonch et al., 2012b). The reason that the stun lasts longer when 90% carbon dioxide is used is probably that the high carbon dioxide content produces stronger acidification of the blood, which takes longer to recover from. The higher the carbon dioxide content, the longer the stun lasts, which is why the exposure time needs to be longer for lower concentrations of carbon dioxide (Llonch et al., 2012b). According to Llonch et al. (2013), when stunning with carbon dioxide and nitrogen mixtures (70-85% nitrogen, 15-30% carbon dioxide), it should be ensured that the pigs are exposed to the gas for at least five minutes, as the pigs have a relatively short recovery phase (compared with pure carbon dioxide). On account of the short duration of the stun, it is not recommended that a mixture of 85% nitrogen and 15% carbon dioxide be used. Electrical head-only stunning initiates a short period of unconsciousness and bleeding should be carried out within 15 seconds (Vogel et al., 2011). Previous observations have shown that the short period of unconsciousness leads to problems with achieving full exsanguination before the stun wears off, particularly at smaller slaughterhouses (Vogel et al., 2011).

The interval between stunning and bleeding increases with increased group size when pigs are stunned in a group, and this can also increase the risk of the animals not remaining stunned right up until they are killed. In a Swedish study, the stun-to-stick time with four pigs per group in carbon dioxide was between 1.12 and 1.29 minutes for the last pig (Atkinson et al., 2015). Previous studies have also shown that the stun-to-stick time is longer in the paternoster system, with 20% of pigs stuck within one minute, whereas the equivalent figure for the dip-lift system is 65%, even when all animals, regardless of the system, are correctly stunned at sticking (Atkinson et al., 2012).

Comparisons between stunning methods and stunning systems

As carbon dioxide is the most common stunning method, despite its aversive nature, it has also been used as the basis for most previous comparisons with other methods.

Carbon dioxide and electricity

Becerril-Herrera et al. (2019) compared electrical stunning with carbon dioxide stunning and concluded that animals stunned with carbon dioxide and with electrical head-only stunning show more physiological signs of stress (hyperglycaemia and acidosis) than on the day prior to slaughter. They also concluded that carbon dioxide stunning produces a higher additional stress load and therefore has a more negative effect on pig welfare.

Jongman et al. (2000) compared carbon dioxide stunning and electrical stunning preceded by restraint in a V-belt restrainer and concluded that stunning with 90% carbon dioxide is considerably less aversive than electrical stunning, as the pigs avoided exposure to electricity to a greater degree after having been stunned by electricity once. The fact that all animals stunned with 90% carbon dioxide lost consciousness may have affected their memory of the treatment, however. Jongman et al. (2000) found no difference in aversion between restraint in the V-belt restrainer and the crate in which the carbon dioxide stunning was carried out, but concluded that this is very much influenced by the design of the system and how the animals are introduced to the crate/restrainer. In situations that can be stressful (at slaughter, for example), those authors suggest that the crate is better from an animal welfare point of view.

Carbon dioxide and argon

Compared with carbon dioxide, inert gases, such as argon, do not interact with other substances and therefore do not irritate the mucous membranes (e.g. Raj & Gregory, 1995). Argon or argon-carbon dioxide mixtures (up to 30% carbon dioxide) are considered to be less aversive than pure carbon dioxide and also produce more stable gas mixtures (EFSA, 2004; Dalmau et al., 2010). Pure argon, or the lowest possible concentration of carbon dioxide, is suggested to be the best stunning method for pigs from an animal welfare point of view (Mota-Rojas et al., 2012). However, it takes a long time for inert gases to induce anaesthesia compared with carbon dioxide (Dalmau et al., 2010).

Kells et al. (2018) compared argon stunning with carbon dioxide stunning in 17-day-old pigs and found that stunning with 100% argon induced less stress than stunning with 100% carbon dioxide. Among other things, the pigs stunned with carbon dioxide exhibited more laboured breathing and earlier and more sustained attempts to escape during the induction phase compared with the pigs that were stunned with argon. Stunning with a mixture of carbon dioxide and argon (60% argon and 40% carbon dioxide) gave similar results to stunning with pure carbon dioxide and was therefore considered to have no benefits from an animal welfare point of view (Kells et al., 2018). Stunning was induced more quickly with pure carbon dioxide compared with pure argon or

a combination of argon and carbon dioxide, but was also considered to have the greatest negative impact on animal welfare, whereas argon was considered to have the least negative impact on animal welfare (Kells et al., 2018).

Raj & Gregory (1996) investigated stunning with argon, carbon dioxide and carbon dioxide-argon mixtures and found that carbon dioxide and carbon dioxide-argon mixtures both induced hyperventilation prior to loss of consciousness. The hyperventilation lasted longer when the carbon dioxide concentration was 20% compared with 30%. No attempts to escape were seen, however, which indicates that the carbon dioxide concentrations were tolerable. The time to LOP was similar for stunning with 50% carbon dioxide and stunning with 2% residual oxygen in argon, but the argon mixture only induced mild respiratory distress, whereas the carbon dioxide caused more respiratory distress (Raj & Gregory, 1996). With a 30% concentration of carbon dioxide in argon, hyperventilation increased but the time to LOP was reduced by 11 seconds without causing breathlessness. Stunning with 2% residual oxygen in argon induced minimal distress, and 30% carbon dioxide with 2% residual oxygen in argon caused moderate distress, while 20-90% carbon dioxide induced severe distress in the pigs (Raj & Gregory, 1996). Stunning with 30% carbon dioxide in argon (with 2% residual oxygen) was therefore deemed less aversive and can be seen as an acceptable stunning method from an animal welfare point of view. Stunning in pure argon (with 2% residual oxygen) was found to be preferable, however, as it results in minimal respiratory distress.

Raj (1999) compared stunning with 90% argon, 90% carbon dioxide and a mixture of 30% carbon dioxide and 60% argon. The time to LOP did not differ between the different gases, but the pigs stunned with 90% argon exhibited convulsions for a longer period after LOP had occurred. Pure carbon dioxide was deemed more effective at killing the animals than either argon or mixtures of argon and carbon dioxide. Based on the results, Raj (1999) recommended that the animals be stunned with argon or a mixture of argon and carbon dioxide for at least three minutes and then killed using an electric current.

Carbon dioxide, nitrogen and argon

Dalmau et al. (2010) compared stunning with argon and carbon dioxide with stunning with nitrogen and carbon dioxide. Compared with a mixture of 70-85% nitrogen and 15-30% carbon dioxide, stunning with pure argon induced fewer escape attempts, a longer time to loss of consciousness, LOP and more muscle spasms after LOP (Dalmau et al., 2010). However, muscle spasms after LOP are associated with unconscious movements and are thus not considered to be an escape attempt. These findings indicate that the pigs showed less aversion to argon compared with carbon dioxide or combinations of carbon dioxide and nitrogen (Dalmau et al., 2010). Although argon induces aversion, the aversion was considered to be lower than with a 15-30% mixture of carbon dioxide (Dalmau et al., 2010). The conclusion drawn was that argon is better for animal welfare than stunning in a high concentration of carbon dioxide, or that the carbon dioxide content should be as low as possible.

Carbon dioxide and nitrogen

Atkinson et al. (2015) compared stunning with 90% carbon dioxide and with a mixture of 20% carbon dioxide and 80% nitrogen. The results did not reveal any difference in the average time to the first reaction to the gas. However, the maximum time to the first reaction was shorter for the nitrogen mixture (12 seconds compared with 21 seconds), which indicates that at least some pigs found the mixture less aversive. No major differences were found for onset of LOP, but the maximum time for LOP was longer with the nitrogen gas mixture, which indicates that it can take longer to induce stunning with that gas mixture than with pure carbon dioxide. The pigs stunned with 90% carbon dioxide exhibited stronger reactions prior to LOP and the pigs stunned with the gas mixture showed stronger reactions after onset of LOP (Atkinson et al., 2015). Gagging was more frequent and lasted longer for pigs in the gas mixture compared with pure carbon dioxide. Overall, both methods were considered to induce a great deal of distress for the pigs.

Llonch et al. (2012b) compared stunning with pure carbon dioxide and stunning with carbon dioxide/nitrogen mixtures. Compared with 90% carbon dioxide, the pigs exhibited fewer escape attempts when stunned with a lower concentration of carbon dioxide (70-85% nitrogen and 15-30% carbon dioxide), probably due to less irritation of the mucous membranes (Llonch et al., 2012b). At a concentration of 15% carbon dioxide, no escape attempts were seen. The amount of gasping also declined with a decreased concentration of carbon dioxide and at a concentration of 15% carbon dioxide no gasping was seen. After stunning for 270 seconds with the different gas mixtures, 91.8% of pigs regained rhythmic breathing and 85.7% regained their corneal reflex before death occurred (Llonch et al., 2012b), which indicates that the low carbon dioxide concentration posed clear problems with stunning quality.

Llonch et al. (2013) investigated stunning with 90% carbon dioxide and different mixtures of nitrogen (70-85%)

and carbon dioxide (15-30%) and found that stunning with pure carbon dioxide induced more aversion, but also more rapid loss of consciousness and a longer-lasting stun than stunning with the gas mixtures. After a long exposure to carbon dioxide (three minutes) all pigs were dead, whereas after five minutes of exposure to the gas mixtures 30% of the pigs were still alive.

Pöhlmann (2018) investigated stunning with nitrogen in foam and found lower levels of catecholamines in the blood after stunning compared with stunning with carbon dioxide, which indicates that the pigs were exposed to less stress.

Carbon dioxide and helium

Machtolf et al. (2014, 2013) compared stunning of slaughter pigs with 90% carbon dioxide and 95% helium. Induction of stunning took roughly the same length of time for both gases (16 seconds for carbon dioxide, 20 seconds for helium). During stunning with helium no aversive behaviours were seen, whereas stunning with carbon dioxide initiated hyperventilation, attempts to escape and vocalisation. Compared with helium, the pigs stunned with carbon dioxide also had significantly higher levels of adrenalin and noradrenalin in their blood, which indicates that those pigs experienced higher levels of stress than those stunned with helium (Machtolf et al., 2013). However, in that study the carbon dioxide stunning was carried out in a commercial Butina system, while the helium stunning was carried out individually, which makes comparisons less reliable. The authors concluded that stunning with helium is better for animal welfare than stunning with carbon dioxide.

Carbon dioxide and LAPS

Engle & Edwards (2011) compared killing of piglets on-farm using carbon dioxide or LAPS and observed no differences in behaviour between the methods, but detected a trend for the pigs killed using LAPS to show more resistance than the animals stunned with carbon dioxide. Furthermore, killing with LAPS took longer than with carbon dioxide (13.4 minutes compared with 7.8 minutes). In addition, not all of the animals were killed with LAPS and therefore the suitability of that method was questioned.

Buzzard (2012) also compared killing of piglets using LAPS and carbon dioxide and found that carbon dioxide killed the animals more quickly (13.8 ±5.1 minutes compared with 27.4 ±6.7 minutes). Behavioural differences between the treatments were also identified, with gasping seen in 100% of the animals killed with carbon dioxide and in 29% of the animals stunned with LAPS. During the first five minutes of treatment, ataxia (involuntary movements) was seen in 57% of the animals stunned with LAPS and in 77% of the animals exposed to carbon dioxide. Overall, LAPS was considered to induce less stress compared with carbon dioxide, as fewer aversive behaviours were seen (Buzzard, 2012), but more studies are needed to confirm this conclusion and it has not been shown whether the method can be recommended for slaughter pigs at all.

Martin et al. (2020) and McKeegan et al. (2020) compared stunning of grower pigs with LAPS or 80% carbon dioxide. Both studies concluded that LAPS cannot be considered a viable stunning method, based on signs of stress and pain displayed by the pigs. Compared with carbon dioxide, LAPS also took longer to induce unconsciousness, which was considered to increase suffering among the pigs. The time to reach LOP was 36.2±0.6 seconds when stunned with carbon dioxide compared with 118.4±1.8 seconds when stunned with LAPS (Martin et al., 2020). Compared with carbon dioxide, pigs in the LAPS treatment showed higher counts of head shaking, head tilts, facial grimaces and high-pitched screams, but a lower number of escape attempts (Martin et al., 2020; McKeegan et al., 2020). Pigs in the carbon dioxide treatment also showed more signs of air hunger. While LAPS was considered more gradual, it also took a longer time to reach unconsciousness and therefore increased the time of suffering for the pigs (McKeegan et al., 2020). Stunning with carbon dioxide and with LAPS were both concluded to affect pig welfare negatively.

McKeegan et al (2020) also compared LAPS stunning with carbon dioxide stunning of slaughter weight pigs. In order to measure aversion to the stunning method, the pigs in that study were taught to push a button if they wanted to leave the stunning chamber or received an edible treat if they stayed. As LAPS was induced, only a small number of pigs pushed the button to leave. However, the researchers suggested that the pigs might have stopped pushing the button as they quickly understood that escape was not possible, and not because they did not want to leave. Stunning with carbon dioxide could also have prevented the pigs from expressing their response, due to the quick induction of the stun. Pathological examinations after slaughter revealed that pigs stunned with LAPS had a higher incidence of congestion and haemorrhages compared with pigs stunned with carbon dioxide and that the symptoms were more severe in the LAPS treatment compared with the carbon dioxide treatment. The majority of the pigs in LAPS also had ruptured ear drums, which probably caused pain if induced while the pigs were still conscious, as indicated by the signs of pain displayed by pigs in the LAPS treatment. McKeegan et al (2020) concluded that stunning with carbon dioxide or LAPS is aversive and

negatively affects several welfare indicators. LAPS was thus not considered a viable alternative to stunning with carbon dioxide from a welfare perspective. Further, the pathological findings could result in condemnation of the carcass at slaughter which, along with higher installation costs for the equipment, also affects the economic viability of the LAPS method.

Carbon dioxide and nitrous oxide (N₂O)

Rault et al. (2013) investigated a two-step procedure for stunning and killing piglets with gas. Pigs exposed to 90% carbon dioxide lost consciousness soonest, followed by pigs exposed to 60% nitrous oxide and 30% carbon dioxide, 60% argon and 30% carbon dioxide, and 60% nitrogen gas and 30% carbon dioxide. Animals exposed to 60% nitrous oxide and 30% oxygen showed no outward signs of stress, but took a very long time to lose consciousness (12 minutes). Despite the long induction period, Rault et al. (2013) suggested that a humane method of killing piglets with gas would be to first stun them with 60% nitrous oxide and 30% oxygen, and then kill them with 90% carbon dioxide.

Other factors

In addition to animal welfare, there are other aspects that influence the choice of stunning method. Such factors were not covered in this report but some deserve a mention, in particular factors affecting the feasibility of the stunning method for commercial use, which are: gas stability and gas handling, working environment and safety, and cost and availability.

Gas stability and gas handling

Many of the alternative gases that have been proposed for stunning are lighter than air. One of the advantages of carbon dioxide is that it is heavier than air and can therefore easily be kept inside a container. This is the basis for systems such as the paternoster and dip-lift systems. The handling of lighter gases such as nitrogen, argon and helium is more difficult and there are currently no commercial systems that can handle these gases without problems.

Earlier studies have indicated difficulties with using certain types of gases due to difficulty from a purely practical point of view in getting the gas to work in the system. According to Dalmau et al. (2010), stunning with carbon dioxide in dip-lift systems cannot be replaced with stunning using free nitrogen gas on its own, because it is difficult to maintain a sufficiently low oxygen level to enable stunning to be induced. Atkinson et al. (2015) also describe the problem of keeping the residual oxygen at a sufficiently low level when stunning using nitrogen, with a longer time required for induction of unconsciousness as a consequence. Therefore, in order to develop an improved stunning method from an animal welfare perspective, technical solutions that can handle the stunning with a high degree of reliability are needed.

Uniformity, that is to say whether the concentration of the gas is the same throughout the space, is better in gas mixtures than with pure carbon dioxide (Dalmau et al., 2010). Gas mixtures of argon and carbon dioxide, 90% argon or mixtures of nitrogen and carbon dioxide where the nitrogen content is similar to the nitrogen content in the atmosphere (~79%) have both high stability and uniformity. Since the gas mixture is uniform, it is not necessary for the pigs to be lowered to the bottom of the pit in order to be exposed to the correct gas mixture. The increased exposure time required (compared with 90% carbon dioxide) can therefore be evened out somewhat due to the fact that exposure begins immediately on entry into the crate. It is still not clear how temperature affects gas uptake by the pigs, but around 17 degrees Celsius seems to be the optimum temperature for 90% carbon dioxide (Atkinson et al., 2015).

Working environment and safety

One risk identified with the use of non-aversive gases with anaesthetising effect is that the gas could potentially leak without it being detected by the staff, resulting in personal injury. If any carbon dioxide were to leak, the leakage would be detected, as the presence of the gas is noticeable to humans on inhalation. The risk of personal injury in connection with restraint and stunning using a captive bolt and electric current should also be taken into account.

Cost and availability

The cost and availability of different gases are referred to in this report. The cost of a gas often reflects its availability and therefore it is not just increased cost that is of relevance for the choice of gas for stunning. For example, the use of xenon for stunning or anaesthesia has been called into question because there is only a small quantity of xenon in the atmosphere and use of large quantities of xenon would result in very high costs. It should therefore be used with caution (Neice & Zornow, 2016).

Previous assessments

EFSA

EFSA (2004) ranks different stunning methods in terms of animal welfare and meat quality in its report. It considers the captive bolt to be best from an animal welfare point of view, followed by gas stunning and electrical stunning methods. However, its report does not discuss the handling of individual pigs and the restraint required for stunning with a captive bolt and electric stunning, which are seen as major hazards for animal welfare in connection with these stunning methods.

According to EFSA (2004), a correctly used pneumatic-powered captive bolt stunner produces the fewest reactions and has the least effect on animal welfare. However, captive bolt stunning is deemed to take too long to enable good production speed to be maintained at large slaughterhouses and pneumatic-powered captive bolt stunners are far too expensive for smaller slaughterhouses. With regard to electrical stunning methods, a regulated current is considered best from an animal welfare point of view (compared with pulsed direct current or constant voltage). Gas is considered to have a very negative effect on the animals for the first 10-12 seconds. In addition, EFSA (2004) deems that stunning for 73 seconds in 80% carbon dioxide does not provide a sufficiently reliable stun in terms of depth or duration, but 90 seconds of exposure causes rapid death. When stunned with 90% carbon dioxide, a large proportion of the pigs die, which means that problems with the depth or duration of the stun are avoided. The EFSA concludes that stunning with gas should be done using a non-aversive gas and loss of consciousness should be induced quickly (EFSA, 2004). According to the report, which is 17 years old, gas stunning should be done with 30% carbon dioxide and 60% argon or nitrogen in air, or with 90% argon, nitrogen or other inert gas in air. This is not entirely supported by more recent research, which indicates that the quantity of oxygen in the air should not exceed 2% (volume). According to EFSA (2004), the gas concentrations should be achieved within 10 seconds for the pigs, which should then remain in the gas for at least three minutes.

Eurogroup for Animals

Eurogroup for Animals (an association of 70 animal welfare organisations in 31 different countries) has written a position paper on stunning and killing animals with high concentrations of carbon dioxide in which it argues for the phasing out of carbon dioxide stunning and reallocation of resources to finding a non-aversive stunning method. Among other things, it considers that non-aversive gas mixtures or two-phase systems (where the animals are first stunned with non-aversive gas mixtures followed by either electrical killing or killing with high concentrations of carbon dioxide) could be alternatives to carbon dioxide stunning (Eurogroup, 2019).

Knowledge gaps and future research

Research carried out in the future should ultimately take the whole slaughter process into account. In research publications on methods that are still not commercially available, information on how the whole slaughter process (from driving to bleeding) affects welfare is often lacking (Table 17). Less well-documented stunning methods may therefore need more research before it is possible to draw conclusions regarding their impact on animal welfare. For example, handling prior to stunning has been shown to be potentially at least as stressful as initiation of the stun (EFSA, 2004), leading to the suggestion that future research should focus on non-aversive gas mixtures for stunning animals (EFSA, 2004). This is in line with the fact that restraint can be the most stress-inducing element of the slaughter process and, with non-aversive gas mixtures, handling prior to slaughter can often be done in groups and with minimal restraint and handling. Future stunning methods should also produce a reliable stun with low risk, and therefore this issue should be given priority in future research. There are currently gaps in knowledge regarding the risks of unsuccessful stunning for several of the alternative gases/gas mixtures that have been investigated to date.

Conclusions

All pig stunning methods that are currently commercially available, in practice mechanical stunning (bolt/bullet), electrical stunning and carbon dioxide stunning, have both advantages and disadvantages from an animal welfare perspective. The relative importance of these advantages and disadvantages is affected by the speed of slaughter, but also by other factors. For some methods, the animal welfare disadvantages lie mainly in the handling required prior to stunning, that is to say they are associated with driving and restraint. In some cases, they relate to the risk of handling errors/the human factor or the risk of the stunning equipment itself failing. In yet other cases the induction of stunning itself is obviously unpleasant for the pigs, or there is a high risk of the pigs regaining consciousness too rapidly. Based on the available scientific evidence, it is not possible to clearly single out a method that is preferable for slaughter of pigs. Of the methods described in this report that are currently at the hypothesis or research stage, none is considered to be ready for commercialisation in the near future as there is a lack of sufficiently extensive research relating to practical application, animal welfare and other relevant aspects.

In purely general terms, a system or stunning method that allows the pigs to be handled and driven in a group without the use of electric prods or another hard driving method, that does not require firm restraint, that provides rapid induction without aversive reactions in the pigs, that is reliable and not to a large extent dependent on the skill of the individual operator, and that produces long-lasting or irreversible stunning would be preferable. No such system is currently available, which means that this area is open for further research.

Acknowledgements

The authors would like to thank the Swedish Board of Agriculture (Jordbruksverket) for funding this review and Rebeca García Pinillos for initiating the translation into English.

References

- Algers, A., Berg, L., Hammarberg, K., Larsen, A., Lindsjö, J., Malmsten, A., Malmsten, J., Mustonen, A., Olofsson, L., Sandström, V. 2012. Utbildning i djurvälstånd i samband med slakt och annan avlivning. <http://disa.slu.se/> – retrieved: 4.11.2020.
- Anderson, K., Ries, E., Backes, J., Bishop, K., Boll, K., Brantner, E., Hinrichs, B., Kirk, A., Olsen, H., Risius, B., Bildstein, C., Vogel, K.D. 2019. Relationship of captive stunning location with basic tissue measurements and exposed cross-sectional brain area in cadaver heads from market pigs. *Transl. Anim. Sci.* 3, pp. 1405-1409.
- Anil, M.H., 1991. Studies on the return of physical reflexes in pigs following electrical stunning. *Meat Science*, 30:13-21.
- Anil, M.H., McKinstry, J.L. 1998. Variations in electrical stunning tong placement and relative consequences in slaughter pigs. *The Veterinary journal*, 155, pp. 85-90.
- Atkinson, S., Larsen, A., Llonch, P., Velarde, A., Algers, B. 2015. Group stunning in pigs during commercial slaughter in a butina pater-noster system using 80% nitrogen and 20% carbon dioxide compared to 90% carbon dioxide. Department of Animal Welfare and Health.
- Atkinson, S., Velarde, A., Llonch, P., Algers, B. 2012. Assessing pig welfare at stunning in Swedish commercial abattoirs using CO₂ group stun methods. *Animal Welfare* 21, pp. 487-495.
- Baumert, J-H. 2009. Xenon-based anesthesia: theory and practice. *Open Access Surgery*. 2009:2, pp. 5-13.
- Becerril-Herrera, M., Alonso-Spilsbury, M., Lemus-Flores, C., Guerrero-Legarreta, I., Olmos-Hernández, A., Ramírez-Necoechea, R., Mota-Rojas, D. 2009. CO₂ stunning may compromise swine welfare compared with electrical stunning. *Meat Science* 81, pp. 233-237.
- Von Borrel, E., Veissier, I. 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – A review. *Physiology and Behaviour* 92(3), pp. 293-316.
- Bouwsema, J.A., Lines, J.A. 2019. Could low atmospheric pressure stunning LAPS (LAPS) be suitable for pig slaughter? A review of available information. *Animal Welfare* 28, pp. 421-432.
- Brandt, P., Aaslyng, M.D. 2015. Welfare measurements of finishing pigs in the day of slaughter: A review. *Meat Science* 103, pp. 13-23.
- Broom, D.M. (1986). Indicators of poor welfare. *British Veterinary Journal*, 142(6), pp. 524-526.
- Buzzard, B. L. 2012. Evaluation of hypobaric hypoxia as a low stress alternative to carbon dioxide euthanasia for use with nursery piglets. Master thesis. Department of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas.
- Correa, J.A., Torrey, S., Devillers, N., Lafort, J.P., Gonyou, H.W., Faucitano, L. 2010. Effects of different moving devices at loading on stress response and meat quality in pigs. *Journal of Animal Science* 88, pp. 4086-4093.
- Dalla Costa, O., Dalla Costa, F.A., Feddern, V., dos Santos Lopes, L., Coldebella, A., Gregory, G.G., Mello Monteiro de Lima, G.J. 2019. Risk factors associated with pre-slaughtering losses. *Meat Science* 155, pp. 61-68.
- Dalmau, A., Rodríguez, P., Llonch, P., Velarde, A. 2010a. Stunning pigs with different gas mixtures: aversion in pigs. *Animal Welfare* 19, pp. 325-333.
- Dalmau, A., Llonch, P., Rodríguez, P., Ruíz-de-la-Torre, J.L., Manteca, X., Velarde, A. 2010b. Stunning pigs with different gas mixtures: gas stability. *Animal Welfare* 19, pp. 315-323.
- Ehlorsson, C-J., Wallgren, P., Leijon, M. 2016. Utredning av orsaker till ökande förekomst av

luftvägsinfektioner i slaktgrisuppfödningen. Gård och Djurhälsan <https://www.gardochdjurhalsan.se/wp-content/uploads/2020/03/utredning-av-orsaker-till-okande-forekomst-av-luftvagsinfektioner-i-slaktgrisuppfodningen-final-version-29-feb.pdf> – retrieved: 4.11.2020.

EUROGROUP. Eurogroup for Animals. 2019. Stunning/killing of pigs with high concentrations of CO₂ [Online]. Available: <https://www.eurogroupforanimals.org/sites/eurogroup/files/2020-03/CO2%20stunning%20EfA%20position%20paper%202019.pdf>

European Commission. 2002. Health and Consumer Protection Directorate-General. Report of the Scientific Committee on Animal Health and Animal Welfare, The welfare of animals during transport (details for horses, pigs, sheep and cattle), pp. 95-101.

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen, SS., Alvarez, J, Bicot, DJ., Calistri, P., Depner, K., Drewe, JA., Garin-bastuji, B., Gonzales Rojas, JL., Gortázar Schmidt, C., Michel, V., Miranda Chueca, MÁ., Roberts, HC., Sihvonen, LH., Spoolder, H., Stahl, K., Viltrop, A., Winckler, C., Candiani, D., Fabris, C., Van der Stede, Y., Velarde, A. 2020. Scientific opinion on the welfare of pigs at slaughter. EFSA journal 2020;18(6)6148. 113pp.

EFSA (European Food Safety Authority). 2019. Hazard identification for pigs at slaughter and during on-farm killing. EFSA Supporting publication 2019:EN-1684. 10 pp.

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013. Scientific Opinion on monitoring procedure at slaughterhouses for pigs. EFSA Journal 2013;11(12):3523, 62pp.

EFSA. 2004. Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main species of animals, The EFSA journal (2004), 45, pp. 1-29.

Engle, T.E. and L.N. Edwards, 2010. Evaluation and application of humane hypoxia euthanasia for nursery pigs. Research report to the National Pork Board, Des Moines, Iowa, USA.

FAWC. (2009). Five Freedoms. <http://www.fawc.org.uk/freedoms.htm> – retrieved: 4.11.2020.

Fraser, D., Weary, D.M., Pajor, E.A., Milligan, B.N. 1997. A scientific conception of animal welfare that reflects ethical concerns. *Animal Welfare* 6, pp. 187-205.

Grandin, T. 2003. The welfare of pigs during transport and slaughter. *Pig News and information*, Vol, 24, No 3.

Grandin, T. 2012. Developing measures to audit welfare of cattle and pigs at slaughter. *Animal Welfare* 21, pp. 351-356.

Gregory, N.G. 1994. Preslaughter handling, stunning and slaughter. *Meat Science* 36, pp. 45-56.

Jongman, E. C., Barnett, J. L. & Hemsworth, P. H. 2000. The aversiveness of carbon dioxide stunning in pigs and a comparison of the CO₂ stunner crate vs. the V-restrainer. *Applied Animal Behaviour Science*, 67, pp. 67-76.

Keeling, L., Rushen, J., Duncan, I.J.H. 2011. Understanding Animal Welfare. In: Appleby, M.C., Mench, J.A., Olsson, A.S. & Hughes, B.O. (eds.), *Animal Welfare* 3rd ed. Oxfordshire, CAB International, pp. 13-26.

Kells, N., Beausoleil, N., Johnson, C., Sutherland, M. 2018. Evaluation of different gases and gas combinations for on-farm euthanasia of pre-weaned pigs. *Animals* 8, 40.

Lindahl, C., Sindhøj, E., Brattlund Hellgren, R., Berg, C., Wallenbeck, A. Responses of pigs to stunning with nitrogen filled high-expansion foam. *Animals* 10(12), 2210.

Llonch, P., Dalmau, A., Rodríguez, Manteca, X., Velarde, A. 2012a. Aversion to nitrogen and carbon dioxide mixtures for stunning pigs. *Animal Welfare* 21, pp. 33-39.

Llonch, P. Rodríguez, Gispert, M., Dalmau, A., Manteca, X., Velarde, A. 2012b. Stunning pigs with nitrogen

- and carbon dioxide mixtures: effects on animal welfare and meat quality. *Animal* 6:4, pp. 668-675.
- Llonch, P., Rodriguez, P., Jospin, M., Dalmau, A., Manteca, X. & Velarde, A. 2013. Assessment of unconsciousness in pigs during exposure to nitrogen and carbon dioxide mixtures. *Animal* 7(3), 492-498.
- Machtolf, M., Moje, M., Troeger, K., Bülte, M. 2013. Stunning slaughter pigs with helium compared to carbon dioxide. *Fleischwirtschaft-Frankfurt*, 93(10), 118-124.
- Machtolf, M., Moje, M., Troeger, K., Bülte, M. 2014. Stunning slaughter pigs using the inert gas helium. 60th International Congress of Meat Science and Technology, 17-22 August 2014, Punta del Este, Uruguay.
- Mackie, N. & McKeegan, D. E. F. 2016. Behavioural responses of broiler chickens during low atmospheric pressure stunning. *Applied Animal Behaviour Science* 174, 90-98.
- Manning, H.L., and Schwartzstein, R.M., 1995. Pathophysiology of Dyspnea. *New England Journal of Medicine*, 333 (23): 1547-1553.
- Martin, J.E., Baxter, E.M., Farish, M., Sparrey, J., Tennant, P., Ritchie, A., McKeegan, D.E.F. 2020. Low atmospheric pressure stunning in pigs: insights from analgesic and anxiolytic interventions. FSVO/UFAW/HSO Online Symposium- Humanely Ending the Life of Animals. 3rd-4th November 2020
- Mason, G., Mendl, M. 1993. Why is there no simple way of measuring animal welfare? *Animal Welfare* 2, pp. 301-319.
- McKeegan, D., Martin, J., Baxter, E. 2020. LAPS in pigs is not a humane alternative to stunning with carbon dioxide. *The Meat Hygienist*, 180, pp. 20-22
- Mota-Rojas, D., Bolanos-Lopez, D., Concepcion-Mendez, M., Ramirez-Telles, J., Roldan-Santiago, P., Flores-Peinado, S., Mora-Medina, P. 2012. Stunning swine with CO₂ gas: Controversies related to animal welfare. *International journal of Pharmacology* 8(3) pp. 141-151.
- Nowak, B., Mueffling, T.V., Hartung, J. 2007. Effect of different carbon dioxide concentrations and exposure times in stunning of slaughter pigs: Impact on animal welfare and meat quality. *Meat Science* 75, pp. 290-298.
- McKinstry, L.J., Anil, M.H. 2004. The effect of repeat application of electrical stunning on the welfare of pigs. *Meat Science* 67, pp. 121-128.
- Neice, A. E. & Zornow, M. H. 2016. Xenon anaesthesia for all, or only a select few? *Anaesthesia*, 71(11), 1259-1272.
- Pöhlmann, V. 2018. Untersuchung zur alternativen Betäubung von Schlachtschweinen mit einem hochexpansiven, Stickstoff-gefüllten Schaum unter Tierschutz- und Fleischqualitätsaspekten [Study on the stunning of slaughter pigs with a nitrogen-filled, high-expansion foam focusing on the aspects of animal welfare and meat quality]. PhD Thesis, Freie Universität, Berlin.
- Raj, A.B.M. 1999. Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. *Veterinary record* 144, pp. 165-168.
- Raj, A.B.M., Gregory, N.G. 1995. Welfare implications of the gas stunning of pigs 1. Determination of aversion to the initial inhalation of carbon dioxide or argon. *Animal Welfare* 4, pp. 273-280.
- Raj, A.B.M., Gregory, N.G. 1996. Welfare implications of the gas stunning of pigs 2. Stress of induction of anaesthesia. *Animal Welfare* 5, pp. 71-78.
- Raj, A.B.M., Johnson, S.P., Wotton, S.B., and McKinstry, J.L., 1997a. Welfare implications of gas stunning of pigs 3. Time to loss of Somatosensory Evoked Potentials and Spontaneous Electroencephalogram of pigs during exposure to gases. *British Veterinary Journal*, 153: 329-340.
- Rault, J. L., McMunn, K. A., Marchant-Forde, J. N. & Lay, D. C. 2013. Gas alternatives to carbon dioxide for euthanasia: a piglet perspective. *Journal of Animal Science* 91, 1874.

Rushen, J. 1991. Problems associated with the interpretation of physiological data in the assessment of animal welfare. *Applied Animal Behaviour Science* 28(4), pp. 381-386.

Shaw, N.A., 2002. The neurophysiology of concussion. *Progress in Neurobiology*, 67: 281-344.

Steiner A, R., Flammer, S.A., Beausoleil, N.J., Berg. C., Bettschart-Wolfensberger, R., García Pinillos, R., Golledge, H.D.R., Marahrens, M., Meyer, R., Schnitzer, T., Toscano, M.J., Turner, P.V., Weary, D.M., Gent, T.C. 2019. Humanely ending the life of animals: research priorities to identify alternatives to carbon dioxide. *Animals* 9, 911; doi:10.3390/ani9110911

Støier, S., Dall Aaslyng, M.D., Olsen, E.V., Henckel, P. 2001. The effect of stress during lairage and stunning on muscle metabolism and drip loss in Danish Pork. *Meat Science* 59, pp. 123-131.

Velarde, A., Cruz, J., Gispert, M., Carrión, Ruiz de la Torre, J.L., Diestre, A., Manteca, X. 2007. Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype. *Animal Welfare* 16, pp. 513-522.

Verhoeven M, Gerritzen M, Velarde A, Hellebrekers H, and Kemp B. 2016. Time to loss of consciousness and its relation to behaviour in slaughter pigs during stunning with 80 or 95% carbon dioxide. *Frontiers in Veterinary Science* 3: 38.

Vogel, K. D., Badtram, G., Claus, J. R., Grandin, T., Turpin, S., Weyker, R. E. & Voogd, E. 2011. Head-only followed by cardiac arrest electrical stunning is an effective alternative to head-only electrical stunning in pigs. *Journal of Animal Science* 89, 1412.

Warner, R.D., Ferguson, D.M., Cottrell, J.J., Knee, B.W. 2007. Acute stress induced by the preslaughter use of electric prodders causes tougher beef meat. *Aust. J. Exp. Agric.* 47, 782-788.

Weary, D.M., Robbins, J.A. 2019. Understanding the multiple conceptions of animal welfare. *Animal Welfare* 28(1), pp. 33-40.

Woodbury, D.M., and Karler, R., 1960. The role of carbon dioxide in the nervous system. *Journal of American Society of Anaesthesiologists* 21: 686-703.

The **Department of Animal Environment and Health** has three publication series:

- * **Dissertations:** Masters and licentiate dissertations are published here.
- * **Reports:** Various types of scientific report from the Department are published here.
- * **Students' work:** Here, various types of student work is published, including examination work, usually comprising 7.5-30 credits. The student work is an obligatory part of various programmes and is intended, with supervision, to give the student training in finding a solution to a problem independently and in a scientific way. The content, results and conclusions of the work should therefore be assessed against this background.

More information about the Department's publications can be found here:
www.slu.se/husdjurmiljohalsa

DISTRIBUTION:

Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och
 husdjursvetenskap
Institutionen för husdjurens miljö och hälsa
Box 234
532 23 Skara
Tel 0511-67000
E-post: hmh@slu.se
Hemsida:
www.slu.se/husdjurmiljohalsa

*Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal
Science
Department of Animal Environment and Health
P.O.B. 234
SE-532 23 Skara, Sweden
Phone: +46 (0)511 67000
E-mail: hmh@slu.se
Homepage:
www.slu.se/animalenvironmenthealth*
