

Could low atmospheric pressure stunning (LAPS) be suitable for pig slaughter? A review of available information

JA Bouwsema* and JA Lines

Silsoe Livestock Systems, Wrest Park, Silsoe, Bedford MK45 4HR, UK

* Contact for correspondence: jenny.bouwsema@silsoereseearch.org.uk

Abstract

Low atmospheric pressure stunning (LAPS) is a slaughter technique which may be less stressful for pigs (*Sus scrofa domestica*) than current commercial stunning and slaughter methods. The main methods used currently for slaughtering pigs are electric and carbon dioxide stunning, both of which are widely recognised as stressful for pigs. There is currently no published research on the use of LAPS for stunning adult pigs, however there is a significant body of relevant experience from investigations into the effects of low pressure and hypoxia on humans, hypoxia for killing pigs and the use of LAPS for killing poultry, rats and piglets. In this paper, the basic physics and biology of LAPS is briefly reviewed and relevant experience from research with humans, poultry, rats and piglets is presented. On the basis of this information, some initial parameters for LAPS trials with pigs are proposed, potential welfare issues identified and an approach to achieving LAPS at a commercially viable speed is outlined. While the effects of LAPS on pigs is, at present, uncertain, the evidence from research with humans and other animals suggests that healthy, fasted pigs undergoing LAPS are unlikely to suffer from air hunger or from pain. Any pigs suffering from upper respiratory tract disease, tooth decay or excess gas in the alimentary canal may, however, experience pain. A total killing cycle is likely to require 9 to 14 min. To implement LAPS in a commercial, high throughput processing plant will require the use of multiple decompression cylinders. The evidence available suggests that LAPS could be commercially viable for pig slaughter and that for most pigs it will be less stressful than current commercial slaughter methods.

Keywords: animal welfare, decompression, domestic pigs, humane slaughter, hypobarica, hypoxia

Introduction

Low atmospheric pressure stunning (LAPS) is a stunning and slaughter method in which the ambient atmospheric pressure is gradually reduced until the partial pressure of oxygen in the atmosphere is insufficient to support brain function. This approach is of interest because it may be less stressful for pigs (*Sus scrofa domestica*) than current commercial stunning and slaughter methods.

There are no published studies directly dealing with LAPS for slaughter weight pigs so this paper provides an overview of current knowledge relevant to the development of LAPS for pigs. It remains the case, however, that actual effects of LAPS on slaughter weight pigs remain conjecture until trials have been carried out. This paper draws upon relevant published literature, grey literature, fundamental engineering principles, and extensive discussions with experts within the fields of aerospace medicine and in the use of low pressure to kill other animals with the specific aims of: (i) clarifying the basic physics and biology of the approach; (ii) identifying potential welfare problems that might be associated with LAPS; (iii) identifying a starting point for research into the use of LAPS for pig slaughter; and (iv) assessing whether LAPS might be practical on a commercial scale.

Initial literature searches were performed using the Web of Science database. Searches included LAPS and pig*; 'hypobaric hypoxia' and pig*; and 'low atmospheric pressure stunning'. Searches were broadened to the grey literature using Google and Google Scholar. References found in relevant publications were assessed and, where informative, included in this review. Further thematic searches were conducted, including for publications on LAPS for other species; controlled atmosphere stunning; and the effects of hypobarica and hypoxia in other species (including humans). Subsequent searches followed themes that emerged. In parallel, textbooks on aviation medicine were consulted, and experts in relevant academic and commercial fields were contacted and their advice sought.

In the European Union, approximately 255 million pigs are slaughtered for food every year (EU 2018). The two main slaughter methods are electrical stunning and immersion in a high concentration carbon dioxide atmosphere (EFSA 2004). The Humane Slaughter Association considers carbon dioxide stunning to be the most reliable method to ensure consistency of welfare for large-scale plants; it is, nevertheless, considered to compromise pig welfare at the time of slaughter because of pigs' aversion to high concentration carbon

Table 1 Responses of pigs exposed to 90% argon in air for 3, 5, and 7 min. Gagging, eyelid reflex and nose prick responses were recorded 50 s after removal from the gas mixture and 5 s after sticking.

	90% argon in air for:		
	3 min	5 min	7 min
Number of pigs tested	26	102	97
Percentage of pigs which gagged	96%	21%	7%
Percentage of pigs with eyelid reflex	88%	11%	4%
Percentage of pigs responding to nose prick	11%	0%	0%
Carcase convulsions	Yes	No	No

Data from Raj (1999).

dioxide (Humane Slaughter Association 2007). The Farm Animal Welfare Council deemed high concentrations of carbon dioxide to be unacceptable for stunning and killing pigs, and recommended that it be phased out by 2008 (Farm Animal Welfare Council 2003). The EU also implicitly accepts the validity of calls to phase out the use of CO₂ for pigs and justifies its continued use only on economic grounds (European Council 2009). Despite these concerns and considerable research effort, there is as yet no suitable alternative.

Electric stunning can cause stress and distress to pigs because of the need to handle the pigs and human error in the application of the stun. The development of automated systems ameliorates the welfare problems to some extent however ongoing carcass quality issues are causing the pig industry to move increasingly to the use of carbon dioxide stunning and slaughter (Velarde *et al* 2000, 2001).

As carbon dioxide can be used to stun pigs in small groups, they can be allowed to associate freely in their group avoiding the need for individual pigs to be manipulated or positioned and without the risk that poor positioning will compromise the stun. This freedom benefits the welfare of the pigs. Exposure to carbon dioxide is, however, aversive to pigs. Carbon dioxide is a pungent gas and becomes acidic as it dissolves in moisture in the eyes and respiratory tract. In humans, breathing carbon dioxide elicits air hunger, which is a sensation so distressing and urgent that it may override pain (Banzett *et al* 2007). Raj and Gregory (1995) found that pigs would avoid entering a 90% carbon dioxide atmosphere to access food such as chopped apples, even after fasting for 24 h. Cantieni (1976) found that pigs previously exposed to 70% carbon dioxide in air would choose to be deprived of water for 72 h rather than endure a second exposure. Slaughter weight pigs immersed in high concentrations of carbon dioxide (80% or higher) exhibit a range of reactions, from mild to severe aversion. Typically, behavioural reactions are noted after the first 10 s, and take the form of breathlessness, hyperventilation, vocalisation and escape attempts. Observations and EEG monitoring of pigs immersed in an atmosphere comprising 80% carbon dioxide suggest there is a period of approximately 15 s for which the animals are conscious (Farm Animal Welfare Council 2003).

Research to reduce the apparent stress experienced by pigs being stunned in carbon dioxide-rich atmospheres has focused on the use of argon and nitrogen either alone or in combination with carbon dioxide. Trials exposing pigs to argon, and mixtures of argon and carbon dioxide found no evidence to suggest that argon-induced hypoxia was aversive to pigs. Pigs offered chopped apples as a food reward inside a box filled with argon would continue to eat until they lost their footing and were forced to retreat from the box. On regaining steadiness, a number of the pigs voluntarily re-entered the box to resume eating. This was in contrast to offering the same reward in a box filled with 90% carbon dioxide, in response to which almost all pigs withdrew within 5 s and were reluctant to return, even after fasting for 24 h (Raj & Gregory 1995). These authors reported that pigs showed 'minimal respiratory distress' as hypoxia-induced unconsciousness set in when exposed to 2% oxygen in argon. The pigs lost posture after 15 s and this was followed by convulsions between 21 and 54 s later. Table 1 shows the results of observations 50 s after a 3, 5 or 7 min exposure to an atmosphere comprising 90% argon in air (ie 2% oxygen). Raj and Gregory (1996) concluded that a 5-min exposure was insufficient to assure permanent insensibility but that 7 min exposure was probably sufficient.

A similar theme emerged from more recent work in which pigs were restrained in a cradle which was lowered into a pit containing either air, or argon, or argon/carbon dioxide mixtures. Aversion was measured by recording retreat attempts, escape attempts and gasping. Although pigs exhibited aversion to 90% argon in atmospheric air compared to atmospheric air only, this aversion was lower than during exposure to gas mixtures containing nitrogen and carbon dioxide. In these trials aversion increased with carbon dioxide concentration (Dalmau *et al* 2010b).

Although exposure to an argon atmosphere with less than 2% residual oxygen appears to distress pigs less than the use of high levels of carbon dioxide, the high cost of argon makes it a poor candidate for commercial use. Current retail prices for food grade gas indicate that argon is currently around six times the price of carbon dioxide (BOC Online 2018).

Nitrogen has been proposed as an alternative to argon for inducing hypoxia. Nitrogen is comparable in price to carbon dioxide. Since the earth's atmosphere comprises 78% nitrogen, the density of nitrogen is very similar to that of air and so it is difficult to contain in a pit, such as those already in use for carbon dioxide stunning. In trials using nitrogen in a pit, it was difficult to reduce residual oxygen below 6% (Centre de Technologica de la Carn 2005). It is more easily contained if it is mixed with carbon dioxide (Centre de Technologica de la Carn 2005; Dalmau *et al* 2010a), but the introduction of carbon dioxide may compromise the welfare benefits of nitrogen (Dalmau *et al* 2010b).

Nitrogen foam has been proposed as a euthanasia method for single piglets (FISA 2015). Marahrens *et al* (2017) report that very long exposure times are needed to stun piglets (10–12 min), but that slaughter weight pigs appear to reach unconsciousness much more quickly, with

convulsions ending 77 s after immersion in the foam. However, the authors note that some of these pigs showed signs of regaining consciousness and conclude that significant refinements must be made to the method and technology before high expansion nitrogen foam could be an acceptable stunning/killing method for pigs. While nitrogen foam may be a solution for single animal euthanasia, it seems unlikely to be practical for large numbers of slaughter weight pigs in a commercial abattoir.

It would be possible to introduce pigs into an enclosure which is then filled with nitrogen, displacing the air. To reduce residual oxygen from 21 to 2% requires that 90% of the chamber air content be replaced with anoxic nitrogen. Assuming there is perfect mixing and no change in pressure, volume or temperature, this would require 2.3 chamber volumes of nitrogen. This volume is given by the relationship:

$$V_{new} = -\ln(V_{old})$$

where $\ln()$ denotes the natural logarithm of the argument, and V_{new} represents the chamber volumes of gas which must be introduced to the chamber to reduce the volume of the existing gas in the chamber to V_{old} . This relationship is a simplification of the basic room purge equation (Wikipedia 2018).

This approach is used by Meyn to stun poultry using carbon dioxide (Meyn 2018), however these birds are stunned using only 65% carbon dioxide which requires, by this calculation, only one chamber volume of new gas [$-\ln(0.35) = 1.0$]. The Meyn system reduces this volume by using a multi-stage approach and re-using the diluted gas. The cost of the significantly larger volume of nitrogen needed to stun pigs by reducing oxygen concentration to 2% may make this approach unattractive, however it is an avenue that could be explored.

The low levels of stress induced by hypoxia in the absence of carbon dioxide but the high cost of achieving this using nitrogen or argon points to the potential for low atmospheric pressure stunning (LAPS) if the potential welfare problems associated with hypobaria can be adequately managed. LAPS for slaughtering poultry is permitted in the USA, where it achieved USDA 'No objection' status in 2010; in Canada (CIFA 2013); and in the European Union (EFSA 2017; European Commission 2018).

Air hunger

Air hunger is central to the discussion of welfare outcomes in controlled atmosphere stunning methods since this is probably the main cause of stress from such systems. Air hunger is the unpleasant and increasingly urgent sensation humans experience when they hold their breath for an extended period. It should be recognised as a significant welfare issue where it occurs in animals (Beausoleil & Mellor 2015). Air hunger together with chest tightness and increased effort to breathe comprise the three sensations of dyspnoea (Nishino 2011). Of these three, air hunger is reported to be the most distressing (Lansing & Banzett 1996). Human subjects exposed to air hunger stimuli describe a feeling of impending death (Banzett *et al* 1990; Lansing *et al* 2009), and subjects in experiments sometimes volunteer that they would prefer pain to air hunger (Banzett & Moosavi 2001).

Air hunger and pain share some neural pathways (von Leupoldt *et al* 2009). It has been proposed that dyspnoea is a counter-irritant, which may detract from the perception of pain. Under experimental conditions, Banzett *et al* (2007) found painful stimuli increased the perception of dyspnoea, whereas dyspnoea was associated with no increase (or even a slight decrease) in the perception of pain. It has been suggested that this mechanism may have developed to aid survival; whilst pain signals a risk to tissue integrity, dyspnoea signals a risk to life. Under circumstances eliciting dyspnoea, temporarily ignoring pain signals may favour survival (Banzett *et al* 2007).

The mechanisms driving air hunger are only partly understood, it seems to arise from a mismatch between ventilatory drive and feedback received from a variety of sources, including the stretch receptors around the lungs. Ventilatory drive increases in response to information that ventilation is unsatisfactory, for example, signals indicating rising carbon dioxide levels in the blood. Air hunger can be elicited by both normocapnic hypoxia (normal blood carbon dioxide level but low oxygen), and by normoxic hypercapnia (normal blood oxygen level but high carbon dioxide) (Moosavi *et al* 2003) showing that air hunger is generated by an imbalance between carbon dioxide and oxygen in the blood, rather than just by elevated carbon dioxide levels. Low inspired partial oxygen pressures do not generate air hunger in healthy human subjects who are able to increase ventilation and so lower partial carbon dioxide pressures (Moosavi *et al* 2003). With free breathing, tidal lung expansion, detected by stretch receptors, will alleviate some aspects of air hunger. Humans ascending to moderate simulated altitude (4,500 m) under experimental conditions experience hypoxaemia without experiencing significant breathlessness (Nakano *et al* 2015), and participants in altitude chamber training report that exposure to hypobaric hypoxia is not unpleasant (D Gradwell, personal communication 2016).

Characteristics of LAPS

LAPS is achieved by reducing the ambient atmospheric pressure until the partial pressure of oxygen in the atmosphere is insufficient to support brain function. This section outlines the basic principles of LAPS and relevant effects of changes in atmospheric pressure on people and animals.

Pressure and altitude and the characterisation pressure profiles

The SI unit of pressure is the pascal, however pressure and pressure trajectories are reported in the literature using a range of units, including kilopascals, bars and torr. Much of the relevant aerospace literature describes pressure and pressure trajectories in terms of equivalent altitude and rates of ascent. Table 2 gives some equivalent values to assist in conversion between these metrics.

The standard atmospheric model used to relate atmospheric pressure to altitude is:

$$p = 101.325(1 - 2.25577 \times 10^{-5} h)^{5.25588}$$

where p is pressure (kPa), and h is altitude (m) (The Engineering Toolbox 2017a).

Table 2 Pressure and altitude equivalences commonly referenced in this review. These calculations are based on the standard atmospheric model given in The Engineering Toolbox (2017a).

Altitude		Pressure		
m	feet	kPa	Barr	Torr
0	0	101.3	1.01	760
1,000	3,280	89.9	0.90	674
2,000	6,560	79.5	0.79	596
3,000	9,840	70.1	0.70	526
4,000	13,120	61.6	0.62	462
5,000	16,400	54.0	0.54	405
6,000	19,680	47.2	0.47	354
7,000	22,960	41.1	0.41	308
8,000	26,240	35.6	0.36	267
9,000	29,520	30.7	0.31	231
10,000	32,800	26.4	0.26	198
11,000	36,080	22.6	0.23	170
12,000	39,360	19.3	0.19	145
13,000	42,640	16.3	0.16	123
14,000	45,920	13.8	0.14	103
15,000	49,200	11.6	0.12	87
16,000	52,480	9.6	0.10	72
17,000	55,760	8.0	0.08	60
18,000	59,040	6.6	0.07	49
19,000	62,320	5.3	0.05	40
20,000	65,600	4.3	0.04	32

It is important to recognise that a constant rate of pressure decrease (kPa s^{-1}) describes a very different pressure trajectory to that given by constant rate of increase in altitude (m s^{-1}) which, in turn, may also differ significantly from the pressure trajectory used in LAPS. The pressure profile of the first phase of LAPS decompression used to stun poultry is described by Martin *et al* (2016) as decompression from atmospheric pressure to 33 kPa in 67 s and by Holloway and Pritchard (2017) as a decompression rate of 1 kPa s^{-1} average. Both fail to alert the reader that the actual rate of decompression starts at over 2 kPa s^{-1} and decreases to less than 0.4 kPa s^{-1} (data derived from a tabulation provided by Holloway & Pritchard [2017]). Since the rate of pressure reduction is likely to impinge on the welfare of the animals (Mackie & McKeegan 2016), a fuller description of pressure reduction rates could be beneficial. Figure 1 shows a plot of this tabulated pressure vs time together with curves representing a constant rate of ascent of (127 m s^{-1}) and a constant decrease in pressure.

Water vapour pressure and oxygen availability

At normal atmospheric pressures the water vapour pressure in the lungs has only a minor influence on the availability of oxygen, however at low atmospheric pressure it becomes significant. The saturated vapour pressure (svp) of water (kPa) can be calculated as:

$$\text{svp} = 0.001T^{8.2} e^{(77.345+0.0057T-7235/T)}$$

where T is the absolute temperature (The Engineering Toolbox 2017b).

From this equation we find that at a temperature of 20°C saturated vapour pressure is 2.3 kPa and at a body temperature of 37°C it is 6.3 kPa.

As air enters the lungs it warms to close to body temperature and becomes saturated with water vapour. The partial vapour pressure is therefore close to 6.3 kPa. At normal atmospheric pressure (101 kPa), the total partial pressure of all the other gases in the air is therefore 94.7 kPa of which oxygen comprises 21%. The partial pressure of oxygen in inspired air is therefore 19.9 kPa.

At low pressure, the partial pressure of water vapour remains constant and so assumes a larger proportion of the whole. Where atmospheric pressure is 10 kPa (10% normal pressure), the water vapour pressure remains at 6.3 kPa, so the total partial pressure of the other gases is 3.7 kPa of which oxygen comprises 21%. Under these conditions the partial pressure of oxygen in inspired air is 0.78 kPa. This means that reducing atmospheric pressure to 10% of normal atmospheric pressure reduces the partial pressure of oxygen to 3.7% of its normal value.

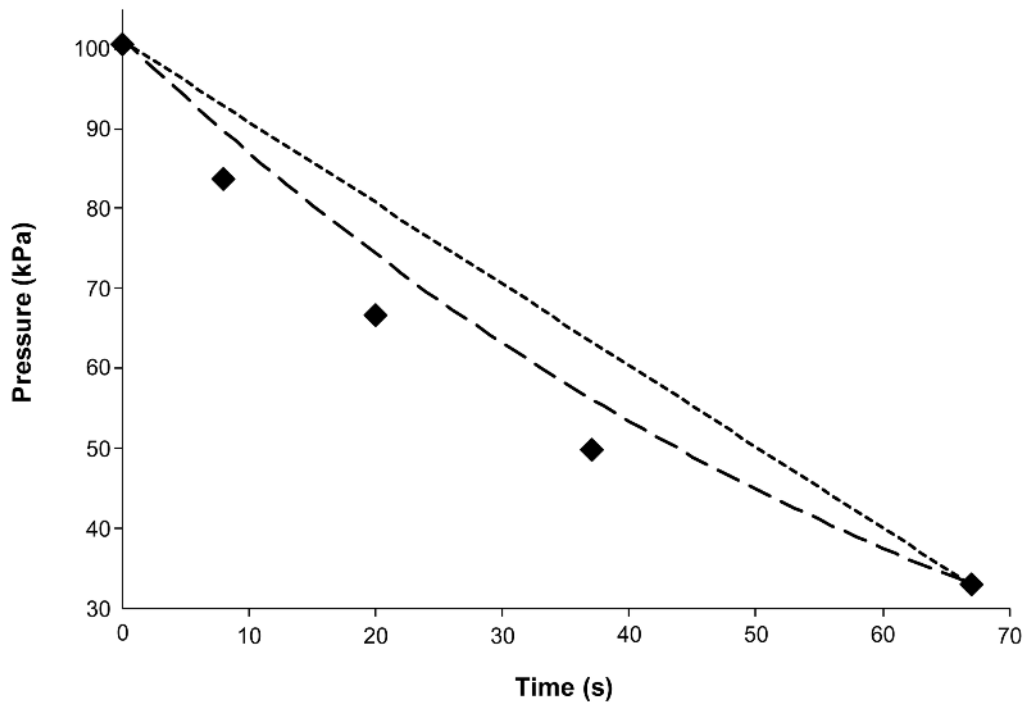
In the trials by Raj (1999) that were discussed earlier, oxygen levels were reduced to 10% of that in atmospheric air by the addition of argon. Due to the effect of the saturated vapour pressure in the lungs, the same partial pressure of oxygen in the lungs would be reached in LAPS at 16% of normal atmospheric pressure (16 kPa).

Effects of hypoxia on humans

Gradwell (2016) describes how humans experiencing hypoxia brought about through exposure to low atmospheric pressure become disoriented, exhibit slow response times, have impaired memory, become unable to complete simple tasks, and lose critical judgment and willpower. This loss of self-criticism means that the subject is usually unaware of their deteriorating condition and the presence of hypoxia. People also often report feeling euphoric, although some become belligerent or anxious. Humans exposed to altitudes above 6,000 m (47 kPa) without supplementary oxygen experience rapidly declining comprehension and mental performance, and unconsciousness sets in without warning (Gradwell 2016).

As pressure decreases, alveolar partial oxygen pressure falls, broadly in line with the air pressure, until a pressure of about 70 kPa is reached at which point the reduced arterial oxygen stimulates increased respiration. (Harding & Gradwell 1999). This reduces alveolar partial carbon dioxide pressure, and consequently increases alveolar partial oxygen pressure,

Figure 1



Pressure trajectories used in the first phase of LAPS for poultry: data points as tabulated by Holloway and Pritchard (2017), with trajectory approximations determined by constant pressure change (dotted line), and equivalent ascent of 127 m s⁻¹ (dashed line).

bringing it closer to the partial oxygen pressure of the inspired air. In humans, consciousness is lost if alveolar partial oxygen pressure falls below 4 kPa, however due to the speed of blood circulation, this loss of consciousness is delayed by at least 12 s (Harding 2002).

The effect of the rate of decrease of atmospheric oxygen pressure on subject response is poorly understood because aviation medicine has focused on human responses at fixed altitudes. Very few subjects have been exposed to gradual decompression under controlled conditions (D Gradwell, personal communication 2016).

There are also very little data on how body mass or composition affects time to unconsciousness or responses to hypoxia (D Gradwell, personal communication 2016). Most experiments and training are carried out on fit military recruits, who are relatively homogeneous in terms of age, build and body mass. There are no known studies comparing hypoxia responses across individuals with differing individual characteristics.

‘Time of Useful Consciousness’ (TUC) is a metric widely used in aviation safety planning to describe the time for which an aviator is conscious enough to take corrective action and its expiry is an early sign of declining consciousness. Accepted values for the time of useful consciousness at various altitudes and pressures are tabulated in Table 3. These data illustrate how the speed at which consciousness declines varies with available oxygen. The loss of useful consciousness however does not indicate the loss of all consciousness.

Effects of hypobaria

The body contains a number of gas-filled cavities. If the escape of this gas is restricted when ambient pressure is reduced, then the excess pressure in the cavities will result in physical stress and so may cause discomfort. Whether this stress depends on the change in absolute pressure or the change in relative pressure will be dependent on the elasticity of the cavity. This can be illustrated by comparing the effects of ascent on divers and aviators. A diver ascending from 9 m depth to the surface and an aviator ascending from the earth’s surface to 15,800 m both experience a decrease in pressure of 91 kPa. The diver experiences the reduction from 192 to 101 kPa and the aviator from 101 to 10 kPa. The force of gas on the walls of a rigid container under these two conditions will be the same, however the volume of an infinitely extendible balloon will increase to 1.9× its original size for the diver but to ten times its original size for the aviator. The effect of gas expansion in the more rigid cavities, such as teeth, may be related to the absolute change of pressure, while the effect on elastic cavities may be more closely related to the relative change in pressure. If most body cavities are elastic, then the percentage decrease in pressure per second might be a more relevant metric than the absolute rate of pressure change.

Semi-closed cavities include the lungs, middle ear and paranasal sinuses. If the Eustachian tube is not blocked then increased pressure in the middle ear relative to the nasopharynx opens the Eustachian tube, allowing

Table 3 Time of Useful Consciousness (TUC) at various altitudes and pressures. Adapted from Skybrary (2014).

Altitude (ft)	Altitude (m)	Pressure (kPa)	TUC
15,000	4,572	57.18	30 min
18,000	5,486	50.60	20–30 min
22,000	6,707	42.79	5–10 min
25,000	7,620	37.60	3–6 min
28,000	8,534	32.93	2.5–3.0 min
30,000	9,144	30.09	1–3 min
35,000	10,668	23.84	30–60 s
40,000	12,192	18.69	10–20 s
43,000	13,106	16.06	9–15 s
50,000	15,240	11.07	6–9 s

expanding air to escape easily to the atmosphere. Difficulty equalising pressure in the middle ear is more common as pressure increases because the higher external pressure can constrict the walls of the Eustachian tube preventing the flow of air into the middle ear (US Department of Transportation, Federal Aviation Administration 2008). The Eustachian tube is relatively similar in pigs and humans (Pracy *et al* 1998). As pigs are expected to become permanently insensible during decompression, there is no reasonable risk of pain being experienced due to middle ear pressure during the pressure increases as pigs are returned to atmospheric pressure at the end of the process.

The paranasal sinuses are connected with the nose via the sinus ostium. If this is inflamed, for example, by disease, then the normal passive ventilation of the sinuses during an increase or decrease of pressure may be obstructed, causing severe pain as the pressure difference between internal sinus pressure and external atmospheric pressure increases (Macmillan 1999a). The major disease of the upper respiratory tract (including sinuses) in pigs in the UK is atrophic rhinitis (Strachan 2004), although it is not widespread among the pig population, and control can be effected by good management including vaccination (Dee 2016). Pain and distress could be experienced by pigs during LAPS if they have upper respiratory tract disease.

The lungs are the largest, semi-closed, gas-filled cavity. In practice, lung damage in humans is only seen when decompression is so rapid that air cannot escape through the bronchi fast enough to equalise the pressure between the alveoli and external environment, or when subjects hold their breath. As very fast decompression is not anticipated and behavioural observations of pigs experiencing hypobaric hypoxia report heavy breathing rather than breath holding (Engle & Edwards 2010; Edwards & Engle 2011; Buzzard 2012), significant gas pressure equalisation problems in the lungs are not expected.

Closed, gas-filled cavities may include parts of the alimentary canal and the teeth. Healthy humans normally have between 0 and 400 ml of gas in the alimentary canal at sea level. As

pressure decreases this gas expands. If individuals are unable to expel this gas through the mouth or anus then a variety of symptoms, from mild discomfort to severe pain may be experienced (Macmillan 1999a). Macmillan (1999a) reports pain for 2–3% of aircrew exposures to pressures below 19 kPa. Lower rates of abdominal pain are cited in other studies; 0.12% of a sample of 885 subjects exposed to pressures between 15 and 38 kPa are reported by Torchia (2007) and 0.15% of a sample of 12,759 subjects exposed to pressures between 30 and 50 kPa is reported by Valdez (1990). In both of these studies, decompression rates varied from relatively gentle (0.5 kPa s^{-1}) to relatively fast (6 kPa s^{-1}).

Gas expansion in the alimentary canal seems to have halted one preliminary unpublished investigation into the application of LAPS for euthanasing piglets (T Whiting, personal communication 2016). These piglets were not fasted before the process. In humans, gas expansion problems are exacerbated if the individual has consumed foodstuffs known to be gas forming (Macmillan 1999a). Gases in the small bowel, which may cause discomfort or pain during decompression, may be reduced by food modification. Food withdrawal, which is routine in pigs prior to stunning, could reduce risk of gas expansion as well help prevent carcass contamination (Eikenbloom *et al* 1990).

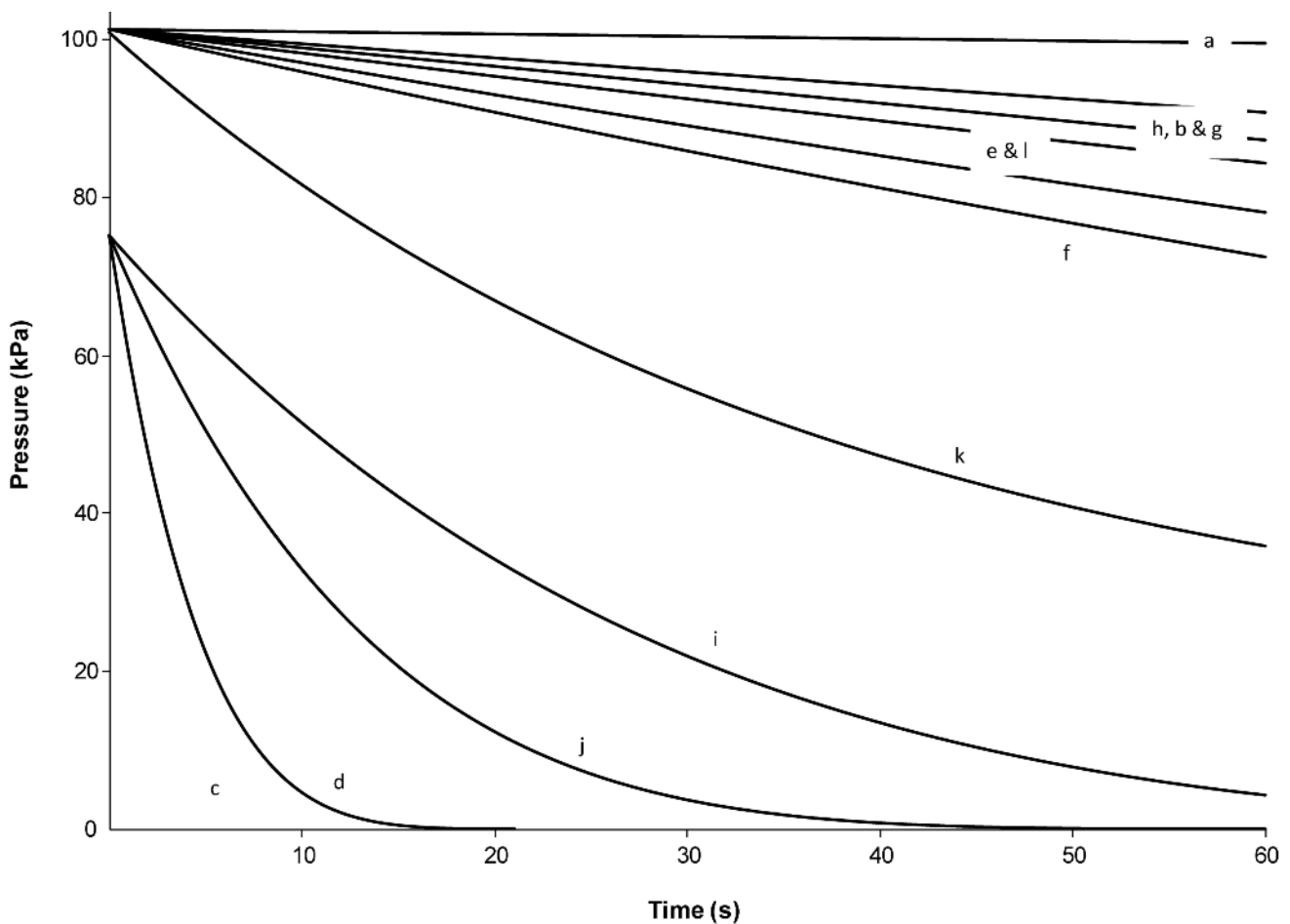
The teeth may contain small pockets of gas if dental caries have led to gas gathering in the apex of a tooth where apical abscesses are present. Lowering atmospheric pressure will lead to a relative increase in pressure within the tooth, which is experienced as pain (Macmillan 1999a). Dental abscesses in pigs tend not to be recorded by the Food Standards Agency but their prevalence appears to be low (S Wotton, personal communication 2016). Closer analysis of this health indicator may be required for a full welfare analysis of LAPS.

Decompression sickness occurs when low atmospheric pressure causes nitrogen dissolved in bodily tissues and fluids to diffuse out of solution forming gas bubbles (Macmillan 1999b). Decompression sickness occurring in divers is relatively well known but is also encountered in aircrew spending periods of time at very high altitudes. It is uncommon at altitudes below 7,500 m (38.3 kPa). Symptoms of decompression sickness are very rare within 5 min of decompression and reach a peak 20–60 min after decompression (Macmillan 1999b). If decompression sickness in pigs follows a similar time course to decompression sickness in humans, then this would be unlikely to present a welfare issue as the signs would develop after the animal has lost consciousness.

The effects of various rates of decompression

A practical decompression profile for LAPS should be as rapid as possible without compromising animal welfare, for example, by exceeding the rate at which gases trapped can escape. This section provides an overview of documented decompression profiles used in aviation with information on the well-being of people exposed to them. These profiles are summarised in Figure 2. The letter identifying each profile description corresponds with the letter shown in Figure 2.

Figure 2



Pressures during the first 60 s of decompression profiles used in aviation, in hypobaric chamber training, in commercial LAPS for chickens and in experimental LAPS for piglets. These profiles and associated responses in subjects are described in the text. In order of increasing speed they are (a) airline passengers; (h) training 0 to 29,000 ft (0 to 8,839 m) at 3,000 ft per min; (b) military pilots 0.23 kPa s⁻¹; (g) training 0–25,000 ft (0 to 7,620 m) in 5 min; (e) training 0–35,000 ft (0 to 10,668 m) in 5 min; (l) LAPS for piglets trials; (f) training 0–45,000 ft (0 to 1,3716 m) in 5 min; (k) LAPS for poultry; (i) training 8,000–29,000 ft (2,438 to 8,839 m) in 22 s; (j) training 8,000–18,000 ft (2,348 to 5,486) m in 5 s; (d) 8,000–25,000 ft (2,438–7,620 m) in 3 s and (c) training 18,000–56,000 ft (5,486–17,069 m) in 3 s.

Airline passengers

Airliner cabins are pressurised to allow them to fly at high altitudes without compromising the safety of the crew and passengers. As an airliner climbs, the pressure in its cabin is slowly dropped until it reaches a pressure of about 79.5 kPa. Many modern airliners limit their cabin ascent to 2.5 m s⁻¹ (a) (Gradwell & Macmillan 2016). This is equivalent to a pressure change of 0.03 kPa s⁻¹ at sea level.

Military pilots

The maximum rate of pressure change recommended for highly trained military pilots is 0.23 kPa s⁻¹ (b), which is equivalent to 19.4 m s⁻¹ at 0 m altitude (Gradwell & Macmillan 2016). This limit seems to refer mainly to descent, as it is on descent that most difficulties equalising pressure in the middle ear occur.

Hypobaric chamber training

During hypobaric chamber training, aircrews are regularly exposed to ascents from (c) 5,486–17,069 m over 3 s which

is equivalent to 21.41 kPa s⁻¹ in the first second, or 1,957.8 m s⁻¹ at 0 m altitude; and to (d) 2,438–7,620 m over 3 s which is equivalent to 14.94 kPa s⁻¹ in the first second, or 1,325.3 m s⁻¹ at 0 m altitude (D Gradwell, personal communication 2016). Such rapid decompressions are also associated with rapid drops in temperature and mist formation which can be alarming to human participants.

Slower decompression profiles are also used in hypobaric chamber training. A report presented to the Italian Association of Aeronautical and Space Medicine (Torchia 2007) details three decompression training profiles, in which subjects (e) ascend from 0 to 10,668 m over 5 min (35.6 m s⁻¹, max 0.43 kPa s⁻¹); (f) ascend from 0 to 1,3716 m over 5 min (43.7 m s⁻¹, max 0.52 kPa s⁻¹); and (g) ascend from 0 to 7,620 m over 5 min (25.4 m s⁻¹, max 0.30 kPa s⁻¹). Of the 885 subjects they report on over five years of testing, they reported only two problems of pain during or following ascent (0.2% prevalence). One case was tooth pain and the other abdominal pain.

Further examples of decompression profiles are given in a United States Federal Aviation Authority (FAA) report (Valdez 1990) detailing complications encountered during altitude training flights for 12,759 students over a 23-year period. The key portions of the ascent profiles involve (h) ascent from 0 to 8,839 m at 15.2 m s^{-1} (max 0.18 kPa s^{-1}); (i) ascent from 2,438 to 8,839 m in 20–24 s (max 2.71 kPa s^{-1} , equivalent to 227.8 m s^{-1} at 0 m altitude); and (j) ascent from 2,348 to 5,486 m in 5 s (max 5.58 kPa s^{-1} , equivalent to 475.3 m s^{-1} at 0 m altitude). Complications experienced by the 12,759 participants include 882 cases of aerotitis media (inflammation of the middle ear, caused by changing air pressures) and 200 cases of aerosinusitis; 15 cases of hyperventilation; 19 cases of abdominal distress, two cases of claustrophobia, and ten cases of decompression sickness. It is not specified whether these complications were encountered on ascent or descent, but inflammation of the middle ear, as experienced by the largest of these groups (totalling 882 cases), is experienced predominantly during descent (Weber *et al* 2014). Neglecting, therefore, half of these cases of ear pain, and those of decompression sickness (probably irrelevant to LAPS due to its time course) gives a total of 675 reported problems and a prevalence of less than 5%. Even when exposed to very rapid ascents humans seldom experience problems due to reduced pressure.

Trials with LAPS

Poultry

The LAPS system for poultry developed by Technocatch LLC uses a 280-s cycle, in two phases. In the first phase, the vacuum chamber pressure is reduced from atmospheric pressure (101 kPa) to 33 kPa (8,500 m equivalent) over 67 s. This is identified as profile (k) in Figure 2. In the second phase, the pumping speed is reduced as the chamber pressure is dropped to a minimum pressure of 20 kPa (Martin *et al* 2016). Evidence of stress and pain has not been found (Mackie & McKeegan 2016). The physical process is described in detail by Holloway and Pritchard (2017). The second stage of this two-stage process seems to be little related to welfare (Mackie & McKeegan 2016) but is claimed to result in better meat quality by reducing clonic spasms and by stabilising tissue pH (Cattaruzzi & Cheek 2008, 2011). The relevance for pigs of this second stage is unclear.

Rats

Rats exposed to decompression from 101 to 20 kPa over 30 s (450 m s^{-1} equivalent) exhibited no observable effects until pressure reached 26 kPa (25 s after the start). The rats exhibited final righting behaviours a maximum of 60 s after behavioural changes were noted and were removed and confirmed as dead 5 min after the behavioural changes were noted. Observations suggested that there was no aversive response to the dropping pressure, although specific aversion tests were not conducted (Talling 2017). No injuries were discovered during post mortem examination. These data illustrate the high tolerance that rats have to hypoxia.

Piglets

A small number of attempts have been made to investigate the use of LAPS for on-farm piglet euthanasia. An investigation into the viability of using hypobaric hypoxia to euthanase unthrifty or moribund piglets is described in a number of sources (Engle & Edwards 2010; Edwards & Engle 2011; Buzzard 2012). This experiment compared hypobaric hypoxia to carbon dioxide euthanasia. The authors report that the piglets were placed in a chamber that was evacuated at a simulated rate of ascent of 36.9 m s^{-1} . This is identified as profile (l) in Figure 2. One of the three accounts reports a peak simulated altitude of 18,000 m (Buzzard 2012). At this point the pressure inside the chamber would have been 6.6 kPa. Based on the evacuation rate reported, this would have been achieved after 10.4 min. Fifty-eight piglets were tested in pairs, one held in a sling, and fitted with EEG electrodes, and one free on the floor of the chamber. Death was confirmed by the observation of an isoelectric EEG for the piglet in the sling, and cessation of movement and breathing for 5 consecutive min for the piglet on the floor. Average treatment time was $27.4 (\pm 6.7)$ min. Sixty percent of the piglets were reported as vocalising during the first few minutes of the treatment and approximately one-third of the piglets were reported as gasping in the first 5 min. All the piglets exposed to carbon dioxide are reported to have vocalised and gasped however gasping in carbon dioxide atmosphere was for a shorter duration, presumably due to the different time courses of the two treatments (Buzzard 2012).

This account can be compared directly with the results of Raj (1999). Raj, working with argon, found that at 2% residual oxygen pigs lost consciousness (loss of posture) within 15 s. Buzzard's (2012) reported evacuation rate equates to a decompression rate of 0.45 kPa s^{-1} in the first seconds. Taking into account the saturated vapour pressure of water in the lungs (6.3 kPa at 37°C), an equivalent inspired partial oxygen pressure to that reported by Raj (1999) would have occurred at a pressure of 16.5 kPa, which would have been reached after approximately 6 min. For humans, the time of useful consciousness at this pressure is 9–15 s (Skybrary 2014). Raj concluded that a further 7-min exposure to these conditions was required to ensure no recovery occurred. This point would have been passed in the piglet experiment at 13 min. Engle and Edwards' (2010) report of the piglet experiment indicates that all activity stopped at between 15.7 and 29.1 min.

The time of 15.7 min appears consistent with Raj (1999), however, the possibility of convulsions continuing for another 13 min is more surprising. This may, however, indicate that the target decompression rate was not achieved consistently throughout the decompression, in which case it may have taken longer than expected to reach the target pressure. Alternatively, these long times may be due to the resilience of young piglets to hypoxic conditions (Arieli *et al* 2008; Marahrens *et al* 2017).

Edwards and Engle (2011) report possible hypobaric injury in the piglet study. Pustules and gas bubbles were noted

beneath the skin of a number of trial animals following euthanasia. Whilst only 1.7% of piglets euthanased using carbon dioxide exhibited post-euthanasia lesions, 21% of pigs euthanased using hypobaric hypoxia displayed post-euthanasia lesions. Lesions noted included small (3–5 mm) pustules in the epidermis, emphysema of subcutaneous tissues, appearing as small air bubbles in the fat and fascia of the subcutis, and lung lesions, ranging from diffuse pulmonary oedema to mild multifocal pleural petechiae.

It seems likely that these pustules were due to the final pressure achieved in the chamber which was close to the Armstrong limit. The normal body temperature for pigs is 39°C (Jackson & Cockcroft 2007) and at this temperature the saturated vapour pressure of water is 7 kPa (The Engineering Toolbox 2017b). Since this exceeds atmospheric pressure (6.6 kPa), it is likely that there was some boiling of fluids which could have caused the pustules. Due to the low pressure the animals were unlikely to have been conscious at this point.

An unrelated and unpublished investigation into LAPS and pigs took place in Manitoba, Canada. Piglets were culled at the end of weaning (4 kg) and at 22 kg. Brief trials were conducted, but not continued, possibly due to gas-filled viscera and colon fermentation. The piglets were not fasted before participation (T Whiting, personal communication 2016). Rates of decompression, final pressures, and timings are not known. No report was published.

A practical LAPS system for pigs

If LAPS is to be considered for the commercial slaughter of pigs, then it is important that it is both practical and cost efficient. In this section, possible LAPS parameters are discussed, with a view to describing how LAPS might be implemented in a commercial abattoir.

Pressure reduction rate, end pressure and dwell time

Raj (1999) concluded that the pigs needed to be held for 7 min in an atmosphere of 2% oxygen if signs of recovery were to be avoided. As previously discussed, this is likely to be equivalent to a pressure of 16.5 kPa. Data from Purswell (2007) show that the time to death (cessation of ventilatory movements) for poultry at low pressure reduced from 115 s at 29.5 kPa to 40 s at 17.8 kPa in an almost linear fashion. It is therefore reasonable to assume that the use of a minimum pressure below 16.5 kPa could reduce this 7-min hold time. The ultimate pressure should however not be below the Armstrong limit which is about 7 kPa to avoid the risk of carcass damage.

The decompression profiles shown in Figure 2 split roughly into two groups. The majority of the aviation pressure profiles are relatively gradual while four of the profiles are faster by an order of magnitude. These fast decompressions are relatively unproblematic for prepared human subjects (Valdez 1990) however for LAPS, initial calculations will focus on the profile which is the fastest of the more gradual group of profiles shown in Figure 2. This uses a rate based on ascent at 45.7 m s⁻¹. This rate is similar to that used by Buzzard (2012) (37 m s⁻¹) and significantly lower than the rate used for poultry

(127 m s⁻¹). Trials suggest it to be safe and pain-free for humans. This therefore represents a cautious starting point for trials where there is little reason to expect problems.

Decompression to 16 kPa at this rate would take 5.7 min. Adding to this time the 7 min to avoid recovery, 1 min to load the chamber and 1 min to decompress and unload, one stun/kill cycle would take 14.7 min.

By way of comparison, if the decompression rate used for poultry was found to be suitable for pigs, and a final pressure of 10 kPa enabled the hold time to be reduced to 5 min, then the total cycle time would reduce to 9.5 min. This much higher rate of decompression is still far lower than is routinely used for humans in hypobaric chamber training.

These cycle times are speculative — experimental observation should inform whether the ascent rate is suitable, observing for signs of aversion in the conscious pigs, and how consciousness is affected during decompression.

LAPS cylinder design

A maximum stocking density for transporting pigs is 235 kg m⁻² (Defra 2006). Assuming an average pig liveweight of 100 kg this indicates a density of 2.35 pigs m⁻². In transport vehicles, pigs can be gathered into groups at this density easily without undue coercion. In Europe, pigs tend to be transported in units of around 15 animals and mixing groups is avoided to prevent pigs from fighting (M Parker, personal communication 2016). These figures suggest that the maximum useful floor area of 6.39 m² per group of 15, and a headroom of 1 m is required in a LAPS chamber. This space is efficiently supplied in a cylinder of diameter 2.3 m and length 3.2 m. This also ensures that the pig space is reasonably square, maximising the possibility of free movement. Such a cylinder suitable for 15 pigs would have a volume of 12.2 m³.

A more efficient 'double decker' design would have two layers of pigs in a cylinder of diameter 2.8 m and a length of 3.2 m. This would hold 30 pigs and have a volume of 20 m³. While 30 pigs could also be contained in a cylinder in a single layer, this would require significantly more space and have a larger volume to be evacuated. Management of the pigs to maintain two separate groups might be no less complex.

The cycle time estimate of 14.7 min results in a processing rate of 122 pigs per hour for a LAPS cylinder accommodating 30 pigs. Using the shorter cycle time estimate of 9.5 min results in a throughput for a single cylinder of 189 pigs per hour. Multiple LAPS cylinders will therefore be required in high throughput plants. Due to the need for large air pumps and tubing these cylinders are likely to be stationary. This will result in a more complex pig-handling system than is currently required for carbon dioxide stunning where all the pigs enter at the same point.

Stun to stick intervals are likely to be longer than with current carbon dioxide systems and may depend on the number of cylinders operating and the re-pressurisation time required. Since LAPS must aim to be irreversible, the stun to stick interval is not a welfare issue, however to preserve the quality of the meat, good bleed-out remains important.

There are no known published studies on whether a delay before bleeding has an effect on the total volume of blood lost and subsequent meat quality. However, as LAPS is not expected to cause any wounds, which would stimulate clotting, a reasonable delay before bleed-out should not affect total blood loss. This conclusion is supported by observations and preliminary unpublished tests by the University of Bristol (S Wotton, personal communication 2017) which found no indication of reduction in carcass quality for stun to stick intervals of up to 30 min.

Animal welfare implications

It is probable that many of the 255 million pigs slaughtered each year in the EU experience stress or suffering at slaughter. The available information indicates that LAPS is likely to cause healthy, well-prepared pigs significantly less stress or suffering than current commercial methods. The development and uptake of LAPS is therefore likely to significantly benefit the welfare of pigs at slaughter.

Conclusion

The literature covered in this review suggests that LAPS for pigs may be both practical and less stressful to most pigs than current commercial slaughter systems. LAPS could provide consistent, irreversible pre-slaughter stunning for pigs whilst they remain in social groups, minimising handling and social stress. It may offer significant welfare advantages over carbon dioxide stunning since it does not result in acidity in the eyes or mucous membranes and appears not to cause air hunger. However, pain and distress may be experienced by pigs with excess intestinal gas, and pigs suffering from inflammation of the upper respiratory tract or tooth decay, therefore, as with all such work, trials should be carried out with due attention to ethical review, and to high welfare standards. LAPS trials should start with a decompression rate of between 0.43 and 0.52 kPa s⁻¹ since piglets and many hundreds of human volunteers have experienced this rate of decompression without significant problems. This rate is lower than the maximum decompression rate currently used for poultry. The ultimate pressure required is likely to be between 16 and 7 kPa to minimise the stunning cycle duration without risking carcass damage. Total cycle times are likely to be 9 to 14 min. The delay between death and bleed-out necessitated by LAPS is unlikely to result in carcass quality issues. A LAPS system would be scalable, adjustable to the needs of small and large plants by varying the number of cylinders. Multiple cylinders would allow large plants to achieve a high throughput using LAPS and these may need to be designed to accommodate two layers of pigs.

Acknowledgements

This review draws on a report prepared for and funded by the Agriculture and Horticulture Development Board (AHDB Pork). The authors are grateful to Katja Stoddart for initiating this review; to Professor David Gradwell for sharing his knowledge on high altitude medicine; to Andy Grist and Steve Wotton for their insight into pig health; and to Peter Kettlewell and Miriam Parker for their advice on pig handling.

References

- Arieli R, Vitenstein A and Peled E** 2008 Acclimation to hypoxia does not improve hypoxic survival of the immature pig in confined atmosphere. *Military Medicine* 173: 107-111. <https://doi.org/10.7205/MILMED.173.1.107>
- Banzett RB, Gracely RH and Lansing RW** 2007 When it's hard to breathe, maybe pain doesn't matter. Focus on 'Dyspnea as a noxious sensation: inspiratory threshold loading may trigger diffuse noxious inhibitory controls in humans'. *Journal of Neurophysiology* 97: 959-960. <https://doi.org/10.1152/jn.00970.2006>
- Banzett RB, Lansing RW, Brown R, Topulos GP, Yager D, Steele SM, Londoño B, Loring SH, Reid M, Adams L and AI E** 1990 'Air hunger' from increased pCO₂ persists after complete neuromuscular block in humans. *Respiration Physiology* 81: 1-17. [https://doi.org/10.1016/0034-5687\(90\)90065-7](https://doi.org/10.1016/0034-5687(90)90065-7)
- Banzett RB and Moosavi SH** 2001 Dyspnea and pain: Similarities and contrasts between two very unpleasant sensations. *American Pain Society Bulletin* 11: 1-8
- Beausoleil NJ and Mellor DJ** 2015 Introducing breathlessness as a significant animal welfare issue. *New Zealand Veterinary Journal* 63: 44-51. <https://doi.org/10.1080/00480169.2014.940410>
- BOC Online** 2018 Gas A-Z. <https://www.boconline.co.uk/shop/en/uk/gas-a-z>
- Buzzard B** 2012 *Evaluation of hypobaric hypoxia as a low stress alternative to carbon dioxide euthanasia for use with nursery piglets*. MSc thesis, Kansas State University, USA
- Cantieni J** 1976 Ein Beitrag zur CO₂-Betaubung von Schlachtschweinen. *Schweiz Arch Tierheilk* 119: 355-37. [Title translation: A contribution to CO₂ stunning of slaughter pigs]
- Cattaruzzi B and Cheek H** 2008 *European patent application: Method for humanely stunning and slaughtering poultry using controlled low atmospheric pressure*. Application number 08150478.9. https://worldwide.espacenet.com/publicationDetails/originalDocument?FT=D&date=20120201&DB=EPODOC&locale=en_EP&CC=EP&NR=2055191B1&KC=B1&ND=5#
- Cattaruzzi B and Cheek H** 2011 *European patent specification: Method for decreasing spastic wing activity of poultry during slaughter*. Application number 11184971.7. https://worldwide.espacenet.com/publicationDetails/originalDocument?FT=D&date=20120418&DB=EPODOC&locale=en_EP&CC=EP&NR=2441331A1&KC=A1&ND=4#
- Centre de Technologica de la Carn** 2005 *Research Project Final Report: Containability and aversiveness of different gas mixtures used for the stunning of slaughter weight pigs*. Project MH0135. DEFRA: London, UK
- CIFA (Canadian Food Inspection Agency)** 2013 *Letter of New Technology or Procedure Final Assessment Track No MPD-NTech/Pro 2013-21-Low Atmospheric Pressure Stunning Technology*. CIFA: Ottawa, Canada
- Dalmau A, Llonch P, Rodríguez P, Ruiz-de-la-Torre J, Manteca X and Velarde A** 2010a Stunning pigs with different gas mixtures: gas stability. *Animal Welfare* 19: 315-323
- Dalmau A, Rodríguez P, Llonch P and Velarde A** 2010b Stunning pigs with different gases: aversion in pigs. *Animal Welfare* 19: 325-333
- Dee SA** 2016 *Overview of respiratory diseases of pigs*. <https://www.msdtvetmanual.com/respiratory-system/respiratory-diseases-of-pigs/overview-of-respiratory-diseases-of-pigs>

- Defra** 2006 *Welfare of Animals (Transport)(England) Order 2006*. Defra: London, UK
- Edwards LN and Engle TE** 2011 Evaluation and application of humane hypoxia euthanasia for nursery pigs. *Allen D Leman Swine Conference* pp 235–241. 17-20 September 2011, St Paul, MN, USA
- 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 commercial species of animals. *The EFSA Journal* 45: 1-29. <https://doi.org/10.2903/j.efsa.2004.45>
- EFSA** 2017 Low atmospheric pressure system for stunning broiler chickens. *EFSA Journal* 15(12): 5056. <https://doi.org/10.2903/j.efsa.2017.5056>
- Eikenbloom G, Bolick AH and Sybesman W** 1990 Effects of feed withdrawal before delivery on pork quality and carcass yield. *Meat Science* 29: 25-30. [https://doi.org/10.1016/0309-1740\(91\)90020-Q](https://doi.org/10.1016/0309-1740(91)90020-Q)
- Engle TE and Edwards LN** 2010 *Evaluation and application of humane hypoxia euthanasia for nursery pigs*. Pork Checkoff. <https://www.pork.org/research/evaluation-and-application-of-humane-hypoxia-euthanasia-for-nursery-pigs/>
- EU** 2018 *Pigmeat production statistics*. <http://agridata.ec.europa.eu/extensions/DashboardPigmeat/PigmeatProductionAnalysis.html>
- European Commission** 2018 *Commission Implementing Regulation (EU) 2018/723 of 16 May 2018 amending Annexes I and II to Council Regulation (EC) No 1099/2009 on the protection of animals at the time of killing as regards the approval of low atmospheric stunning*. EC: Brussels, Belgium
- European Council** 2009 European Council Regulation No 1099/2009 on the protection of animals at the time of killing. *Official Journal of the European Union L303*: 1-30
- Farm Animal Welfare Council** 2003 *Report on the Welfare of Farmed Animals at Slaughter or Killing Part 1: Red Meat Animals*. FAWC: London, UK
- FISA** 2015 *Study on the humane stunning and killing of non-viable piglets with a nitrogen-filled, highly expansive foam on farm level*. http://www.fisaonline.de/index.php?lang=en&act=projects&view=details&p_id=8663
- Gradwell DP** 2016 Hypoxia and hyperventilation. In: Gradwell DP and Rainford DJ (eds) *Ernsting's Aviation and Space Medicine, Fifth Edition* p 904. CRC Press: Boca Raton, FL, USA
- Gradwell DP and Macmillan AJF** 2016 Oxygen systems, pressure cabin and clothing. In: Gradwell DP and Rainford DJ (eds) *Ernsting's Aviation and Space Medicine, Fifth Edition*. CRC Press: Boca Raton FL, USA
- Harding RM** 2002 Pressure changes and hypoxia in aviation. In: Pandolf KB and Burr RE (eds) *Medical Aspects of Harsh Environments, Volume 2* pp 984-1012. The Office of the Surgeon General, The United States Army: Falls Church, Virginia, USA
- Harding RM and Gradwell DP** 1999 Hypoxia and hyperventilation. In: Ernsting J, Nicholson AN and Rainford DJ (eds) *Aviation Medicine, Third Edition* pp 43-58. Butterworth-Heinemann: Oxford, UK
- Holloway PH and Pritchard DG** 2017 Effects of ambient temperature and water vapor on chamber pressure and oxygen level during low atmospheric pressure stunning of poultry. *Poultry Science* 96: 2528-2539. <https://doi.org/10.3382/ps/pex066>
- Humane Slaughter Association** 2007 *Carbon Dioxide Stunning and Killing of Pigs*. HSA: Wheathampstead, UK
- Jackson PGG and Cockcroft PD** 2007 *Handbook of Pig Medicine*. Elsevier: Philadelphia, PA, USA
- Lansing RW and Banzett RB** 1996 Psychophysical methods in the study of respiratory sensation. In: Adams L and Guz A (eds) *Respiratory Sensation, Volume 90* pp 69-100. Marcel Dekker Inc: New York, USA
- Lansing RW, Gracely RH and Banzett RB** 2009 The multiple dimensions of dyspnea: Review and hypotheses. *Respiratory Physiology and Neurobiology* 167: 53-60. <https://doi.org/10.1016/j.resp.2008.07.012>
- Mackie N and McKeegan DEF** 2016 Behavioural responses of broiler chickens during low atmospheric pressure stunning. *Applied Animal Behaviour Science* 174: 90-98. <https://doi.org/10.1016/j.applanim.2015.11.001>
- Macmillan AJF** 1999a The effects of pressure change on body cavities containing gas. In: Ernsting J, Nicholson AN and Rainford D (eds) *Aviation Medicine, Third Edition* pp 13-18. Butterworth-Heinemann: Oxford, UK
- Macmillan AJF** 1999b Sub-atmospheric decompression sickness. In: Ernsting J, Nicholson AN, and Rainford DJ (eds) *Aviation Medicine, Third Edition* pp 19-25. Butterworth-Heinemann: Oxford, UK
- Marahrens M, Pohlmann V, Balzer K, Stehle E, El Amarti A, von Frantzius I, Piotrowski D, Kiezenbrink H and Schwarzlose I** 2017 Killing of slaughter pigs and non-viable piglets with nitrogen gas filled high expansive foam. In: Beausoleil NJ, Golledge HDR, Garcia R and Gibson T (eds) *Welfare Impacts of Controlled Atmosphere Methods for Stunning or Killing Animals* p 15. 29-30 June 2017, Weybridge, UK. UFAW: Wheathampstead, Herts, UK
- Martin JE, Christensen K, Vizzier-Thaxton Y, Mitchell MA and McKeegan DEF** 2016 Behavioural, brain and cardiac responses to hypobaric hypoxia in chickens. *Physiology & Behaviour* 163: 25-36. <https://doi.org/10.1016/j.physbeh.2016.04.038>
- Meyn** 2018 *Meyn multistage stunning system*. <https://www.meyn.com/products/live-bird-handling/multistage-co2-stunning-system>
- Moosavi SH, Golestanian E, Binks AP, Lansing RW, Brown R and Banzett RB** 2003 Hypoxic and hypercapnic drives to breathe generate equivalent levels of air hunger in humans. *Journal of Applied Physiology* 94: 141-154. <https://doi.org/10.1152/jappphysiol.00594.2002>
- Nakano T, Iwazaki M, Sasao G, Nagai A, Ebihara A, Iwamoto T and Kuwahira I** 2015 Hypobaric hypoxia is not a direct dyspnoegenic factor in healthy individuals at rest. *Respiratory Physiology and Neurobiology* 218: 28-31. <https://doi.org/10.1016/j.resp.2015.07.009>
- Nishino T** 2011 Dyspnoea: Underlying mechanisms and treatment. *British Journal of Anaesthesia* 106: 463-474. <https://doi.org/10.1093/bja/aer040>
- Pracy JP, White A, Mustafa Y, Smith D and Perry ME** 1998 The comparative anatomy of the pig middle ear cavity: a model for middle ear inflammation in the human? *Journal of Anatomy* 192: 359-368. <https://doi.org/10.1046/j.1469-7580.1998.19230359.x>
- Purwell JL, Thaxton JP and Branton SL** 2007 Identifying process variables for a low atmospheric pressure stunning-killing system. *Journal of Applied Poultry Research* 16: 509-513. <https://doi.org/10.3382/japr.2007-00026>

- Raj ABM** 1999 Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: welfare implications. *Veterinary Record* 144: 165-168. <https://doi.org/10.1136/vr.144.7.165>
- Raj ABM and Gregory NG** 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: 273-280
- Raj ABM and Gregory NG** 1996 Welfare implication of the gas stunning of pigs 2: stress of induction of anaesthesia. *Animal Welfare* 5: 71-78
- Skybrary** 2014 *Time of useful consciousness*. http://www.skybrary.aero/index.php/Time_of_Useful_Consciousness
- Strachan D** 2004 *The disease status of the UK pig industry*. <http://www.thepigsite.com/articles/1627/the-disease-status-of-the-uk-pigindustry/>
- Talling J** 2017 Challenges of using controlled atmosphere killing for wild animals. In: Beausoleil NJ, Golledge HDR, Garcia R and Gibson T (eds) *Welfare Impacts of Controlled Atmosphere Methods for Stunning or Killing Animals* p 28. 29-30 June 2017, Weybridge, UK. UFAW: Wheathampstead, UK
- The Engineering Toolbox** 2017a *Altitude above sea level and air pressure*. http://www.engineeringtoolbox.com/air-altitude-pressure-d_462.html
- The Engineering Toolbox** 2017b *Water vapour and saturation pressure in humid air*. http://www.engineeringtoolbox.com/water-vapor-saturation-pressure-air-d_689.html
- Torchia F** 2007 *Physiological training in hypobaric chamber*. XX Congresso AIMAS. 4-7 September 2007, Florence, Italy. http://www.aimas.it/convegna/atti2007/06_09_torchia.pdf
- US Department of Transportation: Federal Aviation Administration** 2008 *Aeromedical Factors. Pilot's Handbook of Aeronautical Knowledge* pp 1-20. US Department of Transportation: Oklahoma City, OK, USA. https://www.faa.gov/regulations_policies/handbooks_manuals/aviation/phak/
- Valdez CD** 1990 *The FAA altitude chamber training flight profile: A Survey of Altitude Reactions 1965-1989*. Civil Aeromedical Institute, Federal Aviation Administration: Oklahoma City, OK, USA. https://www.faa.gov/data_research/research/med_humanfacs/oamtechreports/1990s/media/AM90-12.pdf
- Velarde A, Gispert M, Faucitano L, Alonso P, Manteca X and Diestre A** 2001 Effects of the stunning procedure and the halothane genotype on meat quality and incidence of haemorrhages in pigs. *Meat Science* 58: 313-319. [https://doi.org/10.1016/S0309-1740\(01\)00035-3](https://doi.org/10.1016/S0309-1740(01)00035-3)
- Velarde A, Gispert M, Faucitano L, Manteca X and Diestre A** 2000 Survey of the effectiveness of stunning procedures used in Spanish pig abattoirs. *The Veterinary Record* 146: 65-68. <https://doi.org/10.1136/vr.146.3.65>
- von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B and Büchel C** 2009 Dyspnea and pain share emotion-related brain network. *NeuroImage* 48: 200-206. <https://doi.org/10.1016/j.neuroimage.2009.06.015>
- Weber R, Kuehnel T, Graf J and Hosemann W** 2014 *Aerosinusitis: part I: Fundamentals, pathophysiology and prophylaxis*. *HNO* 62: 57-64. <https://doi.org/10.1007/s00106-013-2791-3>
- Wikipedia** 2018 *Dilution*. <https://en.wikipedia.org/wiki/Dilution>